PREVALENCE AND CHARACTERIZATION OF INTESTINAL AND EXTRA-INTESTINAL *ESCHERICHIA COLI*

Thesis Submitted For the Award of the Degree of

DOCTOR OF PHILOSOPHY

In

Microbiology

By

Gulshan Kumar

(Registration No. 41500159)

Supervised by

Dr. Gaurav Kumar

Associate Professor

Lovely Professional University

Phagwara (Punjab)

Co-Supervised by

Dr. Yashwant Kumar

Assistant Director

Central Research Institute

Kasauli (HP)

LOVELY PROFESSIONAL UNIVERSITY

Transforming Education Transforming India

LOVELY PROFESSIONAL UNIVERSITY

PUNJAB

2022

PREVALENCE AND CHARACTERIZATION OF INTESTINAL AND EXTRA-INTESTINAL *ESCHERICHIA COLI*

Thesis Submitted For the Award of the Degree of

DOCTOR OF PHILOSOPHY

In

Microbiology

By

Gulshan Kumar

(Registration No. 41500159)

Supervised by

Dr. Gaurav Kumar

Associate Professor

Lovely Professional University

Phagwara (Punjab)

Co-Supervised by

Dr. Yashwant Kumar

Assistant Director

Central Research Institute

Kasauli (HP)

LOVELY PROFESSIONAL UNIVERSITY

PUNJAB

2022

LOVELY PROFESSIONAL UNIVERSITY

Transforming Education Transforming India

DECLARATION

I hereby declare that this thesis titled **Prevalence and Characterization of Intestinal and Extra-intestinal *Escherichia coli*** is an authentic record of my work carried out at Lovely Professional University and submitted for the award of Degree of Philosophy (Ph. D.) in Microbiology under the guidance of Dr. Gaurav Kumar has not been submitted either in part or full to any degree/diploma awarding Institute/University.

30th Dec. 2022

Gulshan Kumar

Reg. No. 41500159

LOVELY PROFESSIONAL UNIVERSITY

Transforming Education Transforming India

CERTIFICATE

This is to certify that this Ph. D. research work titled **Prevalence and Characterization of Intestinal and Extra-intestinal *Escherichia coli*** was carried out and written by Gulshan Kumar (Registration No. 41500159) under my guidance and has been read and approved by the undersigned as adequate in scope and quality for the award of Doctor of Philosophy Degree in Microbiology. To the best of my knowledge, no part of this thesis was ever submitted either in part or full to any degree/diploma awarding University/Institution.

Laboratory Experimentations involved in this Ph. D. work were carried out by the candidate himself at Central Research Institute, Kasauli under our supervision.

Dr. Gaurav Kumar

Supervisor

Dated: 30.12.2022

Dr. Yashwant Kumar

Co-Supervisor

Dated: 30/12/2022

ABSTRACT

Escherichia coli (*E. coli*) is the most ubiquitous bacterium and can be easily isolated from animal, human, food, or environmental sources. Usually, *E. coli* remains as commensal in the gastrointestinal tract of the animal and humans. In the gastrointestinal tract, it helps avoid colonization of the gut with pathogenic microorganisms and in the absorption of vitamin K. However, pathogenic strains of *E. coli* are also the commonest agents of hospital-acquired and community infections. Pathogenic *E. coli* can infect different host sites and can lead to different disease presentations. *E. coli* infections range from self-limiting diarrhea to severe childhood diarrhea, Urinary tract infections of different severity, septicemia, sepsis, and neonatal meningitis. Pathogenic *E. coli* are also very diverse in terms of epidemiological markers, pathogenesis mechanisms, virulence factors, and drug resistance profiles. *E. coli* is the commonest cause of high mortality, morbidity, and disease burden worldwide. The situation in the developing world including India is more serious due to dense populations, poor socioeconomic conditions, poor hygiene levels, and the unavailability of well-developed healthcare infrastructure. This state is further grieved by emerging drug-resistant strains against pathogenic *E. coli*. Multiple drug-resistant (MDR) *E. coli* strains with resistance to newer and even reserved emergency antimicrobials are reported worldwide. This makes the treatment of drug-resistant *E. coli* infections complicated. Keeping this in consideration World Health Organization (WHO) has placed Extended-spectrum β-lactamase (ESBL) expressing and carbapenems-resistant Enterobacteriaceae (*Klebsiella* and *E. coli)* under "critical pathogens"- the highest priority level, in its list of antibiotic-resistant priority pathogens, Similarly Centre for Disease Control and Prevention (CDC) in its latest antimicrobial resistance report-2019 have kept it under "Urgent Threat" category which is the highest level concern for human health. *E. coli* has also been recognized as a pathogen of public interest in India's National Action Plan on Anti-Microbial Resistance (NAP-AMR). Data on the prevalence of pathogens, their virulence characteristics, and local antimicrobial profiles play a crucial role in deciding appropriate intervention and control measures.

This study was designed to study the prevalence and characteristics of pathogenic intestinal (human diarrheal isolates) and extra-intestinal (human UPEC isolates) *E. coli* in India. This was planned by studying pathogenic *E. coli* isolates from different geographical locations of the country for their prevalent serotypes, Diarrhea-genic *E. coli* (DEC) pathotypes, virulence characteristics, and resistance profiles. To achieve these objectives, *E. coli* human isolates referred to National Salmonella and Escherichia Center, Central Research Institute, Kasauli from different geographical locations of India were taken for the study. A total of 783 isolates referred as suspected to be *E. coli* were initially evaluated by morphological, cultural/growth, and biochemical characterization using standard microbiological techniques i.e. Gram staining, microscopy, and utilization of different biochemical. Out of 783 isolates 534 isolates were identified as *E. coli* which gave results consistent with *E. coli* in biotyping analysis (Gram-negative bacillus, lactose fermenting, Indole producing, Methyl red test positive, Voges Prausker test negative Citrate utilizing, Nitrate reducing, Urease test negative, Catalase test positive, oxidase test negative and Ortho-nitrophenyl-β-D-galactopyranoside (ONPG) test positive). These biochemically confirmed 534 pathogenic *E. coli* isolates were considered for the rest of the study.

All biochemically identified *E. coli* isolates were further subjected to serotyping using specific anti “O” *E. coli* antisera (antisera against somatic cell wall antigen "O"). Pathogenic *E. coli* in circulation in India were found to be very diverse in serotyping. Thirty-six different “O” serotypes were identified among the study samples. The distribution of serotypes in the different geographical areas was not uniform. A large percentage of the isolates were un-typeable by serotyping indicating probable newer serotypes associated with pathogenic *E. coli*. The ten most frequent “O” serogroups detected were O8 (46 isolates; 8.6%), O11 (36 isolates; 6.7%), O22 (33 isolates; 6.2%), O88 (33 isolates; 6.2%), O126 (28 isolates; 5.2%), O83 (25 isolates; 4.7%), O35 (25 isolates; 4.7%), O141 (23 isolates; 4.3%), O149 (22 isolates; 4.1%) and O7 (21 isolates; 3.9%). Serotype O157 associated with STEC/EHEC infections was detected in a very low percentage (7 isolates; 1.3%) in this study.

Serotypes associated with most severe *E. coli* infections and big six serotypes usually associated with foodborne outbreaks i.e. O145, O121, O111, O103, O45, and O26 were not detected in this study.

To study the prevalent entero-pathotypes all 302 intestinal isolates were characterized genotypically. Four pathotype-specific genetic markers (i.e. Enteropathogenic *E. coli* (EPEC) - eae and bfpA genes, Enterotoxigenic *E. coli* (ETEC) - elt and est genes, Enterohemorrhagic *E. coli* (EHEC) - hlyA gene and Enteroaggregative *E. coli* (EAEC) - CVD432 gene) were detected using Diarrheagenic *E. coli* multiplex PCR detection kit. Among intestinal isolates atypical Enteropathogenic *E. coli* (aEPEC; eae gene) and heat-stable Enterotoxigenic *E. coli* (ETEC; est gene) were found to be the most prevalent pathotypes throughout the country [105 (34.8%) and 110 (36.4%) respectively]. EAEC (CVD432 gene) and EHEC (hlyA gene) pathotypes were detected in very low percentages of isolates [25 (8.3%) and 9 (3%) respectively]. All the Enterohemorrhagic *E. coli* (EHEC) isolates detected were non-O157 serotypes.

Colonization is the foremost and the most critical step in *E. coli* infections. The prevalence of virulence factors assisting directly or indirectly the colonization of the *E. coli* (Cell surface hydrophobicity, biofilm, siderophores, colicinogeny, gelatin hydrolysis) was evaluated in study isolates by phenotypic characterization. Cell surface hydrophobicity (CSH) was detected by a salt aggregation test. A very large number of isolates (369; 69.1%) in this study were found to be hydrophobic. The Ability of test isolates to form biofilm was detected using a standard tissue culture plate assay. Two simple techniques viz. the tube method and Congo red agar assay were also used to detect biofilms and results were compared with the results of the TCP method. Both the simple methods were found to be having reasonably good test characteristics in terms of accuracy, specificity, sensitivity, and predictive values when compared with the standard tissue culture plate method. Biofilm-forming ability was detected in a very large number of isolates by all three methods [298(55.8%), 286(53.6%), and 315 (59%) respectively]. Both intestinal and extra-intestinal isolates were found to produce biofilm. Biofilm-forming ability was detected in samples from

all geographical locations. The ability of pathogenic *E. coli* to sequester iron through siderophore release was studied on Chrome Azurol S agar (CAS). Siderophore type i.e. catechol or hydroxamate-type was detected using chemical assays (Arnow's and Csaky assay respectively). Siderophores were observed as the important epidemiological and virulence trait of pathogenic *E. coli* from all over the country. Siderophores expression was detected in a very large percentage (303; 56.7%) of the study isolates expressing one or other type of siderophore. Both hydroxamate (189; 34.3%) and catechol (67; 12.5%) type siderophores were detected among the study isolates. Colicinogenity was detected using the agar overlay method in a comparatively less percentage of isolates (108; 20.2%). Gelatin hydrolysis was detected only in 4.5% (24) of the isolates, indicating less importance of these two virulence characteristics in *E. coli* pathogenesis. Thus Cell surface hydrophobicity, Biofilm formation, and siderophore release were observed as the crucial characteristics of colonization-associated virulence factors.

Co-expression of virulence factors was observed as a common characteristic of *E. coli.* A very large number of isolates (357; 66.9%) expressed more than one type of virulence characteristic evaluated in the present study. Virulence characteristics were expressed in various combinations. The most frequent co-expressed combination was Cell Surface Hydrophobicity, Biofilm, and Siderophore being co-expressed by 114 (21.3%) of the isolates.

The antimicrobial resistance profile of the study samples was evaluated by the Kirby Bauer Disc Diffusion method using 20 different antibiotics representing eight antimicrobial categories. A very high rate of antimicrobial resistance to commonly used antimicrobials including third and fourth-generation cephalosporins and carbapenems was observed among isolates from all geographical locations of the country. Ampicillin with a resistance rate of 72.1% (385) and amikacin with a resistance rate of 8.4% (45) were the most resistant and susceptible antimicrobials respectively. Resistance to all classes of antimicrobials was observed with maximum resistance to the cephalosporin class of drugs (404; 75.7%) and minimum to the furantoin class (58; 10.9%). Isolates resistant to more than two classes of drugs were

marked as resistant to multiple antimicrobials. Overall 73.5% of the isolates were resistant to multiple drugs (MDR) with a rate of 84.8% among intestinal and 64.25% among extra-intestinal isolates respectively.

All the strains were also tested by phenotypic double disc diffusion test for ESBL activity using ceftazidime and ceftazidime-clavulanic acid discs and cefotaxime and cefotaxime-clavulanic acid discs. Isolates were considered ESBL producers when extended-spectrum β-lactamase activity was detected in either of the drug combination discs. ESBL expressing pathogenic *E. coli* in this study was 37.3% (199) of the total isolates. ESBL with a rate of 39.7% (120) among intestinal and 34.1% (79) among extra-intestinal isolates was detected. Results obtained in this study indicate that pathogenic *E. coli* with very high drug resistance and a high rate of MDR and ESBL-producing strains are in circulation in India in all geographical locations of the country.

Biofilms in addition to helping bacterial attachment to various surfaces leading to persistent infections are also known to increase drug resistance due to various mechanisms. However, high resistance rates to antimicrobials were detected in *E. coli* isolates irrespective of biofilm formation in the present study. Detection of a high percentage of MDR, ESBL-producing, and carbapenems-resistant isolates in this study from all geographical locations of the country is a matter of urgent and serious concern.

The findings of this study highlight the prevalence and diversity of pathogenic *E. coli* in circulation in India in terms of epidemiological markers i.e. serotypes, Diarrheagenic *E. coli* pathotypes, and virulence factors. It also highlights the importance of colonization aiding virulence factors i.e. Cell surface hydrophobicity, biofilms, and siderophore release in the pathogenesis of *E. coli* infections and as important intervention targets. This study also reflects the grieved situation of the severe level of antimicrobial resistance in *E. coli* strains in India. More such studies with wider scope from all over the country are required routinely to generate up-to-date epidemiological data to understand the epidemiology of *E. coli*

infections in a better way and to plan effective intervention strategies to contain *E. coli* infections at national, state, and district levels and to formulate effective stewardship plans at local levels for better treatment of drug-resistant infections.

ACKNOWLEDGMENT

First and foremost I would like to thank the almighty God for giving me the courage to think to pursue a doctoral degree at age of late forty and to help complete this research work.

No words can express my gratitude to my mentor and supervisor Dr. Gaurav Kumar, Associate Professor, Lovely Professional University, Phagwara, Punjab, for his tireless efforts in guiding me at every step during this research work and shaping this thesis.

I am thankful from the deepest corner of my heart to my Co-supervisor Dr. Yashwant Kumar, Central Research Institute, Kasauli, for his valuable time and devotion to this research work and his valuable technical and scientific inputs to complete the work.

I will fail in my duty if I don't recognize the help extended by Dr. Ajay Kumar Tahlan, former Director, Central Research Institute, Kasauli for granting permission to pursue this degree and permit me to use laboratory facilities at Central Research Institute, Kasauli.

I also thankfully acknowledge the help extended by all staff members of the National Salmonella and Escherichia Center (Mr. Dev Kumar, Mr. Madan Attri, Mr. Yoginder Singh, and Mr. Bhagwan Dass) throughout the experimentation work.

I am thankful to Dr. Navin Gupta, NCDC, New Delhi, and all the scientists and professionals who submitted isolates to National Salmonella and Escherichia Center.

Lastly, I am thankful to my family members for sparing me to devote my family time to accomplish this work

Dated: 30/12/2022

GULSHAN KUMAR

TABLE OF CONTENTS

LIST OF TABLES

List of Figures

List of appendices

CHAPTER-1

INTRODUCTION

1. INTRODUCTION

Escherichia coli (E. coli) is the most widely studied microorganism in the world as most of the strains are non-pathogenic commensals of the gastrointestinal tract of humans and animals. However, pathogenic *E. coli* strains are also known that can cause infections that vary from self-limiting diarrhea to very severe clinical presentations like hemorrhagic uremic syndrome, hemolytic colitis, or even neonatal meningitis (Makvana and Kirlov, 2015). *E. coli* infections account for 2, 00,000 deaths worldwide affecting mainly young children of age less than 5 years (Havelaar, 2015). It is the most important and most common causative agent of Urinary tract Infections worldwide (Saroswka *et al.,* 2019). *E. coli* infections are very common all over the world affecting both developed as well as developing countries (Torres, 2017; Foster *et al.,* 2015; Frank *et al.,* 2011). Developing countries are affected more severely due to poor hygienic conditions and the non-availability of safe drinking water (Airol *et al.,* 2011; Marmot, 2006). In India, *E. coli* infections are very common and it accounts for an important infectious agent in the total disease burden (Chandra *et al.,* 2012; Lanjewar *et al.,* 2010).

Broadly, pathogenic *E. coli* are categorized as intestinal (primarily infecting the gastrointestinal tract leading to diarrhea) and extra-intestinal pathogenic *E. coli;* (infecting sites other than the gastrointestinal tract causing urinary tract infections (UTI), meningitis, septicemia, etc.). The intestinal pathogenic *E. coli* (IPEC) commonly known as Diarrheagenic *E. coli* (DEC) are categorized into different categories (DEC pathotypes) which vary in their pathogenicity mechanism, disease severity, and biological and virulence profiles. These pathotypes are Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), Shiga toxin-producing *E. coli* (STEC), Enteropathogenic *E. coli* (EPEC), Diffusely adherent *E. coli* (DAEC) and Adherent invasive *E. coli* (AIEC). Similarly, extra-intestinal pathogenic *E. coli*(ExPEC) are also categorized as Uropathogenic *E. coli* (UPEC), Systemic pathogenic *E. coli* (SPEC), and neonatal meningitis (referred to as NMEC), based on the site of infection involved (i.e. UTIs, systemic infections

and meningitis in neonates respectively) and avian pathogenic *E. coli* (APEC) based on the host involved. *E. coli* diarrhea and UTI are the most common disease presentations of *Escherichia coli* infections (Saroswka *et al.,* 2019; George and Manges, 2010).

E. coli is also commonly associated with a wide variety of community and hospital-acquired infections, affecting immune-compromised patients and those with pre-existing conditions (Pitout, 2012). Human and animal feces constitute the commonest source of Diarrheagenic *E. coli* strains which transmits directly by hand-to-mouth transmission or by contaminated water and food (Chekabab *et al.,* 2013; Qadri *et al.,* 2005).

Yearly, intestinal and extra-intestinal *E. coli* strains account for 2 million deaths worldwide, (Russo and Johnson, 2003). Even in this era of modernized medical facilities where the fatality rate due to infections has dramatically reduced, diarrheal diseases continue to be a health hazard worldwide, more importantly in developing countries, where an estimated 2.5 million infant deaths are account for infections per year (Kosek *et al.,* 2003; Avendano *et al.,* 1993). Diarrheagenic *E. coli* pathotypes (DEPs) are the most important cause of pediatric bacterial diarrhea in developing countries, with some responsible for traveler's diarrhea. These strains are also emerging as an important cause of diarrhea in industrialized countries (Cohen *et al.,* 2005; Qadri *et al.,* 2005; Robbins-Browne *et al.,* 2004; Nataro and Kaper, 1998). In the mid-1950s, it was epidemiologically incriminated throughout the world as an important cause of infantile diarrhea (Ewing *et al.,* 1956). The prevalence of Diarrheagenic *E. coli* is different in different parts of the world due to socio-economical and geographical conditions. In developing nations with good socioeconomic conditions, Enterohemorrhagic *E. coli* is the most prevalent DEC pathotype, usually associated with food poisoning. However, in developing countries with poor hygienic conditions, the most frequently reported DEC strains are Enterotoxigenic, Enteropathogenic, or Enteroinvessive *E. coli* mainly transmitted through the fecal-oral route through contaminated water (Platts-Millus, 2018; Guerrant *et al.,* 1990).

Accurate diagnosis of *E. coli* infections is challenging due to the difficulty in differentiating pathogenic and commensal isolates. Various biological and virulence markers are associated with pathogenic *E. coli* which serves as the criteria for diagnosis and epidemiological characterization of these strains. Pathogenic *E. coli* were differentiated using serotyping assay till the methods for the identification of virulence factors were developed. Serotyping scheme for the determination of *E. coli* serotype was proposed by Kauffman in 1944 (Kauffman, 1944), this scheme is used to date in modified form. Three surface antigens that are targeted in *E. coli* serotyping are K (capsular), H (flagellar), and O (somatic) antigen (Lior, 1996). More than 180 types of O-antigens 90 K and 56 H antigens have been reported (Stenutz *et al.,* 2006). The presence of the K antigen results in the masking of the "O" antigen thus making it non-detectable, this requires the K antigen to be destroyed before testing for "O" antigens on the strains. Earlier some other antigens including some fimbrial antigens were also designated as K antigens as they conferred the K phenotype, later the K antigen designation was assigned to only acidic polysaccharides while proteinaceous fimbrial antigens were assigned F designations (Lior, 1996; Orskov and Orskov, 1992).

A specific combination of O, K, and H antigens defines a serotype of an isolate while a single antigenic notation is referred to as a serogroup. The association of serogroups and serotypes with the clinical syndrome has been well documented (Levine, 1987), however, serogroup or serotype themselves are not directly responsible for virulence. Rather, they serve as detectable virulence markers correlating with virulent *Escherichia coli* clones (Whittam *et al.,* 1993).

A broad range of virulence-associated factors is possessed by pathogenic *E. coli* strains that include toxins, adhesions, lipo-polysaccharides, capsular polysaccharides, proteases, and invasins. These virulence factors are known to be encoded by genes in pathogenic islands or these may be encoded by genes in mobile DNA islands. These acknowledged virulence factors though may or may not be directly involved in pathogenesis but are considered to be contributing to bacterial fitness (e.g. adhesins, bacteriocins, proteases, and iron-uptake systems) by increasing

these strain's adaptability to the host environment, competitiveness with other normal flora and capability to colonize the human body and dissemination (Pitout *et al.,* 2012; Dobrindt and Hacker, 2008).

Pathogenesis of *E. coli* is attributed to 1) toxins including exo-toxins (Heat labile, heat-stable toxin, Shiga Toxin, Cytotoxic necrotizing factor) and endo-toxins (lipopolysaccharides), 2) cell attachment and effacement mechanisms, etc. 3) aided by colonization, multiplication and dissemination factors like Biofilm formation, 4) nutrient and metal ion chelators, 5) cell surface hydrophobicity, 6) bacteriocins and enzymes. *E. coli is* primarily involved in intestinal and urinary tract infections and to the onset of infection in these sites, the most important and primary virulence characteristic is adherence and colonization of the gastrointestinal and urinary tract. Biofilm formation is one such important characteristic that helps bacterial colonization and survival in the host environment and helps resist various environmental and immune resistances experienced by pathogens during infections aiding the infectious agent (Flemming *et al.* 2016). Another important factor that indirectly contributes to virulence is the iron-chelating molecule siderophore; which helps iron sequestration in free iron stress conditions in the host environment and thus colonization of the bacterium in the host system (Holden and Batchman, 2015). Cell surface hydrophobicity also aids in the attachment of the bacterial cells to various surfaces and surface colonization. Colicins and enzymes like gelatinase contribute to colonization, surviving host immune defenses, and competition with the normal flora of the host (Krasowska and Sigler, 2014).

The ability of *Escherichia coli* to infect different sites, expression of different virulence factors leading to a variety of disease presentations, and expression of a large number of serogroups, make *E. coli* the most diverse infectious bacterial agent. The diversity of pathogenic *E. coli* in terms of its pathogenicity, bio-markers, and virulence markers makes the management of *E. coli* infections challenging. Studies of the prevalence of pathogenic *E. coli* and its characterization in terms of biological and virulence factors importantly contribute to a better

understanding of its epidemiology and better management of this pathogenic microorganism.

The treatment of illness caused by this bacterium usually does not require antimicrobial therapy for self-limiting diarrhea. However, antimicrobial therapy may be necessarily required in severe diarrhea to effectively reduce the duration of illness and prevent traveler's diarrhea (Nataro and Kaper, 1998). Antimicrobial therapy is also required for UTIs and systemic infections. The emergence of high-level antimicrobial-resistant *E. coli* associated with diarrhea and UTIs has been reported worldwide (Abadi *et al.,* 2019). Pathogenic *E. coli* possessing diverse virulence factors and emerging multidrug-resistant strains are continuously posing a challenge to the management and control of infections caused by this ubiquitous infectious agent. Multiple drug-resistant *E. coli* strains have been widely reported in various studies in India (Malik, 2021; Natrajan *et al.,* 2018; Sudershan *et al.,* 2014). Some of the studies even reported the migration of drug-resistant *E. coli* strains from the Indian subcontinent to the rest of the world (Castanheira *et al.,* 2011a; Leverstein-Van Hall, *et al.,* 2010). Many drugs like trimethoprim-sulfamethoxazole, fluoroquinolones, β-lactams drugs, and aminoglycosides are often used to treat patients infected with community-based or nosocomial *E. coli* infections and many of these and other agents are known to be effective against *E. coli* (Pitout, 2012; Pitout, 2010). However, due to emerging drug-resistant *E. coli* strains the management of *E. coli* infections is complicated (Sudershan *et al.,* 2014; Shephard and Potiger, 2013; Akpaka *et al.,* 2010; Pitout and Laupland 2008). The situation is further grieved by the emergence of strains resistant to third and fourth-generation cephalosporins, carbapenems, and (extended beta-lactamase) ESBL¯producing E. coli strains. This leaves with very limited available treatment thus causing a public health concern as antibiotic resistance results in delayed appropriate therapy and an increase in morbidity and mortality (Tumbarello, *et al.,* 2007; Schwaber and Carmeli, 2007). In India *E. coli* strains with increasing trends of resistance to commonly prescribed antimicrobials such as β-lactams including third and fourth-generation cephalosporins, carbapenems, and the emergence of ESBL-producing *E. coli* are reported routinely. Due to such extensive drug resistance World Health Organization

kept *E. coli* on the list of "critical" bacteria against which new drugs are needed urgently (WHO, 2017). The Center for disease control and prevention (CDC) has also placed carbapenem-resistant Enterobacteriaceae which consists of *Klebsiella* and *E. coli* under the "serious threat" category, the top priority pathogens in its latest report on antimicrobial resistance threats (CDC, 2019) *E. coli* has also been included as a critical pathogen in Indian priority pathogen list by WHO and Department of Biotechnology (DBT) and as important microorganisms that required be closely monitored for its antimicrobial resistance profiles in India's national action plan on antimicrobial resistance (WHO-DBT, 2019).

In *E. coli*, the production of the β-lactamase enzymes, that hydrolyzes and inactivates β-lactam, is known as the most important factor responsible for β -lactam drug resistance (Jacoby, 2009).

Multidrug-resistant infectious diarrhea among children in developing countries has been recognized as one of the important public health problems and is a research priority of the WHO diarrheal disease control program (Vargas *et al.,* 1999). Multidrug resistance (MDR) and extended-spectrum β-lactamases (ESBL)-producing strains are increasingly reported in humans and animals (San *et al.,* 2021; Karaiskos and Giamarellou, 2014). Multiple drug resistance among extraintestinal infectious *E. coli* is also a challenge for the treatment of these infections, especially in nosocomial infections (Poolman and Wacker, 2016). Antimicrobial abuse is considered a main responsible factor that increases the selection pressure for resistant strains and decreases their effectiveness (Laxminarayan *et al.,* 2013). Therefore, screening *E. coli* isolates for their susceptibility to common antibiotics is of very importance in public health. Antibiotic susceptibility testing plays a useful role in the outbreak setting. It also helps in understanding local trends in antimicrobial resistance patterns (O'Connor *et al.,* 2018; McKellar *et al.,* 2004).

Though *Escherichia coli* infections are routinely reported from all over the country, however epidemiological data on the prevalence of various pathotypes of *Escherichia coli* in India is lacking. The main reason for available limited

epidemiological data may be attributed to unreported or under-reported diarrheal and UTI infections in developing countries as most of the patients approach health care practitioners only for severe infections and use antibiotics or alternate therapy without medical prescription. Epidemiological data on infectious organisms can only be generated on isolation and characterization of the causative agents but this facility is not usually available in rural and small-town healthcare facilities. The lack of facilities for proper investigation especially in rural areas is another reason for the limited availability of epidemiological data. Comprehensive data on antimicrobial resistance profiles of pathogenic *E. coli* is also limited which results in ineffective empirical therapy and treatment failures. However, for formulating effective treatment and control strategies for better management of widespread *E. coli* infections there is a need for generating and regularly updating epidemiological data on the prevalence, virulence profiles, and drug susceptibility profiles of infectious *E. coli* strains. Due to the lack of sufficient reliable data on the prevalence of various pathogenic *E. coli* isolates in different parts of the country and the prevalence of drug-resistant *E. coli* strains, the management of infections is very challenging especially when socioeconomic conditions in the country are below satisfaction.

Keeping these facts in view the present study was designed to characterize pathogenic intestinal and extra-intestinal *E. coli* strains in terms of the prevalence of various serotypes among intestinal and extra-intestinal *E. coli* isolates, its virulence markers, and drug susceptibility profiles in various geographical locations in India.

CHAPTER-2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. **Infectious Diseases**

Infectious diseases are a major share of the total disease burden worldwide. Though a declining trend in disease burden due to infectious diseases in adults and children has been observed over the last twenty years, however, six of the top ten causes of global disease burden in children are still attributed to infectious diseases (Murray *et al.* 2020). The problem is severe in low and middle-income countries that have limited resources to provide adequate control and medical care facilities. India being the second-most densely populated country in the world also faces challenges in containing infections due to insufficient healthcare facilities for its huge population. However due to continuous commitment from the Government of India to developing healthcare infrastructure it has witnessed a decreasing trend in the number of deaths due to communicable diseases including infections. Disease burden data from different states and union territories of the country from 1990 to 2016 indicates the declining ratio of deaths from communicable to non-communicable diseases in the country. In 2016 deaths due to communicable diseases was 27.5% % while it was 61.8% due to non-communicable diseases and 10.7% due to injuries; however, among the age group 0-14 years, communicable diseases remain the major cause of 80.8% of deaths in India than 12.0% due to non-communicable diseases and 7.2% because of injuries (Dandona *et al.,* 2017). It is very difficult to eliminate all deaths due to infections, especially in the current scenario of emerging drug resistance and the emergence of newer pathogenic microbial variants. The recent COVID-19 pandemic is one of the examples of microbial potential to affect human civilization both in terms of the healthcare system and economy. Human civilization has seen such massive destructions in many instances in past also when microorganisms have shown their might leading to pandemics, epidemics, or local outbreaks (Bloom and Cadarette; 2019). Both community-acquired and hospital-acquired infections account for a major share of the total disease burden. Among the infectious disease, the majority share is avoidable infections leading to diarrhea, UTI, neonatal infections,

sepsis, lower respiratory infections, etc. mostly involving antimicrobial-resistant infectious organisms. *E. coli* is a leading cause of infections worldwide which were responsible for 9, 29,000 AMR-attributable deaths and 3.57 million AMR-associated deaths globally in 2019 with five other agents i.e. *K. pneumonia, S. aureus, A. baumannii, S, pneumonia,* and *M. tuberculosis* (Murray *et al.,* 2020). This microorganism exists as a commensal in the human and animal gastrointestinal tract and at the same time strains that acquire virulence properties can cause a variety of infections ranging from self-limiting diarrhea to severe infections like hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), neonatal meningitis, UTI and septicemia.

2.2. ***Escherichia coli***

E. coli was actually, isolated initially by Theodor Escherich from the feces of healthy individuals in 1885 and was named *Bacterium coli commune* for its isolation from the colon (Escherich, 1885). The organism is known to typically colonize the infant gastrointestinal (GI) tract after birth within a few hours, and, afterward, survive commensally with the host (Drasar and Hill, 1974). About 10^6 to 10^9 colony-forming units per gram of stool are reported to be present in normal human stool (Tenaillon, 2010). Animals and humans are susceptible to infection by the adapted pathogenic *E. coli* strains. The ability of *E. coli* to become pathogenic to animals and humans is largely considered to be attributed to the flexible gene pool by gaining and losing genetic pathogenic markers (Croxen and Finlay, 2010). Adaptive strains of *E. coli* bear the potential to cause diseases ranging from urinary tract infections in humans, enteric infections, and systemic infections (that include bacteremia, osteomyelitis, nosocomial pneumonia, cellulitis, infectious arthritis, and cholecystitis) to serious infections like neonatal meningitis (Kim, 2012; Foxman, 2010; Kaper *et al.,* 2004). Pathogenic *E. coli* strains can be differentiated from non-pathogenic strains by detecting phenotypic virulence characteristics or virulence marker genes. During earlier periods typing of *E. coli* isolates using standard antisera against different surface antigens (serotyping) was the predominantly used technique to detect virulent *E. coli* strains. Though newer phenotypic and genetic techniques have replaced

serotyping for detecting pathogenic *E. coli* strains, however, it remains one of the most widely used epidemiological criteria for characterizing *E. coli* and screening pathogenic *E. coli.* Serotyping is an important technique to detect the most virulent hemorrhagic *E. coli* involved in most of the outbreaks in the western world including both O157 and non-O157 serotypes.

E. coli is very diverse in terms of its epidemiological markers, expression of virulence properties, site of infection, clinical presentations, and hosts involved. Based on the site of infection by adaptive pathogenic *E. coli* strains these are broadly classified as Intestinal and extra-intestinal pathogenic *E. coli*. Intestinal *E. coli* causes gastroenteritis leading to diarrhea of various severity and many other associated complications including HUS and HC. Different entero-pathotypes of intestinal *E. coli* or Diarrheagenic *E. coli* (DEC) are known which vary in pathogenesis and virulence mechanisms. Extra-intestinal *E. coli* can cause infections at sites other than the intestine i.e. bacteremia, infections of the urinary tract and neonatal meningitis, etc. *E. coli* leading to avian infections is also placed under the extra-intestinal category.

E. coli has gained the focus of healthcare planners worldwide due to the emergence of very high drug resistance levels throughout the world more so in low and lower-middle-income countries. Due to the diverse nature of this ubiquitous bacterial pathogen identification of the pathotypes, characterization in terms of prevalent virulence characteristics, and local antimicrobial susceptibility profiles are the important prerequisites for planning effective treatment and control strategies against this pathogen.

2.2.1. **Intestinal pathogenic *E. coli* (IPEC)**

Intestinal pathogenic *E. coli* possess virulence traits that differentiate these from commensal strains. These pathogenic strains can cause gastro-enteritis of varying severity ranging from self-limiting diarrhea to invasive *E. coli* (EIEC), hemorrhagic uremic syndrome to uremic colitis. Based on virulence factors, disease-causing

mechanism, and disease severity, Intestinal pathogenic *E. coli* are further categorized into the following pathotypes: enterohaemorrhagic *E. coli* (EHEC) [also referred to as Shiga-toxin producing *E. coli* (STEC)], enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), Adherent Invasive *E. coli* (AIEC), diffusely adherent *E. coli* (DAEC) and enterotoxigenic *E. coli* (ETEC), (Croxen *et al.,* 2013; Nataro and Kaper, 1998).

2.2.1.1. **Enteropathogenic *E. coli* (EPEC)**

Some *E. coli* strains have the property to form characteristic lesions on intestinal epithelial cell surfaces, these strains are designated as belonging to EPEC and are collectively referred to as attaching and effacing pathogens (Jerse *et al.,* 1990; Andrade *et al.,* 1989). Two types of EPEC are known i.e. typical EPEC (tEPEC) and atypical (aEPEC) which are distinguished by the presence of *E. coli* adherence factor (EAF) plasmid. Typical EPEC strains carry two adherence factor genes (eae and bfpA) and produce localized adherence (LA). While, atypical EPEC strains carry only one gene (eae) (Trabulsi *et al.,* 2002). These strains produce localized-like (LAL) adherence, diffuse adherence (DA), or aggregative adherence (AA) patterns. The localized-like adherence pattern of aEPEC strains is known to be associated with various *E. coli* adhesins including the common pilus (Scaletsky, *et al.,* 2010). Only humans have recognized reservoirs of tEPEC strains which include symptomatic children and asymptomatic adults and children. The most likely source of tEPEC known is the asymptomatic adults and it is transmitted through the fecal-oral route (Nataro and Kaper, 1998; Levine and Edelman, 1984). Phenotypic and genotypic variations between tEPEC and aEPEC strains have been shown and these strains are also known to differ in their antimicrobial resistance patterns and mechanisms (Scaletsky *et al.,* 2010; Muller *et al.,* 2009; Blanco *et al.*, 2006).

Earlier detection of EPEC was based on serogroups; however, currently, EPEC strains are detected by detecting the presence of specific virulence genes, which are characterized by using molecular techniques. These virulence genes are present in serogroups other than those associated with classical EPEC serogroups and

were earlier classified as non-EPEC strains (Hernandes *et al.,* 2009; Ochoa *et al.,* 2008).

2.2.1.2. **Enterohaemorrhagic *E. coli* (EHEC) [Shiga-toxin producing *E. coli* (STEC)]**

The ability of *E. coli* strains to produce cytotoxins of at least one of the Shiga toxin family is the main virulence characteristic of the STEC pathogroup of *E. coli* (Melton-Celsa, 2014). A variety of infections ranging from very mild and almost unapparent diarrhea to the most severe serious hemorrhagic colitis (HC) are caused by EHEC/STEC (Majowicz *et al.,* 2014). Salvadori and Bertoni (2013) reported EHEC strains producing Shiga-like toxins (Stx) and mediating dysregulation of membrane ion channels in the epithelial membrane of the intestine. They reported that this results in the loss of a massive amount of water and ions from the body. This toxin was also considered to act as a modulator of cell transduction and immune modulation which results in a pro-inflammatory and pro-apoptotic sequel. For sequels of the hemolytic uremic syndrome, endothelial lesions in organs more frequently involving the microvasculature of the kidney are considered the responsible factor. In HUS cases the most affected organs are the gastrointestinal (GI) tract and kidney and in a few cases, involvement of pancreatic, central nervous system (CNS), skeletal, and myocardial systems have also been shown. Verocytotoxin is considered to play a role in changing the state of endothelial cells to a pro-coagulant state from the normal anti-coagulant due to microvascular cell injury. Shiga toxin-producing *or* Vero toxin-producing *E. coli* (VTEC) is known to possess genes designated as Shiga toxin 1 or 2, which are known to be typically acquired by a bacteriophage (Farfan and Torres, 2012). Most STEC strains to cause human infections can attach to enterocytes tightly and can reorganize the underlying cell structure through the intimin gene (Donnenberg *et al.,* 1993). Though large plasmids are present in STEC/EHEC isolates associated with infections, however no equivalent of the *per* locus has been found in these strains (Gomez-Duarte and Kaper, 1995). The STEC plasmid encodes a toxin called enterohemorrhagic *E. coli* hemolysin (hlyA) (EHEC hemolysin) (Schmidt *et al.,* 1995). Genes for hlyA are present in most isolates of EHEC serotypes

(Sandhu *et al.,* 1996), and therefore detection of the hlyA gene has been proposed as a genetic marker for EHEC detection (Beutin *et al.,* 1995). The standard method for Stx phenotypic detection is the demonstration of the cytotoxicity effect of bacterial culture supernatants on eukaryotic cells (Karmali *et al.,* 1983). Numerous molecular assays for STEC/EHEC diagnosis have been developed based on the detection of Stx1, Stx2, and hlyA genes in laboratory culture or directly from stool samples. Serogroup O157:H7 frequently associated with EHEC has a characteristic property that most of these serogroups fail to ferment sorbitol within 24 hours. This property is thus usually used as a preliminary method for screening this serogroup (Center for Disease Control and Prevention 2012; Hunt, 2010; Pawlowski *et al.,* 2009; Leotta *et al.,* 2005).

2.2.1.3. **Enterotoxigenic *E. coli* (ETEC)**

ETEC is a predominant agent of infectious traveler's diarrhea and is endemic in many developing countries with significantly higher mortality rates among children (Isidean *et al.,* 2011). ETEC strains produce characteristic adherence factors called colonization factors (CFs)) for intestinal epithelium cell adherence and at least one of the two enterotoxins: heat-labile; LT and heat stable; ST (Levine, 1987). ETEC is an important causative agent of childhood diarrhea in developing countries and travelers visiting these countries (Gomes *et al.*, 2016). The genes encoding for these enterotoxins and adherence factors are reported to be carried on plasmids (Isidean *et al.,* 2011). *E. coli* LTs are oligomeric toxins in nature that are closely related in structure, protein sequence, enzymatic activity, receptor identity, and activity in animal cell culture to enterotoxin produced by *Vibrio cholerae* (cholera toxin-CT). However, it differs from CT in toxin processing and secretion and responses from helper T-lymphocyte (Dickinson and Clements, 1995; Sixma *et al.,* 1993). Two types of LT toxins are known one LT (LT-I) is generally produced by human isolates and is closely related to cholera toxin, while another LT (LT-II) is mainly released by non-human isolates (Qadri *et al.,* 2005). Initially, Heat stable toxin, ST was detected by rabbit ligated ileal loop assay (Evans *et al.,* 1973), however, due to a lack of standardization and the costs involved, this assay was replaced by the suckling mouse

assay (Gianella, 1976). Several immunoassays including a radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) have been developed for the detection of ST, (Cryan, 1990; Giannella *et al.,* 1981). Traditionally the ETEC was detected using bioassay for the detection of LT cytotoxicity on cell culture. Molecular techniques for the detection of ETEC through the detection of LT and ST-encoding genes using DNA probes were developed as early as 1982 (Moseley *et al.,* 1982).

2.2.1.4. **Enteroinvasive *E. coli* (EIEC)**

EIEC is mainly associated with bacillary dysentery in humans, more importantly in low-income countries (Gomes *et al.,* 2016; Croxen *et al.,* 2013). Chromosomal and plasmid-borne genes conferred invasion of human colonic mucosa is the characteristic pathogenesis of EIEC infections (Sansonetti *et al.,* 1982; Harris *et al.,* 1982). The model of EIEC pathogenesis involves five steps firstly it penetrates the epithelial cells then it lyses the endocytic vacuole and multiplies intracellularly followed by directional movement through the cytoplasm, and finally spreads to adjacent epithelial cells. In severe infections, these events elicit a strong inflammatory reaction leading to ulceration. The disease symptoms of EIEC infection include mild watery diarrhea, malaise, fever, and anorexia followed by dehydration, bloody and mucous stools, and associated complications (Van den Beld and Reubsaet, 2012). Infections of EIEC and *Shigella spp* are quite similar in clinical presentation and many phenotypic properties and virulence characteristics of these bacteria are closely related (Lan *et al.,* 2004; Small and Falkow, 1988; Formal and Hornick, 1978). EIEC grows well in culture routinely used microbiological medium for isolation of *Enterobacteriaceae*. EIEC strains may exhibit the property of late lactose fermentation and may require serotyping complementing routine biochemical utilization tests for differentiation (Levine *et al.,* 1987). Sereny guinea pig eye test is routinely used for demonstrating EIEC invasive capacity which can also be evaluated using tissue culture assays. EIEC trains are confirmed using DNA probes or PCR targeting genes linked with virulence (Pawlowski *et al.,* 2009).

2.2.1.5. **Enteroaggregative *E. coli* (EAEC)**

Enteroaggregative *E. coli* is a pathotype associated with infections leading to acute and chronic diarrhea among populations of both developed and developing countries (Nataro *et al.,* 2006). In EAEC endemic areas it causes persistent diarrhea in children and patients having HIV infections (Mathewson *et al.,* 1998; Wanke *et al.,* 1991). This pathotype has also been shown to be associated with incidences of traveler's diarrhea (Okhuysen and Dupont, 2010).

Pathogenesis of EAEC follows a three-stage model involving colonization of intestinal mucosa with the formation of mucoid biofilm, expression of different enterotoxins and cytotoxins release, and inflammation of intestinal mucosa (Croxen and Finlay, 2010; Navarro-Garcia and Elias, 2011; Kaur *et al.,* 2010; Harrington *et al.,* 2006; Bouzari *et al.,* 1994). Assistance from various adhesins is important for successful colonization and biofilm formation in the first stage of pathogenesis. Biofilm formation at EAEC-embedded sites is favored by excessive secretion of mucus on the intestinal mucosal (Hebbelstrup *et al*., 2014). Production of EAEC toxins leads to cytotoxic effects on the intestinal mucosa in the subsequent steps. The cytotoxic effects include induced microvillus vesiculation, widened cryptopenings, and increased epithelial cell extrusion (Hicks *et al.,* 1996). Different pathogenicity islands like *she* pathogenicity island of *Shigella* which carry enterotoxin and mucinase genes or *Yersinia* high-pathogenicity island which carries the yersiniabactin siderophore gene have been identified in the EAEC group of strains (Weintraub, 2007; Henderson *et al.,* 1999). However, all EAEC strains do not contain these genes, and genes carried may not be specific for the EAEC category which makes it difficult to develop an alternative method to the phenotypic HEp-2 cell adherence assay. Baudry *et al.* (1990) reported a 1kb cryptic diagnostic probe “CVD432” also known as the aggregative probe (AA) for diagnosing EAEC. This probe was obtained from the aggregative plasmid of strain 17-2 (Harrington *et al.,* 2006) However this probe was found to perform variably in different locations (Nataro and Kaper, 1998). Bouzari *et al.,* 2001 found that only 46.9% reacted with the CVD432 probe out of the total 98 EAEC confirmed by HeLa cell assay. PCR method using this probe has been

used in several studies for the detection of EAEC infections in diarrhea patients (Jafari *et al.,* 2009; Jafari *et al.,* 2008). Due to a great degree of heterogeneity among EAEC isolates, laboratory diagnosis of EAEC is a challenging task (Pawlowski *et al.,* 2009) however, the most reliable method is the detection of gene "CVD432" by PCR for EAEC confirmation (Aslani *et al.,* 2011; Baudry *et al.,* 1990).

2.2.1.6. **Diffusely adherent *E. coli* (DAEC)**

The *E. coli* strains, that adhere to HEp-2 cells but the adherence pattern is not similar to typical EPEC-like micro-colonies formation, are referred to as "diffusely adherent *E. coli*" (Arenas-Hernandez *et al.,* 2012). Diffuse adherence has been described to be mediated by surface fimbriae in this strain (Bilge *et al.,* 1989 and 1993). The DAEC is associated with diarrhea only in children which are older than infants (Gunzberg *et al.,* 1993; Baqui *et al.,* 1992; Giron *et al.,* 1991). Symptoms associated with DAEC infections are watery diarrhea without blood or fecal leukocytes (Poitrineau *et al.,* 1995). Based on the expression or non-expression of Afa/Dr adhesins, pathogenic DAEC strains were earlier subdivided into two subclasses. Strains expressing Afa/Dr are known to be associated with infections of the urinary tract, complications during pregnancy, and childhood diarrhea in the age group 18 months to 5 years. However, these strains have also been found in the asymptomatic intestinal microbiota of children and adults (Nataro and Kaper; 1998Servin, 2005). DAEC strains are identified by observing Diffusely Adherence patterns in the HEp-2 cells adherence experiments (Schmidt *et al.,* 1994). DNA probes have been developed for the detection of DAEC and some of which are found to cross-react with a subset of EAEC probes (Snelling *et al.*, 2009).

2.2.1.7. **Adherent Invasive *E. coli* (AIEC)**

Crohn's Disease (CD) affects the small bowel and causes inflammatory bowel disease (IBD) which may lead to cancer. AIEC has been associated with Crohn's Disease (CD) as its causative agent. Although it is suggested that several other factors like genetics, intestinal microbiota, and environmental factors also play an important role

in CD (Dam *et al.,* 2013). Over 30% of patients with CD are shown to be having a connection with adhesive and invasive *E. coli* (Croxen *et al.,* 2013). Serogroups O6 and O22 are more prevalent serogroups associated with AIEC strains. They cluster with the B2 phylogenetic group and are closely related to ExPEC, however, AIEC can be differentiated from ExPEC based on adhesion, invasion, and intracellular traits which are usually absent in ExPEC strains. AIEC adheres and invades epithelial cells further replicating in the epithelial cells. Apart from epithelium invasion, AIEC can invade underlying lymphoid tissue and lamina propria which can result in infecting and replicating inside macrophages leading to macrophage death. High levels of TNF-α are produced due to replication occurring in infected macrophages. This causes inflammation of the intestine and the formation of granuloma in patients with CD (Croxen *et al.,* 2013). Pathogenesis of AIEC is thought to be achieved through various virulence factors that include FimH, OmpR, and VAT (Vacuolating autotransporter toxin). FimH binds to the host receptors which can be glycosylated and non-glycosylated (Sokurenko *et al.,* 1997). Using FimH, AIEC adheres to carcinoembryonic antigen-related cell adhesion molecule-6 (CEACAM-6) receptor that is excessively expressed in patients with CD (Cespedes *et al.,* 2017).

2.2.1.8. **Epidemiology of Intestinal Pathogenic *E. coli***

During the 1940s and 1950s, Neter *et al.* (1995) described numerous *E. coli* strains which were epidemiologically related to infantile diarrhea and used the term Enteropathogenic *E. coli* (EPEC), for describing these strains. A number of case-control studies from different countries were shown to be indicating a strong correlation between EPEC strains and infantile diarrhea in comparison to strains from healthy infants (Levine and Edelman, 1984). Lanata *et al.* (2002) reviewed 266 different studies on *E. coli* DEC pathotypes published from 1990 to 2002 and reported that EPEC were the most important pathogens, with 8.8% median prevalence among community patients, 9.1% among outpatient, and 15.6 % among the inpatient. Depending on the differences in study populations, geographic regions, age distributions, periods, method of detection used (adherence patterns, presence of the

eae gene, and serotyping), and the socioeconomic class involved in the study, the prevalence of EPEC infections varies (Ochoa *et al.,* 2008; Maranhao *et al.,* 2008).

Whereas EPEC is mainly associated with infantile diarrhea EHEC/STEC is associated with infections of all age groups leading to infections of various severities from self-limiting diarrhea to HUS and UC. Two key epidemiological studies were important in the recognition of EHEC as a distinct class of pathogenic *E. coli*. Riley *et al.* (1983) detected a rare *E. coli* serotype, O157:H7 from stool cultures of hemorrhagic colitis patients, who presented with gastrointestinal illness having severe crampy abdominal pain, watery diarrhea followed by grossly bloody diarrhea, with low fever or without fever, from two outbreaks which were associated with eating of undercooked hamburgers from a fast-food restaurant chain. Sporadic cases with clinical presentation of hemolytic uremic syndrome (HUS) due to infection with *E. coli* strains producing cytotoxins were reported by Karmali *et al.* (1983).

The most commonly associated serotype with EHEC/STEC infections is serotype O157:H7 in developed countries, however, this serotype is sporadically isolated from human infections in India and other developing countries (Sehgal *et al.,* 2008). Since the association of the *E. coli* O157:H7 serotype with hemorrhagic colitis in the 1980s, it has been reported to cause many outbreaks and sporadic cases with severe disease presentations. This serotype, therefore, becomes the prototype of the EHEC group of bacteria (Kaper and O'Brien, 2014). Some non-O157 serogroups including O145, O121, O111, O103, O45, and O26, are also frequently found to be associated with EHEC/STEC human outbreaks mainly transmitted through contaminated food (Kaper and O'Brien, 2014; Gould *et al.,* 2013). A large number of STEC mainly *E. coli* O104:H4 outbreaks have been encountered in recent years in Europe including the spring 2011 outbreak when approximately 4000 people were infected with *E. coli* O104:H4 serotype in Central Europe, mainly Germany, which lead to about 900 cases of HUS. It was found that this pathogen harbor virulence traits of two DEC pathotypes simultaneously i.e. EHEC and EAEC (Jhandyala, 2013; Bilinski et al 2012; Karch *et al.* 2012). Higher rates of complications are associated with serotype O157 infections i.e. HUS and HC than those by the non-O157

serotypes (Page and Liles, 2013; Ferens and Hovde, 2011). Epidemic and sporadic ETEC infections have been reported increasingly reported throughout the world (Karmali, 1989). Another highly virulent serotype of EHEC O26:H11/H− is also emerging as the common EHEC outbreak-associated strain in Europe (Vasser *et al.,* 2021; Bielaszewska *et al.,* 2013). The presence of EHEC/STEC strains in the gastrointestinal tract of a wide variety of animals suggests that these infections can have a zoonotic character (Gonzalez *et al.,* 2016). Another concern in EHEC/STEC infections is the occurrence and survival of EHEC/STEC strain in the environment as this pathotype is known to survive in the water, manure, and soil (Lascowski et. al., 2013).

DEC pathotype ETEC is associated with two important clinical syndromes 1) childhood diarrhea especially in developing countries and 2) diarrhea in travelers visiting developing nations. Several factors are involved in the epidemiologic pattern of ETEC disease (i) for establishing a successful infection relatively high infectious dose is required (ii) a protective mucosal immunity develops in those infected or exposed to ETEC and (iii) shedding of pathogenic ETEC organisms in the stool of otherwise asymptomatic and immune individuals is common. It is determined through epidemiologic investigations that contaminated water and food are the two most common vehicles of ETEC infection, thus the implementation of proper sanitization is the most crucial and effective strategy to contain ETEC infection (Nataro and Kaper, 1998; DuPont *et al.,* 1971). ETEC traveler's diarrhea usually occurs in first-time travelers from developed countries to developing nations from contaminated food and water mostly during warm and wet months (Arduino and DuPont, 1993).

The first recognition of ETEC strains as causative agents of diarrhea was after its isolation from newborn piglet lethal diarrheal cases (Nataro and Kaper, 1998). The importance of two enterotoxin types of ETEC in disease mechanisms was also elucidated in piglets. Fluid secretion by human childhood diarrheal ETEC isolates in ligated rabbit intestinal loops was the first description of ETEC in humans (Taylor *et al.,* 1961). Later on, ETEC was shown to cause diarrhea in adults also (DuPont *et al.,* 1971).

EIEC strain was first reported during the 1940s and was initially designated as “para colon bacillus”, however later this strain was identified as *E. coli* serotype O124. Other *E. coli* isolates from bacillary dysentery, with the ability to cause keratoconjunctivitis in guinea pigs during experimentations, were identified during the 1950s and 1960s and were initially designated as *Shigella manolovi*, *S. sofia*, *Shigella* strain 13, and *S. metadysenteriae*, which were later re-designated as EIEC (Ewing, 1986; Marier *et al.* 1973; Manolov, 1959; Ewing and Gravatti, 1947). The biochemical characterization of EIEC strains was first carried out in 1967 (Sakazaki *et al.,* 1967).

Enteroinvasive *E. coli*-infections are known to spread through the fecal-oral route mainly sourced to humans as the reservoirs including the infected asymptomatic patients, and animal reservoirs of EIEC are not known. EIEC infections are reported worldwide; however poor hygienic conditions in low-income countries contribute to the easy spreading of EIEC infections (Beutin *et al.,* 1997; Chatterjee and Sanyal, 1984). EIEC is known as an important diarrheal agent in many countries of Latin America and Asia (Levine *et al.,* 1993; Chatterjee and Sanyal, 1984;). EIEC infections in developing countries have been mainly described among travelers returning from countries with a high incidence of EIEC infections (Wanger *et al.,* 1988). Pathogenic EIEC strains are found to be associated with limited serotypes and some of these are found to be identical to *Shigella* spp. "O" antigens include O167, O144, O143, O124, O121, O112, and O28 (Tozzoli and Scheutz, 2014; Cheasty and Rowe, 1983). The two commonest transmission vehicles for EIEC infections are contaminated food and water usually sourced from human reservoirs (Tozzoli and Scheutz, 2014).

Studies related to the demonstration of EPEC adherence patterns to Hep-2 cells lead to the discovery of EAEC and diffusely adherent *E. coli* (DAEC) pathotypes of DEC. Two types of diarrheal *E. coli* isolates with the ability to adhere to Hep-2 cell but with phenotypic presentation different from EPEC pathotypes i.e. diffuse pattern was detected and categorized as aggregative and true diffuse

adherence phenotypes (Nataro *et al.,* 1987; Nataro *et al.,* 1985; Scaletsky *et al.,* 1984; Cravioto *et al.,* 1979). During an outbreak with EAEC/STEC hybrid O104:H4 strain in Germany in 2011 which encountered more than 5000 cases and about 50 deaths, the attention of medical scientists was drawn to EAEC (Boisen *et al.,* 2015). In a study from 1985 to 2006 of different hospital-based, sporadic, and outbreaks cases in the United Kingdom no known serotype could be associated with a large number (43) of EAEC isolates from a total of 143 EAEC strains (Jenkins *et al.,* 2006).

The main clinical presentations in DAEC infections are watery diarrhea and recurring urinary tract infections, this pathotype is responsible for infections in both developed and developing nations (Servin, 2005). DAEC outbreaks are caused by contaminated water, contact with infected animals, and mainly contaminated food. Consumption of contaminated undercooked ground beef is the main vehicle of DAEC infections (O'Sullivan *et al.,* 2007).

Various studies on the prevalence of DEC pathotypes have been reported throughout the world showing regional variations. The prevalence of ETEC was found to be very low in different studies in high-income countries in comparison to low and middle-income countries. In the mid-Anatolia region of Turkey among children 0-5 years of age ETEC the prevalence was found to be low (4.5%) by Iseri et al (2011) and in a similar study reported earlier in Turkey by Ozerol et al (2005), the prevalence of ETEC was found to be 10% among children of 0-5 years. However, an ETEC prevalence study conducted by Mohammadzadeh *et al.* (2015) in Tehran, Iran found that ETEC is a very common aetiological agent of DEC in diarrheal patients when they evaluated 140 diarrheal stool samples collected during April – September 2013, and detected only lt gene in 5 (3.6%) isolates, both lt and st genes co-amplified in 3 (2.1%) isolates and only st gene in 1 (0.7%) of the isolate. A four-year study conducted by Gonzalez *et al.* (2013) in Bolivia from January 2007 to December 2010 among children less than 60 months of age revealed that the most prevalent DEC pathotypes in diarrheal children were EAEC, ETEC, and EPEC with incidence rates of 11.2 %, 6.6 % and 5.8% respectively which peaked during winters seasons of Bolivia (April–September), while EIEC and EHEC were detected in only 1% of the

samples. A 3-year (Dec 1, 2007, and March 3, 2011), prospective study on 9439 children (0-59 months old) with moderate-to-severe diarrhea was conducted by Kotloff *et al.* (2013) in developing countries (South Asia and sub-Saharan Africa) to understand etiology of diarrhea. Heat-stable toxin-producing ETEC (ST-ETEC strains; expressing ST alone or co-expression ST with heat-labile enterotoxin) was found to be one of the most prevalent diarrhea etiological agents along with rotavirus, Cryptosporidium, and Shigella in this study. ST-ETEC and aEPEC were detected with association to the highest risk of case deaths with a hazard ratio of 1.9; 0.99-3.5 and 2.6; 1.6-4.1 respectively in 0-11 months old children. In six-year surveillance during the year 2011-2016 conducted in Brazil with 5047 clinical *E. coli* samples, 683 DEC were detected. The most frequently detected DEC pathotypes were EPEC, EAEC, and ETEC with prevalence rates of 52.6%, 32.5%, and 6.3% respectively. EIEC and STEC were detected with incidence rates of 4.4% and 4.2% respectively (Ori *et al.,* 2019). A study was conducted in Thi Qar city from Oct 2013 - to Oct 2014 on 200 diarrheal children (< 12 years) by Al-Dulaimi *et al.* (2015). DEC was detected in 44.8% of samples with incidence rates of 64.73%, 19.5%, 10.5%, and 5.27% of EAEC, ETEC, EPEC, and EHEC DEC pathotypes respectively. Newitt *et al.* (2016) also reported two outbreaks of EIEC gastrointestinal infections in the United Kingdom in 2014, these outbreaks involved over 100 infection cases. In one episode the EIEC infections were transmitted through contaminated salad vegetables and, in a second outbreak, the EIEC serotype O96:H19 could be isolated from vegetable samples and some patients. Nataro *et al.* (2006) conducted a study to determine the etiology of diarrheal illness in Baltimore, Maryland, and New Haven, Connecticut. They targeted molecular markers for enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli*, enteropathogenic *E. coli,* Shiga toxin-producing *E. coli,* and cyto-detaching *E. coli*. DEC was detected in 77 (9.4%) of 823 cases and EAEC was the most prevalent cause of diarrhea. Saka *et al.* (2019) studied the distribution of DEC pathotypes in 248 diarrheal children <5 years of age and 33 healthy controls out of a total of 455 cases (400 patients and 55 controls) in Nigeria from April-November 2017. They detected DEC pathotypes in 73.8% of the isolates (183/248) with EAEC (36.3%), ETEC (17.3%), and EPEC (6.0%) identified as the commonest DEC pathotypes in this study. A study on children with acute diarrhea in South Africa from

March 2015-May 2017 detected DAEC (41%), EHEC (17%), EIEC (10%), and EPEC (17%) as the most prevalent DEC pathotypes out of 106 confirmed *E. coli* isolates (Omolajaiye *et al.,* 2020). Eybpoosh *et al.* (2021) examined 1305 stool samples from diarrheal patients from different age groups and detected *E. coli* in 979 samples in Iran from 15 provinces from 2013 to 2014. They detected DEC pathotypes in 659 of the samples more frequently during summer than winter. STEC (35.4%), ETEC (14.0%), and EPEC (13.1%) with incidence rates were identified as the most prevalent pathotypes. EAEC (4.3%) and EIEC (0.3%) were not found to be so prevalent in Iran in this study. DEC Detection rates (73%) among children under five years of age were very high than in adults. Zhou *et al.* (2021) surveyed acute diarrheal patients of all age groups in China from 2009 to 2018 and detected DEC in 6119 (6.68%) of the 91651 patients. They detected DEC more among women (6.97%) than male (6.46%) patients and maximum in patients of the age group 18-59 years (7.88%). EAEC (28.51%), ETEC (24.07%), and EPEC (25.36%) were the commonest DEC pathotypes overall. In children <5 years of age EAEC (2.07%), EPEC (1.81%), and EHEC (0.31%) were detected as the most prevalent pathotypes while among age groups 18-59 ETEC (2.36%) was the most prevalent agent. Jarquin *et al.* (2022) evaluated the DEC burden among hospitalized patients (647) and those who visited clinics (2304) with acute diarrhea at Rosa Santa in Guatemala from 2008 to 2009 and from 2014 to 2015. *E. coli* the prevalence rate was detected as 17.9%. DEC pathotypes were detected in 19% of hospitalized and 21% of clinic patient samples with *E. coli* detection. DEC hospitalization and clinic visit incidences in the age group <5 were 648 and 29.3 per 10,000 persons while in the age group > 5 years it was 36.8 and 0.4 per 10,000 respectively in hospitalized and clinic-visiting patients. ETEC, EPEC, and STEC were the most commonly isolated pathotypes in hospitalized (8.2%, 6.8%, and 0.6% respectively) and clinic visiting (12%, 6%, and 0.6% respectively) patients.

India is very diverse geographically and different studies in different parts of the country have shown different pathotypes being involved in gastrointestinal DEC infections. In another study from Mangalore, Karnataka, between June 2002 to June 2004 on 115 diarrheal stool samples. DEC was detected using PCR in 17.4% (20) of

the samples. Among DEC aEPEC (12 isolates), EAEC (4 isolates), and STEC (4 isolates) were detected. No ETEC was detected in this study (Shetty *et al.,* 2012). In a study conducted at the Postgraduate Institute of Medical Sciences Chandigarh (North India) on hospital-acquired diarrhea among children with ages ranging from 02 months to 14 years during the period January 2008-June 2009, DEC was found to be the most predominant causative agent (47%) while *C. defficle* toxin (9%) and rotavirus (8%) were less prevalent. Among DEC isolates ETEC (22%), EAEC (18%), and EPEC (7%) were isolated in this study using multiplex PCR (Chandra *et al.,* 2012). In a study conducted in Kolkatta, Western India on 648 hospitalized diarrheal patients, EPEC was found to be responsible for 3.2% of diarrheal infections among children less than 5 years of age (Nair *et al.,* 2010). In active surveillance on diarrheal etiology in Kolkotta from 2008 to 2011 in 3826 stool samples, DEC was detected in 11.8% of the samples. EAEC was detected most frequently (5.7%) followed by ETEC (4.2%) and EPEC (1.8%). Different pathotypes were found to be associated with different age groups i.e. EAEC and EPEC (>2 years of age), ETEC (>2 to 5 and >5 to 14 years), and EAEC (>14 to 30 and >30 to 50 years) [Dutta *et al.,* 2013]. Suganya *et al.* (2016) studied the prevalence of DEC pathotypes among DEC samples from children from Tamil Nadu using multiplex PCR with primers for EPEC (eae and bfpA), ETEC (elt and Stla), DAEC (CVD432), EHEC (hlyA) and EAEC (ial). They could detect only ETEC pathotypes in one sample out of a total of 75 DEC isolated. A study conducted by Mandal *et al.* (2017) in Bihar on 633 diarrheal children patients (0-60 months of age) detected 191 (30.2%) DEC using multiplex PCR with 69.1% of DEC being EAEC, 8.4% EPEC, 1.6% EIEC and 2.6% EHEC and 7.8% mixed pathotypes. In a study conducted on 1394 stool samples from diarrheal children of age < 5 years in Andaman and Nicobar from 2013 to 2016, DEC has detected in a total of 95 (6.82%) samples. Among the DEC 70.1% were EAEC, 19.6% were EPEC and 10.3% were ETEC. Of the EPEC 63.2% were atypical EPEC (Raghvan *et al.,* 2017). A total of 334 *E. coli* collected during 2013-2015 from children up to 5 years of age were analyzed for DEC pathotypes EPEC and EIEC detection in Mizoram, North-East India. Incidence rates among diarrheal patients of EPEC and EIEC in this study were detected as 11.38% and 1.8% respectively (Chellapandi *et al.,* 2017). A study conducted at Bhubaneswar (Odisha) on 130 diarrheal children (<5 years of age)

between June 2015-April 2016 by Shrivastava et al (2017) detected DEC in 30.07% of the diarrheal samples with EPEC (21.53%) with the majority of cases, and other pathotypes detected less frequently were STEC, EAEC, 0157, and EHEC with incidence percentages of 10.76%, 6.90%, 4.61%, and 0.77% respectively. A study conducted by Surya *et al.* (2014) on 75 stool samples collected from diarrheal children of age < 15 years in Tamil Nadu detected 17 (23%) DEC isolates. EPEC (12%) was detected as the most prevalent pathotype followed by EAEC and STEC with incidences of 5.33% and 4% respectively. ETEC strains were not at all isolated from the study population in this study. Singh *et al.* (2019) detected DEC pathotypes in 198 (66%) of the samples by microbiological culture methods and 170 (56.6%) samples using the polymerase chain reaction technique respectively of the total 300 samples studied in Karnataka. Among 198 DEC isolates eae, stx, east, elt, est, ipaH, and eagg genes were detected in percentages of 59.5%, 27.7%, 27.2%, 12.6%, 10.6%, 5.5%, and 1.5% respectively. Based on the detection of virulence genes, EPEC (33.8%) was detected as the most frequently isolated pathotype followed by STEC (23.2%), ETEC (13.6%), EIEC (5.5%), and EAEC (0.05%). DEC with a combination of virulence traits from different pathotypes was also detected with different combinations including EAST1EC combination pathotype strains, STEC/ETEC, and STEC/EAEC strain with incidence rates of 4.5%, 3.5%, and 1.0% respectively. Mohanty *et al.* (2021) detected DEC pathotypes in 7.4% of diarrheal children out of 350 cases in Bhubaneswar Odisha, during October 2014-September 2016 [12 (46.2%) in 0-1year age; 11 (42.3%) in 1-5years age and 3 (11.5%) in 5-14 years age group]. ETEC (53.8%) and EPEC (38.5) were the most prevalent pathotypes while EHEC was also detected in 2 (7.7%) samples. However, EAEC, EIEC, and DAEC were not detected in this study. The rate of DEC isolation was more during the rainy (69.2%) and summer seasons (23.1%) than in winter (7.7%). Prasad *et al.* (2022) reported DEC pathotypes in 39 out of 170 acute diarrheal patients and 3 of the 47 control cases of age <18 years in Meghalaya, North-east India during one year (January-December 2015).EAEC (38%) and aEPEC (28.5%) were detected as the most prevalent pathotypes in the study. DEC pathotypes tEPEC (16.6%), ETEC (11.9%), and EIEC (4,7%) were also detected in this study.

2.2.2. **Extra-intestinal pathogenic *E. coli* (ExPEC)**

Extra-intestinal pathogenic *E. coli* (ExPEC) are categorized into different pathotypes i.e. uropathogenic *E. coli* (UPEC), avian pathogenic *E. coli* (APEC), and neonatal meningitis (NMEC). Extra-intestinal pathogenic *E. coli* (ExPEC) is associated with complications like lower urinary tract infections (UTIs), systemic infections, and also neonatal meningitis (referred to as NMEC) (Kim, 2012; Pitout, 2012; Kohler and Dobrindt, 2011; George and Manges, 2010). Though ExPEC is mostly involved in infections of the urinary tract, it can enter and cause infections of any sterile body site extraintestinal and may cause infections leading to myositis, meningitis, osteomyelitis, or epididymal-orchitis. In the USA annually approximately 40000 deaths are attributed to systemic inflammatory response syndrome (SIRS) after bloodstream infections, mainly by *E. coli* (Russo and Johnson 2000 and 2003).

2.2.2.1. **Uropathogenic *E. coli***

UTI refers to the number of bacteria present in the urine which can be >150/ml and symptomatic UTIs can be divided according to the severity caused Upper UTIs are called pyelonephritis (infection in the kidney) or lower UTIs are called cystitis (bacterial infection in the bladder) (Parvez and Rahman, 2018; Terlizzi*et al.,* 2017). UPEC pathogenesis includes UPEC colonization in the periurethral, urethral and vaginal areas, penetration into the lumen of the bladder, *E. coli* planktonic cell growth in urine, its adherence to the surface, and interaction with the defense mechanism of the epithelium of the bladder leading to biofilm formation and further invasion and replication in bladder cells also causing damage to the kidney with increased risk of bacteremia/septicemia (Terlizzi*et al.,* 2017).

A wide range of virulence factors assists in the initiation of the pathogenesis of UPEC such as flagella, pili, non-pilus adhesins, curli, polysaccharide capsule, LPS, OMPs, outer-membrane vesicles, TonB-dependent iron-uptake receptors including siderophore receptors, and secretion systems (Terlizzi*et al.,* 2017). Both types of virulence factors viz. bacterial cell surface and secreted virulence factors are

important in the pathogenesis of the UPEC. Type 1 and P fimbriae belong to bacterial cell surfaces which help in adhesion and tissue invasion in the host cell. Flagellum, capsular lipopolysaccharide, and outer membrane proteins also belong to the bacterial cell surface virulence factors. Hemolysin and siderophores belong to the secreted virulence factors which help bacterial colonization (Shah *et al.,* 2019).

PAIs are the regions on bacterial chromosomes where virulence factors accumulate. Different sets of PAIs have been found associated with three different UPEC strains (*E. coli* 536, *E. coli* J96, and *E. coli* CFT073). In strain 536, PAI's I-IV encodes a variety of virulence factors such as P fimbriae, P-related fimbriae, and the yersiniabactin siderophore system. While in strains IJ96 and IIJ96 PAIs have been identified to encode P fimbriae, P-related fimbriae, and α-hemolysin. On the other hand strains, ICFT073 and IICFT073 PAI were identified to encode P fimbriae, alpha-hemolysin, and aerobactin (Sarowska *et al.,* 2019).

2.2.2.2. **Neonatal meningitis *E. coli***

The neonatal meningitis subtype of ExPEC is a major causative agent of neonatal bacterial meningitis (NBM) which causes disease in newborns <1 month of age (Stoll *et al.,* 2011; Johnson and Russo, 2002). Neonatal meningitis *E. coli* has a mortality rate ranging from 17 to 38%. According to studies, NMEC isolates can be differentiated from other pathotypes based on the presence of certain serotypes (O7:K1, O18:K1:H7, and O83:K1) and some virulence factors like S fimbriae, K1 capsule, and invasion of brain endothelium (ibeA). This strain belongs to phylogenetic group B2, the source of other extraintestinal pathogens (Johnson and Russo, 2002).

It is difficult to distinguish the characteristics of NMEC strains from commensal strains due to phenotypic and genotypic heterogeneity (Sarowska *et al.,* 2019). In pregnant women, mostly pathogenic bacteria colonize the vagina, cervix, or rectum. During later stages of pregnancy, these bacteria via crossing the amniotic membrane reach the fetus or infect the baby during childbirth ((Watt *et al.,* 2003;

Romero *et al.,* 1989). Pathogenesis of NMEC involves three sequential steps first transfer of NMEC from the urinary tract, uretro, or intestine lumen to the bloodstream, and its intravascular survival and multiplication occur, secondly NMEC transfers through the blood-brain barrier and lastly it invades the arachnoidal space (Kim 2012; Bonacorsi and Bingen 2005).

NMEC virulence factors are encoded on plasmids that are not found in any of the UPEC strains (Sarowska *et al.,* 2019). About 80% of the meningitis cases caused due to *E. coli* belong to capsular serotype K1 antigen; mainly serotypes O7:K1 and O18:K1 (Moulin-Schouleur *et al.* 2006; Achtman *et al.* 1983). Various virulence factors are known to aid NMEC infections and include adhesins like p-fimbriae, S-fimbriae, type-1 fimbriae; outer membrane proteins: K1 capsular antigen, and Ompa protein; invasions like ibeA, asl, cnf1 gene; siderophores like iroN, fyuA, and iucC/iutA genes and various others like Iss protein, colV, gimB, traT, cvaC genes (Sarowska *et al.,* 2019; Vila *et al.,* 2016).

The virulence genes of the NMEC plasmid core include several operons like aerobactin (iutA/iucABCD), sit (sitABCD), and salmochelin operon (iroBCDEN). These are the three major iron uptake systems that are associated with ExPEC virulence. Virulent NMEC human isolates are strongly connected with the iss gene which is also found in the genome of the large virulence plasmid of NMEC. The transfer of *E. coli* through the ammonitic membrane is due to a virulence factor IbeA; the gene for IbeA is coded on the PAI GimA. This functions by providing invasive properties for the blood-brain barrier which is a carbon-regulated process (Sarowska *et al.,* 2019).

2.2.2.3. **Avian pathogenic *E. coli***

In poultry, APEC strains cause avian colibacillosis starting in the respiratory tract. APEC causes invasion of the respiratory tract which causes inflammation of the air sacs leading to septicemia and infection (Wasinski, 2019). The strain shows similarities with human ExPEC strains and virulence genes are also similar to the

virulence factors identified in human ExPEC (Moulin-Schouleur*et al.,* 2007)**.** APEC strain's virulence factors include type I fimbriae (FimC), temperature-sensitive hemagglutinin (Tsh), colicins (CvaC), and hemolysins (HlyE), increased serum survival protein (iss), and siderophores (IucC, SitA). Genes like ompTandhlyF also contribute to virulence (Sarowska *et al.,* 2019).

2.2.2.4. **Epidemiology of ExPEC**

UPEC is considered the primary cause of community-acquired Urinary Tract Infections, it is estimated that among women more than 18 years of age about 20% suffer from a UTI during their lifetime (Foxman; 2010). In community-onset UTIs cases 70-95% are attributed to UPEC and among nosocomial UTIs, approximately 50 % are attributed to UPEC. It is one of the most common causes of uncomplicated and complicated UTIs (Shah *et al.,* 2019; Nicolle 2013 and 2014). 75-95% of the *E. coli* strains are responsible for uncomplicated UTIs and 40-50% contribute to complicated cases UTIs are more prevalent in 81% of women aged from 16 to 35 years of age and in men aged more than 60 years the cases of UTI increases due to enlarged prostate (Sarowska *et al.,* 2019). The human intestinal tract is believed to be the primary reservoir of UPEC and it acts as opportunistic pathogens employing a battery of virulence factors for colonization and infecting the urinary tract in an ascending pattern. The Source of community-acquired UTI outbreaks had been traced to UPEC-contaminated food. However, UPEC isolates have also been shown to be spread through sexual activities (Foxman, 2010; George and Manges, 2010).

UPEC infections are the most common among ExPEC infections. It is estimated that over 80% of UTIs are attributed to UPEC as the causative agent worldwide (Sarowska *et al.,* 2019). ExPEC burden of UTIs in the USA is estimated at 85-95% of UTI cases and 25-35% of catheter-associated UTIs (Poolman and Walker, 2016).

E. coli (73.5%) was detected as the most common etiological agent among the 200 community-acquired UTI patients in a study conducted in Tuzla, Bosnia from

January 2006-December 2007 by Piljic *et al.* (2010). A study on pregnant women attending different clinical institutions from January 2012 to December 2012 in Cambodia was re-evaluated by Ruiz-Rodriguez *et al.* (2021). It was observed from the medical records that the prevalence of UTI was 14.5%. *E. coli* (75.53%) and *Klebsiella* (17.02%) were detected most commonly as causative agents while other bacteria with low incidence rates detected were *Staphylococcus* (2.13%), *Enterococcus* (2.13%), *Streptococcus* and *Serratia* (1.06%). Another similar study from Cambodia conducted in 2013-to 2015, detected a UTI prevalence of 29% among pregnant women with *E. coli* and Klebsiella as the most prevalent etiological agents with incidence rates of 57.7% and 11.4% respectively (Sanin-Ramirez *et al.,* 2019). A ten-year global prevalence study of Infections in Urology (GPIU) covering the years 2004- 2013 and data from 17 Asian countries revealed that the UTI prevalence rate was 9.8% among hospitalized patients. *E. coli* with an incidence rate of 38.7% was detected as the predominant cause of hospital-associated UTIs from the database of GPIU during this period (Choe *et al.,* 2018). In a one-year study conducted from September 2017 to August 2018 in Nepal on urine samples collected from 1142 suspected UTI patients, 184 were found to be infected with microbes. Of the confirmed UTI cases *E. coli* (57%) was detected as the most common causative agent. Male and female percentages among the UPEC-infected patients were 47% and 53% respectively (Shah *et al.,* 2019). Out of 504 healthcare-associated infections detected in a survey in 2009-2010 in ten geographically diverse states of the USA, *E. coli* was detected as the causative agent in 47 (9.3%) of the infections sharing a different percentage of infection burdens of healthcare-associated infections being the commonest agent of UTI i.e. pneumonia (3; 2.7%), surgical site infections (14;12.7%), gastrointestinal infection (1;1.2%), UTI (18;27.7%), bloodstream infections (5; 10%) (Magill *et al.,* 2014). In a case-control study conducted in Iran UPEC was detected in 138 out of 702 urine samples evaluated. UPEC isolated in the study were compared with 30 commensal *E. coli* isolates for virulence factors and it was observed that virulence factor scores of the UPEC were more than twice higher than in commensals (Rezatofighi *et al.,* 2021).

UTI is also very common in India and most of the cases are attributed to UPEC infections. UTI cases with UPEC as the causative agent are routinely reported from different parts of the country. A study from February- to September 2014 in Patna, Bihar found *E. coli* and *Pseudomonas aeruginosa* was the most common causative agents of UTIs. Out of the total 700 samples analyzed only 200 showed significant bacteriuria. *E. coli* was isolated from 100 (50%) of the culture-positive samples. Other etiological agents isolated in this study were *P. aeruginosa* (12%), *S. aureus* (9%), *C. koseri* (8%), *Enterococcus spp* (6%), *Klebsiella pneumonia (4*%), *Klebsiella oxytoca (4%), S. aureues* –SCV (4%), *S. saprohyticus* (2%) and *Moraxella catarrhalis* (1%) (Bhattacharyya *et al.,* 2015). UPEC was isolated with highest the prevalence from 510 culture-positive urine samples out of 1054 urine samples from patients during 18 month (January 2013- June 2014) study conducted in Odisha. *E. coli* (109), *Enterococcus fecalis (*82*), Staphylococcus aureus (*65*), Enterococcus aerogenes (*64*), Klebsiella oxytoca (*50*), Klebsiella pneumoniae (*45*), Clostridium freundi (*41*), Pseudomonas aeruginosa (*32*) and Proteus vulgaris (*22*)* were isolated from the culture-positive samples in this study (Mishra *et al.,* 2016). Ghosh and Mukherjee (2016) reported the detection of UPEC in 59 (39.86%) of the 148 urine cultures evaluated and collected from patients suffering from UTI in Kolkata, West Bengal. A study was conducted on 3772 urine samples from in-patients from Ural Kerala from May 2016 to April 2017 to evaluate etiology and antimicrobial resistance patterns. Significant bacteriuria was observed in 1265 samples with *E. coli* (48.9%) as the most common etiological agent. Other isolates identified in this study were *Enterococcus* sp., *Klebsiella* spp, *Pseudomonas* and other non-fermenters, and *Staphylococcus* sp. with percentages of 14%, 14%, 11%, and 7% respectively (Sukumaran and Kumar, 2017). Das *et al.* (2018) reported the detection of *E. coli* in 76.6% of the 47 culture-positive samples out of 205 mid-stream urine samples from women in Haryana, North India. *E. coli* was mainly isolated from urine samples of younger women (61.11%) in the age group 21-40 years. In a study at Ankola Maharashtra on 351, urine samples from suspected UTI patients *E. coli* was detected as the most prevalent isolate with a frequency of 75%. Other bacterial agents isolated in this study include *P. aeruginosa* (8%), *P. mirabilis* (6%), *K. pneumoniae* (4%), *S. aureus* (4%), and *E. faecalis* (3%) (Paralikar *et al.,* 2019). Urine samples from a rural

population in the Solan district of Himachal Pradesh were analyzed during a two-year retrospective study (2014-2015) to evaluate the antimicrobial profile of uropathogens (Mehrishi *et al.,* 2019). A total of 1878 urine samples from suspected UTI patients were analyzed, 184 of the samples indicated significant bacteriuria *E. coli* (59.78%) was isolated as the most prevalent etiological agent followed by *Klebsiella pneumonia* (9.78%) and *Enterococcus spp* (8.69%) other bacteria were isolated with low rates. A comparative study for the prevalence and antimicrobial resistance pattern in suspected UTI cases of children was carried out in New Delhi, North India to compare scenarios in 2009 and 2014. Significant colony counts were observed in 340 (16.15%) of 2140 samples during 2009, while it was observed in 407 (18.39%) out of 2212 samples in 2014. *E. coli* was the predominant cause of UTI during both periods (57.1% and 55.5% respectively) with increasing trends of antimicrobial resistance over the period (Patwardhan *et al.,* 2017). Kulkarni *et al.* (2017) isolated 395 *E. coli* from 1000 suspected UTI patients attending a hospital in Karnataka, India from 2012 to 2015. Of these 395 *E. coli,* 170 isolates were resistant to multiple drugs. *E. coli* and *Klebsiella pneumonia* were also detected as the commonest causative agents of UTI in pregnant women attending their first antenatal visit in October 2015-September 2016 at Hyderabad, South India. Bacterial growth was observed in 133 out of 1841 urine samples, *E. coli* (79/133), *K. pneumonia* (29/133), *Sphigmomonas* (3/133), *Enterobacter* (1/133), and *Citrobacter* (1/133) were isolated from these samples (Kammili *et al.,* 2020).

Infections on sites other than gastrointestinal and urinary tracts are not so common however *E. coli* remains a leading agent of bacteremia worldwide mostly sourced from UTIs (Jackson *et al.,* 2005). Overall incidence rates in the USA adult population are estimated at 30-50 cases per 100,000 adults (Laupland and Church, 2014; Williamson *et al.,* 2013). Postoperative drug-resistant *E. coli* infection is another major cause of *E. coli* bacteremia. The incidence of *E. coli* bacteremia increased by about 70% from 1999 to 2011 in the United Kingdom due to drug-resistant *E. coli* strains (Schlackow *et al.,* 2012). Neonatal bacterial sepsis is one of the important causes of neonatal mortality and morbidity throughout the world. The incidence of neonatal sepsis is 1/1000 and 4/1000 among normal-term and preterm

neonates respectively in underweight neonates it increases to 300/1000 (Stoll *et al.,* 2011). Prophylactic antimicrobial administration to pregnant women during the last antenatal visits to control group B streptococcus neonatal sepsis reduced its incidence rate but increased incidence rates of *E. coli*-associated neonatal sepsis. Lopez Sastre *et al.* (2005) reported a decrease in group B *streptococcus* cases after intra-amniotic prophylaxis (IAP) however an increase in *E. coli* cases from 0.17/1000 to 0.38/1000. Schrag and Stoll (2006) also reported post prophylaxis decrease in cases of group B *streptococcus* from 1.7/1000 to 0.34/1000 but an increase in *E. coli* cases from 3.2 per thousand to 6.8 per thousand. Van den Hoogen *et al.* (2010) reported a post-prophylaxis decrease in cases of group B *streptococcus* from 1.8/1000 to 0.7/1000 but an increase in *E. coli* cases from 1.0/1000 to 0.3/1000. *Escherichia coli* is the second most important agent of neonatal meningitis with a mortality rate of 20%–29% and high morbidity among neonates. The neonatal meningitis incidence rate in developed countries is about 0.1 per 1000 live births. About 90% of neonatal meningitis cases occur in neonates below 28 days of age, the rest 10% of cases are reported among children in the age groups of 1 and 3 months (Vila *et al.,* 2016; Okike *et al.,* 2014; Bonacorsi and Bingen 2005; Unhanand *et al.* 1993).

Studies on NMEC prevalence are very few. In one of the studies, of the 444 bacterial neonatal meningitis cases reported from pediatric wards in France from 2001 to 2007, group B streptococci (59%) and *E. coli* (28%) were found to be the most prevalent bacterial agents (Gaschignard *et al.,* 2011). In a systemic review of neonatal meningitis in developing countries, Furyk *et al.* (2011) observed that mortality rates of neonatal meningitis in developing countries are very high 40-50% in comparison to 10% in developed countries, with the difference in the common etiological agents in developed and developing countries except for *E. coli*, which is a common agent of neonatal meningitis in developed as well as in developing countries. In a study from North-east India from January 2013- to January 2015, Devi *et al.,* (2017) evaluated 303 cerebrospinal fluid (CSF) samples suspected of neonatal meningitis with the detection of pathogens in 67 of the samples. Bacterial agents were detected in 58 samples, gram-negative bacteria being more frequent (32) than gram-positive bacteria (26). *A. baumannii* (18%), *Klebsiella* (12%), *Pseudomonas* (9%), *N.*

meningitidis (3%), and *E. coli*, *Sneathia*, *Cronobacter sakazakii*, and *Roseomonas cervicalis (1.5% each)* were detected among the gram-negative bacteria. *Enterococcus spp.* (15%) was the most commonly detected gram-positive bacteria*, E. coli* K1 was found to be the most common etiological agent of neonatal meningitis with a mortality rate of 10-15% in Britain in a study conducted by Alkeskas *et al.* (2015). Johansson Gudjonsdottir *et al.* (2019) from Sweden reported the highest neonatal meningitis mortality by *Klebsiella* (33%) and *E. coli* (11%). A retrospective study conducted in eastern China by Liu *et al.* (2021) over three different time zones (2001-2006, 2007-2012, 2013-2020) detected *E. coli* neonatal meningitis incidence rates during these time zones as 0.12, 0.26, and 0.23per thousand live births respectively.

Studies from India have reported different common etiological agents of neonatal sepsis and meningitis. In a study *Klebsiella* (33/58) and *E. coli* (11/58) were found to be the most common bacterial agents of neonatal septicemia in a district hospital in West Bengal (Viswanathan *et al.,* 2012). *Pseudomonas* (33.2%), *Klebsiella* (31.4%), *Acinetobacter* (14.4%), *Staphylococcus a*ureus (9.2%), *E. coli* (4.4%), *Enterobacter* (2.2%), *Citrobacter* (3.1%) and *Enterococci* (2.2%) were detected as the causative agents of neonatal sepsis in a study conducted during January 1998- December 2004 at Manipal, South India (Bhat *et al.,* 2011).

A multidrug-resistant ExPEC strain, *E. coli* O25b: H4/ST131 (sequence type 131) is widely distributed in Europe mainly affecting Italy and Spain. This strain causes a broad spectrum of diseases including UTIs (Rogers *et al.,* 2011; Lopez-Cerero *et al.,* 2014). The occurrence of this pathogenic phenotype is also reported in India and these strains were also found to harbor drug-resistant genes CTX-M-15 (Hussain *et al.,* 2012).

2.3. **Virulence Factors of *E. coli***

The ability of microorganisms to infect and cause disease is referred to as virulence. Microbial-associated factors that assist these to colonize the host at the cellular level

are called virulence factors. Virulence factors are of different natures and include secretory, membrane-associated, or cytosolic effects. The membrane-associated virulence factors facilitate adhesion and evasion by microbes of the host cells. Secretory factors are microbial armory that helps them counter host innate and adaptive immune responses, while the cytosolic factors help to undergo quick adaptive—metabolic, physiological, and morphological shifts (Sharma 2017). Usually, virulence factors are encoded on pathogenicity islands (PAIs), plasmids, or other mobile genetic elements. There are a large number of microbial virulence factors such as toxins, enzymes, exopolysaccharides cell wall structures (LPS, capsules, fimbriae, pili), etc. (Sarowska *et al.,* 2019). Microorganisms, including *E. coli,* use a variety of virulence factors and various combinations to evade host defense mechanisms. These virulence factors may be pathotype specific like LT and ST toxins in ETEC or non-specific like biofilm-forming capability or bacteriocins.

The most critical factor in *E. coli* pathogenesis is the colonization of the host system by the pathogenic strain. Colonization in gastro-intestinal and extra-intestinal sites involves attachment and multiplication of *E. coli* in host tissues. Here, these bacteria experience competition from commensals for space and nutrition and also due to the non-availability of free essential nutrients in the host tissue environments. Additionally, the pathogens also experience flushing actions of the host system i.e. gastrointestinal tract and urinary tract (Nielubowicz and Mobley, 2010; Lievin-Le Moal *et al.,* 2006). Various surface and secreted factors are involved in the successful adhesion and colonization of the host, which contributes to colonization through different mechanisms. Important virulence factors involved in the initial adhesion and colonization of pathogenic *E. coli* include cell surface hydrophobicity, biofilm formation, bacteriocins release, gelatin hydrolysis, and siderophore release.

2.3.1. **Biofilm**

Biofilm may be defined as a structured community of bacterial cells of the same or mixed species enclosed in a self-secreted polymeric matrix and attached to a non-living or living surface (Costerton *et al.,* 1999). The extracellular matrix held the

constituent cells together and is composed of exo-polysaccharide (EPS), proteins, and sometimes nucleic acids (Lasa and Penades 2006; Branda *et al.,* 2005; Whitchurch *et al.,* 2002).

In medicine, biofilms are known to adversely affect infection rates due to recurrent persistent infections, medical device-associated infections, and enhanced drug resistance. Around 80% of human bacterial infections are related to biofilm-forming microorganisms. Around 60-70% of nosocomial or hospital-acquired infections that are a leading cause of death are known to be associated with some type of implanted medical device (Bryers, 2008). Microbial biofilms have been observed on most such devices such as prosthetic heart valves, intravenous catheters, cardiac pacemakers, cerebrospinal fluid shunts, urinary catheters, and contact lenses resulting in chronic infections (Abidi *et al.,* 2013; Tran *et al.,* 2012; Santos *et al.,* 2011; Hall-Stoodley *et al.,* 2004; Donlan 2001; Franson *et al.,* 1984; Marrie *et al.,* 1982). Most biofilm infections appear as chronic or recurrent infections and hence are difficult to resolve (Vejborg and Klemm, 2009). These infections pose clinical challenges, including diseases that involve uncultivable species, impaired wound healing, chronic inflammation spread of infectious emboli, and rapidly acquired antibiotic resistance (Bryers, 2008). One of the most important characteristics of biofilms is decreased susceptibility to antimicrobial agents which can be attributed to tolerance and resistance. Tolerance arises once the density of bacteria has aggregated, while resistance mainly develops due to mutations. The resistance of biofilms to antibiotics can mainly be attributed to the fact that bacterial cells in the biofilm are slow-growing, while antibiotics act best against rapidly growing cells, for instance, β-lactam antibiotics act upon rapidly dividing cells (Lewis, 2005; Tuomanen *et al.,* 1986). Another reason for the observed tolerance is that antibiotics have low penetration within the biofilms as aminoglycosides have limited access to the cells, though fluoroquinolones diffuse freely into the biofilm and can kill slow-growing cells (Bjarnsholt, 2013; Ishida *et al.,* 1998; Nichols *et al.,* 1988). In implant-associated biofilm infections removal of such devices is the only option. If the infected device can't be removed, then continued antibiotic treatment to prevent biofilm growth must be given (Wu *et al.,* 2015; Mermel *et al.,* 2009). This may

involve adding high doses of antibiotics or 70% ethanol or HCl (2 mol/L) into the lumen of the Catheter (Tan *et al.,* 2014) In cases of gram-negative bacilli inhabiting device surfaces, Amikacin, Gentamycin, Ciprofloxacin, Ceftazidime are the drugs of choice for antibiotic lock therapy (Fernandez-Hidalgo and Almirante, 2014; Funalleras *et al.,* 2011). Change of cardiac pacemakers or infected heart valves in patients suffering from endocarditis becomes very necessary since if not done at the earliest can lead to cardiac insufficiency and infected embolic complications (Nataloni *et al.,* 2010).

Surfaces with altered physical, chemical, and topographical properties are being developed that prevent bacterial adhesion (Donlan, 2011; Renner and Weibel, 2011). In *vitro* studies have shown successful anti-biofilm treatment in comparison to in-vivo studies. There is a consensus on the fact that for the effective treatment of biofilm infections, a combination of an anti-biofilm compound with an effective antibiotic is required. However, in the current clinical scenario, no anti-biofilm compound is in use (Romling and Balsalobre, 2012).

Biofilms have played a crucial role in *E. coli* epidemiology. Many studies indicating the role of biofilms in *E. coli* infections have been reported relating it to recurrent and difficult-to-treat infections. Martinez-Medina *et al.* (2009) compared the biofilm-forming ability of AIEC and non-AIEC strains isolated from intestinal mucosa and observed that biofilm-forming indices were significantly more in AIEC than in non-AIEC isolates. They detected moderate to strong biofilm in 65.4% of the AIEC strains while 74.4% weak biofilm in non-AIEC strains, thus indicating more efficiency of AIEC to form biofilms in comparison to non-AIEC strains. Pereira *et al.* (2010) demonstrated that *C. deficile* and EAEC isolated from the diarrheal case show synergistic biofilm-forming potential and adherence to HeLa cells. Ahmed *et al.* (2013) detected ETEC as planktonic and biofilms in water reservoirs in Dhaka households. They observed that the detection of biofilms was correlated with seasonal ETEC epidemics. From October 2015-April 2016 study was conducted in Iraq on 390 clinical isolates from different infection sites. On bacterial culture and identification, 50 were identified as *E. coli* (25 UTIs, 18 diarrheal stools, and 7 blood isolates). All

these 50 *E. coli* isolates were producing biofilm, and siderophores and showed adhesion (Jaloob Aljanabi and Hashim Alfaham, 2017). Of the 116 *E. coli,* isolated from children with UTI biofilm was detected in 48 of the isolates with 8 showing strong, 21 moderate, and 19-week biofilm formation in a study conducted by Gonzalez *et al.* (2017). A cross-sectional study was conducted in Nepal on 105 urine samples suspected of catheter-associated UTIs. *E. coli* (56.9%) was found as the most common causative agent and 33.3% of *E. coli* were detected as biofilm producers in the study (Maharjan *et al.,* 2018). A cross-sectional study conducted in Uganda detected biofilm formation in 62.5% (125/200) of *E. coli* isolates from UTI cases and 78% (156/200) of these were multi-drug-resistant (Katongole *et al.,* 2020).

Studies from different parts of India have also shown biofilm-forming pathogenic *E. coli*-associated infections. Biofilm was detected by the Congo red agar method in isolates from various infection sites including UTI, septicemia, soft tissue infections, and post-operative infections from patients in Mumbai Maharashtra, Western India from August-October 2011. A total of 150 isolates were evaluated including 38 *E. coli* (29 UTI; 3 soft tissue, 2 septicemias, and 4 from post-operative infections) for biofilm formation. Biofilm was detected in *E. coli* isolates in 27/29 UTI and 2/2 septicemia isolates (De *et al.,* 2012). Biofilm was detected by three methods viz. TCP, TM, and CRA methods in 107 UPEC isolates from catheter-associated, symptomatic, and asymptomatic UTI patients in Karnataka, South India. All three methods detected biofilm in 89.5% of catheter-associated UPEC isolates, 0-13.3% of asymptomatic UPEC isolates, and 48-72% of symptomatic isolates by different methods used (Golia *et al.,* 2012). *E. coli* (54.01%) and *Klebsiella pneumonia* (11.66%) were the commonest uropathogens detected from 137 urine cultures in a study conducted in Mumbai, Western India. 26% of the *E. coli* isolates were found to produce biofilm in this study (Tayal *et al.,* 2015). A study conducted on 135 *E. coli* isolates from UTI during January- December 2011 from Haryana, North India detected biofilm in 13.5% of the samples only. All the biofilm producers were found to be resistant to multiple drugs in this study (Mittal *et al.,* 2015). A study on 39 *E. coli* isolates collected from UTI cases in Jharkhand in 2014 detected biofilm in 84.61% of the samples without correlation with antibiotic resistance (Singh *et al.,*

2016). A study on 100 *E. coli* isolates from UTI cases in Central India from December 2013- to May 2014 was conducted for the detection of biofilm formation by tube adherence method. Of the 100 isolates, 62 (57non mucoid and 5mucooid) were biofilm producers (Bajpai *et al.,* 2016). Pullanhi *et al.* (2019) detected biofilm in 92% (88% moderate, 4% strong) of the 150 *E. coli* isolates from UTI cases in Kochi, Kerala, South India, during November 2016-October 2017. All *E. coli* (100%) isolates from catheter patients formed biofilm and these patients were found to recover after catheter removal and appropriate antimicrobial treatment. A study on 100 *E. coli* isolates from UTI cases from Karnataka, South India detected biofilm in 49-69% of samples using different methods. Biofilm was detected in 89.7% of catheterized patients in this study. No association between biofilm production and drug resistance was observed (Karigoudar *et al.,* 2019).

2.3.1.1. **Phenotypic methods of biofilm detection**

To manage the problem of biofilm formation and biofilm formation-associated issues; it is of utmost importance to detect the formation of biofilms so that necessary steps can be taken in this direction to tackle the menace. Different studies over the past few years have widely employed three phenotypic methods – The tissue culture plate method, the tube method, and the Congo red agar method for the detection of biofilm formation by different microorganisms. Christensen *et al* 1982 studied adherence to smooth surfaces by slime-producing strains of *Staphylococcus epidermidis*. Adherent growth was considered to be present if a film lined the inner surface of the tube and the presence of this adherent growth was considered evidence of slime formation. Electron microscopy of the specimens was also carried out and transmission electron micrographs of the isolates indicated that the bacteria were enmeshed in extracellular material. It was observed that the polystyrene test tube walls were coated with an adherent film of *S. epidermidis* and the production of this film required static incubation in TSB for 18-24 hours at 37˚C. The coating was apparent in glass as well as polystyrene equally. Christensen *et al.* (1985) studied the adherence to plastic Tissue Culture Plates by Coagulase-negative Staphylococci. Freeman *et al.* (1989) devised a new culture media technique to detect slime production by coagulase-

negative staphylococci. This involves inoculation of the Congo Red Agar and incubation at 37˚C for 24 hours. Slime-producing biofilm-forming organisms were indicated by black colonies with a dry crystalline consistency while non-slime producers remained pink. The results showed complete agreement between the congo red agar method and the tube method in 107 out of 124 isolates. Mathur *et al.* (2006) evaluated these three phenotypic screening methods for the detection of biofilm in clinical isolates of *Staphylococci*. The tube method showed a good correlation with the TCP assay for strong biofilm-forming isolates. No correlation between the congo red agar method with TCP was observed. Aggarwal *et al.* (2011) tested the biofilm-forming ability in different *Salmonella* serovars using the microtitre plate assay. All the *S. Typhimurium* and *S. Enteritidis* isolates were found to be biofilm producers using the tissue culture plate method. Hassan *et al.* (2011) studied the formation of biofilm in clinical isolates by three methods. The tissue culture plate method detected 25 isolates to be strong biofilm producers; the tube method detected 21 strong isolates, while the congo red agar method detected a total of 4 isolates to be strong biofilm producers. Antibiotic resistance was found to be higher in biofilm producers than non-producers. Al-Mulla *et al.* (2013) compared different methods for the detection of biofilm produced by *Staphylococcus epidermidis.* Methods compared in the study were the tissue culture plate method, the tube method, and the Congo red agar method. Sensitivity, specificity, and accuracy values of the tube method and the congo red agar method were calculated considering TCP as the gold standard. The results demonstrated high sensitivity, specificity, and accuracy for the tube method and lower values for the Congo red agar method. In the year 2014, Vasanthi *et al. (2014)* studied the production of biofilms and the antimicrobial resistance pattern of different bacterial isolates from invasive devices. Biofilm detection was carried out by 3 methods – the tube method, the Congo red agar method, and the tissue culture plate method. A total of 4 out of 55 isolates obtained from various invasive devices were positive by all three methods. These methods have been used by various workers in the recent past also (Gayathree *et al.,* 2021; Shrestha *et al.,* 2018; Manandhar *et al.,* 2018; Panda *et al.,* 2016).

2.3.2. **Cell surface hydrophobicity**

Bacterial cells prefer to live as aggregates in the form of biofilms and this form is achieved through many steps including cell immobilization, cell surface hydrophobicity (CSH) is one important characteristic that helps bacterial cells in this activity (Wu *et al.* 2012). CSH is positively found to be associated with adherence as was measured using a quantitative approach by Elfazazi *et al.,* (2021). CSH is thus one of the important factors that contribute to microbial cell attachment to biotic or abiotic surfaces (Van Loosdretch *et al.,* 1990). Many mechanisms including the release of membrane vesicles are involved in cell surface hydrophobicity (Baumgarten *et al.,* 2012). A strong attachment has been observed by hydrophilic cells to hydrophilic surfaces and similarly by the hydrophobic cell to hydrophobic surfaces (Giaouris *et al.,* 2009). Usually, hydrophobic materials like silicon, Teflon, stainless steel, etc. are used as the material of construction in medical implants thus increasing the chances of biofilm formation on these by hydrophobic pathogenic bacteria. The use of non-hydrophobic materials like polystyrene or polymeric substances or coating of medical devices with anti-biofilm material like silver nano-particles can reduce the chances of biofilm development on these devices thus reducing implant-associated infections (Knetsch *et al.,* 2011, Arciola *et al.,* 2012). Outer membrane proteins, oligosaccharides, and adhesins have been described as the responsible bacterial cell factors for CSH in *Escherichia coli* (Krasowska and Sigler, 2014). Fimbriae have also been thought to play an important role in hydrophobicity by overcoming repulsive electrostatic forces between the cell and surface (Corpe, 1980). In one study, the pattern of aggregation in the CSH test was found to be associated with the type of fimbriae present (Gonzalez *et al.,* 1988). Hamadi F *et al.,* (2008) demonstrated that cell surface hydrophobicity in *E. coli* is associated with the (C-(CH) functional group on the cell surface.

Cell surface hydrophobicity is known to play an important application in the environment clearing organic pollutants like toluene and synthetic polymers (Tribedi and Sil, 2014; Kobayashi *et al.,* 1999). CSH also plays important role in wastewater treatment plants, where it facilitates the formation of bio-granules in microbial

granule reactors for clearing various water pollutants (Liu *et al.,* 2009; Adav *et al.,* 2008). CSH is also associated with negative impacts of microbes on the food industry and medicine, where CSH helps bacteria to adhere to and colonize food processing equipment and medical implants, other devices, and host surfaces thus leading to food spoilage or difficult-to-treat infections (Brooks and Flint, 2008; Ly *et al.,* 2006).

Various studies have been carried out to understand the role of CSH in *E. coli* infections and it has been observed that hydrophobic pathogenic *E. coli* are associated with many communities and hospital-acquired infections. CSH was detected in 29.2% of antenatal cases, 33.3% of catheterized patients, and only 9.7% of controls in a study conducted in Bangalore from 2009 to 2010 (Shruthi *et al.,* 2012). Fakruddin *et al.* (2013) during a study in Bangladesh on 65 *E. coli* isolates from different infection sites observed CSH as an important virulence factor of pathogenic *E. coli*. CSH was detected in 76% UTI, 50% Septicemia, 70% peritonitis, 58% abscess, and 58.7% CSF samples. Mittal *et al.* (2014) detected CSH in 61% of the isolates out of a total of 135 UPEC evaluated from patients in Haryana. Annapurna *et al.* (2014) detected CSH in 26.66% of the 15 UPEC isolates from 50 gram-negative isolates from UTI cases in Hyderabad, South India. Gowtham and Gopinath (2016) detected CSH in 70% of UPEC strains out of the total 20 isolates evaluated. A very high percentage (90%) of *E. coli* isolates from UTI was found to be hydrophobic in one study conducted at Coimbatore (Growther and Yasotha, 2016). Only 09% of ExPEC isolates from various infection sites were found to be hydrophobic in one of the studies (Vaish *et al.,* 2016). In another study conducted on UPEC isolates from UTI patients in Chhattisgarh, 38.15% of isolates were found to be hydrophobic (Bawankar, 2018). A similar study conducted from 2011 to 2012 in Mumbai detected 27.64% hydrophobic UPEC (Kaira and Pai, 2018). Abd Al-Baky *et al.* (2020) detected CSH in *E. coli* isolates from various infection sites with different rates. They detected CSH properties in 81.8% of UTI isolates 86.9% of stool isolates, 88.8% of blood isolates, and 60% of the wound swab *E. coli* isolates.

2.3.4. **Colicins**

Bacteriocins are proteins produced by some strains of bacteria which can inactivate or kill related species of bacteria. Bacteriocins of *E. coli* are referred to as colicins after the species that produced them. The gene responsible for the production of colicins under stressed conditions is carried by plasmids. Colicin structure has three domains that are involved 1) in the recognition of receptors, 2) in translocation, and 3) in lethality. Over 30 types of colicins are known to be produced by *E. coli* (Cursino *et al.,* 2002). The number is increasing as newer colicins are identified regularly (Micenkova *et al.,* 2019, Rendueles *et al.,* 2014).

Colicins act on sensitive cells through three different action mechanisms. The most common mechanism is membrane depolarization through plasma membrane pore formation which also leads to cytoplasmic ATP depletion by induction of phosphate and potassium ion efflux. The second common mechanism is endonuclease activity against chromosomal DNA or 16-S RNA. The third mechanism is by degradation of the cell wall, hydrolyzing the β-1,4 bond between NAG and NAM in the glycan backbone of the cell wall, or inhibiting cell wall synthesis. The producing strains are protected from colicins through an immunity protein that inactivates the colicin (Kleanthous *et al.,* 1998). Apart from colicin's role in pathogenesis, it also has some promising applications like use as a natural food preservative, as a therapeutic agent in infections, or as a treatment for tumors (Yang *et al.,* 2014; Lancaster *et al.,* 2007).

Colicins are known to be involved in pathogenicity and have also been associated with other virulence factors. Azpiroz *et al.,* 2009 observed a relationship between colicins virulence in uro-virulent *E. coli*. In one study on commensal and uro-virulent *E. coli,* colicin E1 was identified as a potential virulence factor (Smajs *et al.,* 2010). Colicin activity was observed in *E. coli* isolates from different infection sites by Fakruddin *et al.* (2013). Rates of colicin activity detected in *E. coli* isolates were 69% in UTI isolates, 72% in septicemia, 75% in peritonitis, 75% in abscess, and 0% in CSF isolates. Colicins were found to be associated with ExPEC virulence as

colicin production was observed to correlate positively with other virulence factors (Micenkova *et al.,* 2014). A study on colicin type and the prevalence of ExPEC from various infection sites and commensal intestinal *E. coli* revealed a difference in colicin types in isolates from various sites, ExPEC strains were found to release colicins less frequently than commensal fecal *E. coli* (Micenkova *et al.,* 2016). Sulchz et al (2015) have demonstrated the usefulness of *E. coli* colicins expressed in plants in the control of pathogenic *E. coli* in food.

2.3.4. **Gelatin hydrolysis**

Gelatin hydrolysis is accomplished through an exo-enzyme gelatinase of bacterial origin representing zinc- metalloprotease (Makinen *et al.,* 1989). Gelatinase degrades a broad spectrum of biomolecules i.e. complement components (C3 and C3a), fibrinogen, fibrin, collagen, bradykinin, endothelin, etc. leading to tissue damage and dissemination of the pathogenic organisms (Park *et al.,* 2007 and 2008; Waters *et al.,* 2003; Schmidtchen *et al.,* 2002; Makinen and Makinen, 1994). Gelatinase is also known to assist in biofilm development thus contributing to virulence through this mechanism as well (Thomas *et al.,* 2009). Gelatinase plays an important role in the virulence of both gram-positive and gram-negative bacteria and has been associated with the pathogenesis of severe complications like endocarditis (Thurlow *et al.,* 2010).

In *E. coli* infections gelatin hydrolysis is considered to play important role in its virulence and dissemination, especially in ExPEC strains (Rafaque *et al.,* 2020). Fakruddin *et al.* (2013), detected protease activity among *E. coli* isolates from different infection sites from patients in Bangladesh with rates of 13.3% in UTI isolates, 16.7% septicemia, 8.33% peritonitis, 10% abscess, and 0% CSF isolates. Mittal *et al.* (2014) detected gelatinase in a very large percentage (67.5%) of 135 *E. coli* isolates from UTI patients in a study conducted over one year (January-December 2011) in Haryana, North India. Annapurna *et al.* (2014) detected gelatinase in 5 out of 15 *E. coli* isolates from UTIs in Hyderabad, South India. Vaish *et al.* (2016) detected gelatinase activity in only 2 *E. coli* isolates (1 UTI and 1 pus isolate)

in a study conducted on 100 *E. coli* isolates from various infection sites from patients in Andhra Pradesh from August 2011- to December 2012. Bawankar *et al.* (2018) detected gelatinase activity in 40.13% of UPEC isolates in comparison to 16% of the controls in a study conducted on UTI samples from patients in Chhattisgarh during 2014-2015. In some of the studies, gelatinase was not detected at all among the pathogenic *E. coli* evaluated for this virulence trait. Niyas and Gopinath (2018) did not detect gelatinase activity in 20 pathogenic *E. coli* from UTI cases. No gelatinase activity was detected in 123 *E. coli* isolates from UTI samples in a study conducted by Kaira and Pai (2018) in New Delhi. In a similar study conducted by Shah *et al.* (2019) in Nepal on 105 *E. coli* isolates isolated from 1142 UTI samples, gelatinase activity was not detected among these *E. coli* isolates. Hiremath and Lava (2020) detected gelatinase in 77 (51.3%) of the 150 ExPEC isolates from different clinical samples from patients in Karnataka, South India.

2.3.5. **Siderophores**

Siderophores are the iron-chelating low molecular weight (< 10KD) molecules secreted by many microorganisms to sequester iron under free iron-limiting environments (Lankford and Byers, 1973). In *E. coli*, the expression of siderophores was first described as ferric uptake regulation (FUR). FUR is transcriptionally regulated by negative repressor molecules (Casadevall and Pirofski, 2009). Structurally many types of siderophores are known which can broadly be categorized as hydroxamate, catecholate, and carboxylate types (Neilands *et al.,* 1981). Diversity among siderophores has been used as a criterion for the epidemiological typing of pathogenic organisms (Reissbrodt and Rabsch, 1988). Enterobactin and aerobactin are two siderophores that are produced by *E. coli* and represent catecholate and hydroxamate-type siderophores respectively.

Siderophores play an important role in pathogen virulence as they help fulfill the iron requirements of the pathogen for its normal metabolism in an iron-limited environment of the host system and it has been seen that loss of siderophore activity makes the pathogen attenuated (Schrettl *et al.,* 2010; Fetherston *et al.,* 2010).

Montgomerie *et al.* (1984) found a significant association between aerobactin association and bacteremia and found aerobactin-producing strains as more virulent than aerobactin-non-producing strains in a mouse model. The iron sequester process through siderophores involves sequential steps which are initiated from the excretion of siderophores to outside cells under free ferric iron-limited conditions. Siderophores compete with iron-binding molecules in body fluids and sequester them owing to their high affinity for ferric iron. Iron-bound siderophores specifically attach to receptors i.e. FecA and FepA protein receptors on the outer membrane, FepB in periplasms, and ATP-dependent Fec-CDE Fep CDE proteins and Ton B-ExbB-ExbD protein complex in the inner membrane (Chakravorty *et al.,* 2003 and 2007; Andrews *et al.,* 2003). Inside the cytoplasm, free ferrous iron is released from the iron-siderophore molecules either by reduction, siderophore destruction, or through chemical modification for use by the cell (Neilands *et al.,* 1981).

The role of siderophore in *E. coli* virulence has been studied in various studies. Areobactin was found to be significantly produced by more UTI and ExPEC isolates than control *E. coli* isolates by Demir and Kaleli (2004). Freestone *et al.* (2003) demonstrated the involvement of enterobactin in norepinephrine-mediated iron supply to EHEC. Siderophore production on CAS agar was found in 97.5% of UPEC isolates by Vagarali M.A *et al.* (2008). Caza *et al.* (2011) demonstrated the role of catecholate-type siderophore enterobactin in ExPEC strains by a study on the ΔentD derivative of strain χ7122. Watts *et al.* (2012) demonstrated the importance of different siderophore types in UTI colonization by *E. coli*. Mittal *et al.* (2014) detected siderophores in 199 (88%) of the 135 UPEC isolates from UTI patients in a study conducted in Haryana, North India. Siderophore was detected on chrome azurol S agar in 95% of *E. coli* isolates from the urine of patients with type 2 diabetes mellitus (Saleem and Daniel, 2017). Using the CAS agar test siderophores were detected in 63.81% of UPEC isolates from UTI patients in Chhattisgarh by Bawankar *et al.* (2018). Siderophores-associated genes *iroN* were also identified in STEC isolates indicating the importance of siderophores in these strains (Zhang et al, 2020). Gerner *et al.* (2021) successfully demonstrated the usefulness of siderophore targeting against colonization of the AIEC pathotype of *E. coli* by vaccinating mice with

siderophore and cholera toxoid conjugates eliciting B-cell elicited immune response, thus showing the importance of siderophores as an important virulence factor aiding *E. coli* colonization.

2.4. ***E. coli*** **Serogrouping**

After the discovery of *Escherichia coli,* medical microbiologists faced the problem of distinguishing pathogenic strains of *E. coli* from non-pathogenic *E. coli* strains during the 1920s and 1930s. Several scientists tried to identify specific types of *E. coli* but no significant progress could be made until a serotyping scheme was developed by Kauffmann in 1944 to type *E. coli* into 20 "O" groups based on antigen-antibody interaction (Kauffman, 1944). This scheme was further expanded and consisted of 25 O antigen groups, 55 K antigens, and 20 H antigens (Kauffman, 1947). A comprehensive serotyping scheme comprising 164 "O" groups to investigate epidemiological and surveillance studies was presented by Orskov *et al.* in 1977 (Orskov *et al.,* 1977). Six more "O" serogroups were added to this scheme in 1984 by Orskov and Orskov (1984) who further contributed by adding two more "O" serogroups i.e. O172 and O173 in 1991 (Orskov *et al.,* 1991). Scheutz *et al.* (2004) further added six more (O176 to O181) in the scheme. Presently 188 "O" antigens are known which are designated as O1 to O188 (O31, O47, O67, O72, O94, and O122 being withdrawn) (Joensen *et al.,* 2015). Recently, novel un-typeable strains containing no previously known serotype genome were reported to be associated with human disease in isolates representing the global scenario of enterotoxigenic *E. coli* (Iguchi *et al.,* 2017).

K1 capsular polysaccharide antigen-containing strains of *E. coli* are associated with approximately 40% of septicemia cases and 80% of meningitis cases (Makvana and Krilov, 2015).

The Association of serogroups and serotypes with the clinical syndrome has been well documented (Levine, 1987), however serogroup or serotype themselves do not directly responsible for virulence. Rather, they serve as detectable virulence

markers correlating with virulent *Escherichia coli* clones. (Whittam *et al.,* 1993) "O" serogroups usually associated with different pathotypes are in the below-mentioned table (Table 2.1).

Specific serotypes were found to be involved in specific diseases i.e. serotype O18:K1 was associated with neonatal meningitis and neonatal bacteremia (Pluschke *et al.,* 1983). Similarly, serotype O157:H7 is known to cause hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) (Byrne *et al.,* 2020). Serotypes O145, O121, O111, O103, O45, and O26 are known to be associated with Shiga toxin-producing *E. coli* outbreaks associated with food poisoning (FAO and WHO, 2018; Gyles, 2007; Karmali *et al.,* 2003). Strains un-typeable with the presently known serotyping scheme are also routinely reported to be associated with *E. coli* infections (Bai *et al.,* 2016).

Geographical variations in the distribution of serotypes involved in *E. coli* infections are observed, the commonly involved serotypes in *E. coli* outbreaks in the developed world are usually O157, O104, etc. on the other hand these serotypes are rarely isolated in *E. coli* infections in developing world countries. In the US the most common *E. coli* infections are food-borne STEC infections which were first identified in 1982 with the isolation of two O157:H7 serotypes (Wells *et al.,* 1983). An estimated 1,75,905 STEC infections occur in the US and involve serotype O157 in 64% of food-borne STEC infections and non-O157 serotypes in 82% of foodborne STEC infections. O45, O69, O84, O103, O104, O121, O141, O145, and O169 are frequently isolated in the US (Scallan *et al.,* 2011). Luna-Gierke *et al.* (2014) detected O111 and O26 as the most common non-O157 serotypes involved in more than 60% of infections. In other developed countries, the most commonly associated serotypes with STEC infections are O157, O26, O91, O111, O103, O145, and O146 (Kim *et al.,* 2020; Loconsole *et al.,* 2020; Torti *et al.,* 2021). Though. STEC infections with serotype O157 are not so common in developing countries. In a ten-year survey of samples of 5678 human isolates, only 30 (0.5%) were O157 serotypes (Sehgal et al, 2008). One study from southern India reported non-O157 STEC infection involving serotypes O59, O60, and O69 (Purwar *et al.,* 2016).

Table 2.1: Serogroups associated with different *E. coli* pathotypes

E. coli Pathotype	Associated O-Serogroups
Enterotoxigenic *E. coli* (ETEC)	O169, O167, O159, O153, O148, O139, O128, O115, O85, O80, O78, O63, O27, O25, O20, O15, O8, O6, (Wolf, 1997; Beatty *et al.,* 2004)
Enteropathogenic *E. coli* (EPEC)	O158, O142, O128, O127, O126, O125, O119, O114, O111, O86, O55, O26 (Hernandes *et al.,* 2009).
Enteroinvasive *E. coli* (EIEC)	O173, O167, O164, O159, O152, O144, O143, O136, O135, O124, O121, O115, O112, O96, O29, O28 (Michelacci *et al.,* 2016; Escher *et al.,* 2014; Orskov *et al.,* 1991; Silva *et al.,* 1980; Gomes *et al.,* 1987, Matsushita *et al.,* 1993; Tozzoli and Scheutz, 2014; Newitt *et al.,* 2016; Voeroes *et al.,* 1964).
Enteroaggregative *E. coli* (EAEC)	O44, O73, O86, O92, O104, O111, O126, O136 (Boisen *et al.,* 2015; Jenkins *et al.,* 2006)
Enterohemorrhagic *E. coli* (EHEC)	O157:H-, O157:-H7, O26: H11, O 104, O111:H-, O111:H multiple, O45, O103, O121, O145 (Bilinski *et al.* 2012; Gould *et al.,* 2013; Kaper and O'Brien, 2014)
Diffusely adherent *E. coli* (DAEC)	O162, O151, O142, O137, O130, O128, O127, O126, O125, O119, O117, O114, O111, O86, O75, O65, O55, O36, O26, O25, O23, O21, O20, O18, O11, O10, O7, O4, O2, O1, UT (Giron *et al.,* 1991; Campos *et al.,* 2004; Fujihara *et al.,* 2009)
Adherent-invasive *E. coli* (AIEC)	06;022 (Croxen *et al.,* 2013)

In developing countries, enteropathogenic and enterotoxigenic *E. coli* infections are more prevalent, and different serotypes are associated with these

infections in developing countries. In a retrospective study considering ETEC in developing countries serotypes O6, O8, O78, O128, and O153 were the most common serotypes responsible for over 50% of ETEC infections (Qadri *et al.,* 2005). In one study of diarrheal *E. coli* isolates in Iraq serotypes O6, O7, O9, O25, O29, O78, O80, O111, O112, O128, and O157 were the most prevalent serotypes (Otaiwi *et al.,* 2019). O26 and O111 were found to be the most common serotypes associated with EPEC infections in developing countries by Trabulsi (2002) who also observed a geographical variation in typical and atypical EPEC serotypes pointing out that serotypes could be easily associated with typical or atypical EPEC except few exceptions in developed countries but this is not the case in developing countries. Serotypes O6, O1, and O15 were found as the most common among UPEC isolates in a study conducted in Delhi in India (Sharma *et al.,* 2016).

2.5. **Emerging drug resistance trends among *E. coli***

Drug resistance among pathogenic *E. coli* is a leading cause of concern worldwide as the emergence of highly drug-resistant strains has left limited treatment options and increased disease and death rates by antimicrobial-resistant *E. coli*. In 2019 *E. coli* was responsible for the highest number of deaths due to antimicrobial resistance attributable and associated deaths worldwide followed by *K. pneumonia, S. aureus, A. baumannii, S, pneumonia,* and *M. tuberculosis*. Collectively, these six pathogens were responsible for 9, 29,000 deaths attributable to antimicrobial resistance and 3.57 million deaths associated with antimicrobial resistance globally in 2019 with the maximum contribution from drug-resistant *E. coli* (Murray *et al.* 2020). *E. coli* has shown increasing trends of drug resistance worldwide over the years. Many drugs like trimethoprim-sulfamethoxazole, β-lactams drugs, aminoglycosides, and fluoroquinolones are often used to treat patients infected with community-based or nosocomial *E. coli* infections, and these and other agents are known to be effective against *E. coli* (Pitout, 2010 and 2012). However, due to emerging drug-resistant *E. coli* strains, it is becoming very difficult to manage *E. coli* infections thus causing a public health concern due to this infectious agent as antibiotic resistance results in

delayed appropriate therapy and an increase in morbidity and mortality (Sudershan *et al.,* 2014; Shepherd and Pottinger, 2013; Akpaka *et al.,* 2010; Pitout and Laupland 2008; Schwaber and Carmeli, 2007; Tumbarello *et al.,* 2007).

In *E. coli*, the production of the β-lactamase enzyme, which inactivates β-lactam antibiotics by hydrolysis, is known as the most important factor responsible for β -lactam drug resistance. Various types of β-lactamase enzymes i.e. narrow-spectrum β-lactamases (like TEM-1 and TEM-2), the plasmid-mediated AmpC β-lactamases (like CMY types), extended-spectrum β-lactamases (like TEM, SHV, CTX-M types), and Metallo-β-lactamases (like NDM types) are associated with multi-drug resistance in *E. coli* (D'Andrea *et al.,* 2013; Johnson and Woodford, 2013; Bush and Jacoby, 2010; Jacoby, 2009; Pitout, 2008; Pitout *et al.,* 2005). The origin of such drug-resistant strains has mainly been linked to the Indian subcontinent (Castanheira *et al.,* 2011a and 2011b; Lascols *et al.,* 2011; Leverstein-Van Hall *et al.,* 2010; Peirano and Pitout, 2010; Jacoby, 2009; Laupland *et al.,* 2008). An estimation study conducted using different model approaches based on data from seventeen countries estimated 0.4million to 6.4 million bloodstream infections and 2.7 million to 50.1 million serious infections by cephalosporin drug-resistant *E. coli* and *Klebsiella pneumoniae* in 2014 (Temkin *et al.,* 2018).

Antibiotic-resistant infection burden due to pathogenic *E. coli* strains in both intestinal as well as extra-intestinal categories are found to be increasing worldwide over the years. Increasing trends of *E. coli* infectious strains with drug resistance to commonly used antimicrobials are reported worldwide. Vila *et al.* (1999) studied antibiotic resistance prevalence in DEC isolated from 346 children of age <5 years in Tanzania. They identified that 38% of diarrhea cases were due to multi-drug-resistant ETEC, EPEC, and EAEC strains. All these three DEC pathotypes were found to be highly resistant to ampicillin, tetracycline, co-trimoxazole, and chloramphenicol while highly susceptible to quinolones. Gupta *et al.* (2001) studied a nationwide prevalence during 1998 in 1,03, 232 UTI isolates from women in the USA from 9 geographical regions and isolated *E. coli* as the most prevalent agent of UTI in the

USA in women (72% in age group 15-50 years and 53% in > 50 year age group). Almost all *E. coli* isolates in this study were susceptible to nitrofurantoin and fluoroquinolones. However, 33-44% of *E. coli* isolates were resistant to ampicillin and 16-18% were resistant to co-trimoxazole in two age groups.

In a study conducted from 1998 to 2003 on UTI *E. coli* isolates in Manisa, Turkey, increasing trends in antibiotic resistance to commonly used antimicrobials were observed by Kurutepe *et al.* (2005). In a study, Nguyen *et al.* 2005, reported 100% susceptibility to Imipenem among 162 diarrheagenic *E. coli* isolates, however, a high percentage of isolates were resistant to other antimicrobials i.e. 86.4% to ampicillin, 77.2% to chloramphenicol, 29.6% to cefuroxime, 24.1% to cefotaxime, 19.1% to nalidixic acid, 3.7% to ciprofloxacin and 88.3% were resistant to trimethoprim-sulfamethoxazole. Among the few ciprofloxacin-resistant isolates, 05 were EPEC strains and 01 was ETEC. The traditional antibiotics, including ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole, showed low activity against the diarrheagenic *E. coli* strains. Multidrug resistance was observed in 89.5% of all diarrheagenic *E. coli* strains, strain-wise 100% EIEC, 91.8% EAEC, 86% EPEC, and 78.6% ETEC strains were resistant to multiple antimicrobials. Van de Sande-Bruinsma *et al.* (2008) evaluated the European surveillance system database of 21 European countries for 6 years (2000-2005) and observed an increasing trend of fluoroquinolone-resistant *E. coli* in most of the European countries including Germany, Austria, Hungary, Belgium, Czech Republic, Spain, Bulgaria, Finland, Croatia, Netherland, Luxembourg, Portugal, and Sweden. In a 30-year follow-up study from the year 1979 to 2009, an increasing resistance trend among *E. coli* isolates for common treatment antimicrobials i.e. ampicillin, gentamicin, sulphonamide and was observed (Kronvall, 2010). Ibrahim *et al.* (2012) studied antimicrobial resistance among 232 *E. coli* isolates from various clinical samples from patients in different hospitals in Khartoum state, Sudan from May to August 2011, and observed that 92.2% of the *E. coli* isolates were MDR. Very high resistance rates were recorded against most of the commonly used antimicrobials i.e. amoxicillin (97.7%), cefuroxime (92.5%), co-trimoxazole (88.3%), tetracycline (77.1%), nalidixic acid (72%), ceftriaxone (64%), ciprofloxacin (58.4%), ofloxacin

(55,1%), Amoxyclav (50.4%), ceftazidime (35%), gentamicin (35%), nitrofurantoin (22.4%), tobramycin (18.2%), chloramphenicol (18.2%) and Amikacin (1.9%). In an ECDC report increasing trends of weighted mean percentage of third-generation cephalosporin resistance in *E. coli* from 11.9% to 13.1% in 2012 and 2015 was reported in European Union/ European Economy Area populations (CIDRAP, 2017). In a study conducted during 2014-2015 on 150 *E. coli* isolated from hospital patients in Egypt high drug resistance rates were observed to β-lactam antibiotics i.e. cefoperazone (70.67%), ceftriaxone (64.67%), cefotaxime (42.67%), amoxiclav (39.33%), cefoxitin (58%), ceftazidime (44%), however, resistance to imipenem (1.33%) and meropenem (1.33%) were found to be relatively less (Abbas, 2017). In a study conducted on 775, *E. coli* isolates from UTI cases in six European countries antimicrobial resistance to commonly used first-line treatment antimicrobials in UTIs was found very low i.e. nitrofurantoin (1.2%), fosfomycin (1.3%) and mecillinam (4.1%). Higher resistance rates in this study were observed for ampicillin (39.6%), trimethoprim (23.8%), cotrimoxazole (22.4%), amoxiclav (16.7%), and ciprofloxacin (15.1%), while all isolates were found susceptible to meropenem (Ny *et al.,* 2019). McGough *et al.* (2020) evaluated antimicrobial resistance data of 28 European countries for the period 2000 to 2016 for *E. coli, K. pneumonia, and S. aureus* and increase in ambient minimum temperatures. They observed that *E. coli and K. pneumonia* have remarkably rapidly shown increasing resistance trends (0.33% to 1.2% per year) to all classes of antimicrobials in European countries with 10°C warmer ambient minimum temperatures in comparison to others. Kaye *et al.* (2021) studied resistance patterns against co-trimoxazole, fluoroquinolones, and nitrofurantoin among 15, 13, 822 *E. coli* isolates collected from US women UTI patients between 2011-2019 and observed an increasing trend of antibiotic resistance. Overall non-susceptibility rates were 25.4%, 21.1%, and 3.8% for co-trimoxazole, fluoroquinolones, and nitrofurantoin respectively· ESBL the prevalence was 6.4% among the isolates. On average 7.7% per annum increase rate of ESBL-producing *E. coli* in the US was observed in the study. Halabi *et al.* (2021) also reported increasing trends of antimicrobial resistance in *E. coli* isolates from UTI patients in Morocco, North Africa from January 2016 to June 2019. Out of 438, *E. coli* isolates from 670 urine samples 259 (59%) were ESBL-producing *E. coli* of these 200 (77%) were from

adult (>50 years) patients majority being women patients. All the ESBL-producing *E. coli* isolates were found resistant to third-generation cephalosporins and quinolones but sensitive to carbapenems and fosfomycin. MacKinnon *et al.* (2021) evaluated antimicrobial resistance patterns of 31, 889 *E. coli* isolated from bloodstream infections in Finland, Austria, Sweden, and Canada from the year 2014 to 2018. They observed that overall 7.8% of the *E. coli* isolates were resistant to third-generation cephalosporins and that the incidence rate of third-generation cephalosporins-resistant *E. coli* from bloodstream infections has significantly increased from 2014 to 2016, 2017, and 2018. ECDC (2022) released antimicrobial resistance surveillance data for the year 2020 indicating that *E. coli* (38.4%) was the most commonly reported organism followed by *S. aureus* (17.3%) and *K. pneumoniae* (14.9%). Resistance percentages to fluoroquinolones <10% to > 50% and third-generation cephalosporins 5 to >50% were observed in different countries of data from 40 countries. A resistance rate of 1% or more was observed to carbapenems in six of the total 40 countries reported.

Due to very high drug resistance rates *E. coli* along with other carbapenems and third-generation cephalosporin-resistant Enterobacteriaceae members has been kept by WHO on the list of "CRITICAL" bacteria for which new drugs are urgently needed (WHO, 2017). Center for disease control and prevention (CDC) has also placed carbapenem-resistant Enterobacteriaceae that consist of *Klebsiella* and *E. coli* under the "SERIOUS THREAT" category, the top priority pathogens in its latest report on antimicrobial resistance threats (CDC, 2019).

2.5.1. **Scenario in India**

Drug-resistant infections are estimated to cause 58000 deaths in newborns every year in India (Arinaminpathy *et al.,* 2021). Multiple drug-resistant *E. coli* strains have widely been reported in various studies in India, some of the studies have reported the migration of drug-resistant strains from the Indian subcontinent to the rest of the

world (Castanheira *et al.,* 2011a and 2011b; Leverstein-Van Hall *et al.,* 2010; Peirano and Pitout, 2010; Jacoby, 2009; Lascols *et al.,* 2009; Laupland *et al.,* 2008).

Increasing trends in drug resistance rates among *E. coli* isolates in India have been observed over years. Community-isolated *E. coli* isolates between 2004 to 2007 were found highly resistant to commonly used drugs against *E. coli* i.e. ampicillin -75%, nalidixic acid -73%, cotrimoxazole -59% (WHO, 2009). Hussain *et al.,* (2012) identified 23 ESBL-producing out of a total of 100 *E. coli* isolates from UTI patients in Pune from January-October 2009. Of these 23 strains, 16 indicated the presence of 16 CTX-M-15-O25b-S131 group strains. Of these 16 ST 131 strains 94% were resistant to tetracycline, 81% to ciprofloxacin, 69% to co-trimoxazole and gentamicin, and 6% to chloramphenicol. Twelve of these strains were resistant to multiple drugs. Mukherjee *et al.* (2013) investigated antimicrobial susceptibility patterns of 40 *E. coli* isolates from 200 urine samples from suspected UTI hospitalized patients in Kolkata, Western India. Thirty-seven of the 40 isolates were found as MDR. A very high level of drug resistance was observed to ampicillin (97.5%), cephalexin (95%), nalidixic acid (95%), amoxicillin (92.5%), co-trimoxazole (82.5%), and ciprofloxacin (80%). Nitrofurantoin and Amikacin with susceptibility rates of 72.5% and 70% respectively were found to be the most effective drugs. Thirty-eight out of forty isolates were resistant to third-generation cephalosporins while 18 of these were ESBL-producing.

Diarrhea-genic *E. coli* was isolated predominantly (25.1%) of the pediatric diarrheal samples in a tertiary-care center in New Delhi from October 2010 to March 2012. These strains were found to be highly resistant to commonly used antimicrobials i.e. nalidixic acid (100%), cotrimoxazole (95.8%), ampicillin (90.8%), doxycycline (80.5%), cefotaxime (78.1%), ceftazidime (74.1%), ofloxacin (73.3%) and ciprofloxacin (72.4%). Resistance to at least one of the third-generation cephalosporins was found in 78.1% of these DEC isolates and 64.3% were confirmed as ESBL-producing (Aggarwal *et al.,* 2013). Niranjan and Malini, (2014) studied the

antimicrobial susceptibility of 119 in-patient *E. coli* isolates from Puducherry, South India from August 2011 to July 2012. They observed a very high percentage (76.51%) of MDR *E. coli* in this study. A high level of resistance to commonly used antimicrobials was observed i.e. ampicillin (88.4%), Amoxyclav (74.4%), norfloxacin (74.2%), cefuroxime (72.2%), ceftriaxone (71.4%) and cotrimoxazole (64.2%). The most effective antimicrobials were amikacin, piperacillin-tazobactam, nitrofurantoin, and imipenem with susceptibility rates of 82.6%, 78.2%, 82.1%, and 98.9% respectively. *E. coli* isolates (229 Nos.) from diarrheal cases were studied in Hyderabad from 2010 to 2011 and more than 70% of isolates were found to be resistant to Norfloxacin, ampicillin, amoxicillin, cotrimoxazole, ceftriaxone, cefotaxime, metronidazole (Sudershan *et al.,* 2014). In one study comprising 99 *E. coli* isolates from 150 different food samples in Hyderabad 14.7% antimicrobial resistance rate was observed overall with 6% ESBL-producing *E. coli* detection (Rasheed *et al.,* 2014). Over 2008 to 2013 resistance rates in *E. coli* to third-generation cephalosporins were reported to increase from 70% to 83%, it was reported to increase from 78% to 85% against fluoroquinolones and from 10% to 13% for carbapenems in India (CDDEP, 2015). *E. coli* isolates from various clinical samples were found resistant to ampicillin (95%), amoxiclav (95%), cefotaxime (90%), ceftazidime (80%), ciprofloxacin (75%), norfloxacin (70%) and gentamicin (35%) in a study conducted at Chennai. Resistance to imipenem was also found very high (30%) in this study with 15% confirmed carbapenemase-producing strains (Noor and Gopinath, 2018). In a recent study on the drug resistance index (DRI) of 41 countries, India was found to be the worst in terms of drug resistance among infectious agents with the lowest DRI among the 41 countries evaluated (Klein *et al.,* 2019). Gandra *et al.* (2019) evaluated the mortality burden due to MDR pathogens in India. They evaluated data from 10 hospitals from different geographical regions of India (5 in Northern India, 2 in Western India, 2 in Southern India, and 1 in Eastern India) and observed that out of a total of 5103 MDR culture-confirmed bacterial infections *E. coli* infections were the highest (1907; 37.4%) during the study period (January-December 2015). It was also observed that in comparison to non-MDR *E. coli*, the odds of mortality were many times higher in infections with MDR *E. coli*

(2.63 times), beta-lactam/beta-lactamase inhibitor-resistant *E. coli* (2.23), and XDR *E. coli* (2.34). In a study conducted at Christian Medical College Vellore on 99257 bloodstream infections isolates 1100 were identified as *E. coli* isolates of these 10% were resistant to carbapenems. Whole-genome sequencing of 60 of these MDR carbapenem-resistant *E. coli* isolates was reported to belong to two clades i.e. ST-131 and ST-410 as the prevalent clades of *E. coli* in India. Genotypes blaCTX-m-15 and blaNDM-5 were found to be common among cephalosporin-resistant and carbapenems-resistant isolates respectively in this study (Devanga Ragupathi *et al.,* 2020). Paul *et al.* (2021) studied 103 *E. coli* isolates from 504 urine samples from patients suspected of UTI from November 2018 to October 2019 in Assam, North East India. They detected ESBL production in 26.2% of the *E. coli* isolates and 12.6% were identified as carbapenemase-producing. Malik *et al.* (2021) studied the antimicrobial susceptibility of 53 UPEC patients from September 2017 to April 2018 in Haryana, North India. High antimicrobial resistance rates against ampicillin (88.7%), ofloxacin (83.1%), and amoxiclav (81.1%) were observed in this study, while imipenem, meropenem, nitrofurantoin, fosfomycin, colistin, amikacin, and tigecycline were found to be very effective drugs against UPEC with susceptibility rates of 75.4%, 62.3%, 84.9%, 86.8%, 96.2%, 90.6%, and 100% respectively.

E. coli has also been included among the important microorganisms that are required to be closely monitored for their antimicrobial resistance profiles in India's National Action Plan on Antimicrobial Resistance (National Action Plan on antimicrobial resistance, 2017). It has also been placed under the "Critical Priority" tier the topmost in Indian priority pathogens stratification due to its higher rates of increasing drug resistance trends (WHO, DBT, 2019).

The development of drug-resistant strains is mainly attributed to the overuse and irrational use of antimicrobials. In India sale of 24 antimicrobial agents including third and fourth-generation cephalosporins, carbapenems, and newer fluoroquinolones without prescription by a medical practitioner is banned under

Schedule H of the Drug and Cosmetic Act and Rules (The Drugs and Cosmetic Act, 2016). However, many antimicrobials are sold by pharmacies without any prescription. One study conducted in the corporation area in South India revealed that antimicrobial agents are being available in pharmacies without any medical prescription (Rathnakar *et al.,* 2012). The use of antimicrobials in animal feed is another major contributing factor to the emergence of drug resistance. In a study, *E. coli* resistant to as many as eleven antimicrobials and with ESBL-producing ability was detected in poultry farms in Punjab (Brower *et al.,* 2017). ESBL-producing *E. coli* were isolated from cattle and poultry in Odisha also (Kar *et al.,* 2015). ESBL-producing *E. coli* were also isolated from cattle mastitis in West Bengal (Das *et al.,* 2017). Usage of antimicrobials in animal feed is a major concern for curtailing emerging drug resistance to essential emergency antimicrobials like colistin which is the last resort drug against multidrug-resistant ESBL-producing and carbapenem-resistant *E. coli* and is declared an essential threat to medicines by WHO but is commonly used as an animal growth promoter in India (WHO, 2021; Davies and Walsh, 2018). Though many regulations are made through different agencies like FSSAI in India for regulating the irrational use of antimicrobials in animal and animal food products, however, strict measures are required to be taken (Walia *et al.,* 2019).

Escherichia coli is widely distributed in India and is isolated from humans, animals, food, water, and the environment (Musale *et al.,* 2014; Chandra *et al.,* 2012; Chakraborty *et al.,* 2001). Though *E. coli* pathotypes are known to be one of the leading causes of morbidity and mortality in India, however, significantly informative data on the prevalence of various *E. coli* pathotypes in different parts of the country is lacking as most of the studies from the country usually reports infections at local level sporadically. Studies on the prevalence of infectious agents in different locations and their antimicrobial susceptibility profiles play a crucial role in the management and containment of infections. Thus more studies on *E. coli* pathotype distribution in the country and their virulence and drug susceptibility profiles will be valuable for more understanding and better management of this ubiquitous infectious bacterium.

The present study was designed to understand the prevalence of different *E. coli* pathotypes in various parts of the country their virulence characteristics and drug susceptibility patterns.

CHAPTER-3

OBJECTIVES

3. OBJECTIVES OF THE STUDY

This study was designed with aim of studying the prevalence and characterizing of pathogenic *E. coli* isolates from intestinal and extra-intestinal sites in various geographical regions of India.

The aim of the study was achieved through the following objectives:

3.1. To biotype intestinal and extra-intestinal *E. coli* isolates

3.2. To serotype the confirmed *E. coli* isolates

3.3. To detect different *E. coli* pathotype markers

3.4. To characterize the various detected *E. coli* isolates in terms of their virulence factors

3.5. To study the antimicrobial susceptibility patterns of the *E. coli* isolates

3.6. To detect ESBL-producing *E. coli* isolates

CHAPTER-4

MATERIAL & METHODS

4. MATERIAL AND METHODS

4.1. **Study samples, subculture, and selection of bacterial samples**

E. coli isolates from intestinal (diarrheal) and extra-intestinal infections were taken for the present study. The source of *E. coli* isolates was National Salmonella and Escherichia Center (NSEC) situated at Central Research Institute, Kasauli (HP) which is the reference center for *Escherichia coli* in India. This Center receives suspected *E. coli* samples from all over the country for confirmation, and hence was considered the best source for representative *E. coli* strains from the Indian population. Seven hundred and eighty-three (783) suspected *E. coli* strains from Intestinal and extra-intestinal infections referred to National Salmonella and Escherichia Center, Central Research Institute, Kasauli (HP) from different geographical regions of the country were taken as representative samples of pathogenic *E. coli* strains in circulation, in India. All the 783 bacterial samples were streaked on MacConkey agar plates and pure colonies consistent with *E. coli* (Edwards and Ewing, 1972) were selected and further subjected to morphological and biochemical characterization to confirm as *E. coli*.

4.2. **Morphological, Microscopy, and Biochemical Characterization of study samples for selection of *E. coli* isolates**

Isolates under study were characterized using standard morphological, microscopical, and biotyping techniques (Edwards and Ewing, 1972). The morphology of the strains was examined microscopically after Gram staining under a compound microscope (100X objective). Gram staining was carried out using crystal violet and carbol-fuschin as primary and counterstains respectively with ethyl alcohol as the decolorizing agent (Smith and Hussey, 2005). Biotyping of the isolates was carried out using standard biochemicals as described by Ewing (1986) and Barrow and Felthon (1993) with minor modifications to identify the *E. coli* isolates. Isolates which gave growth characteristics on microbiological media (nutrient broth, nutrient agar, and MacConkey agar) and reactions consistent to *E. coli* on different

biochemical tests i.e. indole, methyl red, Voges Proskauer, citrate utilization (IMViC), sugar (lactose, glucose, sucrose) fermentation, reduction of nitrate, Triple Sugar Iron (TSI), utilization of urea, catalase test, oxidase test, and Ortho-nitrophenyl beta D-galactopyranoside (ONPG) were designated as *E. coli*.

4.2.1. **Catalase Test**

Test organism cultured overnight on a nutrient agar plate was used to test catalase. One drop of catalase reagent (3%H_2O_2) was put on a clean glass slide with a glass dropper. An isolated colony from the nutrient agar plate was picked with a sterile Pasteur pipette and emulsified in the catalase reagent drop. The emergence of effervescence within 10 seconds was observed.

4.2.2. **Oxidase Test**

Test organism cultured overnight on a nutrient agar plate was used to test oxidase. One drop of oxidase reagent (tetramethyl-p-phenylenediamine dihydrochloride) was put on a sterile Whatman paper with a glass dropper. An isolated colony from the nutrient agar plate was picked with a sterile Pasteur pipette and emulsified in the oxidase reagent spot. The development of a dark purple spot at the site of emulsification within 10 seconds was observed.

4.2.3. **Sugar Fermentation tests**

Sugar media (1% sugar in peptone water containing Andrade indicator and Durham's tube) was inoculated with the test. Inoculated media was incubated at 35± 2°C overnight. The next day tubes were observed for color change and accumulation of gas in Durham's tube. The development of pink color in the medium was considered sugar fermentation while no pink color was considered no sugar fermentation. Accumulation of bubbles in Durham's tubes indicated gas formation while no bubble in Durham's tubes was considered as no gas formation.

4.2.4. **Indole Test**

Peptone water was inoculated with the test organism and incubated at 35± 2°C overnight. The next day 2-3 drops of Kovac's reagent were added into the tubes and the appearance of a red ring at the interface of medium and reagent was observed. The development of a red-colored ring at the interface was read as indole positive while, the development of a yellow ring or no ring at all was considered an indole negative result.

4.2.5. **Methyl Red (MR) and Voges Prausker (VP) Tests**

Two tubes of Glucose phosphate medium (GPM) were inoculated with the test organism and incubated at 35± 2°Covernight. The next day 3-4 drops of methyl red reagent were added into the first medium tube and the appearance of red color was observed. The development of red color was read as MR positive test while the development of yellow color was taken as MR negative result.

To the second GPM tube few drops of 40%, KOH was added. After this 3-4 drops of α-naphthol were added and the appearance of the red ring at the interface of the medium and reagent was observed. The development of a red-colored ring at the interface was read as VP positive test while the formation of a yellow or no ring was taken as VP negative result.

4.2.6. **Citrate Utilization Test**

Simmon citrate medium was inoculated with test organism and incubated at 35± 2°C overnight. The next day change in the color of the medium was observed. Development of blue color in medium and bacterial growth was read as citrate positive test. While no change in medium color was considered a citrate negative test.

4.2.7. **Urease test**

The urea medium was inoculated with test organisms by streaking on the surface of the medium. Inoculated media was incubated at 35± 2°C overnight. The next day change in the color of the medium was observed. Development of pink/red color in the medium was read as a urease positive test. While no change in medium color was considered as a urease negative test.

4.2.8. **ONPG test**

ONPG test was performed using ONPG discs from HiMedia Pvt. Ltd. (Mumbai) as per the instructions of the manufacturer. One ONPG disc was placed in sterile tubes. To these tubes 100µl of sterile normal saline was added. Tubes were gently shaken and used immediately for the test. Isolated colonies of the test strains were picked with a sterile loop and emulsified in the saline tubes containing ONPG discs. Tubes were incubated at 35± 2°C and observed for any color change after 1 hour and 6 hours. The development of yellow color in the medium was read as ONPG positive test. While no change in medium color was considered an ONPG negative test.

4.2.9. **Triple Sugar Iron (TSI) Test**

TSI medium was inoculated with test organisms by streaking on the media surface and stabbing the medium using a sterile inoculation needle. Inoculated media was incubated at 35± 2°C overnight. The next day media tubes were observed for changes in the color of the medium in slant and butt, the development of black color, and cracks in the medium/raising of the medium. The development of red color in the medium was considered an indication of sugar fermentation (acid formation), the development of black color production of H_2S, and cracks in the medium/raising of the medium as production of gas CO_2 (Table 4.1).

Table 4.1: Interpretation of results on TSI media

Observations	Interpretation
Pink slant, yellow butt	K/A
Pink slant, pink butt	K/K
Yellow slant, yellow butt	A/A
Black color	H_2S production
Cracks in medium	Gas formation
No cracks in medium	No gas formation

Here; A: Acidic K: Alkaline

4.2.10. **Nitrate Reduction (NR) Test**

Nitrate broth medium was inoculated with test organisms by using a sterile inoculation loop. Inoculated media was incubated at 35± 2°C overnight. The next day NR reagent I (α-naphthylamine) and NR reagent II (sulfanilic acid) were added to media tubes and were observed for change in color of the medium. The development of red color in the medium was read as a nitrate reduction positive test, while no change in color was considered a nitrate reduction negative test.

4.3. **Preparation of Master and Working stocks of the test and standard strains**

Working stocks of the strains were kept on nutrient agar slants and were replaced with new working stock as and when required. The master stocks were kept as stab cultures.

4.4. **Serological Characterization**

E. coli isolates were subjected to the determination of "O" serotypes as per the method adopted in the National Salmonella and Escherichia Center (NSEC) protocol for *E. coli* "O" serotyping, originally described by Orskov et.al. (1977) and Orskov and Orskov (1984).

4.4.1. **Preparation of antigenic suspension**

The nutrient broth was inoculated with a test organism using a sterile inoculating loop. The organism to be tested in the serotyping assay was grown at a temperature of 35±2°C overnight in nutrient broth. The overnight bacterial culture was boiled for 1 hour followed by the addition of formalin to a final concentration of 0.25%. Cell concentration of the antigenic suspension so prepared was diluted to contain $3X10^9$organisms/ml with normal saline and checked by Brown's opacity set. The antigenic suspension was stored at 2-8°C till it was used for serotyping.

4.4.2. **Serotyping**

U-shaped 96-well microtiter plates were used for serotyping. Isolates were first serotyped using pooled *E. coli* anti-O antisera by mixing 50µl of antigenic suspension and 50 µl of pooled (Pool A to Pool P) *E. coli* anti-O antisera (NSEC, Central Research Institute, Kasauli, India). With each set of reactions, a negative control was also put up by adding 50 µl of sterile normal saline instead of antisera. Microtiter plates were gently shaken and covered with aluminum foil. The plates were incubated at 35± 2°C overnight. The next day wells were examined for agglutination. The test was repeated with all constituent monovalent antisera of the pool that gave clear agglutination. Test results were interpreted as given below.

- The strains agglutinated with a particular pool were tested for agglutination with individual antisera constituting the pool, similarly.
- The individual antiserum showing agglutination reaction with an isolate determined the serogroup of that isolate.
- The isolate not showing agglutination reaction with any of the pooled antisera was reheated at 121^0C for 2.5 hours to destroy K antigen and the whole process was repeated. The isolate that still did not show any agglutination was read as un-typeable (UT).

4.5. **Detection of virulence factors**

Virulence factors assisting in colonization (siderophores, biofilm, cell surface hydrophobicity, colicinogeny, gelatin hydrolysis) commonly expressed by pathogenic *E. coli* were phenotypically studied as described below:

4.5.1. **Siderophore Detection**

4.5.1.1. **Siderophore screening**

Screening of siderophore-producing *E. coli* isolates was carried out by inoculating test strains on Chrome Azurol S (CAS) agar.

CAS agar plates were prepared as described by Louden *et al.* (2011). To remove the trace elements from the glassware used in the experiment these were pretreated with 6M HCl and then thoroughly washed with water for injection (WFI).

E. coli isolates were inoculated on Chrome Azurol S (CAS) agar and incubated at 35± 2°C for 48 hours. A change in medium color around the bacterial growth was observed for scoring the plates. Isolates were assigned as siderophore-producing if the color change from blue to orange was observed (Schwyn and Neilands 1987, Shin *et al.* 2001).

4.5.1.2. **Siderophore typing**

All the test isolates were further characterized using chemical assays described by Csaky for the detection of hydroxamate and by Arnow for the detection of catecholate-type siderophores (Arnow, 1937; Csaky, 1948; Maheshwari *et al.* 2019). *E. coli* isolates were grown at 35 ± 2°C overnight in iron-restricted modified Fiss Minimal Medium to express the siderophores. The next day, the culture supernatant was separated from the bacterial mass by centrifugation at 4000 RPM for 10 minutes. Culture supernatant was carefully collected in separate sterile tubes using a sterile

Pasteur pipette and hydroxamate and catecholate siderophores types were detected in the culture supernatant using Csaky and Arnow assays respectively.

4.5.1.2a. **Csaky assay**

Csaky assay was carried out to detect hydroxamate-type siderophores. To 1ml culture supernatant (obtained as described in point no. 5.5.1.2 above) 1ml of 6M H_2SO_4 was added and autoclaved for 30 minutes to completely hydrolyze. To this sequentially added 3 ml of sodium acetate (35%), 1 ml sulphanilic acid (1% in 30% acetic acid), and 0.5 ml of iodine solution (1.3% in 30% acetic acid). After 5 minutes of incubation at room temperature, 1ml sodium arsenite solution (2% w/v) was added to destroy excess iodine. To this 1ml of naphthylamine (0.3% in 30% acetic acid) was finally added and the tubes were kept in dark for 20 minutes to allow the development of pink color. Absorbance was measured at 526 nm using a UV-Visible double-beam spectrophotometer. Deionized water was used as blank.

4.5.1.2b. **Arnow assay**

Arnow assay was carried out to detection of catechol-type siderophores. To 1 ml culture supernatant (obtained as described in point no. 5.5.1.2 above) 1 ml HCl was added. Then 1 ml nitrite-molybdate was added to this followed by the addition of 1 ml of 1N NaOH. Tubes were then incubated at room temperature for 10 minutes in dark to develop the red color. Absorbance was measured at 510 nm using a UV-Visible double-beam spectrophotometer. Deionized water was used as blank.

4.5.2. **Biofilm Formation**

Biofilm-forming potential among the study samples was detected using three common screening assays viz. The tissue culture plate method (TCA), The tube method (TM), and The congo red agar method (CRA). All the tests were performed in triplicate.

4.5.2.1. **Tissue Culture Plate Method**

The tissue culture plate method described by Christensen *et al.* (1985) was used for detecting the biofilm-forming ability of the test isolates. Biofilm-forming isolates were further categorized as strong biofilm producers, moderate biofilm formers, or weak biofilm producers using criteria described by Stepanovic *et al.* (2007). Briefly; 10 ml trypticase soy broth containing 1% glucose was inoculated with a fresh culture of test isolates and incubated at 35 ± 2°C overnight. Before adding the broth culture to the tissue culture plate, it was 100 times diluted using a fresh medium as the diluent. The tissue culture plate (polystyrene tissue culture treated, flat bottom 96 well plate) was then seeded with diluted broth culture of the test isolates with a volume of 200 µl per well, in triplicate. Sterile soy broth containing 1% glucose was used as the negative control. The tissue culture plates were incubated at a temperature of 35 ± 2°C overnight. After incubation, broth and suspended cells were removed from the wells by gently tapping the plate. The plate was washed four times by adding 0.2 ml of sterile phosphate buffer saline (pH 7.2) to each well and then decanting it with gentle tapping of the plate to remove non-adherent bacterial cells. Biofilm formed in the wells was fixed with 2% sodium acetate and then stained with crystal violet (0.1%). To remove excess stain plates were again washed with deionized water and air-dried. The optical density (OD) of stained biofilm was determined after elution with 70% ethanol using a micro ELISA auto reader at wavelength 570 nm.

The results were interpreted according to the criteria of Stepanovic *et al.* (2007) as follows (Table 4.2)

Table 4.2: Criteria for scoring plates in the TCP method

OD values of the test isolate in reference to ODc value	Interpretation
≤ ODc	Negative
> ODc - ≤ 2X ODc	Weak
> 2X ODc - ≤ 4X ODc	Moderate
> 4X ODc	Strong

Here, ODc: Optical Density of control

4.5.2.2. **The tube method**

This test was performed as per the method described by Freeman et al in 1989. In a sterile tube, 10 ml of trypticase soy broth containing 1% glucose was inoculated with a loopful of test isolate. The test isolates inoculated medium was overnight at a temperature of 35 ± 2°C. Broth and suspended bacterial cells were decanted from the tubes and tubes were washed thoroughly with sterile phosphate buffer saline (pH 7.3). Tubes were placed in an inverted position to allow them to dry. Crystal violet solution (0.1%) was added to the tubes to stain the biofilms formed if any. The excess stain was removed by washing again with deionized water. To allow the tubes to dry the tubes were again kept in an inverted position and scored for the presence of biofilm. The presence of biofilm was examined by observing a stained film lining the wall and bottom of the tube. The tubes were scored according to the results of the control tubes.

4.5.2.3. **Congo Red Agar (CRA) method**

This test was performed as per the method described by Costerton *et al.,* in 1995. CRA medium was prepared by dissolving and autoclaving Congo red indicator stock and the rest of the media separately and mixing them just before pouring them onto

media Petri dishes. Congo red indicator stock was prepared by dissolving 0.8g of Congo red indicator in 100 ml of distilled water and autoclaving at 121°C for 15 minutes. The remaining medium was prepared by dissolving 37g of brain heart infusion broth powder, 50g of sucrose, and 10g of agar No. 1 in 900 ml of distilled water. The Congo red indicator stock was aseptically mixed with the rest of the medium constituents at 55°C and poured onto sterile Petri dishes and allowed to settle. To detect the biofilm-forming ability of the test isolates CRA plates were inoculated with test organisms and incubated overnight aerobically at a temperature of 35 ± 2°C. The color of the colonies developed after overnight incubation was observed and results were interpreted. The appearance of grey to black colonies with a dry crystalline consistency was considered biofilm production and scored based on the intensity of pigmentation produced as weak, moderate, and strong.

4.5.3. **Cell Surface Hydrophobicity**

Cell surface hydrophobicity was tested by salt aggregation test (SAT) (Lee, 1996; Ljungh, 1982). Test isolates were inoculated on CFA agar and incubated overnight at 35±2°C. Bacterial growth was harvested in phosphate-buffered saline (PBS, pH 6.8) by scraping. Bacterial concentration was adjusted to $5X10^9$ cells/ml using Macfarland's standard 6. In a V-shaped microtiter plate, 25ul of solutions of ammonium sulfate (5M, 2.5M, 1.25M, 0.625M, and 0.3125M) in water for injection were added and mixed with an equal volume of test bacterial suspension ($5X10^9$/ml) in phosphate-buffered saline (PBS, pH6.8), and incubated for 3 hours at room temperature. Microtiter plates were observed with an unaided eye for bacterial aggregation.

Isolates showing aggregation with ammonium sulfate concentration of $\leq$1.25M were considered as exhibiting cell surface hydrophobicity.

4.5.4. **Colicin production**

The phenotypic soft agar overlay technique was used to detect colicin production by *E. coli* isolates (Parreira, 1998). Luria Bertani (LB) broth was inoculated with test

isolates and incubated overnight at a temperature of 35±2°C. Growth from overnight culture was stabbed on an LB agar plate and incubated overnight at a temperature of 35±2°C for the diffusion of colicin in the growth medium if produced. The plate was exposed to chloroform for 10 minutes for killing bacterial cells by placing chloroform-soaked filter paper on the growth. Colicin-sensitive strain (*E. coli* K12) grown in LB broth overnight was mixed with soft agar (0.4% agar) at 60°C and overlaid on the Petri dish with test organism growth. Plates were left undisturbed to let the soft agar settle and then incubated overnight at a temperature of 35±2°C. A zone of growth inhibition of the colicin-sensitive strain was observed around the test organism growth for colicin activity detection. Zone of growth inhibition of the sensitive strain around the test organism growth was considered a colicin production positive test. No zone of growth inhibition of the sensitive strain was considered as, no colicin production.

4.5.5. **Gelatinase detection**

Gelatinase was detected on gelatinase agar plates (Kaira and Pai, 2018; McDade and Weaver, 1959). Gelatinase media containing 1% extra-pure gelatin was inoculated with the test organism and incubated overnight at a temperature of 35±2°C. Plates were then flooded with acidic mercuric chloride solution (15g$HgCl_2$, Conc. HCl 20ml and WFI 100ml). Plates were observed for the appearance of the zone of clearance around bacterial growth and the cloudiness of the gelatinase medium. The appearance of a zone of clearance around bacterial growth and cloudiness of the gelatinase medium was considered gelatinase production. No zone of clearance around bacterial growth was considered a gelatinase-negative test.

4.6. **Detection of various pathotype markers**

A Diarrheagenic *E. coli* detection kit (Hi-media Pvt. Ltd. Mumbai, India) was used for the detection of virulence marker genes of the four most important diarrheagenic pathotypes viz. ETEC (elt and est genes), EPEC (eae and bfpA genes), EHEC (hlyA gene), and EAEC (CVD432 gene).

4.6.1. **DNA extraction**

The boiled lysis method was used to prepare bacterial DNA for PCR analysis (Chellapandi *et al.,* 2017; Dutta *et al.,* 2013). Isolated colonies of *E. coli* isolates were inoculated in Luria–Bertani (LB) broth and the broth was incubated at 35± 2°C overnight for bacterial growth. The bacterial cells were harvested by centrifuging the bacterial growth at 3000 rpm for 5 minutes. The supernatant was discarded and the bacterial pellet was resuspended in sterile water for injection. The suspension was boiled for 10 minutes. The tubes were immediately chilled by keeping them on ice for 5 minutes for chilling shock. The bacterial lysate so obtained was then centrifuged at 10,000 rpm for 5 minutes. The supernatant was taken as template DNA for multiplex PCR analysis.

4.6.2**. Multiplex PCR**

A volume of 25 µl reaction mixture was prepared as per manufacturer instructions using 5 µl template DNA in two sets one each for primer set I (eae (482bp), bfpA (300bp), CVD432 (194bp)) and primer set II (hlyA (534bp), elt (322bp), est (170bp)). Two sets of the additional PCR reactions were prepared which contained 5 µl positive control DNA (supplied with the kit) as DNA template for primer set I and primer set II respectively (Table 4.3).

Table 4.3: Reaction mixture used in multiplex PCR for detection of DEC pathotypes

Components	Tube 1	Tube 2
2X Taq Mixture	12.5 µl	12.5 µl
Primer Set 1	6 µl	-
Primer Set 2	-	7.5 µl
Molecular Biology Grade water for PCR	1.5 µl	-
Template DNA	5 µl	5 µl
Total Volume	25 µl	25 µl

The PCR cycle was run using a conventional thermocycler (Eppendorf Mastercycler). The cycler was programmed for initial denaturation at 94°C for 10 minutes. Followed by; 30 cycles of denaturation at 94°C for 45 seconds, annealing at 56°C for 45 seconds, and extension at 72°C for 30 seconds. The final extension was carried out at 72°C for 10 minutes. PCR product was held at 4°C and analyzed the same day or stored at -20°C for analysis the next day.

4.6.3. **Agarose gel electrophoresis**

Agarose gel electrophoresis was carried out as per the user instructions contained in the literature provided with a Diarrheagenic *E. coli* detection kit (Hi-media Pvt. Ltd. Mumbai, India). Agarose solution (2%) was prepared by dissolving 2g of agarose per 100 ml of 1X TBE Buffer. Agarose suspension was allowed to heat on flame for melting. Molten agarose was cooled to 50°C and poured into a gel loading tray with combs placed. The agarose was allowed to solidify and then the comb was removed carefully. The DNA samples (10 µl) were mixed with 1.5 µl of tracking dye and were loaded gently into the wells. Two wells were loaded with positive control and DNA samples were loaded into the remaining wells. Then the gel was mounted on the respective electrophoresis apparatus tank filled with 1X TBE buffer. Electrophoresis was carried out at 100 V until the tracking dye reaches two-thirds of the gel length. The agarose gel was stained with EtBr for 15 minutes. The gel was examined and

photographed on the Gel Documentation System. Amplicon bands in the test samples were compared with the positive control of set 1 and set 2. Amplicon size was estimated by comparing it with the 50bp DNA ladder.

4.7. **Determination of antimicrobial susceptibility patterns**

Antibiotic Sensitivity Testing (AST) was performed by Kirby Bauer Disc Diffusion method as per the CLSI guidelines (CLSI, 2014) using 20 drugs representing different antimicrobial classes [amoxyclav (30ug), ampicillin (10ug), amikacin (30ug), chloramphenicol (30ug), ciprofloxacin (5ug), ceftazidime (30ug), cefotaxime(30ug), cotrimoxazole(25ug), ceftriaxone (30ug), cefepime (30ug), cefuroxime (30ug), gentamicin(10ug), kanamycin (30ug), imipenem (10ug), meropenem (10ug), nitrofurantoin (300ug), norfloxacin (10ug), nalidixic acid (30ug), trimethoprim (5ug)and piperacillin-tazobactam (100/10ug)].

A single colony from the media plate was selected and transferred into the tube containing 3ml of LB broth. The culture tube was incubated for appx. 6 hours at a temperature of 35±2°C to obtain a concentration of 10^6-10^8 cells per ml. The turbidity of the actively growing culture was adjusted to match that of the 0.5 Mc Farland standards using LB broth. A sterile cotton swab was dipped into the adjusted suspension and rotated several times. To squeeze out excess inoculum the swab was pressed firmly on the sidewall of the tube above the fluid level. This swab was then used to apply the suspension evenly on the surface of a Mueller- Hinton agar. This procedure was repeated twice by rotating the plate approximately at an angle of 60° each time to ensure an even distribution of inoculum. The rim of the agar was swabbed in the final step. Then the plate was left for 3 to 5 minutes to dry with the lid in place. The antimicrobial discs were dispensed onto the surface of the inoculated agar plate with the help of sterile forceps, in an equidistance fashion with their center approximately 24mm apart and 5 discs were deposited per plate. Each disc was pressed down to ensure complete contact with the agar surface. Plates were then inverted and incubated at 35±2°C overnight. The next day, results were noted by

measuring the zone diameter of the inhibition of bacterial growth, nearest to the millimeter with the help of a scale. Results were interpreted as sensitive, intermediate, and resistant after comparing with the interpretive chart as per CLSI guidelines (CLSI, 2014).

4.8. **ESBL Detection**

ESBL production was detected using disk diffusion assay on Muller Hinton Agar as per the guidelines of CLSI (CLSI, 2014) using cefotaxime and ceftazidime alone and in a combination with clavulanic acid. Test isolates were cultured in LB broth overnight at 35±2°C. The overnight culture was spread on Muller Hinton agar using a sterile cotton swab as described above for AST assay. Antibiotic discs (Cefotaxime, ceftazidime alone, and in combination with clavulanic acid) were seeded onto the agar plate using sterile forceps as described above for AST assay the next day. Plates were incubated overnight at 35±2°C. The next day, a difference in the zone diameter of the drug with clavulanic acid and the drug alone was observed. The difference in zone diameter of the drug with clavulanic acid and the drug alone was taken as the criteria for scoring isolates as ESBL positive and negative. The difference of $\geq$ 5mm was taken as positive while that of <5mm was taken as negative.

4.9. **Statistical Analysis**

SPSS version 22.0 was used to calculate frequencies, mean, percentages, standard deviations, associations, and significances. When assays results were taken in triplicate mean values were considered i.e. optical densities in the TCP method for biofilm detection, and chemical assays for siderophores detection. Wherever association or comparison between two or more groups i.e. intestinal and extra-intestinal isolates, geographical variations, etc., were made, the significance was tested at a 95% confidence level. Accuracy, specificity, sensitivity, and predictive values (positive predictive value and negative predictive value) were calculated when two or more methods were compared i.e. Biofilm detection by TM and CRA methods against the TCP method.

CHAPTER-5

RESULTS

&

DISCUSSION

5. RESULTS AND DISCUSSION

Escherichia coli is the most common etiological agent of urinary tract infection (UTI) worldwide and accounts for more than 80% of UTIs. It is also one of the important etiological agents of diarrhea and can result in diarrhea of variable severity and may also lead to very severe systemic infections like septicemia, neonatal meningitis, hemolytic uremic syndrome (HUS), and hemorrhagic colitis (HC). Though *Escherichia coli* outbreaks are very common worldwide, developing nations including India are the most affected due to poor socioeconomic conditions, poor hygiene, and thick populations. Inadequate healthcare infrastructure, poor hygiene, low awareness, thickly populated habitats, and poor socioeconomic conditions in developing countries not only contribute to high *E. coli* infection rates making its transmission under such conditions easier through the fecal-oral route but also pose a challenge to managing and control of this infectious agent. Thus, *E. coli* makes a major contribution to the total disease burden in low and middle-income countries. Another challenge in the management and control of *E. coli* infections is the emergence of strains resistant to multiple drugs (MDR) including resistance to newer-generation drugs like third and fourth-generation cephalosporins and carbapenems, which makes treatment and management of *E. coli* infections difficult. The prevalence and characterization of *E. coli* isolate are of prime importance for better understanding and better planning of control and management strategies for this common infectious agent.

An attempt was made in the present study to characterize clinical *E. coli* isolates from various geographical locations in India. To incorporate clinical *E. coli* isolates from all geographical regions of the country, the study samples were taken from National Salmonella and Escherichia Center (NSEC); a reference laboratory in India, which receives *Salmonella* and *E. coli* isolates from all parts of the country. In the present study, a total of 783 suspected *E. coli* isolates that were received at the National Salmonella and Escherichia Center (NSEC) during the study period were taken for the study. All the samples were first revived by sub-culturing on

MacConkey agar and nutrient broth then the stock culture of all revived samples was taken on nutrient agar slants. All viable isolates were characterized by bio-typing for confirmation as *E. coli.* Isolates identified as *E. coli* on bio-typing were then further characterized by serotyping, virulence characteristics, and antimicrobial profiles. All intestinal isolates were also characterized genotypically to identify prevalent diarrhea-genic pathotypes.

5.1. **Bio typing**

Study isolates included in the study were identified using standard microbiological techniques (Tables 5.1 and 5.2). Isolates with morphological and biochemical profiles consistent with *E. coli* were designated as confirmed *E. coli* isolates. A total of 534 isolates out of 783 taken for the study were confirmed as *E. coli.* The so-confirmed 534 *E. coli* isolates were from different regions of the country as shown in figure 5.1.

Table 5.1: Morphological characteristics of the confirmed *E. coli* isolates

Study characteristics	**Observations**
Gram staining	Gram-negative rods
Growth characteristics on MacConkey agar	Lactose fermenting colonies
Growth characteristics on Nutrient agar	Smooth, creamish colonies
Growth characteristics on Nutrient broth	Turbidity with or without pellicles

Table 5.2: Biochemical characteristics of the confirmed *E. coli* isolates

Biochemical Test Targeted for	**Test Performed**	**Results**
Enzyme Detection	Catalase test	+ ve
	Oxidase test	- ve
	Nitrate Reduction test	+ ve
	Urea hydrolysis test	- ve
Sugar Fermentation tests	Glucose fermentation (Acid/Gas)	AG
	Lactose fermentation (Acid)	A
	Sucrose fermentation (Acid)	- ve
Substrate Utilization	Triple Sugar Iron (TSI)	A/A with/without gas No H_2S
IMViC	Indole	+ ve
	MR	+ ve
	VP	- ve
	Citrate	- ve
Late lactose fermenter detection	Ortho-nitrophenyl-β-D-galactopyranoside (ONPG)	+ ve

E. coli isolates representing different geographical regions were obtained from different states in the regions i.e. Central India (Madhya Pradesh-59Nos.), Northern India (Chandigarh-98 Nos., Himachal Pradesh-26 Nos., Delhi-22 Nos. and Punjab-02Nos.), Southern India (Andhra Pradesh- 12 Nos., Tamil Nadu-38 Nos. and Karnataka-120Nos.), Eastern India (Mizoram- 134Nos.) and Western India (Maharashtra-23 Nos.).

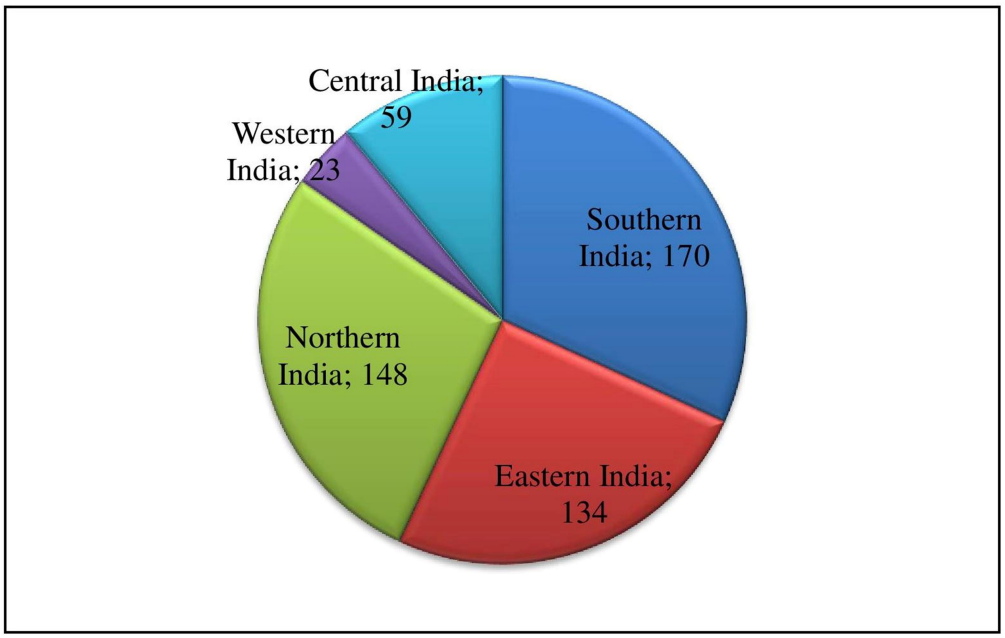


Figure 5.1: Geographical distribution of the study samples

The distribution of the confirmed *E. coli* isolates was non-uniform but representative samples were obtained from all geographical regions. Infection rates due to *E. coli* and many other etiological agents of these infections remain under-reported or under-investigated due to many reasons no medical aid is taken in most of the infections, the majority of reported infections are treated by empirical treatment without characterization of the etiological agent, and poor surveillance laboratory network in developing countries.

E. coli is one of the most diverse microorganisms in terms of its antigenic structure, pathogenesis, and virulence profiles (Lagerstrom and Hadly, 2021; Anderson *et al.,* 2006; Dozois and Curtiss; 1999). All 534 confirmed clinical *E. coli* isolates in the present study were further characterized to see the prevalence of different epidemiological, virulence, and antimicrobial resistance characteristics in different geographical locations of the country.

5.2. **Serotyping**

The Association of some of the serotypes frequently with specific *E. coli* pathotypes makes serotyping one of the most widely used epidemiological and virulence markers (Tamura *et al.,* 1996). Sometimes serotyping is solely used to assign pathotypes to *E. coli* isolates (Otaiwi *et al.,* 2019; Lanjewar *et al.,* 2010). It is the most dependable technique to characterize certain highly pathogenic *E. coli* strains like O157:H7 and the big six group associated with food-borne outbreaks (Wang *et al.,* 2013).

In the present study, *E. coli* were typed using specific anti "O" *E. coli* antisera to evaluate the prevalent "O" serogroups in India and the geographical variability of "O" serogroups, if any, in the country. *E. coli* isolates in the present study were found to be diverse in terms of "O" serogroups as these strains were clustered over 36 different "O" serogroups including 96 isolates that could not be typed using the serotyping and were designated as UT (untypeable). The ten most frequent "O" serogroups detected were O8 (46 isolates; 8.6%), O11 (36 isolates; 6.7%), O22 (33 isolates; 6.2%), O88 (33 isolates; 6.2%), O126 (28 isolates; 5.2%), O83 (25 isolates; 4.7%), O35 (25 isolates; 4.7%), O141 (23 isolates; 4.3%), O149 (22 isolates; 4.1%) and O7 (21 isolates; 3.9%) (Figure 5.2).

Untypeable isolates were frequently encountered isolates from all geographical locations, however, only one isolate was found to be non-typeable from Western India. The Untypeable isolates in the present study may be representing serotypes other than 173 serotypes targeted in the present study or newer emerging serotypes, further research in this regard will be helpful to characterize such isolates. In other similar studies, non-typeable *E. coli* isolates were detected in large numbers which were considered to probably represent emerging serogroups/serotypes associated with pathogenic *E. coli* strains (Iguchi *et al.,* 2017; Bai *et al.,* 2016). Among type-able isolates, the most predominant "O" serogroups in this study were O8, O11, O22, O126, O88, O83, O35, O7, O149, and O141. Similar other studies from different regions of India have reported different serogroups associated with clinical *E. coli* samples (Sai and Chandhar, 2019; Pralhad *et al.,* 2018; Vijayan *et al.,*

2017; Thakur *et al.,* 2016, Verma *et al.,* 2013). Thus, it can be inferred from the present and other studies from India that very diverse serogroups are associated with pathogenic *E. coli* in India.

In the developing world, the most commonly reported *E. coli* outbreaks involve the STEC pathotype which is usually found to be associated with very limited serogroups viz. O157 and big six non-O157 serogroups i.e. O26, O45, O103, O111, O121, and O145 lead to very severe clinical presentations like bloody diarrhea and HUC (CDC, 2011; Brooks *et al.,* 2005). Based on incidence rates, frequency of outbreaks, association with HUS/HC, and serotypes involved STEC has been categorized into five seropathotype classes A-E. Seropathotypes class A is the most severe class linked to human infections (high incidence rate, frequently involved in outbreaks, associated with HUS/HC), and class E is associated with non-human sources only (Amezquita-Lopez, *et al.,* 2018; Karmali *et al.,* 2003). In the recent past, a new serogroup O114 has emerged to be associated with STEC outbreaks in the developed world (Buchcholz *et al.,* 2011). Studies carried out in different parts of India on *E. coli* isolates from human has rarely reported the occurrence of *E. coli* O157, O114, and big six non-O157 STEC serogroups, thus indicating very few incidences of human STEC infections in India (Sai and Chandhar, 2019; Pralhad *et al.,* 2018; Vijayan *et al.,* 2017; Verma *et al.,* 2013; Sehgal *et al.,* 2008; Khan A. *et al.,* 2002). The present study also detected only a few serogroups which are usually linked to STEC/EHEC pathotype in very low percentages i.e. Serogroup O157 which belongs to class A seropathotype was detected in 7(1.3%) isolates only (6 intestinal and 1 extra-intestinal), serogroup O5 belonging to class C seropathotype was detected with very insignificant percentage i.e. 0.2% and few serogroups i.e. O7; 21/534 (3.9%), O119; 10/534 (1.9%), O117; 1/534 (0.2%) belonging to seropathotype class D. The results of this study, other studies carried out in different parts of India and other developing countries thus indicate geographical variations in the prevalence of *E. coli* seropathotypes in developed and developing nations.

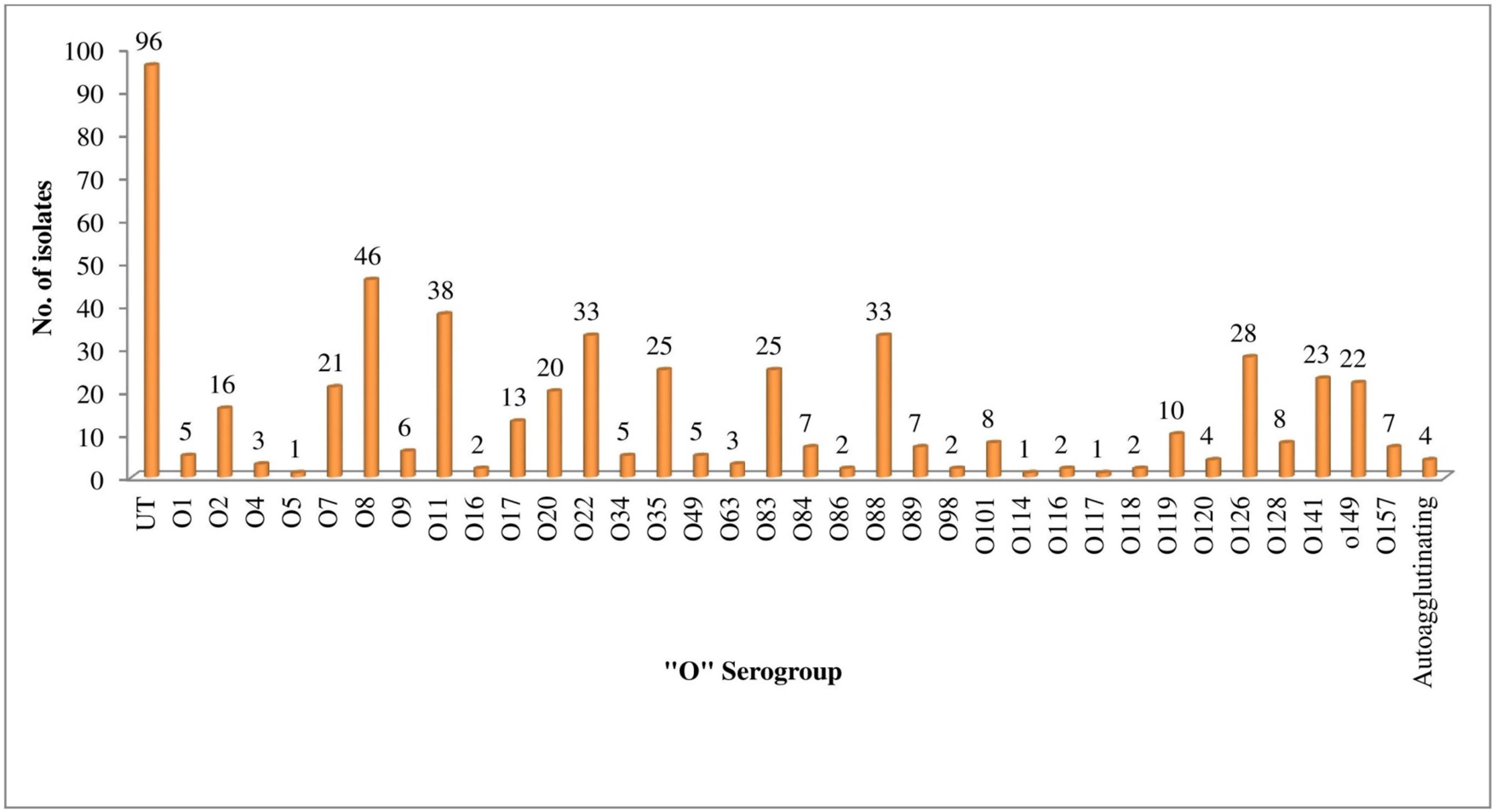


Figure 5.2: Distribution of "O" serogroups in Intestinal (DEC) and Extraintestinal *E. coli (*ExPEC*)*

Here: UT- untypeable, O1 to O157- different “O” serogroups detected

E. coli seropathotypes were not uniform across the country in the present study (Kurskal-Wallis test $p<0.05$) as some of the serogroups were found among isolates from one or two regions only i.e. O1, O4, O5, O16, O89, O98, O101, O114, O116, O117, O118 were detected in isolates from mainly eastern and southern regions. Frequently detected "O" serogroups in different geographical regions were found to be different i.e. Southern India: O8 (21/170; 12.3%), O88 (17/170; 10%), O11 (14/170;8.2%), O20 (12/170; 7.06%), O83 (10/170; 5.9%); Northern India: O83 (14/148; 9.5%), O149 (14/148; 9.5%), O126 (12/148; 8.1%), O35 (10/148; 6.7%); Eastern India: O22 (19/134; 14.2%), O126 (12/134; 9%), O141(12/134; 9%), O8 (11/134; 8.2%), O2 (10/134; 7.5%); Central India: O11 (12/59; 20.3%), O7 (7/59; 12%), O149 (4/59; 7%), and Western India: O88 (5/23;22%), O22 (3/23; 13.04%) [Table 5.3].

The majority of serogroups were detected in both intestinal as well as extra-intestinal isolates. However few serogroups were detected in either of the sources (intestinal or extra-intestinal only) i.e. serogroups O4, O89, and O117 were detected only in extra-intestinal isolates while O5, O84, O86, O98, O114, O126, O128, and auto-agglutinating were detected only in intestinal isolates or were found to be associated significantly with either of the sources (Mann-Whitney U test $P<0.05$) (i.e. intestinal: O2, O22, O119, O149, O157 or extra-intestinal: O7, O11, O35) in the present study (Figure 5.3).

Other studies from different parts of the country reported other "O" serogroups associated with intestinal and extra-intestinal *Escherichia coli* isolates than those detected in the present study (Chellapandi *et al.,* 2017; Maloo *et al.,* 2017; Sharma *et al.,* 2016; Roy *et al.,* 2015; Chhibber *et al.,* 2014; Muni *et al.,* 2014; Lanjewar *et al.,* 2010; Kausar *et al.,* 2009; Maiya *et al.,* 1977). These findings indicate that pathogenic *E. coli* in India are very diverse in terms of the prevalence of seropathotypes as a wide range of "O" serogroups is in circulation among pathogenic intestinal and extra-intestinal isolates in the country.

Table 5.3: Distribution of "O" serogroups in different geographical locations of India

"O" Serogroup	**Frequency of expression of "O" Serogroup in different Geographical Regions (n)**					**Total**
	Central India	**Eastern India**	**Northern India**	**Southern India**	**Western India**	
UT	13	30	24	28	1	96
O1	0	4	0	1	0	5
O2	1	10	2	3	0	16
O4	0	3	0	0	0	3
O5	0	1	0	0	0	1
O7	7	2	3	9	0	21
O8	3	11	9	21	2	46
O9	0	2	1	3	0	6
O11	12	4	7	14	1	38
O16	0	2	0	0	0	2
O17	0	1	5	7	0	13
O20	0	2	6	12	0	20
O22	2	19	6	3	3	33
O34	1	1	1	2	0	5
O35	3	3	10	8	1	25
O49	0	0	3	1	1	5
O63	0	0	1	0	2	3
O83	0	1	14	10	0	25
O84	0	0	6	1	0	7
O86	0	0	2	0	0	2
O88	3	1	7	17	5	33
O89	0	0	0	7	0	7
O98	0	0	0	2	0	2

"O" Serogroup	Frequency of expression of "O" Serogroup in different Geographical Regions (n)					Total
	Central India	Eastern India	Northern India	Southern India	Western India	
O101	0	4	0	2	2	8
O114	0	0	0	1	0	1
O116	0	2	0	0	0	2
O117	0	1	0	0	0	1
O118	0	1	0	1	0	2
O119	0	1	7	2	0	10
O120	1	0	0	1	2	4
O126	3	12	12	1	0	28
O128	0	4	4	0	0	8
O141	2	12	1	6	2	23
O149	4	0	14	4	0	22
O157	3	0	1	2	1	7
Auto-agglutinating	1	0	2	1	0	4
TOTAL	59	134	148	170	23	534

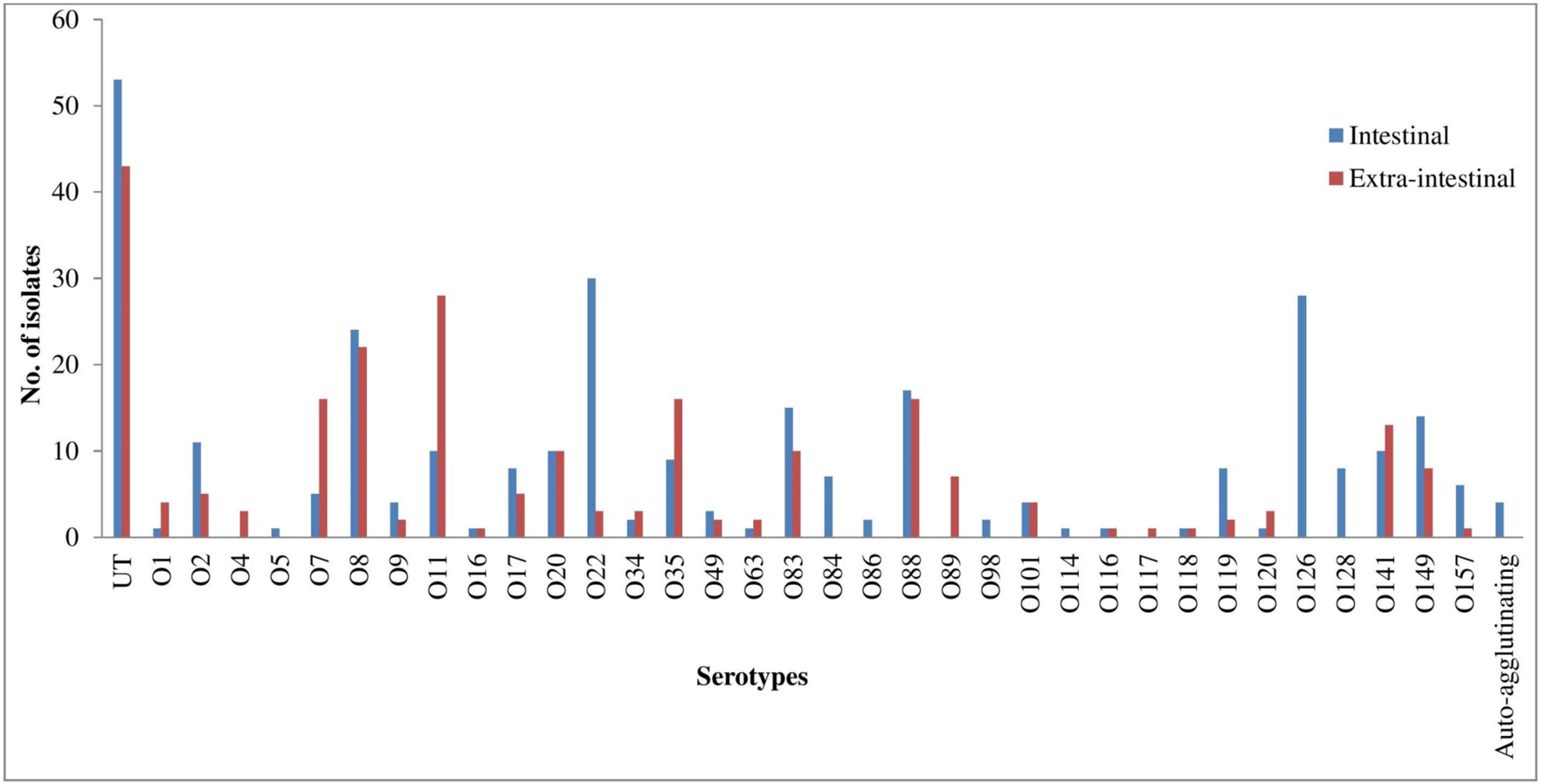


Figure 5.3: Percentage of "O" serogroups among intestinal and Extra-intestinal *E. coli*

Here: UT-untypeable, Auto..-Autoagglutinating, O1 to O157- different "O" serogroups detected.

5.3. **Pathotypes**

Diarrheal *E. coli* (DEC) is known to cause diarrhea using diverse mechanisms and with varying severity. Seven diarrhea-genic *E. coli* pathotypes have been described i.e. ETEC, EPEC, EHEC, EIEC, DAEC, EAEC, and AIEC (Lee, 2019; Gomes, 2016). The prevalence of DEC pathotypes varies with the socio-economic and geographical conditions of an area. Different pathotypes have been reported to be prevalent in the developed and developing world. Whereas EPEC, ETEC, and EAEC are reported predominantly in developing countries; EHEC, EPEC, and EIEC outbreaks are frequently reported in developed countries (Abbasi *et al.,* 2020; EFSA BIOHAZ Panel, 2020; Omolajaiye *et al.,* 2020; Anderson *et al.,* 2019; Brouzerdi *et al.,* 2018; Thakur *et al.,* 2018; Cabal et al, 2016; CFSPH, 2016; Keskimaki *et al.,* 2000;). In the present study, intestinal isolates from various geographical regions of India were characterized using multiplex PCR targeting virulence genes of four prevalent DEC (genes: eae and bfpA for EPEC, est and elt for ETEC, hlyA for EHEC, and CVD432 for EAEC). DEC virulence genes targeted in the present study could be detected in 297 (98.3%) out of 302 confirmed intestinal *E. coli* isolates. The most prevalent DEC pathotypes in the study samples were enterotoxigenic *E. coli* (ETEC) and enteropathogenic *Escherichia coli* (EPEC) with the frequency of 137/302; 45.4% and 126/302 i.e. 41.7% respectively (Figure 5.4). ETEC and EPEC were detected most commonly from all geographical locations of the country (Table 5.4). Other studies from various parts of the country have also reported ETEC and EPEC as the most prevalent pathotypes in the country (Singh *et al.,* 2019; Natrajan *et al.,* 2018; Raghvan *et al.,* 2017).

EPEC is the most prevalent entero-pathotype in childhood diarrhea worldwide with two variants viz. typical and atypical EPEC. Typical EPEC exhibit characteristics of attaching and effacing marked by gene bfpA whereas atypical strains lack this gene. Genetically, atypical strains are characterized by eae genes and typical by the presence of both eae and bfpA (Mare *et al.,* 2021). In this study, among the EPEC isolates atypical EPEC strains (eae gene only-34.8%) were found to be more prevalent than the typical EPEC strains (both eae and bfpA genes)

throughout the country ($p>0.05$). Similar observations have also been made by other workers from some studies in India (Natrajan *et al.,* 2018; Raghvan *et al.,* 2017; Wani *et al.,* 2006). However, in some other studies carried out in other parts of India typical EPEC was found more frequently than atypical strain (Singh *et al.,* 2017; Ghosh and Ali, 2010). Thus the present study and similar other studies from India indicate that both typical and atypical EPEC are prevalent in India.

ETEC strains are also frequently associated with childhood and adult diarrhea and associated with traveler's diarrhea in developing countries. It produces diarrhea through the action of cholera-like heat-labile (LT) or/and heat-stable (ST) enterotoxins (Anderson *et al.,* 2019, Qadri *et al.,* 2005). ST strains (heat-stable ETEC-est gene-36.4%) were found to be more prevalent than LT strains (heat-labile ETEC-elt gene) in the present study. In this study all ETEC strains were found to possess marker genes either for heat-labile (elt) or heat-stable (est) enterotoxin, no isolate was expressing both genes simultaneously. In contrast to this study, the *elt* gene was detected more frequently than the *est* gene by some other workers from different parts of the country (Singh *et al.,* 2019; Raghvan *et al.,* 2017; Singh *et al.,* 2015). Thus suggesting that ETEC strains producing heat-stable as well as heat-labile toxins are prevalent in the country.

All the intestinal isolates except 5 (1.6%) from Northern India belonged to any one of the targeted pathotypes. The pathotypes were not found uniformly distributed throughout the country ($p<0.05$). Seasonal variations in the detection of DEC pathotypes have been observed in some studies with frequent detection in rainy, summer, or winter seasons (Mohanty *et al*., 2021; Gonzalez *et al*., 2013). However, DEC pathotypes in this study were detected in all seasons except in five isolates in the winter season ($p>0.05$).

Enterohemorrhagic *E. coli* is the most pathogenic DEC strain involved in the most severe pathogenic systematic infections leading to hemorrhagic colitis and hemorrhagic uremic syndrome. Both O157:H7 and non-O157:H7 serotypes of this strain are involved in many outbreaks in developed countries (Yang *et al.,* 2017).

EHEC pathotype was found to be very scanty in this study as the hlyA gene could be detected in very few (9 out of 302; 3 % only) study samples ($p>0.05$). The marker gene for hemorrhagic *E. coli* (hlyA) was detected only among isolates from eastern and northern India with very low frequencies (Table 5.4). Similarly, other studies, from India have also detected EHEC with very low-frequency rates (Mandal *et al.,* 2017; Singh *et al.,* 2015; Rajenderan *et al.,* 2009).

These findings show that though EHEC is a very common pathotype in the developed world, outbreaks in developing countries including India due to this DEC pathotype are less frequent. Reasons for low EHEC incidence rates in India and other low and middle-income countries are a matter of further research in the area. One study in Mexico has correlated low incidence rates with the presence of protective antibody levels against EHEC lipopolysaccharides in the normal population against EHEC surface antigens (Navarro *et al.,* 2003).

However, the occurrence of high incidence rates among animals in India and the isolation of EHEC isolates from human infections in certain incidences pose a public health concern due to this most virulent *E. coli* pathotype (Purwar *et al.,* 2016).

All isolates possessing the hlyA gene were found to be non-O157 serotypes in the present study. Thus indicating risks of non-O157 STEC cases similar to non-O157 *E. coli* outbreaks reported from developed countries (Yang *et al.,* 2017; Frank *et al.,* 2011; King *et al.,* 2012).

Recently, enteroaggregative *E. coli* is emerging as one of the important diarrheal agents in developing countries including India (Modgil *et al.,* 2020; Gupta *et al.,* 2016; Kaur *et al.,* 2010). The virulence gene marker for EAEC (CVD432) was also detected in the present study in 8.3% of the isolates (25/302). Though enteroaggregative strains (CVD432 gene) were also found very less frequently; however, it was found to be more prevalent than enterohemorrhagic strains (hlyA gene). EAEC has been reported from different parts of the country with varying frequencies. EAEC was detected as the most prevalent DEC in various studies from

different locations (Raghvan *et al.,* 2017; Mandal *et al.,* 2017; Singh *et al.,* 2015; Dutta *et al.,* 2013) while other studies detected EAEC with a relatively low prevalence rate (Singh *et al.,* 2019; Natrajan *et al.,* 2018).

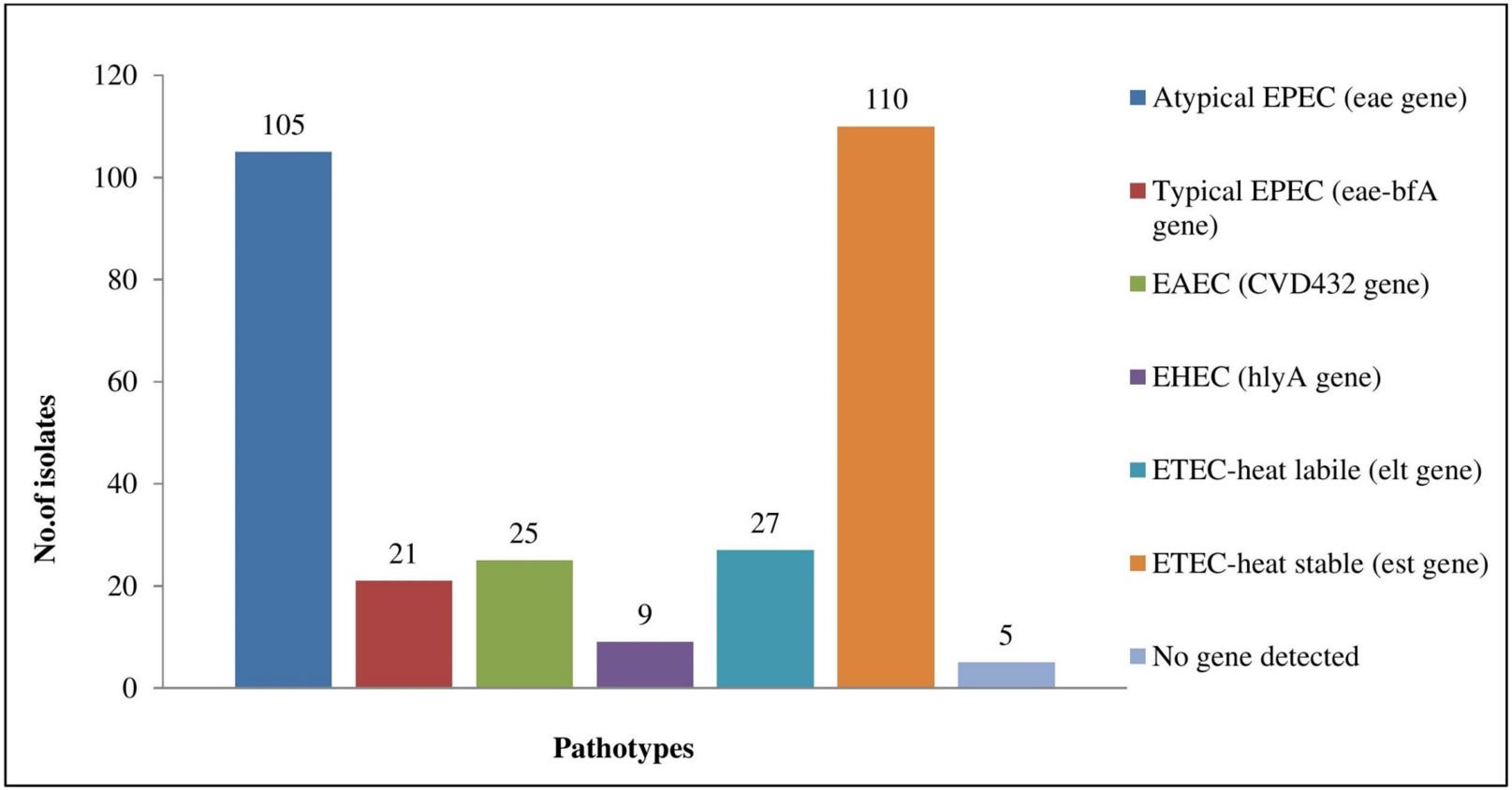


Figure 5.4: Pathotype genetic markers detected among intestinal *E. coli* isolates

Five isolates in which none of the targeted virulence genes were detected may be carrying other virulence genes not covered in the present study and may be representing other less prevalent pathotypes in the country i.e. EIEC (Chellapandi *et al.,* 2017).

DEC pathogenicity is not limited to the established pathotypes as newer variants with hybrid DEC pathotypes are emerging rapidly (Bhave *et al.,* 2018; Nyholm *et al.,* 2015). This study did not notice any hybrid pathotype with the limited range of markers used to screen only the most predominant pathotypes in India.

An association between *E. coli* serotype and pathotype has been shown by Kauffman as early as 1947 and thereafter various studies in support have been published and very few serogroups are known to be associated with different *E. coli* pathotypes (Tamura *et al.,* 1996; Orskov and Orskov, 1984 and 1992; Evans and Evans, 1983; Kauffmann, 1947). In the present study wide range of serotypes was found to be associated with each pathotype detected in the present study (Table 5.5).

No significant association between pathotype and serotype was found in the present study ($p>0.05$). Pathotype assignment merely based on its serotype is thus not a good practice hence confirmation of pathotype characteristics by phenotypic analysis or through the detection of marker genes should be considered.

Thus results of the present study and similar studies from India indicate that EPEC and ETEC are the two most prevalent DEC pathotypes in the country while EAEC is emerging as a common DEC pathotype. Incidences of EHEC human infections are relatively remote however threats of EHEC outbreaks remain as EHEC is routinely reported from animal and human infections though with very low incidence rates.

Table 5.4: DEC pathotypes in different geographical locations in India

Geographical location	**DEC Pathotype n (%)**						
	EPEC		**EAEC**	**EHEC**	**ETEC**		**None**
	eae	**eae+bfpA**	**CVD432**	**hlyA**	**Elt**	**Est**	**None**
Central India	6 (20.7)	0 (0.0)	4 (13.8)	0 (0.0)	0 (0.0)	19 (65.5)	0 (0.0)
Eastern India	44 (45.4)	0 (0.0)	5 (5.2)	3 (3.1)	10 (10.3)	35 (36.1)	0 (0.0)
Northern India	40 (32.0)	16 (12.8)	12 (9.6)	6 (4.8)	12 (9.6)	34 (27.2)	5 (4.0)
Southern India	14 (30.4)	3 (6.5)	3 (6.5)	0 (0.0)	4 (8.7)	22 (47.8)	0 (0.0)
Western India	1 (20.0)	2 (40.0)	1 (20.0)	0 (0.0)	1 (20.0)	0 (0.0)	0 (0.0)
TOTAL	105 (34.8)	21 (7.0)	25 (8.3)	9 (3.0)	27 (8.9)	110 (36.4)	5 (1.7)

Table 5.5: Serotypes associated with different pathotypes

Pathotype	Associated serotype
aEPEC- n=105	UT (24),O2 (5), O5 (1), O8 (11), O9 (1), O11 (5), O17 (2), O20 (4), O22 (6), O34 (1), O35 (5), O83 (6), O84 (3), O86 (2), O88 (2), O101 (1), O114 (1), O119 (3), O126 (13), O128 (1), O141 (2), O149 (4), O157 (1), Rough (1)
tEPEC n=21	UT (2), O7 (1), O8 (2), O11 (2), O20 (1), O22 (1), O49 (1), O83 (3), O88 (1), O101 (1), O119 (2), O126 (2), O128 (1), Rough (1)
EAEC n= 25	UT (4), O2 (2), O8 (2), O17 (3), O22 (2), O35 (1), O83 (2), O84 (1), O88 (1), O126 (3), O128 (2), O149 (2)
EHEC n = 9	UT (1), O7 (1), O22 (1), O35 (1), O88 (1), O119 (1), O126 (2), O149 (1)
ETEC-LT N = 27	UT (3), O8 (1), O9 (2), O20 (2), O22 (4), O84 (2), O88 (2), O98 (1), O119 (1), O126 (6), O141 (3),
ETEC-ST n = 110	UT (19), O1 (1), O2 (3), O7 (3), O8 (7), O9 (1), O11 (3), O16 (1), O17 (3), O20 (3), O22 (16), O34 (1), O35 (2), O49 (2), O63 (1), O83 (3), O88 (10), O98 (1), O101 (2), O116 (1), O118 (1), O119 (1), O120 (1), O126 (2), O128 (4), O141 (5), O149 (6), O157 (5), Rough (2)
None n = 5	O2 (1), O8 (1), O83 (1), O84 (1), O149 (1)

5.4. **Virulence Factors in intestinal and extra-intestinal isolates**

The most critical common virulence characteristics in intestinal and extra-intestinal *E. coli* infections are the adhesion and colonization of bacteria to host cells and tissues. Among the virulence characteristics evaluated in the present study Cell surface hydrophobicity, biofilm, colicin, and siderophores are directly or indirectly associated with adhesion and colonization of *E. coli* in the host environment and further pathogenicity. All these virulence factors were expressed by a very large percentage of isolates in the present study among isolates from all over the country.

5.4.1. **Cell surface hydrophobicity**

Cell surface hydrophobicity is an indicator of the adhesion capability of microorganisms to hydrophobic biotic and abiotic surfaces. Due to this characteristic, microorganisms, on one hand, are beneficial as they can effectively adhere to hydrophobic pollutants and their effective decomposition, on the other hand, due to the same characteristic; **microorganisms become a nuisance as it is associated with enhanced pathogenicity due to their adhesion to cellular and non-cellular surfaces resulting in colonization of the bacteria and tissue invasion** (Heilmann, 2011; Obuekwe 2009; Goulter, 2009). **Aggravated adhesive nature due to CSH help in the initiation of biofilm formation and resistance to biological and biochemical factors in the host thus is considered an important virulence factor** (Mirani, 2018). In the present study Salt aggregation test (SAT) was used to study the hydrophobicity of the pathogenic *E. coli* under evaluation. SAT is considered a rapid and simple assay to detect hydrophobicity and adhesion of bacterial cells to surfaces (Mythreyi *et al.,* 2011). In this study, this virulence characteristic among study samples with a 69.1% positivity rate was detected. Pathogenic *E. coli* exhibiting CSH in large percentages has been reported in various other studies also indicating the importance of this virulence property among pathogenic *E. coli* (Varshney and Dimri, 2021; Gowthan and Gopinath, 2016; Fakruddin *et al.,* 2012).

Cell surface hydrophobicity is an important factor in nosocomial infections as microorganisms with hydrophobic characteristics can easily colonize medical implants like catheters, pacemakers, or prosthetic heart valves which are made of hydrophobic materials like; Teflon, polyvinyl chloride, silicone, steel, etc. Colonization of these biomaterials results in persistent, recurrent, and drug-resistant nosocomial infections. Strategies such as the use of anti-microbial colonization of natural materials like gluten, silk, fibroin, fibrinogen, etc., use of polymeric nanofibers on polystyrene, and use of surface-modified material by coating the surfaces with noble nanoparticles i.e. silver nano-particles have been found very useful in decreasing microbial adhesion and biofilm formation on surfaces of these materials (Krawsoska and Sigler, 2014).

The isolates in this study were from cases of diarrhea (intestinal) and UTI (extra-intestinal) and in both the infection sites adhesion to the host cell surface is the first and foremost requirement for successful infection, more importantly in the gastrointestinal tract as here pathogenic bacteria experience competition with commensal organisms in addition to flushing actions due to fecal discharge and peristaltic movements of the bowel. Cell surface hydrophobicity has been commonly observed in the majority of intestinal as well as extra-intestinal pathogenic *E. coli* in various studies (Varshney and Dimri, 2021; Abd El-Baky *et al.* 2020; Gowthan and Gopinath, 2016). In the present study, a significantly more percentage of intestinal isolates (75.2%) were found to be hydrophobic in comparison to extra-intestinal isolates (61.2%) ($p<0.05$) [Table 5.6].

Cell surface hydrophobicity characteristic was found in large percentages among both intestinal as well as extra-intestinal isolates irrespective of geographical locations ($p>0.05$) [Table 5.7]. Cell surface hydrophobicity with a positivity rate ranging from 56.5% in isolates from Western India to 74.6% among isolates from Eastern India was observed. Other studies from different geographical locations in India have also reported varying positivity rates of cell surface hydrophobicity (Bawankar, 2018; Kaira and Pai, 2018; Growther and Yasotha, 2016; Shruthi, 2012).

Table 5.6: Frequencies of isolates showing cell surface hydrophobicity (CSH)

Study isolates	**CSH Positive n (%)**	**CSH Negative n (%)**	**Total N (%)**
Intestinal isolates	227 (75.2%)	75 (24.8%)	302 (100%)
Extra-intestinal isolates	142 (61.2%)	90 (38.8%)	232 (100%)
Total	369 (69.1%)	165 (30.9%)	534 (100%)

Table 5.7: Region-wise frequencies of isolates showing cell surface hydrophobicity (CSH)

Geographical location	**Intestinal Isolates**		**Extra-intestinal isolates**		**Total CSH positive**
	CSH positive	**CSH negative**	**CSH positive**	**CSH negative**	
Central India	21(72.4%)	8 (27.6%)	20 (66.7%)	10 (33.3%)	41 (69.5%)
Eastern India	74 (76.3%)	23 (23.7 %)	26 (70.3%)	11 (29.7%)	100 74.6%)
Northern India	93 (74.4%)	32 (25.6%)	11 (47.8%)	12 (52.2%)	104 70.3%)
Southern India	36 (78.3%)	10 (21.7%)	75 (60.5%)	49 (39.5%)	111 65.3%)
Western India	3 (60%)	2 (40%)	10 (55.6%)	8 (44.4%)	13 (56.5%)
Total	227 (75.2%)	75 (24.8%)	142 61.2%)	90 (38.8%)	369 69.1%)

Cell surface hydrophobicity and "O" serogroup antigens are associated with cell surface moieties i.e. cell membrane and cell wall respectively (Singleton, 2005). Studies demonstrating the association between serotype and cell surface hydrophobicity are lacking. In some of the studies, though, study samples have been subjected to evaluate both these characteristics the study reports are silent about any association between these two virulence characteristics (Growther and Yasotha, 2016). In the present study, a wide range of "O" serogroups were found to be associated with intestinal and extra-intestinal hydrophobic isolates (Table 5.8). No preference among different serotypes for cell surface hydrophobicity was observed among intestinal as well as extra-intestinal isolates which indicates that these two cell surface characteristics are not associated at all. All six O157 intestinal isolates were found to be hydrophobic. O157 infections are usually associated with food-borne infections hydrophobicity is of major importance in these isolates as this property can effectively aid the attachment of such isolates to food processing, packaging, and transport equipment and thus food contamination and transmission to the consumers.

A high *E. coli* infection incidence rate is observed during summer, rainy and post-monsoon seasons as the transmission of *E. coli* through contaminated food and water becomes easy during these seasons (Deeny *et al.,* 2015; Schwab *et al.,* 2014; Al-Hasan, 2009).

The expression of virulence factors by isolates during these seasons can further increase the chances of colonization of this microorganism to establish infection. Cell surface hydrophobicity was detected in a very high percentage in *E. coli* isolates irrespective of the seasons ($p>0.05$) [Table 5.9].

Table 5.8: Serotype-wise expression of Cell Surface Hydrophobicity

"O" Serogroup	**Overall CSH positive isolates n (%)**	**CSH positive Intestinal isolates n (%)**	**CSH positive Extra-Intestinal isolates n (%)**
UT; N=96	65 (67.7)	40 (41.7)	25 (26.0)
O1; N=5	2 (40.0)	0 (0)	2 (40.0)
O2; N=16	13 (81.3)	8 (50.0)	5 (31.3)
O4; N=3	0 (0.0)	0 (0)	0 (0)
O5; N=1	1 (100.0)	1 (100.0)	0 (0)
O7; N=21	15 (71.4)	3 (14.3)	12 (57.1)
O8; N=46	33 (71.7)	15 (32.6)	18 (39.1)
O9; N=6	5 (83.3)	4 (66.7)	1(16.7)
O11; N=38	19 (50.0)	8 (21.1)	11 (28.9)
O16; N=2	2 (100.0)	1 (50.0)	1 (50.0)
O17; N=13	9 (69.2)	7 (53.8)	2 (15.4)
O20; N=20	11 (55.0)	6 (30.0)	5 (25.0)
O22; N=33	26 (78.8)	25 (75.8)	1 (3.0)
O34; N=5	1 (20.0)	1 (20.0)	0 (0)
O35; N=25	21 (84.0)	8 (32.0)	13 (52.0)
O49; N=5	4 (80.0)	3 (60.0)	1 (20.0)
O63; N=3	3 (100.0)	1 (33.3)	2 (66.7)
O83; N=25	14 (56.0)	7 (28.0)	7 (28.0)
O84; N=7	5 (71.4)	5 (71.4)	0 (0)
O86; N=2	2 (100.0)	2 (100.0)	0 (0)
O88; N=33	21 (63.6)	13 (39.4)	8 (24.2)
O89; N=7	4 (57.1)	0 (0)	4 (57.1)
O98; N=2	1 (50.0)	1 (50.0)	0 (0)
O101; N=8	5 (62.5)	3 (37.5)	2 (25.0)

"O" Serogroup	**Overall CSH positive isolates n (%)**	**CSH positive Intestinal isolates n (%)**	**CSH positive Extra-Intestinal isolates n (%)**
O114; N=1	0 (0.0)	0 (0)	0 (0)
O116; N=2	2 (100.0)	1 (50.0)	1 (50.0)
O117; N=1	1 (100.0)	0 (0)	1 (100.0)
O118; N=2	0 (0.0)	0 (0)	0 (0)
O119; N=10	9 (90.0)	8 (80.0)	1 (10.0)
O120; N=4	3 (75.0)	1 (25.0)	2 (50.0)
O126; N=28	20 (71.4)	20 (71.4)	0 (0)
O128; N=8	7 (87.5)	7 (87.5)	0 (0)
O141; N=23	20 (87.0)	8 (34.8)	12 (52.2)
O149; N=22	16 (72.7)	11 (50.0)	5 (27.7)
O157; N=7	6 (85.7)	6 (85.7)	0 (0)
Auto-agglutinating; N=4	3 (75.0)	3 (75.0)	0 (0)
TOTAL; N=534	369 (69.1)	227 (42.5)	142 (26.6)

Table 5.9: Seasonality and Cell surface hydrophobicity

Season	**Intestinal isolates**			**Extra-intestinal isolates**			**Overall**		
	CSH positive	**CSH negative**	**Total**	**CSH Positive**	**CSH negative**	**Total**	**CSH positive**	**CSH negative**	**Total**
Summer	30 (81.1)	7 (18.9)	37	39 (65.0)	21 (35.0)	60	69 71.1%)	28 (28.9%)	97
Winter	60 (71.4)	24 (28.6)	84	10 (55.6)	8 (44.4)	18	70 68.6%)	32 (31.4%)	102
Monsoon	119 (76.3)	37 (23.7)	156	93 (60.4)	61 (39.6)	156	212 68.4%)	98 (31.6%)	310
Post Monsoon	18 (72.0)	7 (28.0)	25	-	-	-	18 (72.0%)	7 (28.0%)	25

5.4.2. **Biofilm**

Microbial biofilms have received the attention of researchers due to their economic, ecological, and medical importance. Biofilm provides multi-factorial advantages for survival in adverse environments and further propagation of micro-organisms. Biofilms shield bacteria from the negative influence of the surrounding environment (Abdallah *et al.* 2014; Juhna *et al.* 2007) help in its disperse (Petrova and Sauer 2016) and from inactivation by antibiotics, chemical agents, and attack by predators (Moreira *et al.* 2015; DePas *et al.* 2014; Fux *et al.* 2005). Biofilm-forming capabilities of microbes have both beneficial effects as well as harmful effects on human society. Microbial biofilms have been utilized to the benefit of human beings in many industrial applications, especially in the field of biotechnology (Maksimova, 2014) however, biofilms have also created a nuisance in some industries and medicine (Galie *et al.* 2018; Jamal *et al.* 2018; Srivastava and Bhargava 2016). **Biofilms have played a detrimental role in medicine leading to many difficult-to-treat recurrent severe chronic infections such as chronic prostatitis, chronic pneumonia in patients with cystic fibrosis, chronic osteomyelitis, chronic otitis media chronic cystitis. The use of biofilm colonized biomaterial i.e. contact lenses, vocal cord prosthesis, intravenous and urethral catheters prosthetic heart valves, orthopedic devices, etc. is also an important reason for biofilm-associated nosocomial infections** (Toretta *et al.* 2019; Hoiby *et al.* 2017; Kackar *et al.* 2017; Sabir *et al.* 2017; Somogyi-Ganss *et al.* 2017; Zimmerli and Sendi 2017; Bartoletti *et al.* 2014; Tenke *et al.* 2012; Brady *et al.* 2008; Donlan 2001). Recurrent nosocomial urogenital infections are widely related to biofilms colonized surfaces mainly surgical articles and catheters (Karigoudar *et al.* 2019; Dash *et al.* 2018; Sharma *et al.* 2016; Sanchez *et al.* 2013).

In the present study, a large percentage (53.6% to 59%) of isolates was found to form biofilm as detected using three different methods. The capability of the *E. coli* isolates to form biofilm was seen irrespective of their geographical source in this study. The percentage of isolates from different regions in the present study ranged from 74-91% from western India, 59- 69% from eastern, southern (52-66%), and

central India (59-63%) to 28-55% in northern India ($p<0.05$), data is depicted in Table 5.10. Biofilm-forming characteristics have also been reported as an important virulence mechanism of pathogenic *E. coli* in various other studies in different parts of the country (Atray and Atray 2015; Suman *et al.* 2007).

Table 5.10: Percentages of biofilm-producing *E. coli* isolates from different regions of the country

Geographical Area	**Frequency of Biofilm producing *E. coli* isolates**		
	TCP method n (%age)	**TM method n (%age)**	**CRA method n (%age)**
Central India	37 (63%)	35(59%)	35(59%)
Eastern India	86 (64%)	79 (59%)	93 (69%)
Northern India	42 (28%)	59 (40%)	82 (55%)
Southern India	112 (66%)	95 (56%)	88 (52%)
Western India	21 (91%)	18 (78%)	17 (74%)
TOTAL	298 (55.8%)	286 (53.6%)	315 (59%)

Studies on biofilm formation by pathogenic *E. coli* are mostly reported on extra-intestinal *E. coli* especially associated with uropathogenic *E. coli*. However, pathogenic intestinal *E. coli* strains were also observed to be biofilm producers in some studies (Pereira *et al.* 2010; Bokranz *et al.* 2005). Biofilm formation in this study was detected in both intestinal as well as extra-intestinal isolates. Biofilm-forming potential among extra-intestinal isolates was detected in more number isolates than in intestinal isolates by TCP and TM methods ($p < 0.05$), however, the reverse was observed in the CRA method which was however not significant ($p>0.05$). Formation of biofilms involving more than single bacterial species i.e. mixed biofilms are known to produce strong synergetic associations with a very high level of drug and disinfectant resistance than biofilms involving a single bacterial species (Burmolle *et al.* 2006; Kara *et al.* 2006; Al-Bakri *et al.* 2005; Leriche *et al.* 2003). Intestinal pathogenic *E. coli* strains may encounter many intestinal

commensal and other pathogenic bacterial species during intestinal infections. It would be an interesting matter of research if intestinal pathogenic *E. coli* strains from synergetic mixed biofilms with other species, their role to resist intestinal peristaltic movements, defense mechanisms, and antimicrobial treatment.

The strength of biofilm in the majority of the isolates was found moderate among extra-intestinal as well as intestinal isolates (Table 5.11). The serotype of the strains is specifically reported to be associated with biofilm formation and its strength (Noie Oskouie *et al.* 2019; Wang *et al.* 2012; Martinez-Medina *et al.* 2009).

No association between serotype and biofilm formation was observed in the present study (Man Whitney U test; p-value > 0.05), though isolates with some of the "O" serogroups i.e. O7, O8, O22, O35, O88, O141, O157 were found to produce biofilms more frequently than isolates of other serotypes (Table 5.12 and 5.13). "O" serogroups O125, O101, O25, O16, O15, and O8 are highly biofilm-forming in some earlier studies from India (Tomar *et al.* 2018; Saikia *et al.* 2016). More studies on this subject are sought.

Isolates belonging to serotype O157, which is strongly linked to severe STEC infections, were also found to form moderate to strong biofilms in the present study. As STEC infections involving serotype O157 are usually acquired through food contaminated from biofilms formed on surfaces of food processing utensils and equipment, the use of validated equipment cleaning and sanitization processes is crucial in the control of biofilms on their surfaces and thus avoiding associated foodborne outbreaks.

Table 5.11: Percentage of strong, moderate, or weak biofilm producers among intestinal and extra-intestinal *E. coli* isolates.

Biofilm Detection Method	Frequency of biofilm-producing *E. coli* isolates											
	Intestinal *E. coli* isolates N (%)				Extra-intestinal *E. coli* isolates N (%)				Overall N (%)			
	Weak	Moderate	Strong	Total	Weak	Moderate	Strong	Total	Weak	Moderate	Strong	Total
TCP	7 (2.3)	94 (31.1)	52 (17.2)	153 (50.7)	7 (3.0)	105 (45.3)	33 (14.2)	145 (62.5)	14 (2.6)	199 (37.3)	85 (15.9)	298 (55.8)
TM	62 (20.5)	81 (26.8)	7 (2.3)	150 (49.7)	51 (22.0)	80 (34.5)	5 (2.2)	136 (58.6)	113 (21.2)	161 (30.1)	12 (2.2)	286 (53.6)
CRA	44 (14.6)	80 (26.5)	61 (20.2)	185 (61.3)	20 (8.6)	58 (25.0)	52 (22.4)	130 (56.0)	64 (12.0)	138 (25.8)	113 (21.2)	315 (59.0)

Seasonal biofilm-related and hospital infections have been reported, increasing number of hospital infections are found to be in summer (Ahmed *et al.,* 2013; Eber *et al.,* 2011). In the present study, *E. coli* isolates (intestinal and extra-intestinal collectively) were detected to form biofilms in all the seasons with varying frequencies, biofilm was detected in more percentages of isolates from summer, monsoon, and post-monsoon seasons than in isolates from the winter season ($p<0.05$). This trend was the same among intestinal isolates, however, the frequency of biofilm producers in winter isolates among extra-intestinal *E. coli* was also found to be very high (77.8%-94.4%) as depicted in table 5.14. Detection of biofilm-forming isolates in high percentages in serogroups frequently associated with *E. coli* infections and during summer and post-monsoon seasons which are peak seasons of *E. coli* infections is suggestive of the importance of biofilms in *E. coli* epidemiology.

Table 5.12: Serotype-wise expression of Biofilm formation

"O" Serogroup	**Biofilm-TCP method n (%)**	**Biofilm Formation in Intestinal isolates n (%)**	**Biofilm Formation in Extra-Intestinal isolates n (%)**
UT; N=96	53 (55.2)	31 (32.3)	22 (22.9)
O1; N=5	2 (40.0)	0 (0)	2 (40.0)
O2; N=16	9 (56.3)	5 (31.3)	4 (25.0)
O4; N=3	2 (66.7)	0 (0)	2 (66.7)
O5; N=1	1 (100.0)	1 (100.0)	0 (0)
O7; N=21	17 (81.0)	4 (19.0)	13 (61.9)
O8; N=46	29 (63.0)	12 (26.1)	17 (36.9)
O9; N=6	2 (33.3)	1 (16.7)	1 (16.7)
O11; N=38	20 (52.6)	6 (15.8)	14 (36.8)
O16; N=2	2 (100.0)	1 (50.0)	1 (50.0)
O17; N=13	6 (46.2)	2 (15.4)	4 (30.8)
O20; N=20	10 (50.0)	4 (20.0)	6 (30.0)
O22; N=33	20 (60.6)	17 (51.5)	3 (9.1)
O34; N=5	2 (40.0)	2 (40.0)	0 (0)
O35; N=25	15 (60.0)	4 (16.0)	11 (44.0)
O49; N=5	2 (40.0)	0 (0)	2 (40.0)
O63; N=3	3 (100.0)	1 (33.3)	2 (66.7)
O83; N=25	10 (40.0)	3 (12.0)	7 (28.0)
O84; N=7	0 (0.0)	0 (0)	0 (0)
O86; N=2	0 (0.0)	0 (0)	0 (0)
O88; N=33	20 (60.6)	7 (21.2)	13 (39.4)
O89; N=7	2 (28.6)	0 (0)	2 (28.6)
O98; N=2	2 (100.0)	2 (100.0)	0 (0)
O101; N=8	3 (37.5)	2 (25.0)	1 (12.5)

"O" Serogroup	**Biofilm-TCP method n (%)**	**Biofilm Formation in Intestinal isolates n (%)**	**Biofilm Formation in Extra-Intestinal isolates n (%)**
O114; N=1	0 (100.0)	0 (0)	0 (0)
O116; N=2	1 (50.0)	1 (50.0)	0 (0)
O117; N=1	1 (100.0)	0 (0)	1 (100.0)
O118; N=2	1 (50.0)	1 (50.0)	0 (0)
O119; N=10	5 (50.0)	3 (30.0)	2 (20.0)
O120; N=4	3 (75.0)	0 (0)	3 (75.0)
O126; N=28	18 (64.3)	18 (64.3)	0 (0)
O128; N=8	3 (37.5)	3 (37.5)	0 (0)
O141; N=23	16 (69.6)	9 (39.1)	7 (30.4)
O149; N=22	10 (45.5)	6 (27.3)	4 (18.2)
O157; N=7	6 (85.7)	5 (71.4)	1 (14.3)
Auto-agglutinating; N=4	2 (50.0)	2 (50.0)	0 (0)
TOTAL; N=534	298 (55.8)	153 (28.7)	145 (27.1)

Table 5.13: Serogroups with predominant biofilm-forming potential

Serogroup	**TCP Method Biofilm producers n/N (%)**	**TM Method Biofilm producers n/N (%)**	**CRA Method Biofilm producers n/N (%)**
O7	17/21 (81.0)	13/21 (61.9)	14/21 (66.7)
O8	29/46 (63)	32/46 (69.6)	35/46 (76.1)
O22	20/33 (60.6)	25/33 (75.8)	28/33 (84.8)
O35	15/25 (60.0)	18/25 (72.0)	15/25 (60.0)
O88	20/33 (60.6)	20/33 (60.6)	21/33 (63.6)
O141	16/23 (69.6)	19/23 (82.6)	17/23 (73.9)
O157	6/7 (85.7)	4/7 (57.1)	5/7 (71.4)

Table 5.14: Seasonality and biofilm formation among pathogenic *E. coli* isolates

Season	**Overall Frequency of biofilm formation in different methods n (%)**			**Frequency of biofilm formation among intestinal isolates in different methods n (%)**			**Frequency of biofilm formation among extra-intestinal isolates in different methods n (%)**		
	TCP	**TM**	**CRA**	**TCP**	**TM**	**CRA**	**TCP**	**TM**	**CRA**
Summer	68 (70.1)	69 (71.1)	68 (70.1)	27 (73)	31 (83.8)	31 (83.8)	41 (68.3)	38 (63.3)	37 (61.7)
Winter	49 (48)	49 (48)	57 (55.9)	32 (38.1)	33 (39.3)	43 (51.2)	17 (94.4)	16 (88.9)	14 (77.8)
Monsoon	160 (51.6)	154 (49.7)	181 (58.4)	73 (46.8)	72 (46.2)	102 (65.4)	87 (56.5)	82 (53.2)	79 (51.3)
Post-monsoon	21 (84)	14 (56)	9 (36)	21 (84)	14 (56)	9 (36)	0	0	0
TOTAL	298 (55.8)	286 (53.6)	315 (59)	153 (50.7)	150 (49.7)	185 (61.3)	145 (62.5)	136 (58.6)	130 (56)

Detection of biofilms may play an important role in managing hospital-acquired infections because biofilm-associated infections tend to be more difficult to treat due to enhanced antimicrobial resistance and escape from the host's immune defenses and medicinal strategies, thus biofilms require special consideration for appropriate management and treatment of such infections. Various methods like microtiter plates, biofilm coupon techniques, bioluminescent assay, Calgary device, PCR, and scanning electron microscopic examination are used for the detection of biofilms in clinical laboratories (Triveni *et al.* 2018). The three most frequently used phenotypic biofilm screening methods in clinical isolate are Tissue culture microtiter plate assay (TCP), tube adherence method (TM), and congo red agar (CRA), TCP is considered the gold standard (Dhanalakshmi *et al.* 2018; Magna *et al.* 2018; Triveni *et al.* 2018; Ruchi *et al.* 2015; Hassan *et al.* 2011). However, the TCP method has multiple steps and requires plate readers, tissue culture plates, stains, and reagents to perform the test, which makes it not a very favorable method for routine screening of clinical isolates, especially in resource-limited laboratories. TM and CRA methods on the other hand advantage of the use of limited reagents and media and no special equipment and their ease of performance, which make these suitable for routine screening even in resource-limited laboratories. These methods have been shown to detect biofilms in some organisms with varying sensitivity and specificity in different studies (Dhanalakshmi *et al.* 2018; Magna *et al.* 2018; Triveni *et al.* 2018; Tayal *et al.* 2015; Hassan *et al.* 2011; Garcia *et al.* 2004). Pathogenic *E. coli* isolates in the present study were subjected to these three phenotypic biofilm detection methods and test characteristics of TM and CRA methods to detect biofilms in intestinal and extra-intestinal infectious *E. coli* were compared with the TCP method. Test characteristics (accuracy, sensitivity, specificity, and predictive values) of both TM and CRA methods were found to be reasonably good (Table 5.15). The tube method was found to be more accurate (70.79) and specific (68.07) and have better predictive values in comparison to the CRA method in this study (Table 5.15). Other studies have also reported CRA and TM methods possessing reasonably good test characteristics, thus suggesting that these methods can be employed in routine screening of biofilms in pathogenic *E. coli* isolates especially in resource-limited laboratories (Tayal *et al.,* 2015; Hassan *et al.,* 2011).

Table 5.15: Test Characteristics of Tube and The congo red agar methods in reference to the TCP Method

Test Method	Test Characteristics				
	Accuracy (%)	**Sensitivity (%)**	**Specificity (%)**	**Positive predictive value (%)**	**Negative predictive value (%)**
Tissue culture Plate (TCP) Method	100	100	100	100	100
Tube Method (TM)	70.79	71.14	68.64	74.13	65.32
Congo Red Agar (CRA) Method	64.98	71.48	56.78	67.62	61.19

5.4.3. **Siderophores**

The affinity of siderophores for iron molecules has led to their wide applicability in different fields, in medicine potential of siderophores has been seen as a vaccine candidate, drug delivery system (Trojan Horse) anti-infectious agent, and epidemiological tool (Fan and Fang, 2021; Ghosh *et al.,* 2020; Prabhakar, 2020; Shah *et al.,* 2018; Vila *et al.,* 2016; Ali and Vidhale, 2013; Nagoba and Vedpathak, 2011). **Siderophore production is an important virulence factor of pathogenic *E. coli* which helps it to survive and colonize under iron stress host environment for establishing different clinical manifestations** (Sarowska *et al.,* 2019; Robinson *et al.,* 2018; Saleem *et al.,* 2017; Watts *et al.,* 2012; Demir *et al.,* 2004). Micro-organisms are diverse in the expression of the type of siderophores and mainly two structural types of siderophores i.e. various structural types of siderophores catecholate and hydroxamate are expressed by pathogenic *E. coli* strains (Khan *et al.,*

2018; Miethke and Marahiel, 2007). In this study, the prevalence of this virulence factor and its type among *E. coli* isolates was also evaluated.

On CAS agar 45.1% of isolates were found to express siderophores which were significantly more ($p<0.05$) in extra-intestinal *E. coli* isolates than the intestinal isolates (Fig.5.5). Detection of siderophores on CAS agar was evident among isolates from all geographical regions thus indicating its importance as an important *E. coli* virulence factor. Various other studies have also found extra-intestinal *E. coli strains producing* siderophores in very large percentages (Sarowska *et al.,* 2019; Vagrali, 2009).

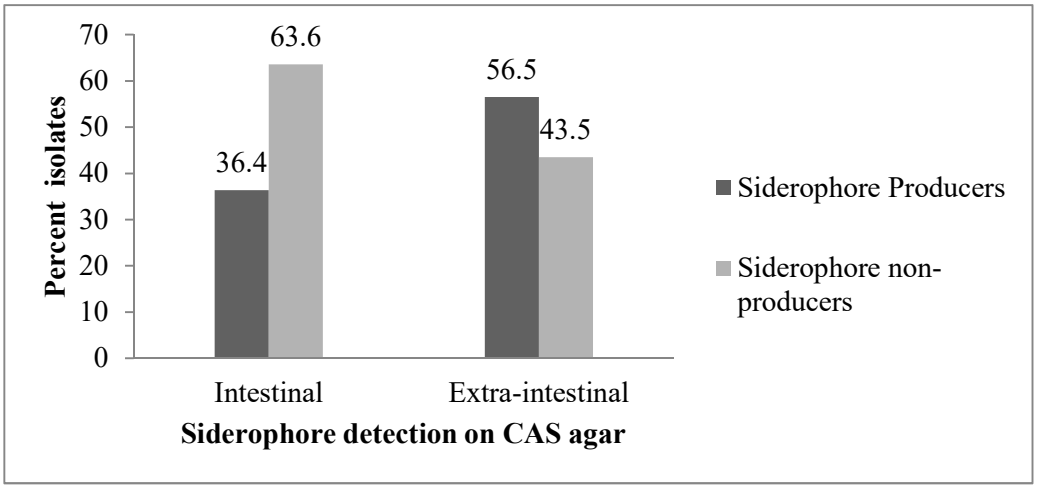


Figure 5.5: Rates of Siderophore Producing intestinal and extra-intestinal pathogenic *E. coli* isolates on CAS agar

Though regional variations ($p<0.05$) in the expression of siderophores i.e. percentages of isolates releasing siderophores among Southern, Eastern, and Central region isolates than in Western and Northern isolates were observed in this study (Table 5.16), however, other studies have reported a very large percentage of isolates from Western and Northern India expressing siderophores (Bawankar, 2018; Mital *et al.,* 2014). Thus *E. coli* isolates in all geographical locations of India seem to possess this virulence characteristic.

Table 5.16: Siderophore-producing pathogenic *E. coli* isolates from different geographical regions.

Geographical Area	**Siderophore detection by chrome-azurol agar method**		**Total No. of isolates**
	Siderophore Producers n (%)	**Siderophore Non-Producers n (%)**	
Central India	31 (52.5%)	28 (47.5%)	59
Eastern India	75 (56%)	59 (44%)	134
Northern India	33 (22.3%)	115 (77.7%)	148
Southern India	99 (58.2%)	71 (41.8%)	170
Western India	3 (13%)	20 (87%)	23
TOTAL	241 (45.1%)	293 (54.9%)	534

On chemical characterization, pathogenic *E. coli* were found to express hydroxamate-type(35.4% i.e. 34.4% hydroxamate alone and 1.1% with catechol) siderophore more frequently than the catechol-type siderophores (12.5% i.e. 11.4% catechol alone and 1.1% along with hydroxamate) [$p<0.005$]. Within intestinal isolates frequency of detection of hydroxamate-type siderophore was expressed more than catechol-type siderophore among intestinal isolates ($p<0.005$) with rates of 36.1% and 3.3% respectively. While both types of siderophores were expressed in a large percentage (34.5% hydroxamate-type, and 24.6% catechol-type) in extra-intestinal isolates (Table 5.17, Figure 5.6, and Figure 5.7). Comparing isolates from two sites, no significant difference was observed in the expression of hydroxamate-type siderophore between intestinal and extra-intestinal *E. coli* isolates ($p>0.05$); however extra-intestinal isolates expressed catechol-type siderophore more frequently than intestinal isolates ($p<0.05$). Detection of hydroxamate and catecholate in large percentages among pathogenic *E. coli* strains has been reported in other studies also and catechol-type siderophore has also been recognized as an important virulent factor associated with extra-intestinal *E. coli* (Searle, *et al.,* 2015; Caza *et al.,* 2011;

Demir and Kaleli, 2004). The results of the present study are thus consistent with earlier studies.

Overall, siderophores could be detected in 56.7% of the total 534 isolates, including 62 of 293 isolates in which siderophores were not detected on CAS agar. No siderophore could be detected in a total of 43.3% of isolates by any of the methods. No hydroxamate or catechol siderophore types could be detected in 53 of the 241 isolates in which siderophores were detected on CAS agar (Table 5.17).

E. coli and other bacteria are known to express very diverse kinds of siderophores and this characteristic has been employed in the epidemiological typing of bacterial isolates (McRose *et al.,* 2018; Grass, 2006; Demir and Kaleli, 2004; Reissbrodt and Rabsch, 1988).

Based on results obtained in this study on CAS agar and chemical characterization five types of *E. coli* isolates could be identified viz .1) Isolates in which only hydroxamate was detected 2) Isolates in which catechol was detected 3) isolates in which both catechol and hydroxamate were detected 4) isolates in which siderophores was detected on CAS but no hydroxamate and catechol detected and 5) isolates in which siderophore not detected at all. Isolates in which siderophores were detected on CAS but no hydroxamate and catechol were detected may be producing some other type of siderophores. Fulfillment of iron requirements by isolates in which no siderophore was detected can be through other mechanisms like hemolytic cytotoxins to free iron from hemoglobin or heme, enzymes for release of iron from iron complex molecules, or direct utilization of iron-bound host molecules (Page, 2019; Payne, 1993). Detection of siderophores in chemical tests among CAS-negative isolates can be due to the assay limitation of CAS agar (Shin *et al.,* 2001).

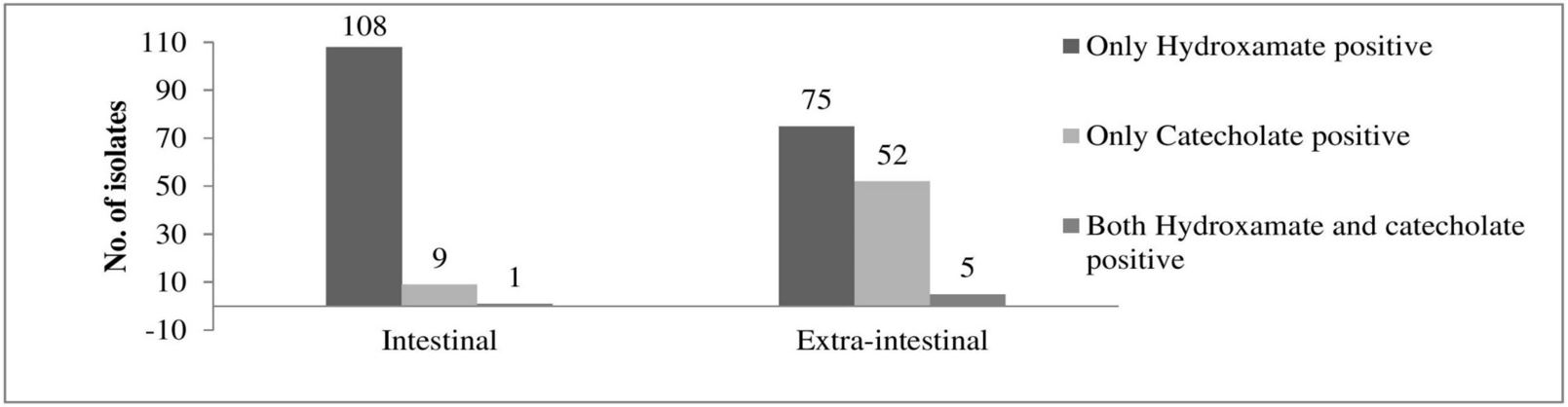


Figure 5.6: Frequency of Siderophore Types among pathogenic *E. coli* isolates

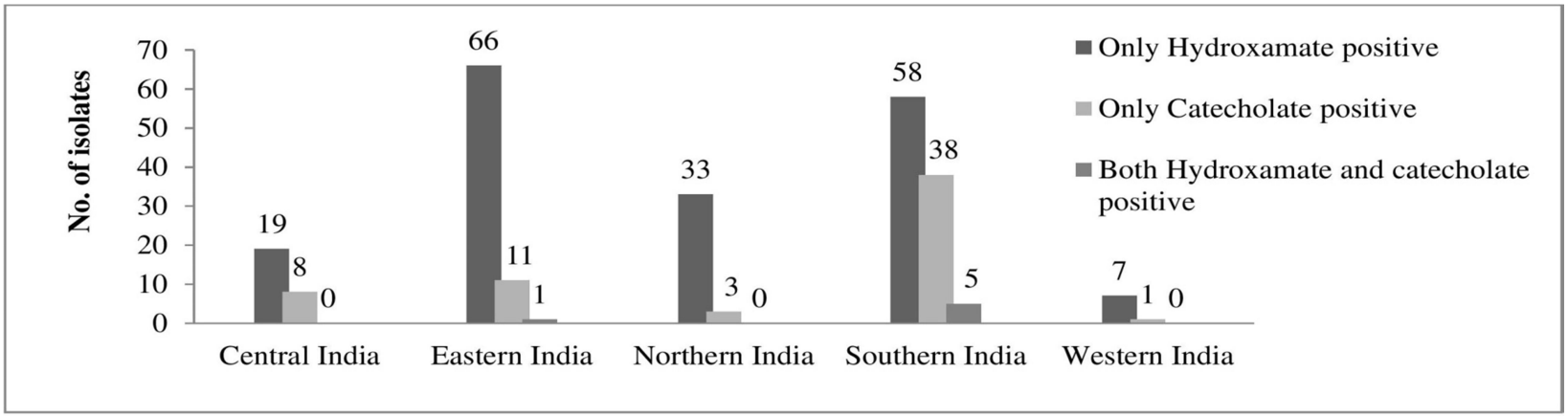


Figure 5.7: Frequency of Siderophore types Geographically

Table 5.17: Type of Siderophore expressed by intestinal and extra-intestinal *E. coli* isolates

SOURCE	**Among CAS agar-positive isolates n=241** (intestinal:110, extrta-intestinal:131)				**Among CAS agar negative isolates n=293** (intestinal:192, extrta-intestinal:101)			**Overall n=534**		
	1 Hydroxamate only	**2 Catechol only**	**3 Both Hyd. and Cat.**	**4 None detected**	**5 Hydroxamate only**	**6 Catechol only**	**7 Both Hyd. and Cat.**	**8 (1+3+5) Hyd. Detected**	**9 (2+3+6) Cat. Detected**	**10 (8+9+4-3) Sid. Detected**
Intestinal	76 UT(16), O1(1), O2(2), O5(1), O7(1), O8(6), O9(2), O11(4), O16(1), O17(3), O20(1), O22(11), O34(2), O35(1), O83(2), O88(3), O98(1), O119 (1), O126(9), O128(3), O141(2), O149(2), O157(1)	5 UT(1), O22(1), O88(2), O157(1)	1 O7(1)	28 UT(2), O2(3), O7(2), O8(1), O11(1), O20(1), O22(5), O35(1), O83(1), O101(1), O119 (1), O126(4), O141(5)	32 UT(10), 2(2), O8(7), O20(2), O22(1), O88(2), O98(1), O119 (2), O126(1), O128(3), O141(1)	4 UT(1), O88(1), O149(1), O157(1)	0	**109**	**10**	**146**

SOURCE	Among CAS agar-positive isolates n=241 (intestinal:110, extrta-intestinal:131)				Among CAS agar negative isolates n=293 (intestinal:192, extrta-intestinal:101)			Overall n=534		
	1 Hydroxamate only	**2 Catechol only**	**3 Both Hyd. and Cat.**	**4 None detected**	**5 Hydroxamate only**	**6 Catechol only**	**7 Both Hyd. and Cat.**	**8 (1+3+5) Hyd. Detected**	**9 (2+3+6) Cat. Detected**	**10 (8+9+4-3) Sid. Detected**
Extra-intestinal	56 UT(13), O1(2), O2(2), O7(1), O8(5), O9(1), O11(6), O17(1), O20(5), O22(1), O35(2), O63(1), O83(1), O88(6), O117(1), O118 (1), O141(5), O149(2)	45 UT(2), O7(6), O8(7), O11(8), O16(1), O17(2), O34(2), O35(3), O83(3), O88(3), O89(1), O101 (1), O119(1), O141(4), O149(1)	5 O7(1), O11(1), O35(1), O83(1), O88(1)	25 UT(4), O7(6), O8(1), O11(6), O20(1), O35(6), O141(1)	19 UT(1), O1(1), O2(1), O4(1), O7(1), O8(2), O11(1), O22(2), O34(1), O35(1), O88(1), O101(1), O141(3), O149(1), O157(1)	7 UT(1), O4(1), O8(1), O20(1), O49(1), O83(2),	0	**80**	**57**	**157**
TOTA	**132**	**50**	**6**	**53**	**51**	**11**	**0**	**189**	**67**	**303**

Here; Hyd.- Hydroxamate, Cat.- Catechol and Sid.-Siderophore

Siderophores and serogroups seem to be two independently expressed traits of pathogenic *E. coli* as siderophores expressing and non-expressing isolates were observed within the same serogroup in a wide variety of serogroups. Though all isolates of some “O” serogroups i.e. O120, O116, O114, O86, O84, and auto-agglutinating did not express siderophores in CAS and chemical assays, and all isolates in some serogroups were found to produce siderophores i.e. O16, O117, and O5. Significantly more isolates of serogroups O141, O11, and O7 was siderophores producers ($p<0.05$). These serogroups are frequently associated with extra-intestinal *E. coli* infections (Poolman and Wacker, 2016). On comparing the type of siderophores and "O" serogroups no significant association was observed. Both hydroxamate and catechol-types of siderophores expressing serogroups were few (1.1% including O88, O83, O35, O11, and O7) which expressed both types of siderophores in very low proportions [Table 5.18].

EHEC/STEC strains are also known to produce siderophores as their important virulence traits (Zhang *et al.,* 2020; Freestone *et al.,* 2003). Four of the seven *E. coli* serogroup O157 isolates were also detected to express siderophores (two hydroxamate types and two catechol-types) in this study. As EHEC/STEC strains usually represented by this serogroup is known to cause serious infections like HC and HUS (Silva *et al.,* 2020; Croxen, *et al.,* 2013), siderophores seem to be important virulence characteristics of this pathotype.

Significantly more percentages ($p<0.05$) of *E. coli* isolates from summer (84.5%), monsoon (51.6%), and post-monsoon (88%) seasons than winter season (38.2%) were found to express siderophores production. A similar trend was observed was seen in intestinal as well as extra-intestinal isolates (Table 5.19). Infection rates of agents including *E. coli* infections transmitting through fecal-oral routes from contaminated food and water are known to increase during summer and monsoon/post-monsoon seasons (Ahmed *et al.,* 2013; Eber *et al.,* 2011), detection of a higher percentage of siderophores production during these seasons is of important as siderophores further assist bacteria in colonization in infected hosts.

The importance of siderophores in the epidemiology of pathogenic *E. coli* is indicated by the detection of siderophores in a large percentage of isolates (56.7%), diversity of *E. coli* isolates in expressing siderophore types, detection of siderophores in clinically important serogroups including *E. coli* O157 and seasonal trends in siderophore expression in this study.

Table 5.18: Different *E. coli* "O" serogroups expressing siderophores

"O" Serogroup	**Siderophore Screening on CAS agar N (%age)**			**Hydroxamate/Catechol Detection N (%age)**				
	Positive	**Negative**	**Total**	**Hydroxamate Positive**	**Catechol Positive**	**Both Positive**	**Both Negative**	**Total**
UT	38(39.6)	58(60.4)	96	40(41.7)	5(5.2)	0	51(53.1)	96
O1	3(60)	2(40)	5	4(80)	0	0	1(20)	5
O2	7(43.8)	9(56.3)	16	7(43.8)	0	0	9(56.3)	16
O4	0	3(100)	3	1(33.3)	1(33.3)	0	1(33.3)	3
O5	1(100)	0	1	1(100)	0	0	0	1
O7	18(85.7)	3(14.3)	21	3(14.3)	6(28.6)	2(9.5)	10(47.6)	21
O8	20(43.5)	26(56.5)	46	20(43.5)	8(17.4)	0	18(39.1)	46
O9	3(50)	3(50)	6	3(50)	0	0	3(50)	6
O11	26(68.4)	12(31.6)	38	11(29)	8(21)	1(2.6)	18(47.4)	38
O16	2(100)	0	2	1(50)	1(50)	0	0	2
O17	6(46.2)	7(53.8)	13	4(30.8)	2(15.4)	0	7(53.8)	13
O20	8(40)	12(60)	20	8(40)	1(5)	0	11(55)	20
O22	18(54.5)	15(45.5)	33	15(45.5)	1(3)	0	17(51.5)	33

"O" Serogroup	Siderophore Screening on CAS agar N (%age)			Hydroxamate/Catechol Detection N (%age)				
	Positive	Negative	Total	Hydroxamate Positive	Catechol Positive	Both Positive	Both Negative	Total
O34	4(80)	1(20)	5	3(60)	2(40)	0	0	5
O35	14(56)	11(44)	25	4(16)	3(12)	1(4)	17(68)	25
O49	0	5(100)	5	0	1(20)	0	4(80)	5
O63	1(33.3)	2(66.7)	3	1(33.3)	0	0	2(66.7)	3
O83	8(32)	17(68)	25	3(12)	5(20)	1(4)	16(64)	25
O84	0	7(100)	7	0	0	0	7(100)	7
O86	0	2(100)	2	0	0	0	2(100)	2
O88	15(45.5)	18(54.5)	33	12(36.4)	6(18.2)	1(3)	14(42.4)	33
O89	1(14.3)	6(85.7)	7	0	1(14.3)	0	6(85.7)	7
O98	1(50)	1(50)	2	2(100)	0	0	0	2
O101	2(25)	6(75)	8	1(12.5)	1(12.5)	0	6(75)	8
O114	0	1(100)	1	0	0	0	1(100)	1
O116	0	2(100)	2	0	0	0	2(100)	2
O117	1(100)	0	1	1(100)	0	0	0	1
O118	1(50)	1(50)	2	1(50)	0	0	1(50)	2

"O" Serogroup	**Siderophore Screening on CAS agar N (%age)**			**Hydroxamate/Catechol Detection N (%age)**				
	Positive	**Negative**	**Total**	**Hydroxamate Positive**	**Catechol Positive**	**Both Positive**	**Both Negative**	**Total**
O119	3(30)	7(70)	10	3(30)	1(10)	0	6(60)	10
O120	0	4(100)	4	0	0	0	4(100)	4
O126	13(46.4)	15(53.6)	28	10(35.7)	0	0	18(64.3)	28
O128	3(37.5)	5(62.5)	8	6(75)	0	0	2(25)	8
O141	17(73.9)	6(26.1)	23	11(47.8)	4(17.4)	0	8(34.8)	23
O149	5(22.7)	17(77.3)	22	5(22.7)	2(9.1)	0	15(68.2)	22
O157	2(28.6)	5(71.4)	7	2(28.6)	2(28.6)	0	3(42.9)	7
Auto-agglutinating	0	4(100)	4	0	0	0	4(100)	4

Table 5.19: Frequency of siderophore-producing *E. coli* isolates during different seasons

Seasons	**Among CAS agar-positive isolates n=241** (summer:72, winter:33, Monsoon:122, Post-monsoon:14)				**Among CAS agar negative isolates, n=293** (summer:25, winter:69, Monsoon:188, Post-monsoon:11)			**Overall n=534** (summer:97, winter:102 Monsoon:310 Post-monsoon:25)		
	1 Only hydroxamate detected	2 Only Catechol detected	3 Both detected	4 None 1 detected	5 Only hydroxamate detected	6 Only Catechol detected	7 Both detected	8 (1+3+5) Hydroxamate Detected	9 (2+3+6) Catechol Detected	10 (8+9+4-3) Siderophores Detected
Summer	28 (IPEC:17 ExPEC:11)	22 (IPEC:01 ExPEC:21)	6 (IPEC:01 ExPEC:05)	16 (IPEC:08 ExPEC:08)	5 (IPEC:04 ExPEC:01)	5 (IPEC:01 ExPEC:04)	0	**39**	**33**	**82** (IPEC:32 ExPEC:50)
Winter	24 (IPEC:22 ExPEC:02)	3 (IPEC:2 ExPEC:01)	0	6 (IPEC:06 ExPEC:00)	6 (IPEC:01 ExPEC:05)	0	0	**30**	**3**	**39** (IPEC:31 ExPEC:08)

Seasons	Among CAS agar-positive isolates n=241 (summer:72, winter:33, Monsoon:122, Post-monsoon:14)				Among CAS agar negative isolates, n=293 (summer:25, winter:69, Monsoon:188, Post-monsoon:11)			Overall n=534 (summer:97, winter:102 Monsoon:310 Post-monsoon:25)		
	1 Only hydroxamate detected	2 Only Catechol detected	3 Both detected	4 None 1 detected	5 Only hydroxamate detected	6 Only Catechol detected	7 Both detected	8 (1+3+5) Hydroxamate Detected	9 (2+3+6) Catechol Detected	10 (8+9+4-3) Siderophores Detected
Monsoon	70 (IPEC:27 ExPEC:43)	24 (IPEC:01 ExPEC:23)	0	28 (IPEC:11 ExPEC:17)	32 (IPEC:19 ExPEC:13)	6 (IPEC:03 ExPEC:03)	0	**102**	**30**	**160** (IPEC:61 ExPEC:99)
Post-monsoon	10 (IPEC:10 ExPEC:00)	1 (IPEC:01 ExPEC:00)	0	3 (IPEC:03 ExPEC:00)	8 (IPEC:08 ExPEC:00)	0	0	**18**	**1**	**22** (IPEC:22 ExPEC:00)
TOTAL	**132**	**50**	**6**	**53**	**51**	**11**	**0**	**189**	**67**	**303**

5.4.5. **Colicin**

To achieve sustained colonization and establishment of infection competition with commensals for available resources in the host environment is critical, especially where the host environment is full of commensals like the gastrointestinal tract. To colonize such infection sites and to counter the competing microbial populations' virulent agents adopt various mechanisms and **bacteriocin is one of these mechanisms which help eliminate sensitive commensal/competitors to create a suitable environment for its colonization and growth. Colicin is a class of bacteriocins produced by Enterobacteriaceae family members including *E. coli* thus colicin production is one of the important virulence characteristics of the pathogenic microbes of Enterobacteriaceae** (Budic *et al.,* 2011; Smajs *et al.,* 2010). In the present study, 20.2% of the total isolates were found to be colicin producers which were comparatively less than other virulence factors i.e. CSH, biofilm, and siderophores. Contrary to this colicin production was reported in a very high percentage of isolates from different infection sites (Abd El-Baky *et al.,* 2020; Fakruddin *et al.,* 2012).

Though colicin production is known as a virulence characteristic providing a survival advantage to pathogens in competitive environments like the gastro-intestinal tract however n this study, interestingly, colicin producers were detected in more percentage among extra-intestinal isolates (31%) than intestinal isolates (11.9%) ($p< 0.05$) (Table 5.20). In many other studies also, colicin has been detected in large numbers among extra-intestinal isolates. It is known to have a toxic effect on eukaryotic cells and is considered a virulence factor in extra-intestinal *E. coli,* especially UPEC strains (Armstrong, 2013; Petkovsek *et al.,* 2012; Budic *et al.,* 2011; Smajs *et al.,* 2010; Azpiroz *et al.,* 2009; Chumchalova and Smarda, 2003). Detection of colicin producers among UTI isolates may be attributed to the fact that pathogenic intestinal and UPEC isolates are actually of commensal intestinal origin which becomes pathogenic after acquiring various pathogenic traits (Finlay and Falkow, 1997; Ochman and Selander, 1984).

The percentage of colicin-producing isolates was low among isolates from all the geographical areas ranging from 4 to 30% ($P<0.05$). No intestinal isolate from western India was found to produce colicin and no extra-intestinal isolate from northern India produced colicin (Table 5.21). Low colicin-producing *E. coli* isolates indicate a limited role of colicin as a virulence factor in *E. coli* pathogenicity.

Table 5.20: Frequencies of isolates producing colicin

Study isolates	**Colicin Positive n (%)**	**Colicin Negative n (%)**	**Total N (%)**
Intestinal isolates	36 (11.9%)	266 (88.1%)	302 (100%)
Extra-intestinal isolates	72 (31.0%)	160 (69.0%)	232 (100%)
Total	108 (20.2%)	426 (79.8%)	534 (100%)

Table 5.21: Region-wise frequencies of isolates producing colicin

Geographical location	**Intestinal Isolates**		**Extra-intestinal isolates**		**Total Colicin positive n (%)**
	Colicin positive n (%)	**Colicin negative n (%)**	**Colicin positive n (%)**	**Colicin negative n (%)**	
Central India	2 (6.9%)	27 (93.1%)	11 (36.7%)	19 (63.3%)	13 (22.0%)
Eastern India	9 (9.3%)	88 (90.7 %)	20 (54.1%)	17 (45.9%)	29 (21.6%)
Northern India	11 (8.8%)	114 (91.2%)	0 (0%)	23 (100%)	11 (7.4%)
Southern India	14 30.4%)	32 (69.6%)	37 (29.8%)	87 (70.2%)	51 (30.0%)
Western India	0 (0%)	5 (100%)	4 (22.2%)	14 (77.8%)	4 (17.4%)
Total	36 11.9%)	266 (88.1%)	72 (31.0%)	160 (69.0%)	108 (20.2%)

Colicin production was associated with many “O” serogroups. Only one out of seven O157 isolates was found to produce colicin (Table 5.22).

The colicin production rate among isolates from the post-monsoon season was comparatively more than among isolates from other seasons ($p<0.05$) when most of the infections are usually reported (Table 5.23).

Table 5.22: Serotype-wise expression of Colicinogeny

"O" Serogroup	**Overall Colicin Positive isolates n (%age)**	**Colicin Positive Intestinal isolates n (%age)**	**Colicin Positive extra-intestinal isolates n (%age)**
UT; N=96	16 (16.7)	6 (6.3)	10 (10.4)
O1; N=5	2 (40.0)	0 (0.0)	2 (40.0)
O2; N=16	3 (18.8)	2 (12.5)	1 (6.3)
O4; N=3	3 (100.0)	0 (0.0)	3 (100.0)
O5; N=1	0 (0.0)	0 (0.0)	0 (0.0)
O7; N=21	5 (23.8)	3 (14.3)	2 (9.5)
O8; N=46	9 (19.6)	4 (8.7)	5 (10.9)
O9; N=6	2 (33.3)	1 (16.7)	1 (16.7)
O11; N=38	18 (47.4)	0 (0.0)	18 (47.4)
O16; N=2	0 (0.0)	0 (0.0)	0 (0.0)
O17; N=13	6 (46.2)	1 (7.7)	5 (38.5)
O20; N=20	1 (5.0)	1 (5.0)	0 (0.0)
O22; N=33	2 (6.1)	0 (0.0)	2 (6.1)
O34; N=5	2 (40.0)	0 (0.0)	2 (40.0)
O35; N=25	4 (16.0)	1 (4.0)	3 (12.0)
O49; N=5	0 (0.0)	0 (0.0)	0 (0.0)
O63; N=3	0 (0.0)	0 (0.0)	0 (0.0)
O83; N=25	2 (8.0)	2 (8.0)	0 (0.0)
O84; N=7	1 (14.3)	1 (14.3)	0 (0.0)
O86; N=2	0 (0.0)	0 (0.0)	0 (0.0)
O88; N=33	10 (30.3)	3 (9.1)	7 (21.2)
O89; N=7	0 (0.0)	0 (0.0)	0 (0.0)
O98; N=2	2 (100.0)	2 (100.0)	0 (0.0)
O101; N=8	3 (37.5)	0 (0.0)	3 (37.5)
O114; N=1	0 (0.0)	0 (0.0)	0 (0.0)

“O” Serogroup	**Overall Colicin Positive isolates n (%age)**	**Colicin Positive Intestinal isolates n (%age)**	**Colicin Positive extra-intestinal isolates n (%age)**
O116; N=2	0 (0.0)	0 (0.0)	0 (0.0)
O117; N=1	0 (0.0)	0 (0.0)	0 (0.0)
O118; N=2	0 (0.0)	0 (0.0)	0 (0.0)
O119; N=10	5 (50.0)	3 (30.0)	2 (20.0)
O120; N=4	0 (0.0)	0 (0.0)	0 (0.0)
O126; N=28	0 (0.0)	0 (0.0)	0 (0.0)
O128; N=8	0 (0.0)	0 (0.0)	0 (0.0)
O141; N=23	10 (43.5)	5 (21.7)	5 (21.7)
O149; N=22	1 (4.5)	0 (0.0)	1 (4.5)
O157; N=7	1 (14.3)	1 (14.3)	0 (0.0)
Auto-agglutinating; N=4	0 (0.0)	0 (0.0)	0 (0.0)
TOTAL; N=534	108 (20.2)	36 (6.7)	72 (13.5)

Table 5.23: Seasonality and colicin production

Season	**Intestinal n (%)**			**Extra-intestinal isolates n (%)**			**Overall n (%)**		
	Colicin positive	**Colicin negative**	**Total**	**Colicin positive**	**Colicin negative**	**Total**	**Colicin positive**	**Colicin negative**	**Total**
Summer	1 (2.7)	36 (97.3)	37	13 (21.7)	47 (78.3)	60	14 (14.4)	83 (85.6)	97
Winter	5 (6.0)	79 (94.0)	84	4 (22.2)	14 (77.8)	18	9 (8.8)	93 (91.2)	102
Monsoon	16 10.3)	140 (89.7)	156	55 (37.7)	99 (64.3)	154	71 (22.9)	239 (77.1)	310
Post - Monsoon	14 (56.0)	11 (44.0)	25	-	-	-	14 (56.0)	11 (44.0)	25

5.4.6. **Gelatin Hydrolysis**

Gelatin hydrolysis activity is the measure of gelatinase release by the pathogenic microorganism. **This enzyme contributes to microbial virulence through the degradation of a wide range of host substances like fibrin, fibrinogen, collagen, bradykinin, and components of complement** (C3 and C3a) (Park *et al.,* 2008; Waters *et al.,* 2003; Park *et al.,* 2007; Makinen and Makinen, 1994; Makinen *et al.,* 1989).

Gelatinase activity was shown by only 4.5% of the total isolates. Though the frequency of gelatinase-producing *E. coli* was more among intestinal isolates than the extra-intestinal isolates ($p<0.05$), however in overall gelatin hydrolysis was the least expressed virulence factor of intestinal as well as extra-intestinal *E. coli* isolates (Table 5.24).

Gelatin hydrolysis was also recorded with very low rates even as low as 0.0% among *E. coli* isolates by other workers, thus indicating the little role of this virulence factor in *E. coli* pathogenicity at least among diarrheal and UTI-causing *E. coli* (Shah, 2019; Kaira and Pai, 2018; Niyas and Gopinath, 2018). On the other hand, gelatin hydrolysis was detected at a relatively high rate in a few studies (Bawankar, 2018; Priya, 2015).

Table 5.24: Frequencies of isolates showing gelatin hydrolysis

Study isolates	**Positive n (%)**	**Negative n (%)**	**Total N (%)**
Intestinal isolates	21 (7.0%)	281 (93.0%)	302 (100%)
Extra-intestinal isolates	3 (1.3%)	229 (98.7%)	232 (100%)
Total	24 (4.5%)	510 (95.5%)	534 (100%)

Gelatin hydrolysis was the least expressed virulence characteristic of the tested isolates in this study in all geographical locations (P>0.05). None of the isolates from Western India was found to hydrolyze gelatin. Similarly, none of the extra-intestinal isolates from northern India was found to hydrolyze gelatin [Table 5.25].

Table 5.25: Region-wise frequencies of isolates showing gelatin hydrolysis

Geographical location	**Intestinal Isolates n (%)**		**Extra-intestinal isolates n (%)**		**Total positive N (%)**
	Positive	**Negative**	**Positive**	**Negative**	
Central India	5 (17.2%)	24 (82.8%)	1 (3.3%)	29 (96.7%)	6 (10.2%)
Eastern India	8 (8.2%)	89 (91.8 %)	1 (2.7%)	36 (97.3%)	9 (6.7%)
Northern India	4 (3.2%)	121 (96.8%)	0 (0%)	23 (100%)	4 (2.7%)
Southern India	4 (8.7%)	42 (91.3%)	1 (0.8%)	123 (99.2%)	5(2.9%)
Western India	0 (0%)	5 (100%)	0 (0%)	18 (100%)	0 (0%)
Total	21 (7.0%)	281 (93.0%)	3 (1.3%)	229 (98.7%)	24 (4.5%)

Twenty-four gelatin hydrolyzing isolates in the present study were represented by eight different “O” serogroups. Among intestinal isolates, 57% of gelatin hydrolyzing isolates were associated with O22 and UT isolates. None of the O157 isolates were found to hydrolyze gelatin (Table 5.26).

The rate of gelatin hydrolyzing isolates was low during all seasons (p>0.05). All three gelatin hydrolyzing extra-intestinal isolates were from the monsoon season (Table 5.27).

Table 5.26: Serotypes associated with gelatinase-*positive Escherichia coli* isolates

"O" serogroups associated with gelatinase-positive isolates in overall Total gelatinase positive n = 24	"O" serogroups associated with gelatinase-positive intestinal isolates Total gelatinase positive n = 21	"O" serogroups associated with gelatinase-positive extra-intestinal isolates Total gelatinase positive n = 03
UT (6), O22 (7), O35 (1), O88 (3), O101 (1), O114 (1), O126 (3), O149 (2)	UT (5), O22 (7), O35 (1), O88 (2), O114 (1), O126 (3), O149 (2)	UT (1), O88 (1), O101 (1)

Table 5.27: Seasonality and gelatin hydrolysis

Season	Intestinal			Extra-intestinal isolates			Overall		
	Gelatinase positive	**Gelatinase negative**	**Total**	**Gelatinase positive**	**Gelatinase negative**	**Total**	**Gelatinase positive**	**Gelatinase negative**	**Total**
Summer	5 (13.5)	32 (86.5)	37	0 (0.0)	60 (100.0)	60	5 (5.2%)	92 (94.8%)	97
Winter	4 (4.8)	80 (95.2)	84	0 (0.0)	18 (100.0)	18	4 (3.9%)	98 (96.1%)	102
Monsoon	10 (6.4)	146 (93.6)	156	3 (1.9)	151 (98.1)	154	13 (4.2%)	297 (95.8%)	310
Post-Monsoon	2 (8.0%)	23 (92.0%)	25	-	-	-	2 (8.0%)	23 (92.0%)	25

Co-expression of virulence factors was found to be a common characteristic among intestinal as well as extra-intestinal isolates. 91.2% of the isolates expressed at least one of the virulence characteristics evaluated in this study. Multiple virulence factors were expressed by 38.6% of the isolates (Table 5.28). Among intestinal isolates 31.7% and extra-intestinal isolates, 47.4% of the isolates expressed multiple virulence factors.

The expression of multiple virulence factors is important for entry and colonization in host cell surfaces and breaching the host immune mechanisms to establish infections and disease. Microbial strains expressing multiple virulence factors are considered to be more pathogenic than those expressing one or two virulence factors. *E. coli* pathogenicity is also known to be attributed to the co-expression of many virulence factors (Shruthi *et al.* 2012, Katouli *et al.* 2005; Foxman *et al.,* 1995). In the present study co-expression of virulence factors was observed in a large percentage of intestinal and extra-intestinal isolates. Co-expression of cell surface hydrophobicity (CSH) and siderophore production with biofilm formation was observed as the most commonly expressed combination of multi-virulent traits with significantly positive correlation values ($p<0.05$) [Figure 5.8 and Table 5.29]. Co-expression of these virulence factors may be because CSH and siderophore are associated with initial adhesion and colonization which are prerequisites for the formation of biofilms (Pi *et al.,* 2012; Rosenberg, and Kjelleberg, 1986).

Table 5.28: Co-expression of virulence factors by *E. coli* isolates

No. of virulence factors expressed	**Intestinal isolates**	**Extra-intestinal isolates**	**Overall**
Zero	29 (9.6%)	18 (7.8%)	47 (8.8%)
One	88 (29.1%)	42 (18.1%)	130 (24.3%)
Two	89 (29.5%)	62 (26.7%)	151 (28.3%)
Three	72 (23.8%)	88 (37.9%)	160 (30%)
Four	23 (7.6%)	22 (9.5%)	45 (8.4%)
Five	1 (0.3%)	0 (0%)	1 (0.2%)

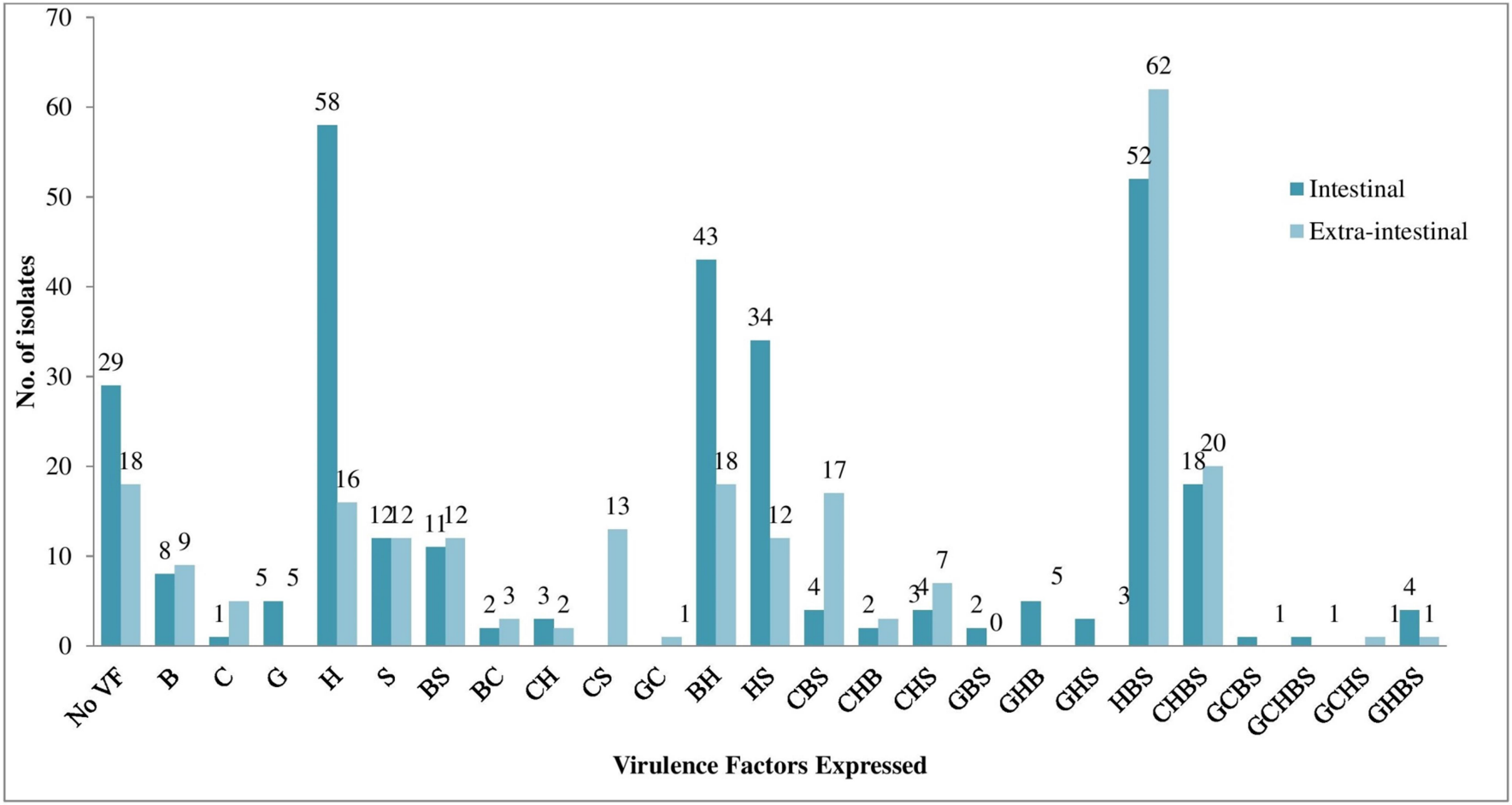


Figure 5.8: Virulence factor pattern of Escherichia coli isolates

Here; VF- Virulence factor ; B-biofilm; C- colicin; G- Gelatinase; H- CSH; S-Siderophore

Table 5.29: Correlation between virulence factors expression by *E. coli* isolates

Virulence factor	**Correlation % and p values (at 95% confidence level)**				
	Biofilm (TCP)	**Gelatinase**	**CSH**	**Colicin**	**Siderophore**
Biofilm (TCP)	-	0.11 (0.799)	18.8 (0.000)	10.1 (0.02)	27.3 (0.000)
Gelatinase	0.11(0.799)	-	-3.1 (0.475)	-1.9 (0.658)	-1.1 (0.795)
CSH	18.8 (0.000)	-3.1 (0.475)	-	-13.8 0.001)	7.9 (0.069)
Colicin	10.1 (0.02)	-1.9 (0.658)	-13.8 0.001)	-	23.3 (0.000)
Siderophore	27.3 (0.000)	-1.1 (0.795)	7.9 (0.069)	23.3 (0.000)	-

The occurrence of diverse isolates of *E. coli* makes management and control of this infectious agent difficult. The problem is further complicated by the emergence of multiple drug-resistant strains. The emergence of multiple drug resistance and persistent and recurrent *E. coli* infections has complicated the treatment and management of *E. coli* infections. Multiple drug resistance among pathogenic *E. coli* has been reported extensively in India (Kulkarni *et al.,* 2017; Niranjan and Malini, 2014) the spread of resistant *E. coli* worldwide is thought to be Indian-originated (Kumarasamy *et al.,* 2010). Considering the increasing trends in *E. coli* infection rates worldwide and the emergence of strains resistant to multiple antimicrobials; W.H.O. has placed *E. coli* among the critical microorganisms that require immediate attention as a health threat globally and against which there is an urgent need to develop newer antimicrobials (WHO, 2017).

5.5. **Antibiogram**

In the present study, 20 antibiotics from different antimicrobial classes were tested for susceptibility and only 11% of isolates were found to be sensitive to all the antibiotics. High resistance rates to ampicillin, nalidixic acid, cefotaxime, and amoxiclav were found and this was observed in isolates from all geographical locations of the country. Earlier studies from different parts of India also reported high resistance rates in *E. coli* to these drugs with increasing trends, which is mainly attributed to the non-judicious use of antibiotics and lack of antimicrobial stewardship programs (AMSP) at healthcare institutions (Malik *et al.,* 2021; Prasada *et al.,* 2019). On the other hand amikacin, nitrofurantoin, chloramphenicol, imipenem, and meropenem were found to be the most effective drugs. The frequency of drug resistance exhibited by *E. coli* isolates under study is shown below in Table 5.30 and Figure 5.9.

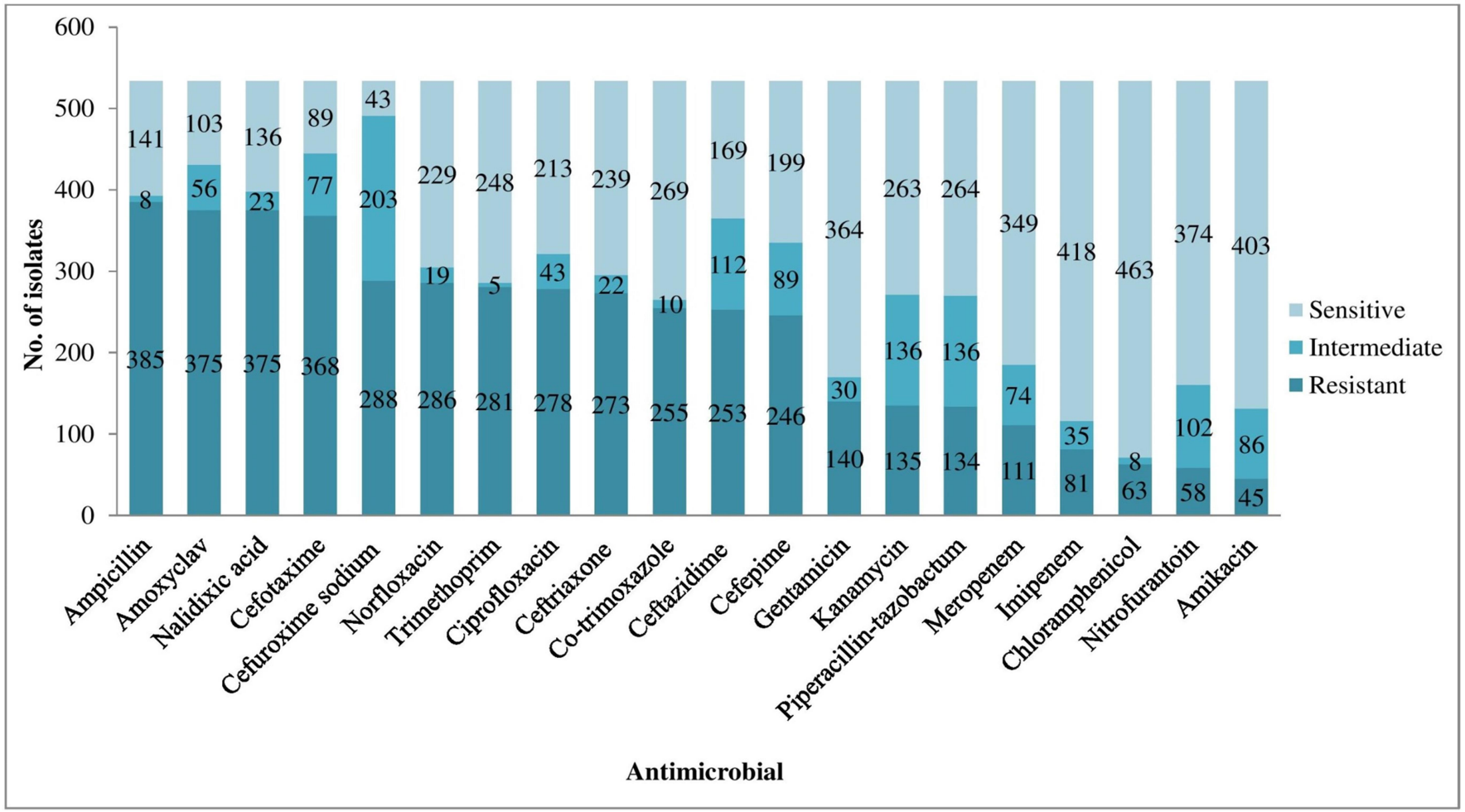


Fig. 5.9: Antimicrobial susceptibility pattern of intestinal and extraintestinal *E. coli*

Table 5.30: Antimicrobial resistance Pattern of intestinal and extra-intestinal *Escherichia coli* isolates

Name of the antimicrobial agent	**Resistance pattern of intestinal and extraintestinal E. coli N (%)**					
	Intestinal *E. coli* isolates, Total=302			**Extra-intestinal *E. coli* isolates, Total=232**		
	R	I	S	R	I	S
Amoxiclav	237 (78.5)	23 (7.6)	42 (13.9)	138 (59.5)	33 (14.2)	61 (26.3)
Ampicillin	238 (78.8)	4(1.3)	60 (19.9)	147 (63.4)	4 (1.7)	81 (34.9)
Amikacin	25 (8.3)	72 (23.8)	205 (67.9)	20 (8.6)	14 (6.0)	198 (85.3)
Chloramphenicol	38 (12.6)	6 (2)	258 (85.4)	25 (10.8)	2 (0.8)	205 (88.4)
Ciprofloxacin	175 (57.9)	24 (7.9)	103 (34.1)	103 (44.4)	19 (8.2)	110 (47.4)
Co-trimoxazole	160 (53)	8 (2.6)	134 (44.4)	95 (40.9)	2 (0.8)	135 (58.2)
Ceftriaxone	180 (59.6)	12 (4)	110 (36.4)	93 (40.1)	10 (4.3)	129 (55.6)
Cefotaxime	224 (74.2)	35 (11.6)	43 (14.2)	144 (62.1)	42 (18.1)	46 (19.8)
Cefuroxime sodium	189 (62.6)	97 (32.1)	16 (5.3)	99 (42.7)	106 (45.7)	27 (11.6)
Cefepime	163 (54)	45 (14.9)	94 (31.1)	83 (35.8)	44 (19)	105 (45.3)
Gentamicin	69 (22.8)	16 (5.3)	217 (71.9)	71 (30.6)	14 (6.0)	147 (63.4)
Imipenem	46 (15.2)	24 (7.9)	232 (76.8)	35 (15.1)	11 (4.7)	186 (80.2)
Kanamycin	71 (23.5)	88 (29.1)	143 (47.4)	64 (27.6)	48 (20.7)	120 (51.7)

Name of the antimicrobial agent	**Resistance pattern of intestinal and extraintestinal E. coli N (%)**					
	Intestinal *E. coli* isolates, Total=302			**Extra-intestinal *E. coli* isolates, Total=232**		
	R	I	S	R	I	S
Meropenem	65 (21.5)	51 (16.9)	186 (61.6)	46 (19.8)	23 (9.9)	163 (70.3)
Nalidixic acid	228 (75.5)	16 (5.3)	58 (19.2)	147 (63.4)	7 (3.0)	78 (33.6)
Nitrofurantoin	32 (10.6)	61 (20.2)	209 (69.2)	26 (11.2)	41 (17.7)	165 (71.7)
Norfloxacin	188 (62.3)	8 (2.6)	106 (35.1)	98 (42.2)	11 (4.7)	123 (53.0)
Piperacillin-tazobactum	79 (26.2)	91 (30.1)	132 (43.7)	55 (23.7)	45 (19.4)	132 (56.9)
Trimethoprim	178 (59)	4 (1.3)	120 (39.7)	103 (44.4)	1 (0.4)	128 (55.2)
Ceftazidime	156 (51.7)	70 (23.2)	76 (25.1)	97 (41.8)	42 (18.1)	93 (40.1)

Out of 534 total isolates studied 60 (11.2%) were sensitive to all twenty antibiotics while the rest were resistant to one or more antibiotics. The distribution of resistant isolates among the study samples is shown below (Fig 5.10).

Twenty antimicrobials used in the present study belong to different drug classes (penicillins, cephalosporins, carbapenems, aminoglycosides, phenicols, quinolones, and sulphonamides). The level of drug resistance in study samples was found to be very high as 71% of the isolates were MDR. 11% of the isolates were found to be sensitive to all the classes of antimicrobials included in the study, 9% were resistant to two different classes of antimicrobials (TDR) and 9% were resistant to only one class of antimicrobials as shown below in figure 5.11.

A comparative evaluation of the level of drug resistance is shown in the following figure (Fig 5.12). A very high % age of MDR isolates was found in the various geographical area of the country as shown in the following figure (Fig 5.13).

Antibiotic susceptibility results of the present study show that drug resistance is a common characteristic among pathogenic *E. coli* isolates in the country and that MDR strains are in circulation throughout the country. MDR and resistance to newer-generation antibiotics are throwing challenges to the management and treatment of *E. coli* infection. The emergence of *E. coli* strains resistant to multi drugs and extensively drug-resistant strains is a matter of concern for healthcare practitioners worldwide as the treatment options remain very limited to control infections with such strains (Sheu *et al.,* 2019).

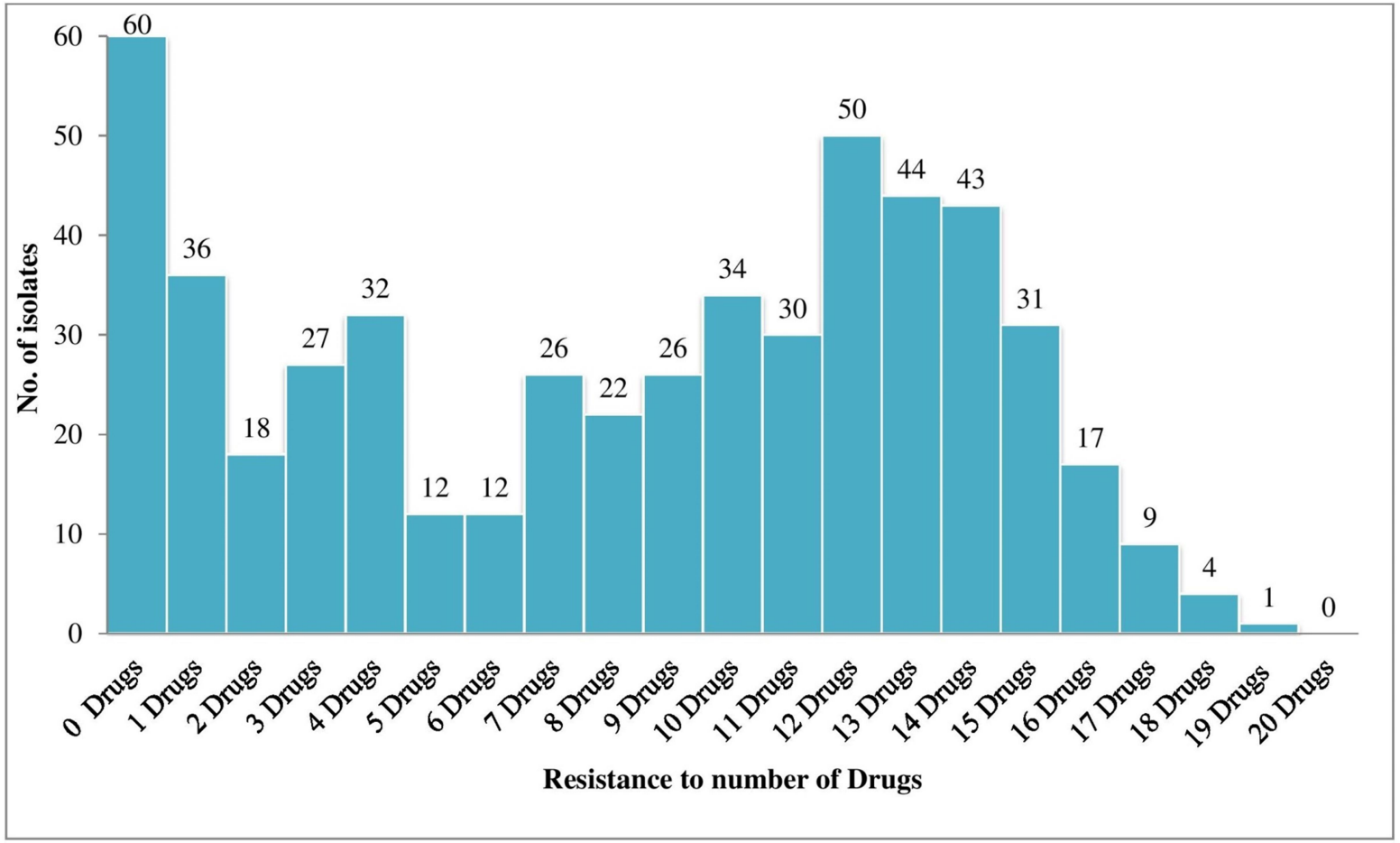


Fig 5.10: *E. coli* isolates resistant to number of antimicrobials

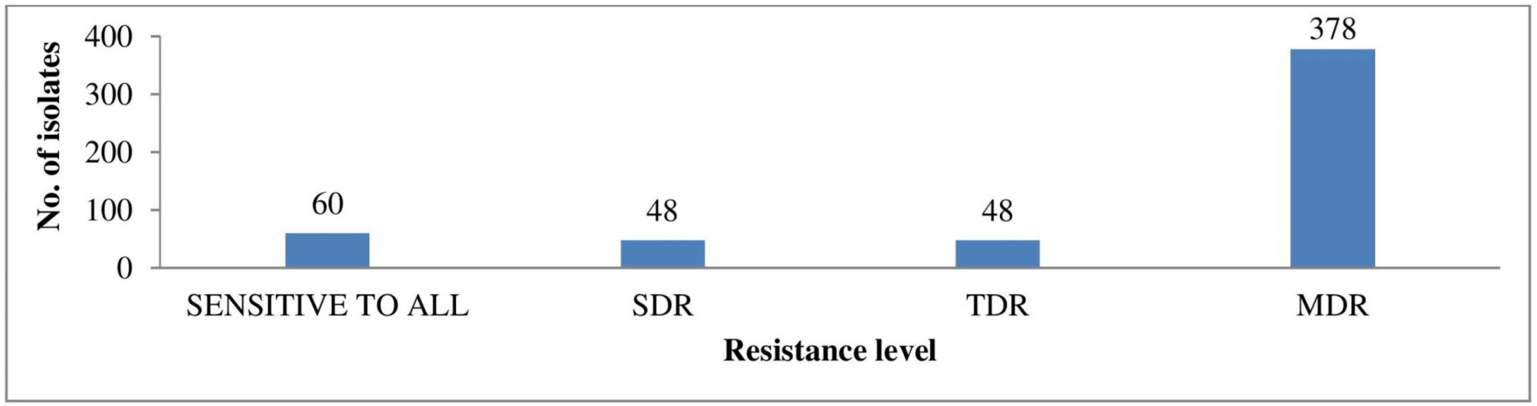


Fig 5.11: Drug resistance level in *E. coli* isolates

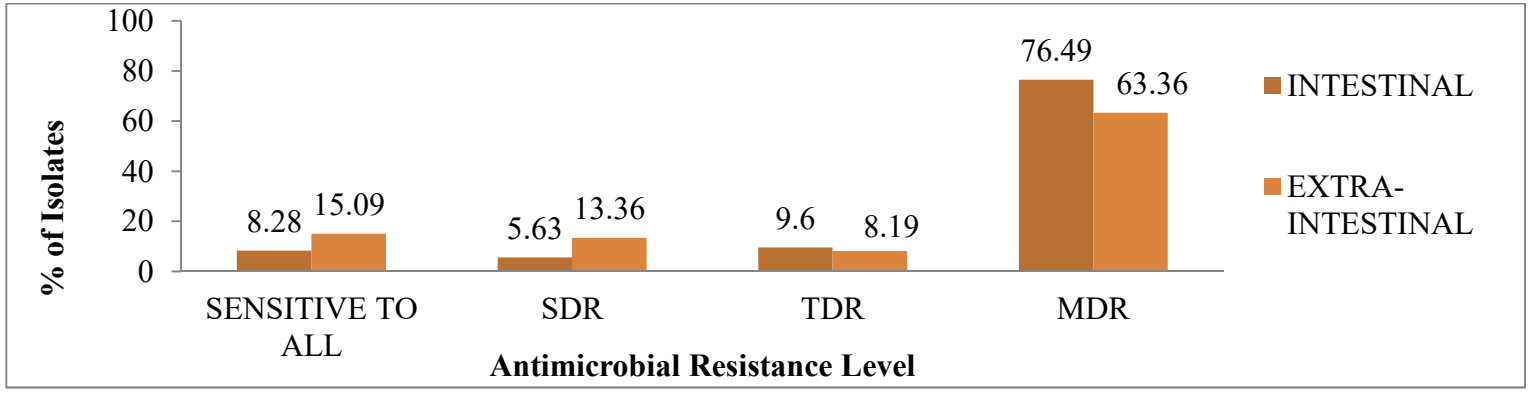


Fig 5.12: Comparative level of antimicrobial resistance among intestinal and extra-intestinal *E. coli*

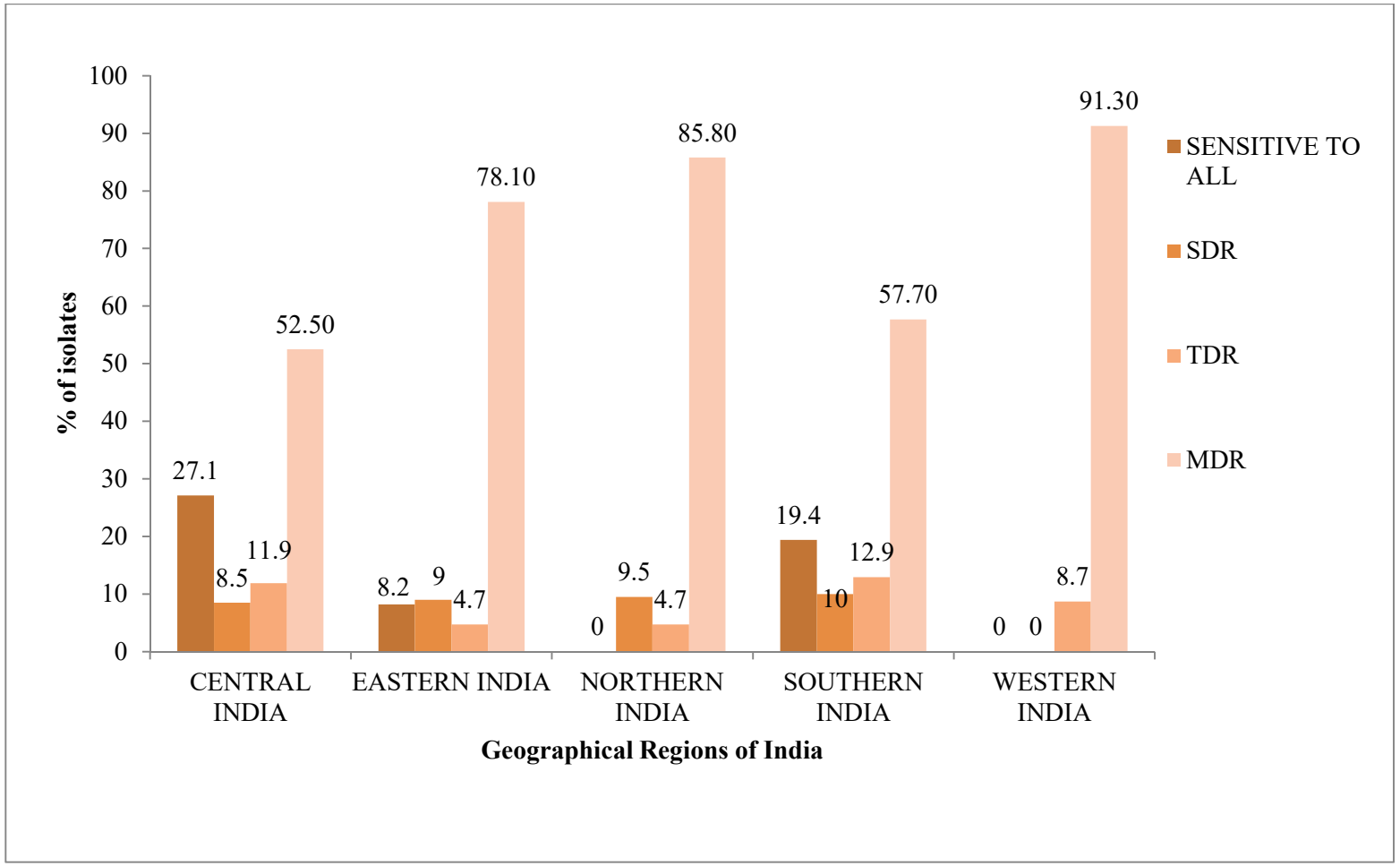


Fig 5.13: Region-wise distribution of resistant isolates

In the present study resistance to β-lactam drugs was observed in a very large percentage (82.8%), which was found high among intestinal (85.4%) as well as extra-intestinal isolates (79.3%) with no significant difference among the groups ($p>0.05$). Among β-lactam drugs, resistance rates to penicillins, cephalosporins, and carbapenem classes of drugs were observed in 74.2%, 75.7%, and 24.3% respectively. The resistance rate to these β-lactam classes of drugs was found high among intestinal (81.1%, 79.8%, and 23.8% respectively) as well extra-intestinal isolates (65.1%, 70.3%, and 25% respectively). The difference among groups was significant for resistance to penicillin and cephalosporin groups of drugs ($p<0.05$), however, there was no significant difference among the groups in resistance rates to carbapenems, ($p>0.05$).

β-lactam antibiotic-resistant strains are prevalent worldwide and resistance to carbapenems is also reported frequently from all over the world (Malchione *et al.,* 2019; Abbas *et al.,* 2017). β-lactam drugs are the choice of drug for treating *E. coli* infections and contribute a major share of empirical treatment prescriptions. Overuse of β-lactam drugs is considered to have resulted in selection pressure for resistance among clinical isolates to this group of drugs. Increasing trends in resistance rate to these drugs is a matter of concern and warrant an urgent need to wisely plan and implement stewardship programs throughout the country at the level of healthcare facilities.

Penicillin is the most widely used antibiotic; however, most bacteria including *E. coli* acquire resistance to this drug through the production of the enzyme β-lactamase which hydrolyses the β-lactam ring of the antibiotic. Penicillin combination with β-lactamase inactivating compounds like clavulanic acid and tazobactam are found to be effective against some β-lactamase producing organisms; however, resistance to β-lactam- β-lactamase inactivating combination drugs are also observed routinely (Waltner-Toews *et al.,* 2011; Bonnet *et al.,* 2009, Perez-Llarena and Bou, 2009). In the present study, one pure penicillin (ampicillin) and two penicillin with β-lactamase inhibitors i.e. amoxiclav (amoxicillin + clavulanic acid) and piperacillin with tazobactam were evaluated.

Overall 74.2% of the isolates were resistant to penicillins. The resistance rate to penicillins among isolates from various geographical locations was significantly different ($p<0.05$) with 55.9% among isolates from Central India, 61.2% in Southern India, 77.6% in eastern India, 89.2% in Northern India, and 100% among isolates from western India. Overall 81.1% intestinal and 65.1% extra-intestinal isolates were resistant to the penicillin group of drugs, with a significant difference ($p<0.05$). The percentages of resistance to the penicillin group of antimicrobials in different geographical regions are shown in Figure 5.14. Individually, piperacillin-tazobactam was the most effective penicillin drug against intestinal as well as extra-intestinal isolates while the resistance rate to ampicillin and amoxiclav was higher (Figure 5.15). Resistance to the penicillin group of drugs has been reported in various studies in different parts of the world (Edwards *et al.,* 2020; Zhou *et al.,* 2019; Vranic and Uzunovic, 2016). The resistance rate in intestinal isolates was significantly more to ampicillin and amoxiclav in comparison to extra-intestinal isolates ($p<0.05$). However, no significant difference in resistance rate to piperacillin-tazobactam was found among the two groups of isolates ($p>0.05$). Resistance to penicillins and its combination with β-lactamase inactivating compounds is attributed to selective pressure due to over usage of these drugs over decades and to treatment strains resistant to other drugs (Vranic and Uzunovic, 2016; Lee *et al.,* 2013). Comparatively low resistance to piperacillin-tazobactam than amoxiclav and ampicillin may be attributed to the inactivation of beta-lactamase by tazobactam and comparatively less usage of piperacillin-tazobactam as empirical therapy in diarrheal and UTI infections in India.

A total of 24.3% of isolates were resistant to carbapenem antimicrobials. The resistance rate to the carbapenem class of antimicrobials among intestinal and extra-intestinal isolates was 23.8% and 25% respectively. The resistance rate to carbapenems was observed relatively low in isolates from Central (5.1%) and Southern India (10.6%) than from other parts of the country (Eastern India 29.1%, Northern India 36.5%, and Western India 69.6%) ($p<0.05$). Overall no difference in resistance rates to carbapenem among intestinal and extra-intestinal isolates was

observed ($p>0.05$) (Figure 5.16). The resistance rate to carbapenems is high in various studies in different regions of the country (Jaggi *et al.,* 2019; Mahalingam *et al.,* 2018) This shows that Carbapenem-resistant *E. coli* is prevalent throughout India, which is a major health concern and needs to be addressed urgently. Individually resistance rate to imipenem was 15.2% overall with almost equal rates among intestinal (15.2%) as well as extra-intestinal (15.1%) isolates ($p>0.05$). Similarly, no significant difference in resistance rate to meropenem among intestinal and extra-intestinal isolates (21.5 % and 19.8% respectively) was observed ($p>0.05$) and collectively 20.8% of the isolates were resistant to meropenem ((Figure 5.17).

Carbapenems are a last resort drug for ESBL-producing isolates because of their low resistance rates reported worldwide however, escalating resistance trends have been observed more so in Asian countries (Mahalingam et al.,2018; Laxminarayan and Chaudhary, 2016; Xu et al.,2015). Keeping in view the threat due to emerging carbapenems-resistant Enterobacteriaceae (CRE-*Klebsiella* and *E. coli*) this has been listed as an "Urgent threat" at the most critical level by CDC in its 2019 antibiotic resistance threat report (CDC, 2019). Resistance to carbapenems in this study is relatively high than reported earlier (Jaggi *et al.,* 2019; Ny *et al.* 2019; Abbas *et al.* 2017; Ali *et al.,* 2016; Niranjan and Malini, 2014). Increasing usage of carbapenems for the treatment of probable ESBL infections is usually considered the most likely reason for the development of resistance to these emergency drugs (Sheu *et al.,* 2019).

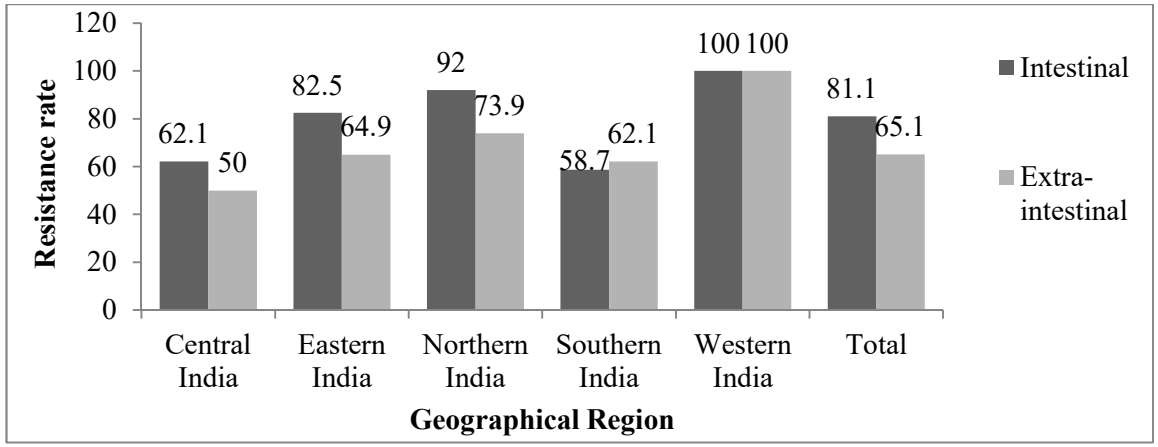


Figure 5.14: Region-wise resistance rates to the penicillin class of antimicrobials

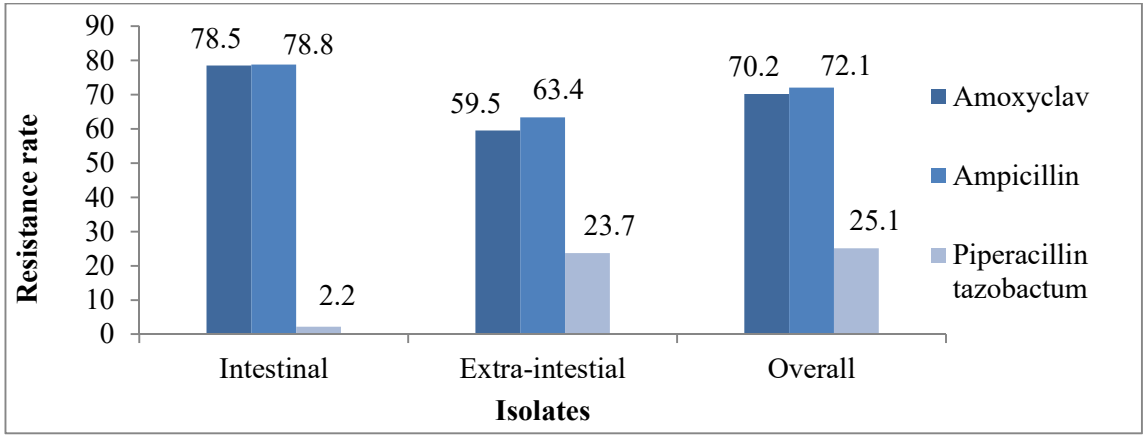


Figure 5.15: Resistance rates of *E. coli* isolates to individual penicillins

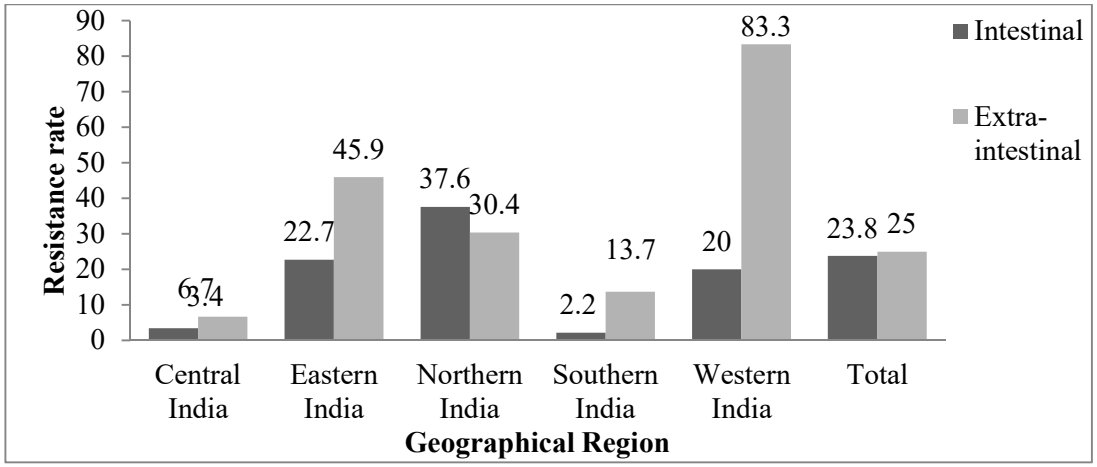


Figure 5.16: Region-wise resistance rates to carbapenems class of antimicrobials

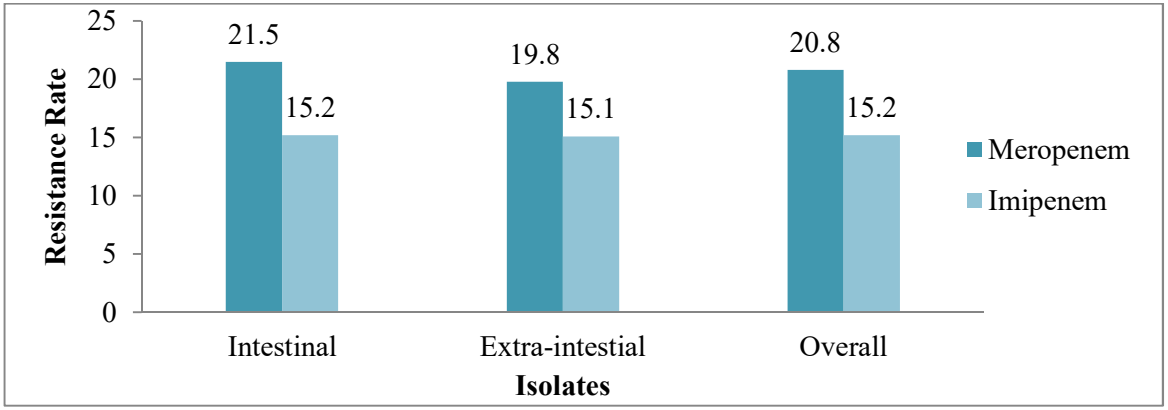


Figure 5.17: Resistance rates of *E. coli* isolates to individual carbapenems

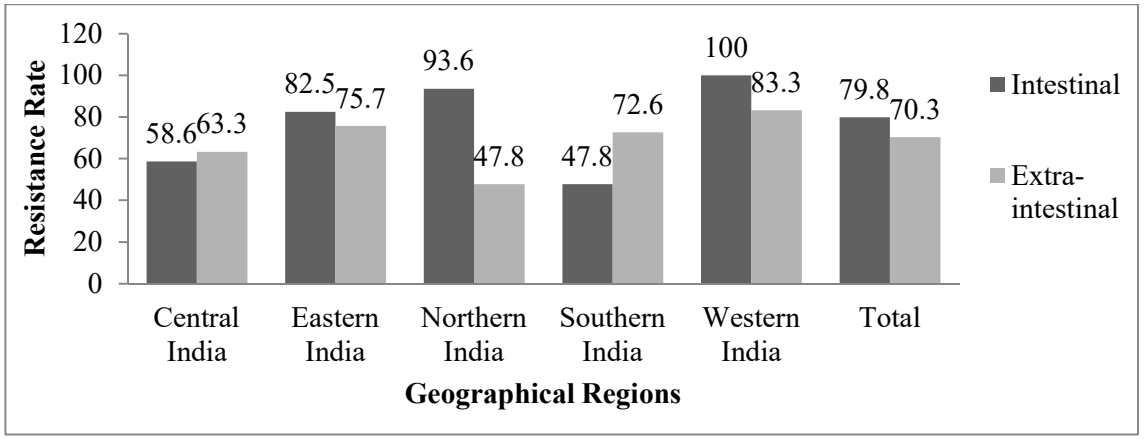


Figure 5.18: Region-wise resistance rates to the cephalosporin class of antimicrobials

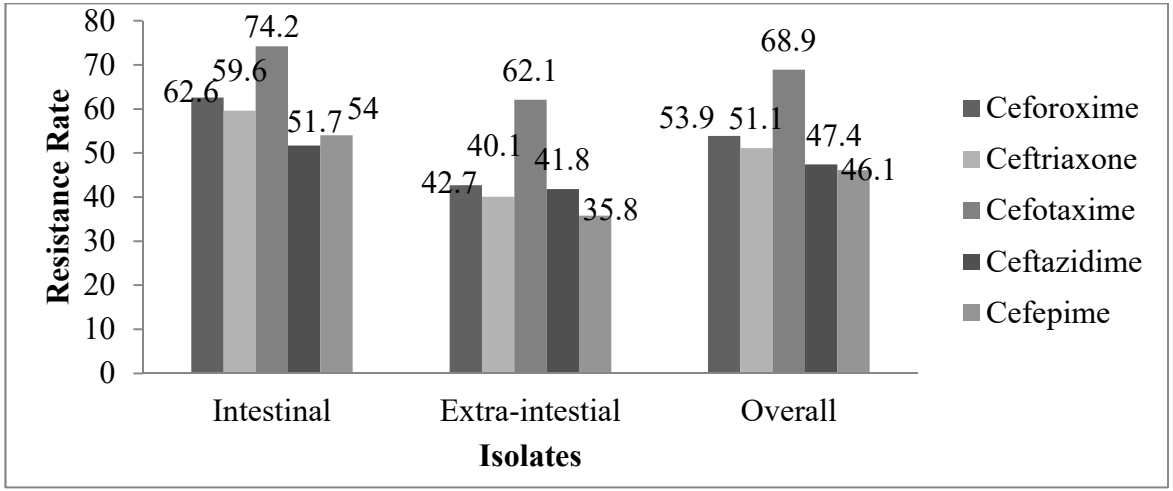


Figure 5.19: Resistance rates of *E. coli* isolates to individual cephalosporins

Resistance to cephalosporins in the *E. coli* isolates overall was 75.7%. It was significantly more among intestinal (79.8%) than extra-intestinal (70.3%) isolates ($p<0.05$). Individually, the resistance rate to drugs belonging to cephalosporins was maximum for cefotaxime (68.9%), followed by cefuroxime (53.9%), ceftriaxone (51.1%), ceftazidime (47.4%) and cefepime (46.1%). The resistance rate to all the individual drugs tested in this group was found to be significantly different ($p<0.05$) among intestinal and extra-intestinal isolates, with resistance rates being more among intestinal than extra-intestinal isolates. Resistance to cephalosporins was very high in *E. coli* isolates from all over the country i.e. 61% from Central India, 65.9% from Southern India, 80.6% from Eastern India, 86.5% from Northern India, and 87% from Western India ($p<0.05$) (Figure 5.18).

Resistance to different generations of cephalosporins among clinical *E. coli* isolates is reported routinely worldwide with rising trends (Lin *et al.,* 2019; Gashe *et al.,* 2018; Sudershan *et al.* 2014). Resistance to all generations of cephalosporins tested was observed in *E. coli* isolates with high rates in this study (Figure 5.19). A high resistance rate to two carbapenems and a fourth-generation cephalosporin (cefepime) evaluated in this study, was also observed (46.1%). Whereas carbapenems are a choice of drug to treat ESBL strains, cefepime is a reserved antimicrobial for the treatment of very severe infections with ESBL and carbapenemase-producing agents and treatment of patients with low white blood cell counts. Resistance to cefepime has also been reported among *E. coli* in the recent past (Isac *et al.,* 2021; Dona, 2019). Increasing incidences of resistance to these reserved drugs is a matter of serious concern as this not only results in persistent and recurrent infections; increasing hospital stays and associated costs of treatment but also poses a threat of severe infections by these pathogens, due to limited treatment choices available.

In the aminoglycoside group of drugs, three antimicrobials used were (amikacin, gentamicin, and kanamycin), collectively 39.3% of isolates (37.7% of the intestinal; 41.4% of the extra-intestinal) were resistant to one or more aminoglycosides. The resistance rate to aminoglycosides was more among extra-intestinal isolates than intestinal isolates ($p<0.05$). Different rates of resistance to the

aminoglycoside group of antimicrobials were observed among isolates from different geographical regions i.e. Central India-23.7%, Southern India- 33.5%, Eastern India-41%, Northern India 47.3%, and Southern India- 60.8% ($p<0.05$). Individually, amikacin was the most effective drug among intestinal as well as extra-intestinal isolates with a minimum drug resistance rate among the aminoglycoside group of drugs. While the resistance rate to Kanamycin and gentamicin was relatively more (Figure 5.20-5.21). The difference in resistance rates to kanamycin and amikacin was insignificant among the intestinal and extra-intestinal groups of isolates ($p>0.05$). On the other hand difference in resistance rate to gentamicin was significant among the two groups ($p<0.05$). Amikacin has also been found the most effective drug among various aminoglycosides in many other studies (Fernandez-Martinez et al, 2018; Lindemann *et al.,* 2012; Gad *et al.,* 2011). Resistance to aminoglycosides has been reported among clinical *E. coli* from India in various other studies with the least resistance to amikacin (Ranjini *et al.,* 2015; Ray *et al.,* 2015; Shahid *et al.,* 2008). Better sensitivity of amikacin than other antibiotics i.e. gentamicin, kanamycin, etc. is mainly attributed to the fact that the primary resistance mechanism against aminoglycosides is through enzymatic modification by aminoglycosides-modifying enzymes (AMEs) and akimacin being a semisynthetic aminoglycoside have limited susceptibility to these enzymes. However, now amikacin-resistant gram-negative organisms are known which are acquired through acetylation by aminoglycoside 6`-N-acetyltransferase type 1b [AAC(6`)-1b] enzymes (Ramirez and Tolmasky, 2017).

Quinolones are used as empirical therapy against diarrheal as well as UTI infections and the emergence of drug resistance to these drugs is widely reported (Recacha *et al.,* 2019). Increasing trends in quinolone-resistant *E. coli* infections have been observed worldwide which is closely associated with the spread of the ST131-SH30 clone of *E. coli* (Spellberg and Doi, 2015). The overall resistance rate to quinolones in this study was 71.7% with high resistance rates among intestinal (76.2%) as well as extra-intestinal (65.9%) isolates which were more among intestinal isolates in comparison to extra-intestinal isolates ($p<0.05$) (Figure 5.22). A resistance rate to quinolones was observed with very high rates among isolates from Western (100%), Northern (83.8%), and Eastern India (75.4%) in comparison to isolates from

Central (50.8%) and Southern India (61,8%) ($p<0.05$). *E. coli* with high resistance rates to quinolones have earlier been observed in various geographical locations of the country (Singh *et al.,* 2019; Banukumar et al, 2017; Arundhati *et al.,* 2016; Rath and Padhy, 2015). Individually, resistance to nalidixic acid was very high followed by norfloxacin and ciprofloxacin (Figure 5.23). Intestinal isolates were more resistant to all three quinolones in comparison to extra-intestinal isolates ($p<0.05$). Higher resistance to nalidixic acid in comparison to other quinolones has also been observed in other studies (Malekzadegan *et al.,* 2018; Mukherjee *et al.,* 2013).

In the sulphonamide class, trimethoprim and cotrimoxazole (trimethoprim + sulfamethoxazole) were evaluated. Out of 281 trimethoprim-resistant isolates, 11.7% were found sensitive to combination drugs (cotrimoxazole). Among 253 trimethoprim-sensitive isolates, 2.8% were resistant to cotrimoxazole (Table 5.31). The difference in the resistance pattern of trimethoprim and cotrimoxazole was found to be significant ($p<0.05$). Sulfonamides-resistant strains were detected in large percentages of isolates from all geographical locations of the country [Central India-35.6%, Southern India-40%, Eastern India-64.2%, Northern India- 65.5%, and Western India-69.6%), with a rate of 53.9% of the total study samples ($p<0.05$)] (Figures 5.24 and 5.25). The percentage of resistant isolates was more among intestinal (60.9%) than the extra-intestinal (44.8%) isolates ($p<0.05$). Sulfonamides-resistant *E. coli* has also been reported in India by other workers (Natrajan *et al.,* 2018; Singha *et al.,* 2015).

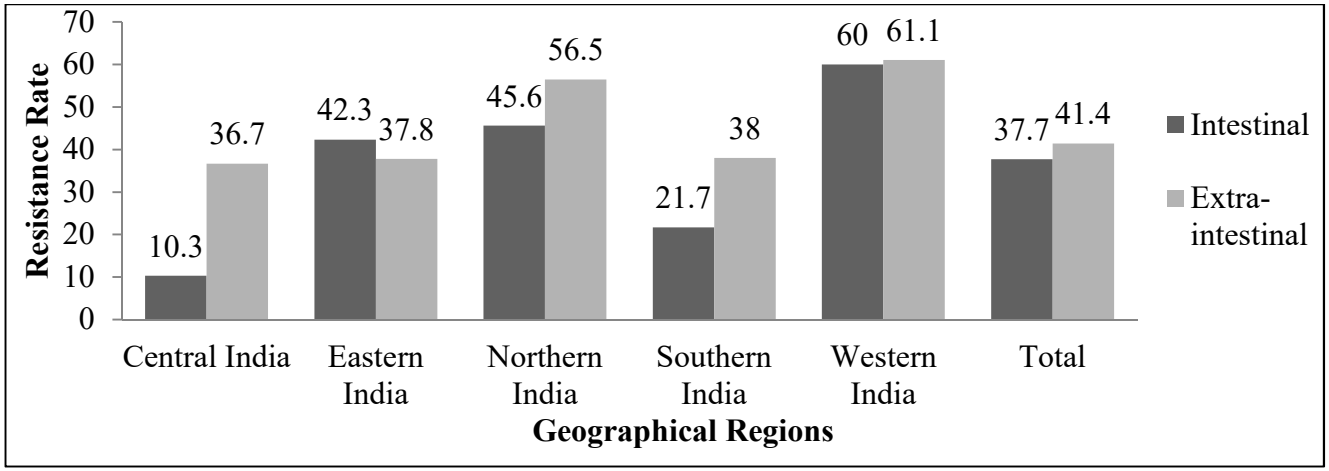


Figure 5.20: Region-wise resistance rates to aminoglycoside class of antimicrobials

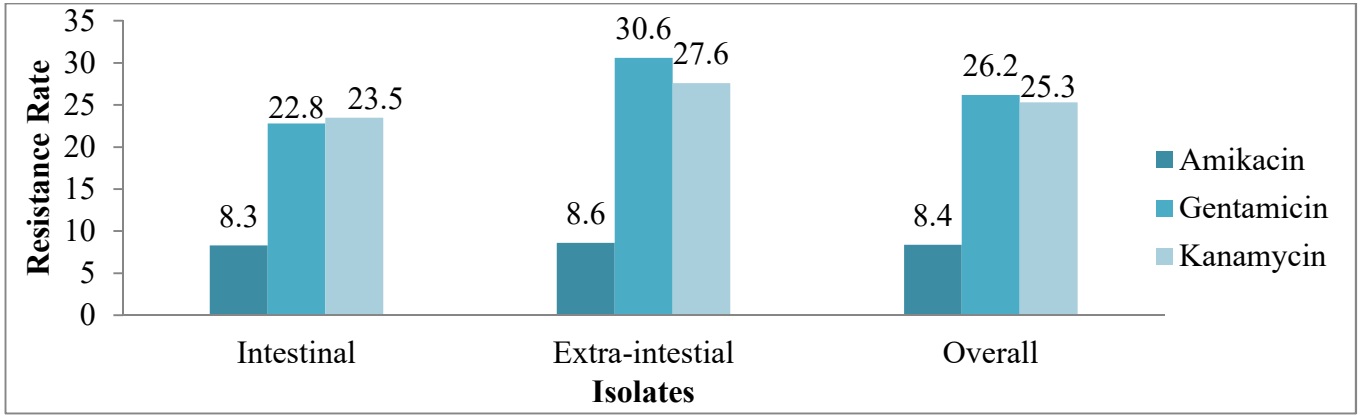


Figure 5.21: Resistance rates of *E. coli* isolates to individual aminoglycosides

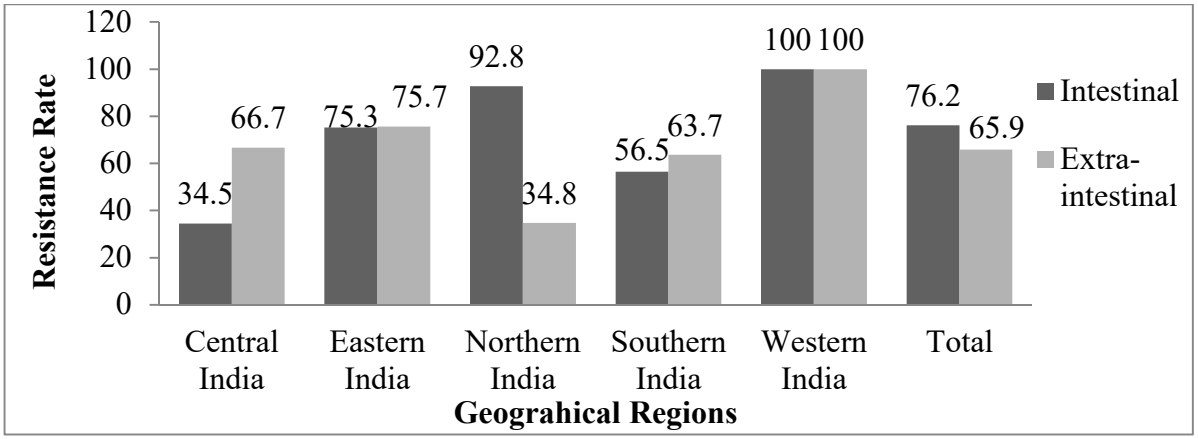


Figure 5.22: Region-wise resistance rates to quinolones class of antimicrobials

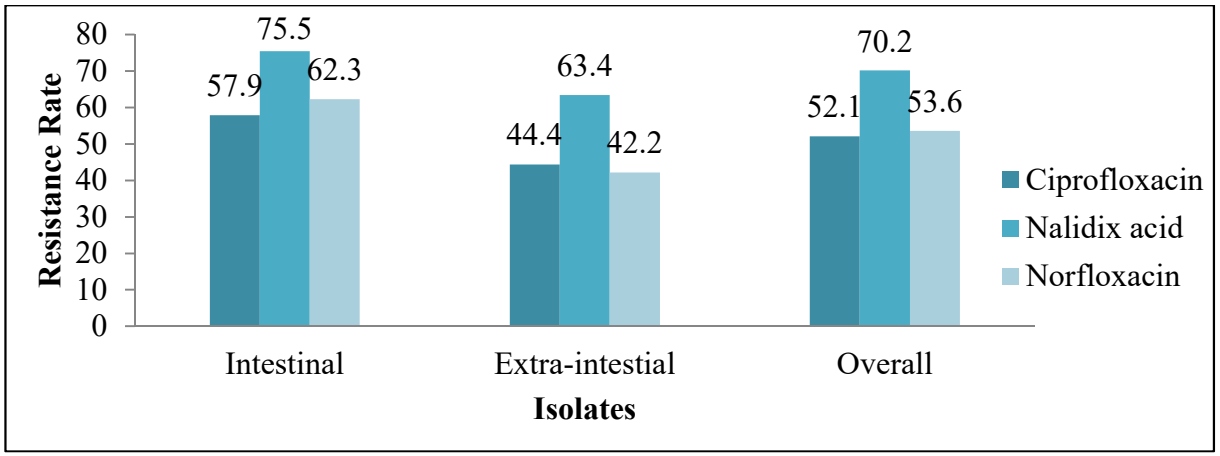


Figure 5.23: Resistance rates of *E. coli* isolates to individual quinolones

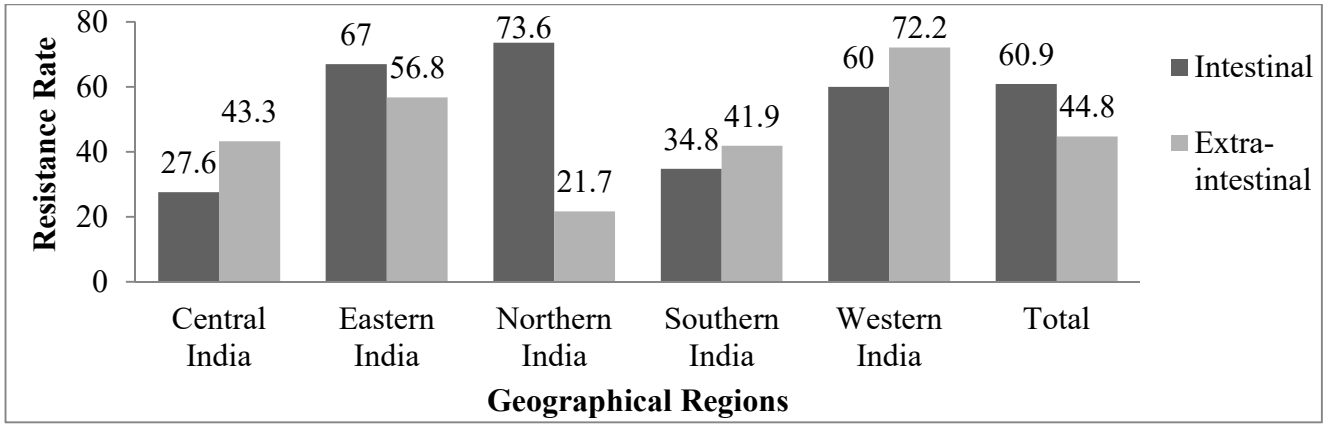


Figure 5.24: Region-wise resistance rates to sulfonamides class of antimicrobials

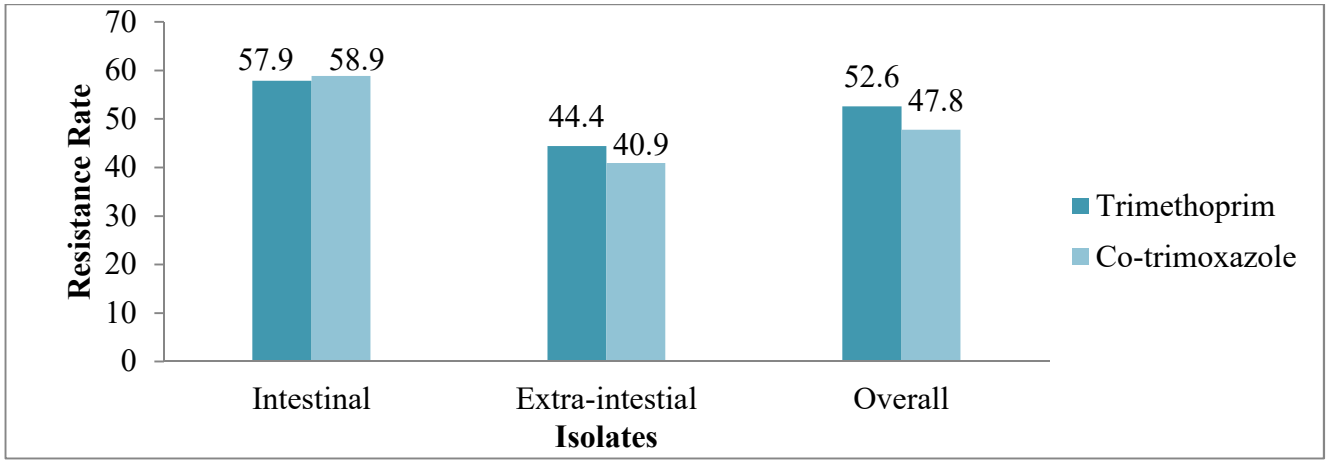


Figure 5.25: Resistance rates of *E. coli* isolates to individual sulfonamides

Table 5.31: Resistance pattern of cotrimoxazole to trimethoprim-resistant and sensitive isolates

Trimethoprim resistance pattern		**Susceptibility to Cotrimoxazole**		**Total**
		Resistant	**Sensitive**	
Resistant	Intestinal	154 (86.5)	24 (13.5)	178
	Extra-intestinal	94 (91.3)	9 (8.7)	103
	Total	248 (88.3)	33 (11.7)	281
Sensitive	Intestinal	6 (4.8)	118 (95.2)	124
	Extra-intestinal	1 (0.8)	128 (99.2)	129
	Total	7 (2.8)	246 (97.2)	253
Total	Intestinal	160 (53.0)	142 (47.0)	302
	Extra-intestinal	95 (40.9)	137 (59.1)	232
	Total	255 (47.8)	279 (52.2)	534

Trimethoprim and sulfamethoxazole-resistant isolates are sensitive to cotrimoxazole (trimethoprim+sulfonamide) due to the synergetic effect of the two drugs (Minato *et al.,* 2018). Sensitivity to cotrimoxazole among trimethoprim-resistant strains in intestinal and extra-intestinal isolates could be observed which may either be due to sensitivity to sulfonamide or the synergetic effect of the combination drug. On the other hand, some of the trimethoprim-sensitive isolates were also found to be resistant to combination drugs. Similar observations were also made by Amyes and Brunton (1981).

Among phenicols chloramphenicol was evaluated. A very large percentage (88.2%) of isolates was found to be sensitive to chloramphenicol. The resistance rate to chloramphenicol in all geographical regions was low [Central India-3.4%, Southern India 8.8%, Eastern India 12.7%, Northern India-16.9%, and Western India-17.4% ($p<0.05$)]. The resistance rate among intestinal and extra-intestinal isolates to chloramphenicol in different regions and resistance to chloramphenicol overall is shown in figures 5.26 and 5.27 respectively. Resistance rates were found to be low among both intestinal (12.6%) as well as extra-intestinal (10.8%) overall isolates. ($p>0.05$). Chloramphenicol resistance became evident a few decades after its use among clinical isolates and later various studies have shown reversal trends of

chloramphenicol sensitivity. This could be due to low selection pressure for chloramphenicol resistance as extensive use of chloramphenicol was banned due to toxicity associated with the usage of this drug (Eliakim-Raz *et al.,* 2015). High sensitivity rates to chloramphenicol during the recent decade indicate promising therapy for infections with chloramphenicol, however, its usage is limited because of its toxic characteristics which have led to more research on synthesizing newer chloramphenicol derivatives with better pharmacological properties (Tevyashova, 2021).

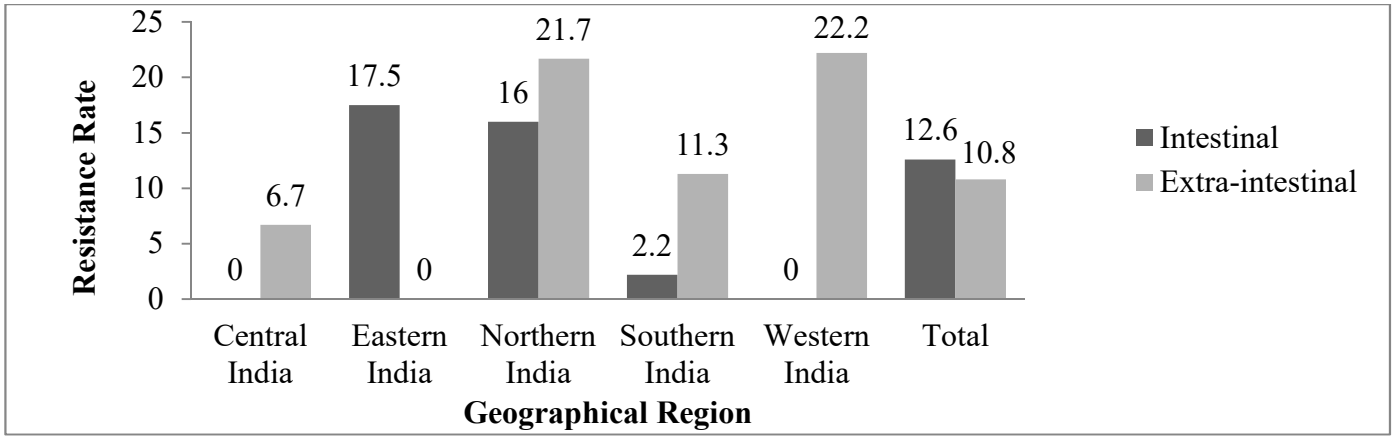


Figure 5.26: Region-wise resistance rates to chloramphenicol (phenicol class of antimicrobial)

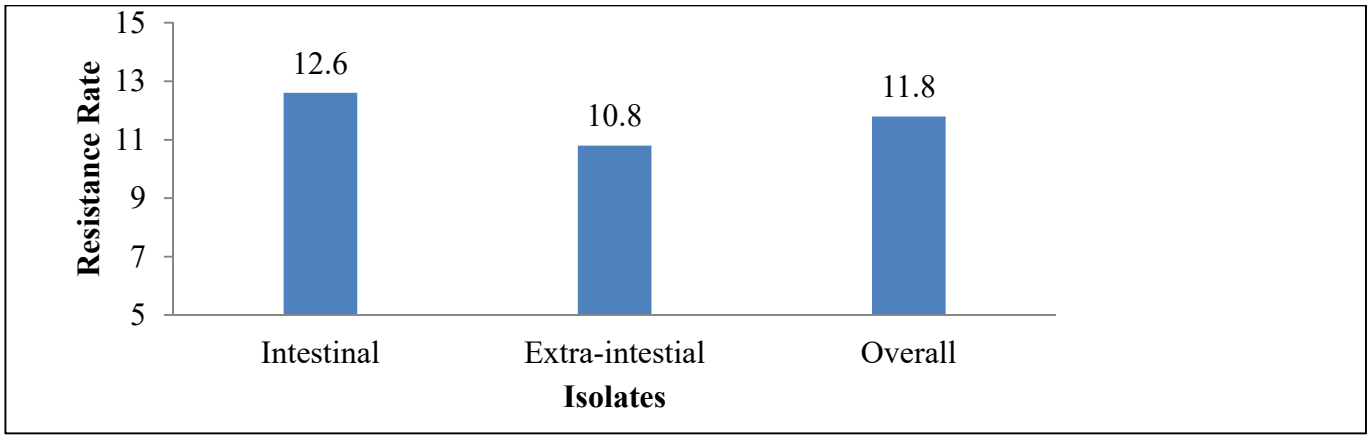


Figure 5.27: Resistance rates of *E. coli* isolates to chloramphenicol

Nitrofurantoin was found to be the second most sensitive drug after amikacin among the twenty antimicrobials evaluated in the present study. Overall least resistance of 10.9% was observed to this drug. Resistance among intestinal and extra-intestinal isolates was 10.6% and 11.2% respectively ($p>0.05$). The resistance rate to nitrofurantoin was found low among intestinal as well as extra-intestinal isolates from all the geographical locations of the country ($p<0.05$) (Figures 5.28 and 5.29).

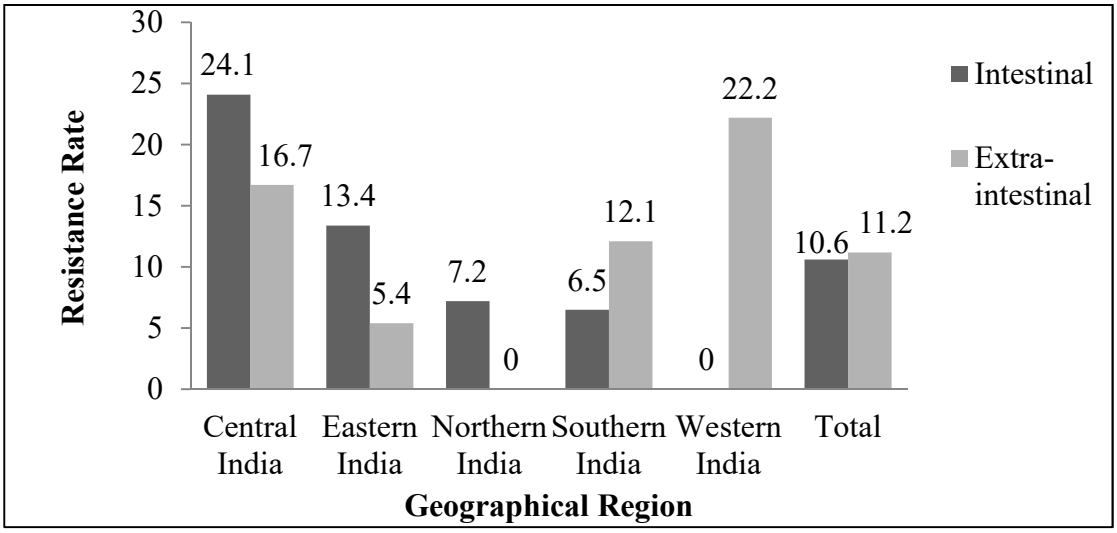


Figure 5.28: Region-wise resistance rates to nitrofurantoin (furantoin class of antimicrobial)

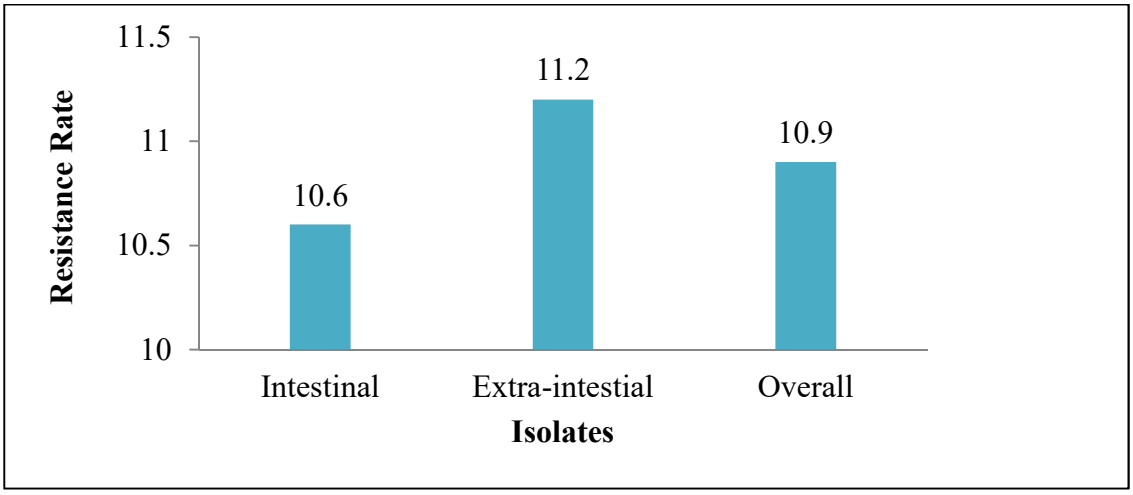


Figure 5.29: Resistance rates of *E. coli* isolates to nitrofurantoin

Nitrofurantoin is one of the important drugs to treat UTI as it is excreted in urine unaltered in very high concentrations making it easy to availability of the drug in the urinary tract. A low resistance rate to nitrofurantoin among *E. coli* is reported worldwide and is thus considered a promising antibiotic against MDR *E. coli* strains (Lee *et al.,* 2018; Sanchez *et al.,* 2014).

5.5.1. **ESBLProduction**

CDC has listed ESBL-producing Enterobacteriaceae among the "serious threats" in its latest antibiotic resistance threats report (CDC, 2019). ESBL-producing *E. coli* strains are becoming a global threat as these strains are resistant to different classes of β-lactam drugs including third and fourth-generation cephalosporins (Vachvanichsanong *et al.,* 2021; Day *et al.,* 2019; Falgenhauer *et al.,* 2019). All 534 isolates were tested for ESBL production using a confirmatory disc diffusion assay using cefotaxime and ceftazidime alone and with clavulanic acid. ESBL using cefotaxime and cefotaxime-clavulanic acid discs was detected in 198 (37.1%) isolates, while it could be detected in only 188 (35.2%) of the isolates using ceftazidime and ceftazidime-clavulanic acid discs. Isolates were considered ESBL producers when ESBL was detected in either of the drug combination discs. ESBL in the present study was detected in 199 (37.1%) of the isolates with very large numbers in both intestinal (120; 39.7%) and extra-intestinal (79; 34.1%) isolates ($p>0.05$) (Table 5.32).

ESBL-producing *E. coli* were detected among isolates from all geographical regions with a range varying from 28.2% in southern India to 60.9% in western India ($p<0.05$) (Table 5.33). Varying rates of ESBL-producing *E. coli* have been reported from different geographical locations in India in different earlier studies (Gautam *et al.,* 2019; Ravikant *et al.,* 2016; Varkey *et al.,* 2014; Sharma *et al.,* 2013; Goyal *et al.,* 2009; Aggarwal *et al.,* 2008).

Table 5.32: Comparison of ESBL detection rates using cefotaxime and ceftazidime.

The drug used to detect ESBL production	**ESBL rate detected n (%)**		
	Intestinal isolates n= 302	**Extra-intestinal isolates; n=232**	**Total N = 534**
Cefotaxime/cefotaxime-clavulanic acid	119 (39.4)	79 (34.1)	198 (37.1)
Ceftazidime/ ceftazidime-clavulanic acid	115 (38.1)	73 (31.5)	188 (35.2)
Overall ESBL producers	120 (39.7)	79 (34.1)	199 (37.3)

Table 5.33: Extended-spectrum β-lactamase activity in *E. coli* isolates

Geographical location	**ESBL rate detected N (%)**		
	Intestinal isolates	**Extra-intestinal isolates**	**Overall**
Central India	5 (17.2)	13 (43.3)	18 (30.5)
Eastern India	34 (35.1)	16 (43.2)	50 (37.3)
Northern India	67 (53.6)	2 (8.7)	69 (46.6)
Southern India	10 (21.7)	38 (30.6)	48 (28.2)
Western India	4 (80.0)	10 (55.6)	14 (60.9)
Total	120 (39.7)	79 (34.1)	199 (37.3)

Among the total 199 ESBL-producing isolates, 193 were MDR. Among ESBL producers 74 were also resistant to carbapenems while 125 were sensitive to carbapenems. Out of the total 534 isolates, a large number of isolates (73/534; 13.6%) were found to be having all three resistance characteristics i.e. MDR, ESBL

production, and carbapenem resistance. These highly resistant *E. coli* were detected among intestinal as well as extra-intestinal isolates and from all geographical locations. Virulence factors, serogroup, and DEC pathotypes (among intestinal isolates) associated with these highly drug-resistant strains are shown in figure 5.30. No significant correlation between ESBL production and carbapenem resistance could be established ($p>0.05$), as 125 ESBL producers but carbapenems sensitive isolates 120 were also found to be MDR (Table 5.34).

Carbapenems are used as a last resort drug for the treatment of ESBL-producing bacteria including ESBL-producing *E. coli* (Vardakas *et al.,* 2012). Resistance to carbapenems in ESBL-producing MDR (73/534; 13.6%) isolates observed in this study is thus a matter of concern. ESBL-producing MDR CRE is reported worldwide including in countries where carbapenems are not even prescribed i.e. Ethiopia, which is a serious concern regarding its spread (Tshitshi *et al.,* 2020; Legese *et al.,* 2017; Hara *et al.,* 2013; Amjad *et al.,* 2011).

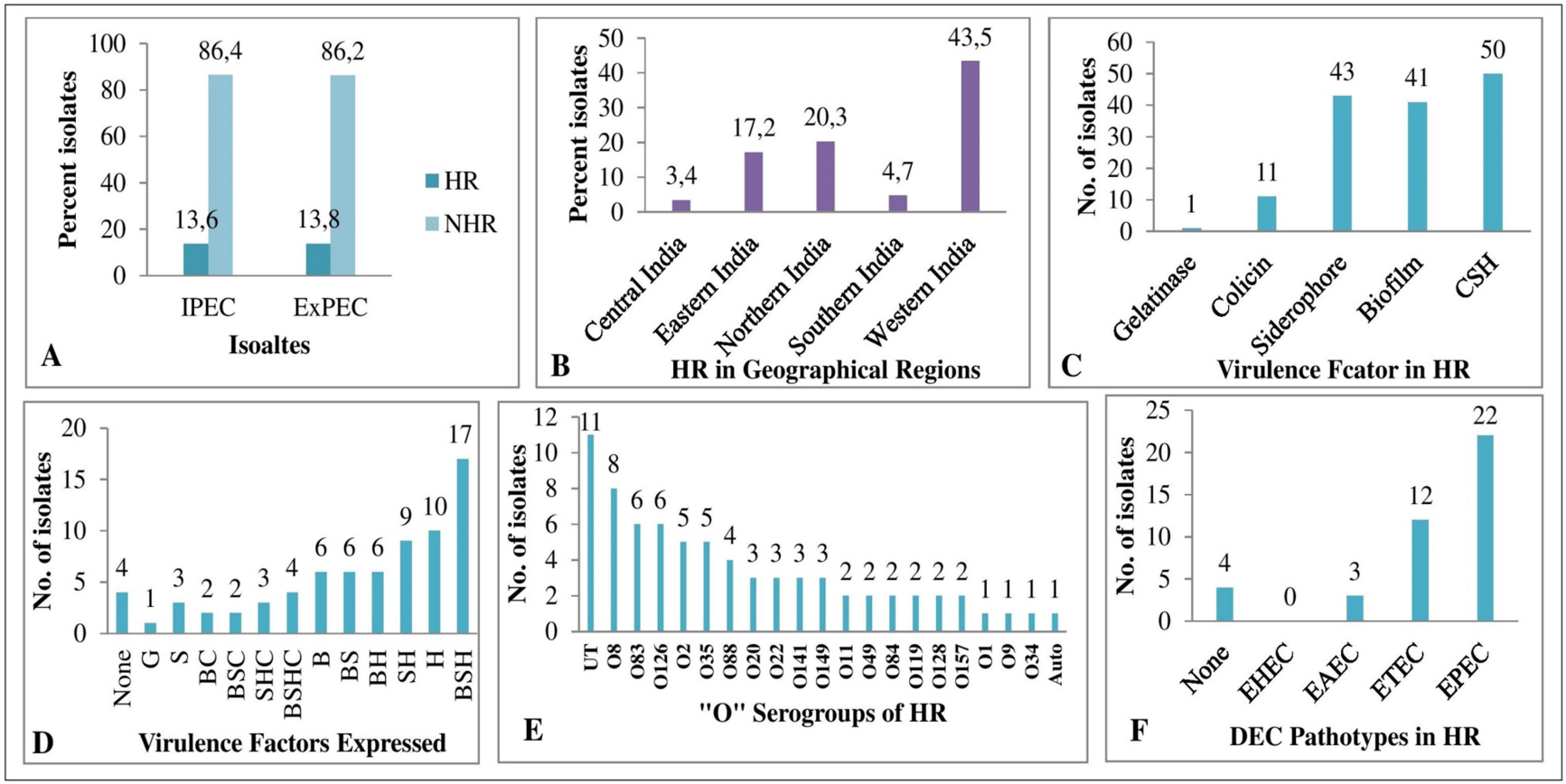


Figure 5.30: Highly drug-resistant isolates - A: Percentage in IPEC and ExPEC isolates; B: Frequency of Virulence factors expressed; C: Region-wise percentage; D: Combination of virulence factors expressed; E: Associated serogroups and F: Associated DEC pathotypes Here: HR- Highly resistant strains, NHR- Non-highly resistant strains, G-gelatinase, B- biofilm, S-siderophore, H-CSH, C- Colicin; UT-untypeable, Auto-agglutinating.

Table 5.34: Relationship between ESBL production, MDR, and carbapenem resistance

ESBL producers	**Carbapenem resistance N**		**Resistance to multiple drug classes**	
			MDR	**Non-MDR**
N= 199	Resistant - 74	Intestinal - 41	41	0
		Extra-intestinal - 33	32	1
		Total - **74**	**73**	**1**
	Sensitive - 125	Intestinal - 79	77	2
		Extra-intestinal - 46	43	3
		Total - 125	**120**	**5**
	Overall among ESBL producers	Overall - 199	**193**	**6**

In medicine, biofilm plays an important role because of its association with drug resistance and recurrent infections. *E. coli* biofilms have strongly and positively been found to be correlated to multiple drug resistance in different studies (Karigoudar *et al.,* 2019; Pavlickova *et al.* 2017). A large number of biofilm-producing isolates in this study were also found to be MDR, ESBL-producing, and carbapenem-resistant, however no statistically significant difference between biofilm producers and biofilm non-producers was observed in resistance rates to these drug resistance characteristics ($p>0.05$) as MDR, ESBL producers, and carbapenem resistance isolates were also found to be in very large numbers among biofilm non-producing isolates (Table 5.35). Though biofilm does increase drug resistance, however other factors like mutation, enzyme production (β-lactamase and extended-spectrum β-lactamase), etc. seem to be more critical drug resistance mechanisms among clinical *E. coli*.

Table 5.35: Biofilm and MDR, ESBL producers, and carbapenem resistance

Biofilm characteristic	MDR n (%)		ESBL n (%)		Carbapenem Resistance n (%)	
	MDR	Non-MDR	Positive	Negative	Resistant	Sensitive
Biofilm forming - 298	96 (32.2)	202 (67.8)	112 (37.6)	186 (62.4)	65 (21.8)	233 (78.2)
Biofilm non-forming - 236	61 (25.8)	175 (74.2)	87 (36.9)	149 (63.1)	65 (27.5)	171 (72.5)

E. coli is the common pathogen associated with hospital-acquired as well as community-associated infections. The prevalence of higher rates of drug resistance to commonly used antimicrobials i.e. beta-lactams including cephalosporins, the newer generation of cephalosporins, and emergency drugs i.e. Cefepime and carbapenems with resistance to multiple drugs is a matter of immediate concern. Two common antimicrobial resistance containment strategies i.e. rapid detection with quick containment responses to resistant cases and screening and use of transmission-based precautions seem to be the best available options in this aspect. The former is effective in low-prevalence areas, while the latter can be employed in higher-prevalence areas like India (Rizzo *et al.,* 2019; Woodworth *et al.,* 2018). Keeping in view the importance of MDR *E. coli* infection in humans and animals and its spread through various sources i.e. food and water "one health" approach suggested by WHO for containment of antimicrobial resistance is critical in reference to MDR *E. coli* infections (McEwen and Collignon, 2018). The government of India has also stressed regular surveillance of microbes including *E. coli* with rapidly increasing resistance rates through the formulation of India's antimicrobial resistance action plan emphasizing on rational use of antimicrobials and strengthening of the surveillance network (National Action Plan on Antimicrobial resistance India, 2017). Antimicrobial resistance is triggered by excessive usage of antimicrobials and hospital-acquired resistance is associated with hospitals' antimicrobial regimes, thus

requiring the implementation of stewardship programs strictly at healthcare levels (Cusini *et al.,* 2018).

Containment of infections requires a good understanding of the prevalence of the pathogen, its transmission and pathogenesis mechanisms, and local resistance patterns through extensive surveillance programs for effective interventions and the selection of appropriate treatment options (Ammenti *et al.,* 2020; Gajdacs *et al.,* 2019). The present study attempted to highlight the prevalence of *E. coli*, different DEC pathotypes, common colonization virulence factors, and drug resistance patterns in different parts of the country. Keeping in view the importance of *E. coli* infections in animals, and humans and its wide occurrence in the environment i.e. food and water sources and the spread of MDR *E. coli* among various sources, more such studies are required on regular basis for better understanding and generate up to date databases for better control and management of this infectious agent and reduce morbidity, mortality and treatment costs associated with it.

CHAPTER-6

SUMMARY

&

CONCLUSION

6. SUMMARY AND CONCLUSION

Escherichia coli is the most common etiological agent of urinary tract infection (UTI) worldwide and accounts for more than 80% of UTIs. It is also one of the commonest etiological agents of diarrhea and can result in diarrhea of variable severity and may also lead to very severe systemic infections like septicemia, neonatal meningitis, hemolytic uremic syndrome (HUS), and hemorrhagic colitis (HC). Though *Escherichia coli* outbreaks are very common worldwide, developing nations including India are the most affected due to poor socioeconomic conditions, poor hygiene, thick populations, and inadequate healthcare infrastructure. These factors in developing countries not only contribute to high *E. coli* infection rates but also aid in the rapid spread of antimicrobial-resistant pathogenic strains making treatment and management of *E. coli* infections a very challenging job. Due to the emergence of MDR *E. coli* strains with resistance to even newer and emergency antimicrobial agents i.e. carbapenems and colistin, WHO and CDC have listed CRE including *E. coli* among “Critical Pathogens” and “Serious Threat” pathogens respectively in their list of priority pathogens of public health that require serious urgent attention for containment strategies and development of newer antimicrobials against them.

The prevalence and characterization of *E. coli* isolate are of prime importance for better understanding and better planning of control and management strategies for containing this common infectious agent. Such studies generate important epidemiological data regarding prevalent serotypes, prevalent pathotypes, most important virulence mechanisms and drug resistance profiles, etc. which serves as important factors in designing containment and control strategies.

E. coli clinical isolates from different geographical regions of India were characterized in the present study. To incorporate clinical *E. coli* isolates from all geographical regions of the country, the study samples were taken from National Salmonella and Escherichia Center (NSEC) a reference laboratory in India, which receives *Salmonella* and *E. coli* isolates from all parts of the country. In this study, a

total of 783 suspected *E. coli* isolates that were received at National Salmonella and Escherichia Center (NSEC) during the study period were taken for the study. All the samples were first revived by sub culturing on MacConkey agar and nutrient broth then the stock culture of all revived samples was taken on nutrient agar slants. All viable isolates were characterized by biotyping for confirmation as *E. coli.* Isolates identified as *E. coli* on biotyping were then further characterized by serotyping, virulence characteristics, and antimicrobial profiles. All intestinal isolates were also characterized genotypically to identify prevalent diarrhea-genic pathotypes.

6.1. **Biotyping**

Cultural characteristics of the test isolates were examined on Nutrient agar, MacConkey agar, and nutrient broth media. The morphology of the isolates was examined microscopically after Gram staining. Study isolates were identified using standard microbiological techniques using a set of biochemical tests. Isolates that had morphological and biochemical profiles consistent with *E. coli* (i.e. Gram-negative rods, IMViC: +ve +ve, -ve, -ve, Glucose: AG, Lactose: A, TSI: A/A with or without gas, ONPG: +ve, NR: +ve, Urease: -ve, Catalase: +ve, Oxidase: -ve) were designated as confirmed *E. coli* isolates. A total of 534 isolates out of 783 taken for the study were confirmed as *E. coli.* Geographically the distribution of the confirmed *E. coli* isolates was not uniform but representative samples were obtained from all geographical regions. These 534 confirmed *E. coli* isolates were from different regions of India (Northern India: 170 isolates, Eastern India: 134 isolates, Central India: 59 isolates, Western India: 23 isolates, and Southern India: 170 isolates). These 534 isolates consisted of both intestinal as well as extra-intestinal isolates i.e. 302 were intestinal isolates from human diarrhea (DEC) and the rest 232 were extra-intestinal isolates from human UTI (UPEC).

All 534 confirmed clinical *E. coli* isolates in the present study were further characterized to see the prevalence of different epidemiological, virulence, and resistance characteristics in different geographical locations of the country.

6.2 **Serotyping**

E. coli were typed using specific anti "O" *E. coli* antisera to evaluate the prevalent "O" serogroups and geographical variability of "O" serogroups if any among study isolates from different parts of the country. *E. coli* isolates in the present study were found to be diverse in terms of "O" serogroups as these strains were clustered over 36 different "O" serogroups including 96 isolates that could not be typed using the serotyping and were designated as UT (untypeable). The ten most frequent "O" serogroups detected were O8 (46 isolates; 8.6%), O11 (36 isolates; 6.7%), O22 (33 isolates; 6.2%), O88 (33 isolates; 6.2%), O126 (28 isolates; 5.2%), O83 (25 isolates; 4.7%), O35 (25 isolates; 4.7%), O141 (23 isolates; 4.3%), O149 (22 isolates; 4.1%) and O7 (21 isolates; 3.9%). In the present study serogroup O157 which is associated with the most severe type of *E. coli* infection that leads to bloody diarrhea and HUC was detected in only 7 (1.3%) isolates (6 intestinal and 1 extra-intestinal). Serotypes frequently isolated during *E. coli* outbreaks in developed countries including i.e., O104, O145, O121, O111, O103, O45, and O26, and those described under severe infections associated categories of the five STEC seropathotypes A to E described by Karmali *et al.,* were not detected in this study except serotype O157 and O5 placed with very insignificant percentage i.e. 1.3% and 0.2% respectively. While few serogroups associated with class D (low incidence rate, rarely involved in outbreaks, not associated with HUS/HC) were detected i.e. O7; 21/534 (3.9%), O119; 10/534 (1.9%), O117;1/534 (0.2%). "O" serogroups among *E. coli* isolates from different regions were not uniform. The majority of serogroups were detected in both intestinal as well as extra-intestinal isolates except O4, O89, and O117 which were detected only in extra-intestinal isolates, and O5, O84, O86, O98, O114, O126, O128, and auto-agglutinating which were detected only in intestinal isolates. Some of the "O" serogroups were found to be associated with its source i.e. intestinal: O2, O22, O119, O149, O157, or extra-intestinal: O7, O11, O35.

6.3. **Pathotypes**

In the present study intestinal isolates from various geographical regions of India were characterized using multiplex PCR targeting virulence genes of four prevalent DEC [genes: eae, bfpA (for EPEC), est, elt (for ETEC), hlyA (for EHEC), and CVD432 (for EAEC). DEC virulence genes targeted in the present study could be detected in 297 (98.3%) out of 302 confirmed intestinal *E. coli* isolates. The most prevalent DEC pathotypes in the study samples were enterotoxigenic *E. coli* (ETEC) and enteropathogenic *Escherichia coli* (EPEC) with the frequency of 137/302; 45.4% and 126/302 i.e. 41.7% respectively. Among the EPEC isolates atypical EPEC strains (eae gene only-34.8%) were found to be more prevalent than the typical EPEC strains (both eae and bfpA genes) throughout the country. Among ETEC ST strains (heat-stable ETEC-est gene-36.4%) were found to be more prevalent than LT strains (heat-labile ETEC-elt gene) in the present study. In this study all ETEC strains were found to possess marker genes either for heat-labile (elt) or heat-stable (est) enterotoxin, no isolate was expressing both genes simultaneously. None of the pathotypes were found uniformly distributed throughout the country.

EHEC pathotype was found to be very scanty in this study as the hlyA gene could be detected in very few (9 out of 302; 3 % only) study samples. The marker gene for hemorrhagic *E. coli* (hlyA) was detected only among isolates from eastern and northern India with very low frequencies.

All isolates possessing the hlyA gene were non-O157 serotypes in the present study but did not belong to any serious seropathotype category.

The virulence gene marker for EAEC (CVD432) was also detected in the present study only in 8.3% of the isolates (25/302).

No hybrid pathotype was detected in the present study. No significant association between pathotype and serotype was found in the present study.

6.4. **Cell surface hydrophobicity**

In the present study Salt aggregation test (SAT) was used to study the hydrophobicity of the pathogenic *E. coli* under evaluation. In this study, this virulence characteristic among study samples with a 69.1% positivity rate was detected. More percentage of intestinal isolates (75.2%) were found to be hydrophobic in comparison to extra-intestinal isolates (61.2%). Cell surface hydrophobicity characteristic was found in large percentages among both intestinal as well as extra-intestinal isolates irrespective of geographical location.

A wide range of "O" serogroups were found to be associated with intestinal and extra-intestinal hydrophobic isolates. No preference among different serotypes for cell surface hydrophobicity was observed among intestinal as well as extra-intestinal isolates. All six O157 intestinal isolates were found to be hydrophobic. Cell surface hydrophobicity was detected in a very high percentage in *E. coli* isolates irrespective of the seasons.

6.5 **Biofilm**

To detect a biofilm-forming property of the isolates standard tissue culture plate technique was used and two simple techniques i.e. the tube method and congo red agar were also performed to evaluate these techniques against the standard TCP method. A large percentage (53.6% to 59%) of isolates were found to form biofilm as detected using three different methods. Irrespective of the geographical regions from where the samples were isolated biofilm-forming characteristic was observed in the majority of *E. coli* isolates among intestinal as well as extra-intestinal isolates. TCP and TM methods detected biofilms in more of the extra-intestinal isolates than intestinal isolates. The strength of biofilm detected in the majority of the biofilm-forming isolates was moderate. *E. coli* isolates were found to form biofilms irrespective of serogroup. Isolates of serotype O157 were also found to form moderate to strong biofilms.

Large percentages of isolates from summer (70.1-71.1%), monsoon (49.7-58.4%), and post-monsoon (36-84%) were found to produce biofilms in comparison to isolates from winter (48%-55.9%). This seasonal trend was also observed among intestinal isolates however among extra-intestinal *E. coli* isolates biofilm was detected in a very high (77.8%-94.4%) percentage of isolates in the winter season also.

TM and CRA methods were found to be having good test characteristics i.e. accuracy, sensitivity and specificity, and predictive values, especially the tube method which was found more accurate (70.79) and specific (68.07) than CRA (64.98 and 56.78 respectively) and having better positive and negative predictive values (73.61 and 65.85 respectively).

6.6. **Siderophores**

In this study, the prevalence of this virulence factor and its type among *E. coli* isolates was also evaluated. Initially, siderophore screening was carried out by Chrome azurol S (CAS) agar culture and then by chemical characterization to detect the two most widely associated siderophores with pathogenic *E. coli* viz. hydroxamate and catechol-types by Arnow's and Csaky assays respectively.

Culture on CAS agar could detect siderophores in 45.1% of the isolates with more percentages in extra-intestinal than the intestinal *E. coli* isolates. Taking into consideration of results of CAS agar and chemical tests siderophores could be detected in 56.7% of the isolates overall with larger percentages of hydroxamate-type siderophores in all geographical regions. Hydroxamate-type siderophore (36.1%) was also detected more frequently than catechol-type siderophore (3.3%) in intestinal isolates. Both hydroxamate and catechol-types of siderophores were detected with high rates in extra-intestinal isolates i.e. 34.5% and 24.6% respectively. Catechol-type siderophore was detected more frequently among extra-intestinal isolates than intestinal isolates. Both types of siderophores were expressed simultaneously in 6 isolates (1 intestinal and 5 extra-intestinal).

E. coli isolates could be typed on basis of siderophore characterization into five categories viz. 1) Isolates producing hydroxamate only 2) isolates producing catechol only 3) isolates producing both catechol and hydroxamate 4) isolates producing siderophores other than hydroxamate and catechol-types and 5) siderophore non-producers.

A significant association between the "O" serogroup and the type of siderophore was not observed. Very high percentages of isolates among some of the serogroups i.e. O7, O11, and O141 were siderophores producers. Isolates (4/7) of serotype O157 were also found to produce.

Isolates from summer (84.5%) and monsoon/post-monsoon (51.6%/88%) seasons were found to produce siderophores more frequently than from the winter season (38.2%).

6.7. **Colicin**

Colicin production was detected in *E. coli* isolates by phenotypic soft agar overlay method using colicin-sensitive strain *E. coli* K12. In the present study, 20.2% of the total isolates were found to be colicin producers which were comparatively less than other virulence factors i.e. CSH, biofilm, and siderophores. Colicin producers were detected in more percentage among extra-intestinal isolates (31%) than intestinal isolates (11.9%). The percentage of colicin-producing isolates was low among isolates from all the geographical areas ranging from 4 to 30%. No intestinal isolate from western India was found to produce colicin and no extra-intestinal isolate from northern India produced colicin. Different "O" serogroups were found to produce colicin. Only one out of seven O157 isolates was found to produce colicin. The colicin production rate among isolates from the post-monsoon season was comparatively more than among isolates from other seasons.

6.8. **Gelatin Hydrolysis**

Gelatin hydrolysis was detected by studying gelatinase activity on gelatin agar. Gelatinase activity was shown by only 4.5% of the total isolates. Though the frequency of gelatinase-producing *E. coli* was more among intestinal isolates than the extra-intestinal isolates, however in overall gelatin hydrolysis was the least expressed virulence factor of intestinal as well as extra-intestinal *E. coli* isolates in all geographical locations. None of the isolates from Western India was found to hydrolyze gelatin. Similarly, none of the extra-intestinal isolates from northern India was found to hydrolyze gelatin. Twenty-four gelatin hydrolyzing isolates in the present study belonged to eight different "O" serogroups. Among intestinal isolates, 57% of gelatin hydrolyzing isolates were associated with O22 and UT isolates. None of the O157 isolates were found to hydrolyze gelatin. The rate of gelatin hydrolyzing isolates was low during all seasons. All three gelatin hydrolyzing, extra-intestinal isolates were from the monsoon season.

6.9. **Co-expression of virulence factors**

38.6% of the isolates were found to express multiple virulence factors (more than three virulence factors). Among intestinal isolates 31.7% and extra-intestinal isolates, 47.4% of the isolates expressed multiple virulence factors. Co-expression of cell surface hydrophobicity (CSH) and siderophore production with biofilm formation was observed as the most commonly expressed combination of the multi-virulent trait.

6.10. **Antibiogram**

In the present study, 20 antibiotics representing seven different antimicrobial classes were tested for susceptibility. Isolates were assigned as resistant, intermediate, and sensitive as per CLSI guidelines High resistance rates to ampicillin, nalidixic acid, cefotaxime, and amoxiclav were found and this was observed in isolates from all

geographical locations of the country. On the other hand amikacin, nitrofurantoin, chloramphenicol, imipenem, and meropenem were found to be the most effective drugs. The level of drug resistance in study samples was found to be very high as only 11% of the isolates were found to be sensitive to all the classes of antimicrobials, 9% were resistant to only one class of antimicrobials, 9% were resistant to two different classes of antimicrobials (TDR) and71% of the isolates were MDR. MDR isolates were found from the various geographical area of the country in large percentages.

Resistance to β-lactam drugs was observed in a very large percentage (82.8%) among intestinal (85.4%) as well as extra-intestinal isolates (79.3%). Resistance was observed to all types of β-lactam drugs i.e. penicillins (74.2%), cephalosporins (75.7%), and carbapenem (24.3%). The resistance rate to these β-lactam classes of drugs was found high among intestinal (81.1%, 79.8%, and 23.8% respectively) as well extra-intestinal isolates (65.1%, 70.3%, and 25% respectively).

6.10.1. **Penicillin class of drugs**

Among the penicillin class of a β-lactam group of drugs three antimicrobials, i.e. one pure penicillin (ampicillin) and two penicillins with β-lactamase inactivators i.e. amoxiclav (amoxicillin + clavulanic acid) and piperacillin with tazobactam were evaluated.

Overall 74.2% of the isolates were resistant to penicillins. The resistance rate to penicillins among isolates from various geographical locations was significantly different ranging from 55.9% among isolates from Central India to 100% among isolates from western India.

Individually, piperacillin-tazobactam was the most effective penicillin drug against intestinal as well as extra-intestinal isolates while the resistance rate to ampicillin and amoxiclav was higher. The resistance rate in intestinal isolates was

significantly more for ampicillin and amoxiclav in comparison to extra-intestinal isolates however, no significant difference in resistance rate to piperacillin-tazobactam was found among the two groups of isolates.

6.10.2 **Carbapenem class of drugs**

The resistance rate to carbapenems was observed relatively low in isolates from Central and Southern India than from other parts of the country in the present study. Individual resistance rate to imipenem was 15.2% overall with almost equal rates among intestinal (15.2%) as well as extra-intestinal (15.1%) isolates. Similarly, no significant difference in resistance rate to meropenem among intestinal and extra-intestinal isolates (21.5 % and 19.8% respectively) was observed. Collectively 20.8% of the isolates were resistant to meropenem.

6.10.3. **Cephalosporin class of drugs**

Resistance to cephalosporins in the *E. coli* isolates overall was 75.7%. It was significantly more among intestinal (79.8%) than extra-intestinal (70.3%) isolates. Individually, the resistance rate to drugs belonging to cephalosporins was maximum for cefotaxime (68.9%), followed by cefuroxime (53.9%), ceftriaxone (51.1%), ceftazidime (47.4%), and cefepime (46.1%). The resistance rate to all the individual drugs was more among intestinal than extra-intestinal isolates. Resistance to cephalosporins was very high in *E. coli* isolates from all over the country ranging from 61% to 87% with maximum resistance among isolates from Western, Northern, and Eastern India.

High resistance rates to all generations of cephalosporins evaluated, including Cefepime (46.1%) a fourth-generation cephalosporin, was observed among the *E. coli* isolates in this study.

6.10.4 **Aminoglycoside class of drugs**

In the aminoglycoside group of drugs, three antimicrobials were used (amikacin, gentamicin, and kanamycin), collectively 39.3% of isolates (37.7% intestinal; 41.4% extra-intestinal) were resistant to one or more aminoglycosides.

Individually, amikacin was the most effective drug among intestinal as well as extra-intestinal isolates. While the resistance rate to Kanamycin and gentamicin was relatively more. The difference in resistance rate to gentamicin was significant among the intestinal and extra-intestinal isolates.

6.10.5 **Quinolones class of drugs**

In the quinolone class nalidixic acid, ciprofloxacin and norfloxacin were used. The overall resistance rate to quinolones in this study was 71.7% with high resistance rates among intestinal as well as extra-intestinal isolates which were more among intestinal isolates in comparison to extra-intestinal isolates. A resistance rate of 50.8% to 100% was observed in various parts of the country, with very high rates among isolates from Western, Northern, and Eastern India.

Individually, resistance to nalidixic acid was very high followed by norfloxacin and ciprofloxacin. Intestinal isolates were more resistant to all three quinolones in comparison to extra-intestinal isolates.

6.10.6. **Sulfonamide class of drugs**

In the sulphonamide class, trimethoprim and cotrimoxazole (trimethoprim + sulfamethoxazole) were evaluated. Sulfonamide-resistant strains were detected in large percentages (53.9%) of isolates and resistant isolates were detected from all geographical locations of the country.

Out of 281 trimethoprim-resistant isolates, 11.7% were found sensitive to combination drugs (cotrimoxazole). Among 253 trimethoprim-sensitive isolates, 2.8% were resistant to cotrimoxazole. The difference in the resistance pattern of trimethoprim and cotrimoxazole was found to be significant ($p<0.05$).

6.10.7. **Phenicol class of drugs**

Among phenicols chloramphenicol was evaluated. A very large percentage (88.2%) of isolates was found to be sensitive to chloramphenicol. Resistance rates were found to be low among both intestinal as well as extra-intestinal isolates in all geographical locations.

6.10.8. **Nitrofurantoin class of drugs**

Nitrofurantoin was found to be the second most sensitive drug after amikacin among the twenty antimicrobials evaluated in the present study. Overall least resistance of 10.9% was observed to this drug. The resistance rate to nitrofurantoin was found low among intestinal as well as extra-intestinal isolates from all the geographical locations of the country.

6.10.9**. ESBLProduction**

All 534 isolates were tested for ESBL production using a confirmatory disc diffusion assay using cefotaxime and ceftazidime alone and with clavulanic acid. ESBL in the present study was detected in 199/534 (37.1%) of the isolates with very large numbers in both intestinal (120/302; 39.7%) and extra-intestinal (79232; 34.1%) isolates. ESBL-producing *E. coli* were detected among isolates from all geographical regions with a range varying from 28.2% in southern India to 60.9% in western India.

Out of 199 ESBL-producing isolates, 193 were MDR, and 74 were resistant to carbapenems. Seventy-three isolates (73/534;13.6%) were having all three resistance characteristics i.e. MDR, ESBL production, and carbapenem resistance.

A large number of biofilm-producing isolates in this study were also found to be MDR, ESBL-producing, and carbapenem-resistant however no statistically significant difference between biofilm producers and biofilm non-producers was observed in resistance rates to either of these drug resistance characteristics.

The present study highlights that pathogenic *E. coli* in circulation in India are very diverse in terms of epidemiological markers i.e. serotypes, DEC pathotypes, and virulence factors. The most common DEC pathotypes are EPEC and ETEC in the country. Virulence factors aiding colonization of pathogenic *E. coli* i.e. Cell surface hydrophobicity, biofilms, and siderophore release seem to play a very important role in the pathogenesis of *E. coli* infections. The results in this study also reflect the grieved situation of the severe level of antimicrobial resistance in *E. coli* strains in India as a large number of strains were resistant to commonly used first and second-line antimicrobials. MDR, ESBL-producing, and resistance to carbapenems and Cefepime were also detected in a large number of isolates. Keeping in view the importance of *E. coli* infections in animals, and humans and its wide occurrence in the environment i.e. food and water sources and the spread of MDR *E. coli* among various sources, concrete steps need to be taken to alleviate this problem like:

1. Conducting surveillance studies on regular basis for better understanding and to generate up-to-date databases at national, state, and district levels for better control and management of this infectious agent and reduce morbidity, mortality, and treatment costs associated with it and to formulate effective stewardship plans at local levels for better treatment of drug-resistant infections; 2. formulation of guidelines on rationale use of antimicrobials in treatment and animal feed and strictly implementing these guidelines; 3. Implementing appropriate intervention strategies for containment of the spread of such infections i.e. practicing proper hygiene, availability of clean drinking water, development of effective laboratory diagnosis and treatment facility network; 4. Discovering newer antimicrobials and exploring alternative treatment options i.e. phage therapy, phytochemicals, vaccines, probiotics, stem cell antimicrobial peptides, etc.

BIBLIOGRAPHY

Abadi, A. T. B., Rizvanov, A. A., Haertle, T., and Blatt, N. L. (2019). World Health Organization report: current crisis of antibiotic resistance. *BioNanoScience*, *9*(4), 778-788.

Abbas, H. A., Kadry, A. A., Shaker, G. H., and Goda, R. M. (2017). Resistance of *Escherichia coli* and Klebsiella pneumoniae isolated from different Sources to β-lactam Antibiotics. Research Journal of Pharmacy and Technology, 10(2), 589-591.

Abbasi, E., Mondanizadeh, M., van Belkum, A., and Ghaznavi-Rad, E. (2020). Multi-drug-resistant diarrheagenic *Escherichia coli* pathotypes in pediatric patients with gastroenteritis from central Iran. Infection and Drug Resistance, 13, 1387-1396.

Abd El-Baky, R. M., Ibrahim, R. A., Mohamed, D. S., Ahmed, E. F., and Hashem, Z. S. (2020). The prevalence of virulence genes and their association with antimicrobial resistance among pathogenic *E. coli* isolated from Egyptian patients with different clinical infections. Infection and Drug Resistance, 13, 1221-1236.

Abdallah, M., Benoliel, C., Drider, D., Dhulster, P., and Chihib, N. E. (2014). Biofilm formation and persistence on abiotic surfaces in the context of food and medical environments. Archives of microbiology, 196(7), 453-472.

Abidi, S. H., Sherwani, S. K., Siddiqui, T. R., Bashir, A., and Kazmi, S. U. (2013). Drug resistance profile and biofilm-forming potential of Pseudomonas aeruginosa isolated from contact lenses in Karachi-Pakistan. BMC ophthalmology, 13(1), 1-6.

Achtman, M., Mercer, A., Kusecek, B., Pohl, A., Heuzenroeder, M., Aaronson, W., ... and Silver, R. P. (1983). Six widespread bacterial clones among *Escherichia coli* K1 isolates. Infection and immunity, 39(1), 315-335.

Adav, S. S., Lee, D. J., Show, K. Y., and Tay, J. H. (2008). Aerobic granular sludge: recent advances. Biotechnology Advances, 26(5), 411-423.

Agarwal, R. K., Singh, S., Bhilegaonkar, K. N., and Singh, V. P. (2011). Optimization of microtitre plate assay for the testing of biofilm formation ability in different Salmonella serotypes. International Food Research Journal, 18(4), 1493.

Aggarwal, P., Uppal, B., Ghosh, R., Prakash, S. K., and Rajeshwari, K. (2013). Highly-resistant *E. coli* as a common cause of pediatric diarrhea in India. Journal of Health, Population, and Nutrition, 31(3), 409-412

Agrawal, P., Ghosh, A. N., Kumar, S., Basu, B., and Kapila, K. (2008). The prevalence of extended-spectrum β-lactamases among *Escherichia coli* and Klebsiella pneumoniae isolates in a tertiary care hospital. Indian Journal of Pathology and Microbiology, 51(1), 139-142.

Ahmed, D., Islam, M. S., Begum, Y. A., Janzon, A., Qadri, F., and Sjoling, Å. (2013). Presence of enterotoxigenic *Escherichia coli* in biofilms formed in water containers in poor households coincides with epidemic seasons in D haka. Journal of Applied Microbiology, 114(4), 1223-1229.

Airol E., Getaz L., Stoll B., Chappuis F. Loutan L. (2011). Urbanization and infectious diseases in a globalized world. The Lancet Infectious Diseases, 11, 131–141.

Akpaka P.E., Legall B. and Padman J. (2010). Molecular detection and epidemiology of Extended-Spectrum beta-lactamase genes prevalent in clinical isolates of *Klebsiella pneumoniae* and E coli from Trinidad and Tobago. West Indian Medical Journal, 59 (6), 591-596

Al-Bakri, A. G., Gilbert, P., and Allison, D. G. (2005). Influence of gentamicin and tobramycin on binary biofilm formation by co-cultures of Burkholderia cepacia and Pseudomonas aeruginosa. Journal of Basic Microbiology: An International Journal on Biochemistry, Physiology, Genetics, Morphology, and Ecology of Microorganisms, 45(5), 392-396.

Al-Dulaimi, T. H., Aziz, H. W., Al-Marzoqi, A. H., Al-Aziz, S. A., and Mohsin, S. A. A. (2015). Molecular characterization and antibiotic susceptibility of diarrheagenic *Escherichia coli* from Children. Medical Journal of Babylon, 12(2), 541-550.

Al-Hasan, M. N., Lahr, B. D., Eckel-Passow, J. E., and Baddour, L. M. (2009). Seasonal variation in *Escherichia coli* bloodstream infection: a population-based study. Clinical Microbiology and Infection, 15(10), 947-950.

Ali, I., Rafaque, Z., Ahmed, S., Malik, S., and Dasti, J. I. (2016). The prevalence of multi-drug-resistant uropathogenic *Escherichia coli* in Potohar region of Pakistan. Asian Pacific Journal of Tropical Biomedicine, 6(1), 60-66.

Ali, S. S., and Vidhale, N. N. (2013). Bacterial siderophore and their application: a review. International Journal of Current Microbiology and Applied Scieces, 2(12), 303-312.

Alkeskas, A., Ogrodzki, P., Saad, M., Masood, N., Rhoma, N. R., Moore, K., Farbos A., Paszkiewicz K. and Forsythe, S. (2015). The molecular characterization of *Escherichia coli* K1 isolated from neonatal nasogastric feeding tubes. BMC Infectious Diseases, 15(1), 1-14.

Al-Mulla, A. F., Al-Khafaji, Z. M., and Al-Kareemy, K. K. (2013). Comparison between Methods for Detection of Biofilm Produced by Staphylococcus epidermidis. International Journal of Biological and Pharmaceutical Research, 4(12), 1010-1014.

Amezquita-Lopez, B. A., Soto-Beltran, M., Lee, B. G., Yambao, J. C., and Quiñones, B. (2018). Isolation, genotyping, and antimicrobial resistance of Shiga toxin-producing *Escherichia coli*. Journal of Microbiology, Immunology and Infection, 51(4), 425-434.

Amjad, A., Mirza, I. A., Abbasi, S. A., Farwa, U., Malik, N., and Zia, F. (2011). Modified Hodge test: A simple and effective test for detection of carbapenemase production. Iranian Journal of Microbiology, 3(4), 189-193.

Ammenti, A., Alberici, I., Brugnara, M., Chimenz, R., Guarino, S., La Manna, A., ... and Italian Society of Pediatric Nephrology. (2020). Updated Italian recommendations for the diagnosis, treatment and follow-up of the first febrile urinary tract infection in young children. Acta Paediatrica, 109(2), 236-247.

Amyes, S. G. B., and Brunton, W. T. (1981). Co-trimoxazole sensitivity testing: comparison of separate and combined disk agar diffusion techniques. Journal of Clinical Pathology, 34(2), 199-202.

Anderson IV, J. D., Bagamian, K. H., Muhib, F., Amaya, M. P., Laytner, L. A., Wierzba, T., and Rheingans, R. (2019). Burden of enterotoxigenic *Escherichia coli* and shigella non-fatal diarrheal infections in 79 low-income and lower-middle-income countries: a modeling analysis. The Lancet Global Health, 7(3), e321-e330.

Anderson, M. A., Whitlock, J. E., and Harwood, V. J. (2006). Diversity and distribution of *Escherichia coli* genotypes and antibiotic resistance phenotypes in feces of humans, cattle, and horses. Applied and Environmental Microbiology, 72(11), 6914-6922.

Andrade J. R., Da Veiga V. F., De Santa Rosa M. R. and Suassuna I. (1989). An endocytic process in HEp-2 cells induced by enteropathogenic *Escherichia coli*. Journal of Medical Microbiology, 28(1), 49-57.

Andrews, S. C., Robinson, A. K., and Rodriguez-Quiñones, F. (2003). Bacterial iron homeostasis. FEMS Microbiology Reviews, 27(2-3), 215-237.

Annapurna, Y. V., Reddy, B. S., and Lakshmi, V. V. (2014). Multidrug resistance and virulence phenotypes among uropathogenic *Escherichia coli*. International Journal of Current Microbiology and Applied Sciences, 3(6), 222-229.

Arciola, C. R., Campoccia, D., Speziale, P., Montanaro, L., and Costerton, J. W. (2012). Biofilm formation in Staphylococcus implant infections. A review of molecular mechanisms and implications for biofilm-resistant materials. Biomaterials, 33(26), 5967-5982.

Arduino, R.C., and Dupont, H. (1993). Travelers' diarrhea. Bailliere's Clinical Gastroenterology, 7 2, 365-85.

Arenas-Hernandez M.M., Martinez-Laguna Y., Torres A.G. (2012). Clinical implications of enteroadherent *Escherichia coli*. Current Gastroenterology Repprts, 14, 386-394.

Arinaminpathy, N., Sinha, A., Anvikar, A., Joseph, A.K., Kang, G., Frost, I., Joshi, J., Wattal, C., Goel, N., Kotwani, A., Hira, S., Pawar, S. and Laxminarayan, R. (2021). Infectious diseases in the South-East Asia Regin. Available at: https://cddep.org/wp-content/uploads/2021/02/infectious-diseases-in-the-south-east-asia-region-1.pdf

Armstrong, G. D. (2013). Uropathogenic *Escherichia coli* colicin-like usp and associated proteins: their evolution and role in pathogenesis. Journal of Infecious Diseases, 208 (10), 1539-1541.

Arnow, L. E. (1937). Colorimetric determination of the components of 3, 4-dihydroxyphenylalanine-tyrosine mixtures. The Journal of Biological Chemistry, 118(2), 531-537.

Arundathi, H. A., Koppad, M., Halesh, L. H., and Siddesh, K. C. (2016). Coexistence of quinolone resistance and extended spectrum beta lactamase production in urinary isolates of *Escherichia coli* - an emerging challenge to antimicrobial prescribing pattern. Indian Journal of Microbiology Research, 3(4), 359-362.

Aslani, M. M., Alikhani, M. Y., Zavari, A., Yousefi, R., and Zamani, A. R. (2011). Characterization of enteroaggregative *Escherichia coli* (EAEC) clinical isolates and their antibiotic resistance pattern. International Journal of Infectious Diseases, 15(2), e136-e139.

Atray, D., and Atray, M. (2015). Correlation between biofilm production and antibiotic resistance pattern in uropathogenic *Escherichia coli* in tertiary care hospital in Southern Rajasthan, India. International Journal of Current Microbiology and Applied Sciences, 4(7), 640-646.

Avendano P, Matson D O, Long J, Whitney S, Matson C C and Pickering L K (1993). Costs associated with office visits for diarrhea in infants and toddlers. Pediatric Infectious Diseases Journal 12, 897-902.

Azpiroz, M. F., Poey, M. E., and Lavina, M. (2009). Microcins and urovirulence in *Escherichia coli*. Microbial pathogenesis, 47(5), 274-280.

Sai, B.C. and Balachandhar B. (2019). The prevalence of Shiga-like toxin producing *Escherichia coli* strain (*E. coli* O157) in freshly consumed vegetables and its characterization. Journal of Food Safety, 39(1), e12577.

Bai, X., Hu, B., Xu, Y., Sun, H., Zhao, A., Ba, P., Fu, S., Fan. R., Jin, Y., Wang, H., Guo, Q., Xu. X., Lu, S., and Xiong, Y. (2016). Molecular and phylogenetic characterization of non-O157 Shiga toxin-producing *Escherichia coli* strains in China. Frontiers in Cellular and Infection Microbiology, 6, 143.

Bajpai, T., Varma, M., Bhatambare, G., and Pandey, M. (2016). *Escherichia coli* biofilms: Accepting the therapeutic challenges. International Journal of Health and Allied Sciences, 5(4), 204-204.

Banukumar, S., Kannan, I., and Sukumar, R. (2017). Fluoroquinolone resistance pattern among the pathogens causing urinary tract infection in a tertiary care hospital in Kanchipuram district, Tamil Nadu, India. Asian Journal of Pharmaceutical and Clinical Research, 10(11), 292-294.

Baqui A. H., Sack R. B. and Black R. E. (1992). Enteropathogens associated with acute and persistent diarrhea in Bangladeshi children <5 years of age. Journal of Infectious Diseases, 166, 792–796.

Barrow G. I. and Feltham R. K. A. (1993). Cowan and Steel's manual for the identification of medical bacteria. 3rd Ed. Cambridge, Cambridge University Press.

Bartoletti, R., Cai, T., Nesi, G., Albanese, S., Meacci, F., Mazzoli, S., and Naber, K. (2014). The impact of biofilm-producing bacteria on chronic bacterial prostatitis

treatment: results from a longitudinal cohort study. World Journal of Urology, 32(3), 737-742.

Baudry, B., Savarino, S. J., Vial, P., Kaper, J. B., and Levine, M. M. (1990). A sensitive and specific DNA probe to identify enteroaggregative *Escherichia coli*, a recently discovered diarrheal pathogen. Journal of Infectious Diseases, 161(6), 1249-1251.

Baumgarten, T., Sperling, S., Seifert, J., von Bergen, M., Steiniger, F., Wick, L. Y., and Heipieper, H. J. (2012). Membrane vesicle formation as a multiple-stress response mechanism enhances Pseudomonas putida DOT-T1E cell surface hydrophobicity and biofilm formation. Applied and Environmental Microbiology, 78(17), 6217-6224.

Bawankar S. Urovirulence markers of uropathogenic *Escherichia coli* and its antimicrobial susceptibility. International Journal of Applied Research. 2018;4(4), 245–248

Beatty, M. E., Bopp, C. A., Wells, J. G., Greene, K. D., Puhr, N. D., and Mintz, E. D. (2004). Enterotoxin-producing *Escherichia coli* O169:H41, United States. Emerging Infectious Diseases, 10(3), 518–521.

Beutin, L., Geier, D., Zimmermann, S., and Karch, H. (1995). Virulence markers of Shiga-like toxin-producing *Escherichia coli* strains originating from healthy domestic animals of different species. Journal of Clinical Microbiology, 33(3), 631-635.

Beutin, L., Gleier, K., Kontny, I., Echeverria, P., and Scheutz, F. (1997). Origin and characteristics of enteroinvasive strains of *Escherichia coli* (EIEC) isolated in Germany. Epidemiology and Infection, 118(3), 199-205.

Bhat Y, R., Lewis, L. E. S., and KE, V. (2011). Bacterial isolates of early-onset neonatal sepsis and their antibiotic susceptibility pattern between 1998 and 2004: an audit from a center in India. Italian Journal of Pediatrics, 37(1), 1-6.

Bhattacharyya, S., Sarfraz, A., Ansari, M. A. A., and Jaiswal, N. (2015). Characterization and antibiogram of uropathogenic *Escherichia coli* from a tertiary care hospital in Eastern India. International Journal of Current Microbiology and Applied Sciences, 4(2), 701-705.

Bhave, S., Kolhe, R., Bhong, C., Jadhav, S., Nalband, S., Ranjan, M., ... and Deshpande, P. (2018). Isolation of Diarrheagenic *Escherichia coli* from Poultry Feces and Raw Chicken. Journal of Animal Research, 8(5), 915-923.

Bielaszewska, M., Mellmann, A., Bletz, S., Zhang, W., Köck, R., Kossow, A., Prager R., Fruth A., Orth-Holler D., Marejkova M., Morabito S., Caprioli A., Pierard D., Smith G., Jenkins C., Curova K.and Karch, H. (2013). Enterohemorrhagic *Escherichia coli* O26: H11/H−: a new virulent clone emerges in Europe. Clinical Infectious Diseases, 56(10), 1373-1381.

Bilge S. S., Apostol J. M., Jr, Aldape M. A. and Moseley S. L. (1993). mRNA processing independent of RNase III and RNase E in the expression of the F1845 fimbrial adhesin of *Escherichia coli*. Proceedings of National Academic of Sciences of the USA, 90,1455–1459.

Bilge S. S., Clausen C. R., Lau W., Moseley S. L. (1989). Molecular characterization of a fimbrial adhesin, F1845, mediating diffuse adherence of diarrhea-associated *Escherichia coli* to HEp-2 cells. J ournal of Bacteriology, 171, 4281–4289.

Bilinski, P., Kapka-Skrzypczak, L., Posobkiewicz, M., Bondaryk, M., Holownia, P., and Wojtyla, A. (2012). Public health hazards in Poland posed by foodstuffs contaminated with *E. coli* O104: H4 bacterium from the recent European outbreak. Annals of Agricultural and Environmental Medicine, 19(1), 3-10.

Bjarnsholt, T. (2013). The role of bacterial biofilms in chronic infections. APMIS, 121, 1-58.

Blanco, M., Blanco, J. E., Dahbi, G., Mora, A., Alonso, M. P., Varela, G., ... and Blanco, J. (2006). Typing of intimin (eae) genes from enteropathogenic

Escherichia coli (EPEC) isolated from children with diarrhea in Montevideo, Uruguay: identification of two novel intimin variants (μB and ξR/β2B). Journal of Medical Microbiology, 55(9), 1165-1174.

Bloom, D. E., and Cadarette, D. (2019). Infectious Disease Threats in the Twenty-First Century: Strengthening the Global Response. Frontiers in Immunology, 10, 549.

Boisen, N., Melton-Celsa, A. R., Scheutz, F., O'Brien, A. D., and Nataro, J. P. (2015). Shiga toxin 2a and enteroaggregative *Escherichia coli*–a deadly combination. Gut Microbes, 6(4), 272-278.

Bokranz, W., Wang, X., Tschäpe, H., and Romling, U. (2005). Expression of cellulose and curli fimbriae by *Escherichia coli* isolated from the gastrointestinal tract. Journal of Medical Microbiology, 54(12), 1171-1182.

Bonacorsi, S., and Bingen, E. (2005). Molecular epidemiology of *Escherichia coli* causing neonatal meningitis. International Journal of Medical Microbiology, 295(6-7), 373-381.

Bonnet, C., Diarrassouba, F., Brousseau, R., Masson, L., Topp, E., and Diarra, M. S. (2009). Pathotype and antibiotic resistance gene distributions of *Escherichia coli* isolates from broiler chickens raised on antimicrobial-supplemented diets. Applied and Environmental Microbiology, 75(22), 6955–6962.

Bouzari, S., Jafari, A., Azizi, A., Oloomi, M., and Nataro, J. P. (2001). Characterization of enteroaggregative *Escherichia coli* isolates from Iranian children. The American Journal of Tropical Medicine and Hygiene, 65(1), 13-14.

Bouzari, S., Jafari, A., Farhoudi-Moghaddam, A. A., Shokouhi, F., and Parsi, M. (1994). Adherence of non-enteropathogenic *Escherichia coli* to HeLa cells. Journal of Medical Microbiology, 40(2), 95-97.

Brady, R. A., Leid, J. G., Calhoun, J. H., Costerton, J. W., and Shirtliff, M. E. (2008). Osteomyelitis and the role of biofilms in chronic infection. FEMS Immunology and Medical Microbiology, 52(1), 13-22.

Branda, S. S., Vik, Å., Friedman, L., and Kolter, R. (2005). Biofilms: the matrix revisited. Trends in Microbiology, 13(1), 20-26.

Brooks, J. D., and Flint, S. H. (2008). Biofilms in the food industry: problems and potential solutions. International Journal of Food Science and Technology, 43(12), 2163-2176.

Brooks, J. T., Sowers, E. G., Wells, J. G., Greene, K. D., Griffin, P. M., Hoekstra, R. M., and Strockbine, N. A. (2005). Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983–2002. The Journal of Infectious Diseases, 192(8), 1422-1429.

Broujerdi, S. M., Ardakani, M. R., and Rezatofighi, S. E. (2018). Characterization of diarrheagenic *Escherichia coli* strains associated with diarrhea in children, Khouzestan, Iran. The Journal of Infection in Developing Countries, 12(08), 649-656.

Brower, C. H., Mandal, S., Hayer, S., Sran, M., Zehra, A., Patel, S. J., Kaur, R., Chatterjee, L., Mishra, S., Das, B.R., Singh, P., Singh R., Gill, J.P.S, and Laxminarayan, R. (2017). The prevalence of extended-spectrum beta-lactamase-producing multidrug-resistant *Escherichia coli* in poultry chickens and variation according to farming practices in Punjab, India. Environmental Health Perspectives, 125(7), 077015.

Bryers, J. D. (2008). Medical biofilms. Biotechnology and Bioengineering, 100(1), 1-18.

Buchholz, U., Bernard, H., Werber, D., Bohmer, M. M., Remschmidt, C., Wilking, H., Delere, Y., an der Heiden, M., Adlhoch, C., Dreesman, J., Ehlers, J.,Ethelberg, S., Faber, M., Frank, C., Fricke, G., Greiner, M., Hohle, M., Ivarsson, S., Jark, U., Kirchner, M., Koch, J., Krause, G., Luber, P., Rosner, B., Stark, K., and Kuhne, M. (2011). German outbreak of *Escherichia coli* O104: H4 associated with sprouts. New England Journal of Medicine, 365(19), 1763-1770.

Budic, M., Rijavec, M., Petkovsek, Z., and Zgur-Bertok, D. (2011). *Escherichia coli* bacteriocins: antimicrobial efficacy and the prevalence among isolates from patients with bacteremia. PLoS One, 6(12), e28769.

Burmolle, M., Webb, J. S., Rao, D., Hansen, L. H., Sørensen, S. J., and Kjelleberg, S. (2006). Enhanced biofilm formation and increased resistance to antimicrobial agents and bacterial invasion are caused by synergistic interactions in multispecies biofilms. Applied and Environmental Microbiology, 72(6), 3916-3923.

Bush K. and Jacoby G.A. (2010). Updated functional classification of beta-lactamases. Antimicrobial Agents and Chemotherapy, 54:969-976.

Byrne, L., Adams, N., and Jenkins, C. (2020). Association between Shiga Toxin–producing *Escherichia coli* O157: H7 stx gene subtype and disease severity, England, 2009–2019. Emerging Infectious Diseases, 26(10), 2394-2400.

Cabal, A., Garcia-Castillo, M., Cantón, R., Gortazar, C., Dominguez, L., and Alvarez, J. (2016). The prevalence of *Escherichia coli* virulence genes in patients with diarrhea and a subpopulation of healthy volunteers in Madrid, Spain. Frontiers in Microbiology, 7, 641.

Campos, L. C., Franzolin, M. R., and Trabulsi, L. R. (2004). Diarrheagenic *Escherichia coli* categories among the traditional enteropathogenic *E. coli* O serogroups: a review. Memorias do Instituto Oswaldo Cruz, 99(6), 545-552.

Casadevall, A., and Pirofski, L. A. (2009). Virulence factors and their mechanisms of action: the view from a damage–response framework. Journal of Water and Health, 7(S1), S2-S18.

Castanheira M., Deshpande L.M., Mathai D., Bell J.M., Jones R.N. and Mendes R.E. (2011a). Early dissemination of NDM-1- and OXA-181-producing Enterobacteriaceae in Indian hospitals: report from the SENTRY Antimicrobial Surveillance Program, 2006-2007. Antimicrobial Agents Chemotherapy, 55, 1274-1278.

Castanheira M., Mendes R.E., Woosley L.N. and Jones R.N. (2011b). Trends in carbapenemase-producing *Escherichia coli* and Klebsiella spp. from Europe and the Americas: report from the SENTRY antimicrobial surveillance programme (2007-09). Journal of Antimicrobial Chemotherapy, 66, 1409-1411.

Caza, M., Lepine, F., and Dozois, C. M. (2011). Secretion, but not overall synthesis, of catecholate siderophores, contributes to virulence of extraintestinal pathogenic *Escherichia coli*. Molecular Microbiology, 80(1), 266-282.

CDC (2011) Outbreak of Shiga toxin-producing *E. coli* O104 (STEC O104:H4) Infections Associated with Travel to Germany (FINAL UPDATE). Available at: https://www.cdc.gov/ecoli/2011/travel-germany-7-8-11.html

CDC (2019). Antibiotic resistance threats in the united states 2019. Available at: https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf

CDDEP (Center for Disease Dynamics, Economics, and Policy). (2015). Resistance map. Washington DC: Center for Disease Dynamics, Economics and Policy; 2015

Centers for Disease Control and Prevention (CDC) (2012). National Shiga toxin-producing *Escherichia coli* (STEC) Surveillance Overview. Atlanta, Georgia: US Department of Health and Human Services, CDC,.

Cespedes, S., Saitz, W., Del Canto, F., De la Fuente, M., Quera, R., Hermoso, M., Muñoz, R., Ginard, D., Khorrami, S., Girón, J., Assar, R., Rosselló-Mora, R., and Vidal, R. M. (2017). Genetic Diversity and Virulence Determinants of *Escherichia coli* Strains Isolated from Patients with Crohn's Disease in Spain and Chile. Frontiers in Microbiology, 8, 639.

CFSPH -Centre for Food Security and Public Health (2016). Enterohemorrhagic *Escherichia coli* and other *E. coli* causing hemolytic uremic syndrome. Available at: https://www.cfsph.iastate.edu/Factsheets/pdfs/e_coli.pdf

Chakraborty S., Deokule J. S, Garg P., Bhattacharya S. K., Nandy R. K., Nair G. B., Yamasaki S., Takeda Y., and Ramamurthy T. (2001). Concomitant infection of

enterotoxigenic *Escherichia coli* in an outbreak of cholera caused by *Vibrio cholerae* O1 and O139 in Ahmedabad, India. Journal of Clinical Microbiology, 39**,** 3241-3246.

Chandra B.K., Singh G., Taneja N., Pahil S., Singhi . and, Sharma M. (2012). Diarrheagenic *Escherichia coli* as a predominant cause of paediatric nosocomial diarrhea in India. Journal of Medical Microbiology, 61(6), 830-836.

Chatterjee, B. D., and Sanyal, S. N. (1984). Is it all shigellosis?. The Lancet, 324(8402), 574.

Cheasty, T., and Rowe, B. (1983). Antigenic relationships between the enteroinvasive *Escherichia coli* O antigens O28ac, O112ac, O124, O136, O143, O144, O152, and O164 and Shigella O antigens. Journal of Clinical Microbiology, 17(4), 681-684.

Chekabab, S. M., Paquin-Veillette, J., Dozois, C. M., and Harel, J. (2013). The ecological habitat and transmission of *Escherichia coli* O157: H7. FEMS Microbiology Letters, 341(1), 1-12.

Chellapandi, K., Dutta, T. K., Sharma, I., De Mandal, S., Kumar, N. S., and Ralte, L. (2017). The prevalence of multi drug-resistant enteropathogenic and enteroinvasive *Escherichia coli* isolated from children with and without diarrhea in Northeast Indian population. Annals of Clinical Microbiology and Antimicrobials, 16(1), 1-9.

Chhibber S, Bhardwaj SB and Aghi P (2014). Serotype profile and acid resistance of *Escherichia coli* strains isolated from different Indian food. Asian Journal of Microbiology, Biotechnology and Environmental Sciences Paper. 2014;16 (2), 395-398.

Choe, H. S., Lee, S. J., Cho, Y. H., Çek, M., Tandoğdu, Z., Wagenlehner, F., Bjerklund-Johansen, T. E., Naber, K., and GPIU Asian Investigators (2018). Aspects of urinary tract infections and antimicrobial resistance in hospitalized urology patients in Asia: 10-Year results of the Global The prevalence Study of

Infections in Urology (GPIU). Journal of Infection and Chemotherapy, 24(4), 278–283.

Christensen, G. D., Simpson, W. A., Bisno, A. L., and Beachey, E. H. (1982). Adherence of slime-producing strains of Staphylococcus epidermidis to smooth surfaces. Infection and Immunity, 37(1), 318-326.

Christensen, G. D., Simpson, W. A., Younger, J. J., Baddour, L. M., Barrett, F. F., Melton, D. M., and Beachey, E. H. (1985). Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. Journal of Clinical Microbiology, 22(6), 996-1006.

Chumchalova, J., and Smarda, J. (2003). Human tumor cells are selectively inhibited by colicins. Folia Microbiologica, 48(1), 111-115.

CIDRAP (2017). Report, Antibiotic resistance rising in Europe. Available at: https://www.cidrap.umn.edu/news-perspective/2017/02/report-antibiotic-resistance-rising-europe

Clinical and Laboratory Standards Institute (CLSI) (2014) Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI Document M100-S24, Wayne, 34(1).

Cohen, M. B., Nataro, J. P., Bernstein, D. I., Hawkins, J., Roberts, N., and Staat, M. A. (2005). The prevalence of diarrheagenic *Escherichia coli* in acute childhood enteritis: a prospective controlled study. The Journal of Pediatrics, 146(1), 54-61.

Corpe, W. A. (1980). Microbial surface components involved in adsorption of microorganisms onto surfaces. In: Adsorption of microorganisms to surfaces. New York: John Wiley and Sons, 105-144.

Costerton, J. W., Lewandowski, Z., Caldwell, D. E., Korber, D. R., and Lappin-Scott, H. M. (1995). Microbial biofilms. Annual Review of Microbiology, 49(1), 711-745.

Costerton, J. W., Stewart, P. S., and Greenberg, E. P. (1999). Bacterial biofilms: a common cause of persistent infections. Science, 284(5418), 1318-1322.

Cravioto, A., Gross, R. J., Scotland, S. M., and Rowe, B. (1979). An adhesive factor found in strains of *Escherichia coli* belonging to the traditional infantile enteropathogenic serotypes. Current Microbiology, 3(2), 95-99.

Croxen, M. A., and Finlay, B. B. (2010). Molecular mechanisms of *Escherichia coli* pathogenicity. Nature Reviews Microbiology, 8(1), 26-38.

Croxen, M. A., Law, R. J., Scholz, R., Keeney, K. M., Wlodarska, M., and Finlay, B. B. (2013). Recent advances in understanding enteric pathogenic *Escherichia coli*. Clinical Microbiology Reviews, 26(4), 822-880.

Cryan, B. (1990). Comparison of three assay systems for detection of enterotoxigenic *Escherichia coli* heat-stable enterotoxin. Journal of Clinical Microbiology, 28, 792 - 794.

Cursino, L., Smarda, J., Chartone-Souza, E., and Nascimento, A. (2002). Recent updated aspects of colicins of Enterobacteriaceae. Brazilian Journal of Microbiology, 33, 185-195.

Cusini, A., Herren, D., Bütikofer, L., Pluss-Suard, C., Kronenberg, A., and Marschall, J. (2018). Intra-hospital differences in antibiotic use correlate with antimicrobial resistance rate in *Escherichia coli* and Klebsiella pneumoniae: a retrospective observational study. Antimicrobial Resistance and Infection Control, 7(1), 1-11.

Czaky, T. (1948). Estimation of bound hydroxylamine in biological materials. Acta Chemica Scandinavica, 21, 450-454.

Dam, A. N., Berg, A. M., and Farraye, F. A. (2013). Environmental influences on the onset and clinical course of Crohn's disease—Part 1: An overview of external risk factors. Gastroenterology and Hepatology, 9(11), 711.

Dandona, L., Dandona, R., Kumar, G. A., Shukla, D. K., Paul, V. K., Balakrishnan, K., ... and Thakur, J. S. (2017). Nations within a nation: variations in

epidemiological transition across the states of India, 1990–2016 in the Global Burden of Disease Study. The Lancet, 390(10111), 2437-2460.

D'Andrea M.M., Arena F., Pallecchi L. and Rossolini G.M. (2013). CTX-M-type beta-lactamases: a successful story of antibiotic resistance. International Journal of Medical Microbiology, 303:305-317.

Das, A., Guha, C., Biswas, U., Jana, P. S., Chatterjee, A., and Samanta, I. (2017). Detection of emerging antibiotic resistance in bacteria isolated from subclinical mastitis in cattle in West Bengal. Veterinary World, 10(5), 517.

Das, B., Mittal, N., Goswami, R., Adhana, D., and Rathore, N. (2018). The prevalence of multidrug resistance (MDR) and extended spectrum beta-lactamases (ESBLs) among uropathogenic *Escherichia coli* isolates from female patients in a tertiary care hospital in North India. International Journal of Reproduction, Contraception, Obstetrics and Gynecology, 7(12), 5031-5037.

Dash, D., Sarangi, G., Patro, P., and Chayani, N. (2018). Study of biofilm production in *Escherichia coli* causing urinary tract infection and its correlation with antimicrobial resistance. Journal of The Academy of Clinical Microbiologists, 20(2), 88-91.

Davies, M., and Walsh, T. R. (2018). A colistin crisis in India. The Lancet Infectious Diseases, 18(3), 256-257.

Day, M. J., Hopkins, K. L., Wareham, D. W., Toleman, M. A., Elviss, N., Randall, L., ... and Livermore, D. M. (2019). Extended-spectrum β-lactamase-producing *Escherichia coli* in human-derived and foodchain-derived samples from England, Wales, and Scotland: an epidemiological surveillance and typing study. The Lancet Infectious Diseases, 19(12), 1325-1335.

De, A., Deshpande, D., Baveja, S.M. and Taklikar, S. (2012). Detection of bifilm formation in bacteria from cases of urinary tract infections, septicemia, skin and soft tissue infections and post-operative infections by The congo red agar method. Journal of Academic Medical Sciences., 2 (1), 46-47.

Deeny, S. R., Van Kleef, E., Bou-Antoun, S., Hope, R. J., and Robotham, J. V. (2015). Seasonal changes in the incidence of *Escherichia coli* bloodstream infection: variation with region and place of onset. Clinical Microbiology and Infection, 21(10), 924-929.

Demir, M., and Kaleli, I. (2004). Production by *Escherichia coli* isolates of siderophore and other virulence factors and their pathogenic role in a cutaneous infection model. Clinical Microbiology and Infection, 10(11), 1011-1014.

DePas, W. H., Syed, A. K., Sifuentes, M., Lee, J. S., Warshaw, D., Saggar, V. Csankovszki, G and Chapman, M. R. (2014). Biofilm formation protects *Escherichia coli* against killing by Caenorhabditis elegans and Myxococcus xanthus. Applied and Environmental Microbiology, 80(22), 7079-7087.

Devanga Ragupathi, N. K., Veeraraghavan, B., Muthuirulandi Sethuvel, D. P., Anandan, S., Vasudevan, K., Neeravi, A. R., Daniel, J., Sathyendra, S., Iyadurai, R., and Mutreja, A. (2020). First Indian report on genome-wide comparison of multidrug-resistant *Escherichia coli* from bloodstream infections. PloS One, 15(2), e0220428.

Devi, U., Bora, R., Malik, V., Deori, R., Gogoi, B., Das, J. K., and Mahanta, J. (2017). Bacterial aetiology of neonatal meningitis: A study from north-east India. Indian Journal of Medical Research, 145(1), 138-143.

Dhanalakshmi, T. A., Venkatesha, D., Nusrath, A., and Asharani, N. (2018). Evaluation of Phenotypic Methods for Detection of Biofilm Formation in Uropathogens. National Journal of Laboratory Medicine, 7(4), 6-11.

Dickinson, B. L., and Clements, J. D. (1995). Dissociation of *Escherichia coli* heat-labile enterotoxin adjuvanticity from ADP-ribosyltransferase activity. Infection and Immunity, 63(5), 1617-1623.

Dobrindt U.and Hacker J. (2008). Targeting virulence traits: potential strategies to combat extraintestinal pathogenic *E. coli* infections. Current Opinion in Microbiology, 11:409-413.

Donà, V., Scheidegger, M., Pires, J., Furrer, H., Atkinson, A., and Babouee Flury, B. (2019). Gradual in vitro evolution of cefepime resistance in an ST131 *Escherichia coli* strain expressing a plasmid-encoded CMY-2 β-lactamase. Frontiers in Microbiology, 10, 1311.

Donlan, R. M. (2001). Biofilms and device-associated infections. Emerging Infectious Diseases, 7(2), 277-281.

Donlan, R. M. (2011). Biofilm elimination on intravascular catheters: important considerations for the infectious disease practitioner. Clinical Infectious Diseases, 52(8), 1038-1045.

Donnenberg, M. S., Tzipori, S., McKee, M. L., O'Brien, A. D., Alroy, J., and Kaper, J. B. (1993). The role of the eae gene of enterohemorrhagic *Escherichia coli* in intimate attachment in vitro and in a porcine model. The Journal of Clinical Investigation, 92(3), 1418-1424.

Dozois, C. M., and Curtiss, R. (1999). Pathogenic diversity of *Escherichia coli* and the emergence of exotic islands in the gene stream. Veterinary Research, 30(2-3), 157-179.

Drasar B. S. and Hill M. J. (1974). Human intestinal flora. London, United Kingdom: Academic Press, Ltd.; pp. 36–43.

DuPont, H. L., Formal, S. B., Hornick, R. B., Snyder, M. J., Libonati, J. P., Sheahan, D. G., ... and Kalas, J. P. (1971). Pathogenesis of *Escherichia coli* diarrhea. New England Journal of Medicine, 285(1), 1-9.

Dutta, S., Guin, S., Ghosh, S., Pazhani, G. P., Rajendran, K., Bhattacharya, M. K., Takeda, Y., Nair, G.B., and Ramamurthy, T. (2013). Trends in the prevalence of diarrheagenic *Escherichia coli* among hospitalized diarrheal patients in Kolkata, India. PLoS One, 8(2), e56068.

Eber, M. R., Shardell, M., Schweizer, M. L., Laxminarayan, R., and Perencevich, E. N. (2011). Seasonal and temperature-associated increases in gram-negative

bacterial bloodstream infections among hospitalized patients. PloS One, 6(9), e25298.

ECDC (2022). Antimicrobial resistance surveillance in Europe 2022 - 2020 data. Available at: https://www.ecdc.europa.eu/en/publications-data/antimicrobial-resistance-surveillance-europe-2022-2020-data

Edwards, P. R., and Ewing, W. H. (1972). Identification of Enterobacteriaceae. Identification of Enterobacteriaceae., (Third edition). Burgess Publishing Co.; Minneapolis, Minn.

Edwards, T., Heinz, E., van Aartsen, J., Howard, A., Roberts, P., Corless, C., Fraser, A.J, Williams, C.T., Bulgasim, I., Cuevas, L.E., Parry, C.M., Roberts, A.P., Adams, E.R., Mason, J. and Hubbard, A. (2020). Piperacillin/tazobactam resistant, cephalosporin susceptible *Escherichia coli* bloodstream infections driven by multiple resistance mechanisms across diverse sequence types. BioRxiv. 2020.09.18.302992;

EFSA Biohaz Panel, Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bover-Cid, S., Chemaly, M., ... and Bolton, D. (2020). Pathogenicity assessment of Shiga toxin-producing *Escherichia coli* (STEC) and the public health risk posed by contamination of food with STEC. EFSA Journal, 18(1), e05967.

Elfazazi, K., Zahir, H., Tankiouine, S., Mayoussi, B., Zanane, C., Lekchiri, S., Ellouali, M., Mliji, E.M, and Latrache, H. (2021). Adhesion Behavior of *Escherichia coli* Strains on Glass: Role of Cell Surface Qualitative and Quantitative Hydrophobicity in Their Attachment Ability. International Journal of Microbiology, 2021, 5580274.

Eliakim-Raz, N., Lador, A., Leibovici-Weissman, Y., Elbaz, M., Paul, M., and Leibovici, L. (2015). Efficacy and safety of chloramphenicol: joining the revival of old antibiotics? Systematic review and meta-analysis of randomized controlled trials. Journal of Antimicrobial Chemotherapy, 70(4), 979-996.

Escher, M., Scavia, G., Morabito, S., Tozzoli, R., Maugliani, A., Cantoni, S., Fracchia, S., Bettati, A., Casa, R., Gesu, G.P., Torresani, E. and Caprioli, A. (2014). A severe foodborne outbreak of diarrhea linked to a canteen in Italy caused by enteroinvasive *Escherichia coli*, an uncommon agent. Epidemiology and Infection, 142(12), 2559-2566.

Escherich, T. (1885). Die darmbakterien des neugeborenen und säuglings. Fortschr. Med, 3(515-522), 547-554.

Evans Jr, D. J., and Evans, D. G. (1983). Classification of pathogenic *Escherichia coli* according to serotype and the production of virulence factors, with special reference to colonization-factor antigens. Reviews of Infectious Diseases, 5(4), S692-S701.

Evans Jr, D. J., Evans, D. G., and Gorbach, S. L. (1973). Production of vascular permeability factor by enterotoxigenic *Escherichia coli* isolated from man. Infection and Immunity, 8(5), 725-730.

Ewing, W. H. (1986). Edwards and Ewing's identification of Enterobacteriaceae. Edwards and Ewing's Identification of Enterobacteriaceae., (Edition 4).

Ewing, W. H., and Gravatti, J. L. (1947). Shigella types encountered in the mediterranean area. Journal of Bacteriology, 53(2), 191-195.

Ewing, W. H., Tanner, K. E., and Tatum, H. W. (1956). Investigation of *Escherichia coli* O group 18 serotypes isolated from cases of infantile diarrhea. Public Health Lab, 14, 106-115.

Eybpoosh, S., Mostaan, S., Gouya, M. M., Masoumi-Asl, H., Owlia, P., Eshrati, B, Khorasan, M.R.M.R. and Bouzari, S. (2021). Frequency of five *Escherichia coli* pathotypes in Iranian adults and children with acute diarrhea. PloS One, 16(2), e0245470.

Fakruddin, M. D., Mazumdar, R. M., Chowdhury, A., and Mannan, K. S. B. (2012). Comparative analysis of virulence factors of *Escherichia coli* from non-enteric infections. Journal of Medical Sciences, 12(2), 37-44.

Fakruddin, M., Mazumdar, R. M., Chowdhury, A., and Mannan, K. S. B. (2013). A preliminary study on virulence factors and antimicrobial resistance in extra-intestinal pathogenic *Escherichia coli* (ExPEC) in Bangladesh. Indian Journal of Medical Research, 137(5), 988-90.

Falgenhauer, L., Imirzalioglu, C., Oppong, K., Akenten, C. W., Hogan, B., Krumkamp, R., Poppert, S., Levermann, V., Schwengers, O., Sarpong, N., Owusu-Dabo, E., May, J. and Eibach, D. (2019). Detection and characterization of ESBL-producing *Escherichia coli* from humans and poultry in Ghana. Frontiers in Microbiology, 9, 3358.

Falkow, S. (1988). Molecular Koch's postulates applied to microbial pathogenicity. Reviews of Infectious Diseases, S274-S276.

Fan, D., and Fang, Q. (2021). Siderophores for medical applications: Imaging, sensors, and therapeutics. International Journal of Pharmaceutics, 597, 120306.

FAO and WHO. (2018). Shiga toxin-producing *Escherichia coli* (STEC) and food: attribution, characterization, and monitoring . Report ISSN 1726-5274 (Vol. 19). World Health Organization.

Farfan M.J., Torres A.G. (2012). Molecular mechanisms that mediate colonization of Shiga toxin-producing *Escherichia coli* strains. Infection Immunity, 80, 903-913.

Ferens W.A. and Hovde C.J. (2011) *Escherichia coli* O157:H7: animal reservoir and sources of human infection. Foodborne Pathogen Diseases, 8, 465-487.

Fernandez-Hidalgo, N., and Almirante, B. (2014). Antibiotic-lock therapy: a clinical viewpoint. Expert Review of Anti-infective Therapy, 12(1), 117-129.

Fernandez-Martinez, M., Ruiz del Castillo, B., Lecea-Cuello, M. J., Rodriguez-Bano, J., Pascual, A., Martínez-Martinez, L., and Spanish Network for the Research in

Infectious Diseases (REIPI) and the Spanish Group for Nosocomial Infections (GEIH). (2018). The prevalence of aminoglycoside-modifying enzymes in *Escherichia coli* and Klebsiella pneumoniae producing extended spectrum β-lactamases collected in two multicenter studies in Spain. Microbial Drug Resistance, 24(4), 367-376.

Fetherston, J. D., Kirillina, O., Bobrov, A. G., Paulley, J. T., and Perry, R. D. (2010). The yersiniabactin transport system is critical for the pathogenesis of bubonic and pneumonic plague. Infection and immunity, 78(5), 2045-2052.

Finlay, B. B., and Falkow, S. (1997). Common themes in microbial pathogenicity revisited. Microbiology and Molecular Biology Reviews, 61(2), 136-169.

Flemming, H. C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., and Kjelleberg, S. (2016). Biofilms: an emergent form of bacterial life. Nature Reviews Microbiology, 14(9), 563-575.

Formal, S. B., and Hornick, R. B. (1978). Invasive *Escherichia coli*. Journal of Infectious Diseases, 137(5), 641-644.

Foster, M. A., Iqbal, J., Zhang, C., McHenry, R., Cleveland, B. E., Romero-Herazo, Y., *et al.* (2015). Enteropathogenic and enteroaggregative *E. coli* in stools of children with acute gastroenteritis in Davidson County, Tennessee. Diagnostic Microbiology and Infectious Disease, 83(3), 319-324.

Foxman B. (2010). The epidemiology of urinary tract infection. Nat Rev Urol,7, 653-660.

Foxman, B., Zhang, L., Palin, K., Tallman, P., and Marrs, C. F. (1995). Bacterial virulence characteristics of *Escherichia coli* isolates from first-time urinary tract infection. Journal of Infectious Diseases, 171(6), 1514-1521.

Frank, C., Werber, D., Cramer, J. P., Askar, M., Faber, M., an der Heiden, M., *et al.* (2011). Epidemic profile of Shiga-toxin-producing *Escherichia coli* O104:H4 outbreak in Germany. New England Journal of Medicine. 365, 1771–1780.

Franson, T. R., Sheth, N. K., Rose, H. D., and Sohnle, P. G. (1984). Scanning electron microscopy of bacteria adherent to intravascular catheters. Journal of Clinical Microbiology, 20(3), 500-505.

Freeman, D. J., Falkiner, F. R., and Keane, C. T. (1989). New method for detecting slime production by coagulase negative staphylococci. Journal of Clinical Pathology, 42(8), 872-874.

Freestone, P. P., Haigh, R. D., Williams, P. H., and Lyte, M. (2003). Involvement of enterobactin in norepinephrine-mediated iron supply from transferrin to enterohaemorrhagic *Escherichia coli*. FEMS Microbiology Letters, 222(1), 39-43.

Fujihara, S., Arikawa, K., Aota, T., Tanaka, H., Nakamura, H., Wada, T., Hase, A., and Nishikawa, Y. (2009). The prevalence and properties of diarrheagenic *Escherichia coli* among healthy individuals in Osaka City, Japan. Japanese Journal of Infectious Diseases, 62(4), 318-323.

Funalleras, G., Fernandez-Hidalgo, N., Borrego, A., Almirante, B., Planes, A. M., Rodriguez, D., Ruiz, I., and Pahissa, A. (2011). Effectiveness of antibiotic-lock therapy for long-term catheter-related bacteremia due to Gram-negative bacilli: a prospective observational study. Clinical Infectious Diseases, 53(9), e129-e132.

Furyk, J. S., Swann, O., and Molyneux, E. (2011). Systematic review: neonatal meningitis in the developing world. Tropical Medicine and International Health, 16(6), 672-679.

Fux, C. A., Costerton, J. W., Stewart, P. S., and Stoodley, P. (2005). Survival strategies of infectious biofilms. Trends in Microbiology, 13(1), 34-40.

Gad, G. F., Mohamed, H. A., and Ashour, H. M. (2011). Aminoglycoside resistance rates, phenotypes, and mechanisms of Gram-negative bacteria from infected patients in upper Egypt. PloS One, 6(2), e17224.

Gajdacs, M., Abrók, M., Lazar, A., and Burian, K. (2019). Comparative epidemiology and resistance trends of common urinary pathogens in a tertiary-care hospital: a 10-year surveillance study. Medicina, 55(7), 356.

Galie, S., Garcia-Gutierrez, C., Miguelez, E. M., Villar, C. J., and Lombó, F. (2018). Biofilms in the food industry: health aspects and control methods. Frontiers in Microbiology, 9, 898.

Gandra, S., Tseng, K. K., Arora, A., Bhowmik, B., Robinson, M. L., Panigrahi, B., Laxminarayan, R., and Klein, E. Y. (2019). The Mortality Burden of Multidrug-resistant Pathogens in India: A Retrospective, Observational Study. Clinical Infectious Diseases, 69(4), 563–570.

Garcia, M. I., Gounon, P., Courcoux, P., Labigne, A., and Le Bouguenec, C. (1996). The afimbrial adhesive sheath encoded by the afa-3 gene cluster of pathogenic *Escherichia coli* is composed of two adhesins. Molecular Microbiology, 19(4), 683-693.

Garcia, P., Benitez, R., Lam, M., Salinas, A. M., Wirth, H., Espinoza, C., Garay, T., Depix, M.S., Labarca, J., and Guzman, A. M. (2004). Coagulase-negative staphylococci: clinical, microbiological and molecular features to predict true bacteraemia. Journal of Medical Microbiology, 53(1), 67-72.

Gaschignard, J., Levy, C., Romain, O., Cohen, R., Bingen, E., Aujard, Y., and Boileau, P. (2011). Neonatal Bacterial Meningitis: 444 Cases in 7 Years. The Pediatric Infectious Disease Journal, 30(3), 212–217.

Gashe, F., Mulisa, E., Mekonnen, M., and Zeleke, G. (2018). Antimicrobial Resistance Profile of Different Clinical Isolates against Third-Generation Cephalosporins. Journal of Pharmaceutics, 2018, 5070742-5070742.

Gautam, V., Thakur, A., Sharma, M., Singh, A., Bansal, S., Sharma, A., Kapil, A., Das, B. K., Sistla, S., Parija, S.C., Veeraraghavan, B., Prakash, J. A. J., Walia, K., Ohri, V. C., and Ray, P. (2019). Molecular characterization of extended-spectrum β-lactamases among clinical isolates of *Escherichia coli* and Klebsiella pneumoniae: a multi-centric study from tertiary care hospitals in India. Indian Journal of Medical Research, 149(2), 208-215.

Gayathree, N. R. R., Parvathi, T., and Rao, P. A. (2021). Comparative evaluation of methods for detection of Biofilm formation in urinary catheter tips and their antibiogram. Journal of Medical Science and Clinical Research. 9 (1) , 25-31.

George D.B. and Manges A.R. (2010). A systematic review of outbreak and non-outbreak studies of extraintestinal pathogenic *Escherichia coli* causing community-acquired infections. Epidemiology and Infections, 138, 1679-1690.

Gerner, R., Hossain, S., Siada, K., Sargun, A., Neumann, W., Nolan, E., and Raffatellu, M. (2021). Targeting siderophores to reduce colonization of adherent invasive *E. coli* in Crohn's disease. Gastroenterology, 160(3), S45.

Ghosh, B., and Mukherjee, M. (2016). Emergence of co-production of plasmid-mediated AmpC beta-lactamase and ESBL in cefoxitin-resistant uropathogenic *Escherichia coli*. European Journal of Clinical Microbiology and Infectious Diseases, 35(9), 1449-1454.

Ghosh, P. K., and Ali, A. (2010). Isolation of atypical enteropathogenic *Escherichia coli* from children with and without diarrhea in Delhi and the National Capital Region, India. Journal of Medical Microbiology, 59(10), 1156-1162.

Ghosh, S. K., Bera, T., and Chakrabarty, A. M. (2020). Microbial siderophore–A boon to agricultural sciences. Biological Control, 144, 104214.

Gianella RA (1976). Suckling mouse model for detection of heat stable *Escherichia coli* enterotoxin: characteristics of the model. Infection and Immunity, 14(1), 95–99.

Giannella, R. A., Drake, K. W., and Luttrell, M. (1981). Development of a radioimmunoassay for *Escherichia coli* heat-stable enterotoxin: comparison with the suckling mouse bioassay. Infection and Immunity, 33(1), 186-192.

Giaouris, E., Chapot-Chartier, M. P., and Briandet, R. (2009). Surface physicochemical analysis of natural Lactococcus lactis strains reveals the existence of hydrophobic and low charged strains with altered adhesive properties. International Journal of Food Microbiology, 131(1), 2-9.

Giron J. A., Jones T., Millan Velasco F., Castro Munoz E., Zarate L., Fry J., Frankel G., Moseley S. L., Baudry B. and Kaper J. B. (1991). Diffuse-adhering *Escherichia coli* (DAEC) as a putative cause of diarrhea in Mayan children in Mexico. Journal of Infectious Diseases, 16, 507–513.

Golia, S., Hittinahalli, V., Karjigi, S. K., and Reddy, K. M. (2012). Correlation between biofilm formation of uropathogenic *Escherichia coli* and its antibiotic resistance pattern. Journal of Evolution of Medical and Dental Sciences, 1(3), 166-175.

Gomes, T. A., Elias, W. P., Scaletsky, I. C., Guth, B. E., Rodrigues, J. F., Piazza, R. M., ... and Martinez, M. B. (2016). Diarrheagenic *Escherichia coli*. Brazilian Journal of Microbiology, 47, 3-30.

Gomes, T. A., Toledo, M. R., Trabulsi, L. R., Wood, P. K., and Morris Jr, J. G. (1987). DNA probes for identification of enteroinvasive *Escherichia coli*. Journal of Clinical Microbiology, 25(10), 2025-2027.

Gomez-Duarte, O. G., and Kaper, J. B. (1995). A plasmid-encoded regulatory region activates chromosomal eaeA expression in enteropathogenic *Escherichia coli*. Infection and Immunity, 63(5), 1767-1776.

Gonzales, L., Joffre, E., Rivera, R., Sjoling, Å., Svennerholm, A. M., and Iniguez, V. (2013). The prevalence, seasonality and severity of disease caused by pathogenic *Escherichia coli* in children with diarrhea in Bolivia. Journal of Medical Microbiology, 62(11), 1697-1706.

Gonzalez, A. G. M., Cerqueira, A. M. F., Guth, B. E. C., Coutinho, C. A., Liberal, M. H. T., Souza, R. M., and Andrade, J. R. C. (2016). Serotypes, virulence markers and cell invasion ability of Shiga toxin-producing *Escherichia coli* strains isolated from healthy dairy cattle. Journal of Applied Microbiology, 121(4), 1130-1143.

Gonzalez, E. A., Blanco, J., Baloda, S. B., and Wadstrom, T. (1988). Relative cell surface hydrophobicity of *Escherichia coli* strains with various recognized

fimbrial antigens and without recognized fimbriae. Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene. Series A: Medical Microbiology, Infectious Diseases, Virology, Parasitology, 269(2), 218-236.

Gonzalez, M. J., Robino, L., Iribarnegaray, V., Zunino, P., and Scavone, P. (2017). Effect of different antibiotics on biofilm produced by uropathogenic *Escherichia coli* isolated from children with urinary tract infection. Pathogens and Disease, 75(4). June 2017, ftx053

Gould, L. H., Mody, R. K., Ong, K. L., Clogher, P., Cronquist, A. B., Garman, K. N., Lathrop, S., Medus, C., Spina, L. N., Webb, T. H., White, P. L., Wymore, K., Gierke, R. E., Mahon, B. E. and Griffin, for the Emerging Infections Program FoodNet Working Group, P. M. (2013). Increased recognition of non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States during 2000–2010: epidemiologic features and comparison with *E. coli* O157 infections. Foodborne Pathogens and Disease, 10(5), 453-460.

Goulter, R. M., Gentle, I. R., and Dykes, G. A. (2009). Issues in determining factors influencing bacterial attachment: a review using the attachment of *Escherichia coli* to abiotic surfaces as an example. Letters in Applied Microbiology, 49(1), 1-7.

Gowtham, R., and Gopinath, P. (2016). Detection of cell surface hydrophobicity among uropathogenic isolates of *Escherichia coli*. Research Journal of Pharmacy and Technology, 9(11), 1883-1885.

Goyal, A., Prasad, K. N., Prasad, A., Gupta, S., Ghoshal, U., and Ayyagari, A. (2009). Extended spectrum beta-lactamases in *Escherichia coli* and Klebsiella pneumoniae and associated risk factors. Indian Journal of Medical Research, 129(6), 695-700.

Grass, G. (2006). Iron transport in *Escherichia coli*: all has not been said and done. Biometals, 19(2), 159-172.

Growther, L., and Yasotha, M. (2016). Isolation of Uropathogenic *Escherichia coli* and Study of Its Virulence Traits. International Journal of Medical Science and Clinical Inventions. 3 (11), 2393-2396.

Guerrant, R. L., Hughes, J. M., Lima, N. L., and Crane, J. (1990). Diarrhea in developed and developing countries: magnitude, special settings, and etiologies. Reviews of Infectious Diseases, 12(1), S41-S50.

Gunzberg S. T., Chang B. J., Elliott S. J., Burke V. and Gracey M. (1993). Diffuse and enteroaggregative patterns of adherence of enteric *Escherichia coli* isolated from aboriginal children from the Kimberley region of Western Australia. Journal of Infectious Diseases, 167,755–758.

Gupta, D., Sharma, M., Sarkar, S., Thapa, B. R., and Chakraborti, A. (2016). Virulence determinants in enteroaggregative *Escherichia coli* from North India and their interaction in in-vitro organ culture system. FEMS Microbiology letters, 363(17). fnw189,

Gupta, K., Sahm, D. F., Mayfield, D., and Stamm, W. E. (2001). Antimicrobial resistance among uropathogens that cause community-acquired urinary tract infections in women: a nationwide analysis. Clinical Infectious Diseases, 33(1), 89-94.

Gyles, C. L. (2007). Shiga toxin-producing *Escherichia coli*: an overview. Journal of Animal Science, 85(13), E45-E62.

Halabi, M. K., Lahlou, F. A., Diawara, I., El Adouzi, Y., Marnaoui, R., Benmessaoud, R., and Smyej, I. (2021). Antibiotic Resistance Pattern of Extended Spectrum Beta Lactamase Producing *Escherichia coli* Isolated From Patients With Urinary Tract Infection in Morocco. Frontiers in Cellular and Infection Microbiology, 11. 720701

Hall-Stoodley, L., Costerton, J. W., and Stoodley, P. (2004). Bacterial biofilms: from the natural environment to infectious diseases. Nature Reviews Microbiology, 2(2), 95-108.

Hamadi, F., Latrache, H., Zahir, H., Elghmari, A., Timinouni, M., and Ellouali, M. (2008). The relation between *Escherichia coli* surface functional groups' composition and their physicochemical properties. Brazilian Journal of Microbiology, 39(1), 10-15.

Hara, G. L., Gould, I., Endimiani, A., Pardo, P. R., Daikos, G., Hsueh, P. R., Mehtar, S., Petrikkos, G., Casellas, J. M., Daciuk, L., Paciel, D., Novelli, A., Saginur, R., Pryluka, D., Medina J., and Savio, E. (2013). Detection, treatment, and prevention of carbapenemase-producing Enterobacteriaceae: recommendations from an International Working Group. Journal of Chemotherapy, 25(3), 129-140.

Harrington, S. M., Dudley, E. G., and Nataro, J. P. (2006). Pathogenesis of enteroaggregative *Escherichia coli* infection. FEMS Microbiology Letters, 254(1), 12-18.

Harris, J. R., Wachsmuth, I. K., Davis, B. R., and Cohen, M. L. (1982). High-molecular-weight plasmid correlates with *Escherichia coli* enteroinvasiveness. Infection and Immunity, 37(3), 1295-1298.

Hassan, A., Usman, J., Kaleem, F., Omair, M., Khalid, A., and Iqbal, M. (2011). Evaluation of different detection methods of biofilm formation in the clinical isolates. Brazilian Journal of Infectious Diseases, 15(4), 305-311.

Havelaar, A.H., Kirk, M. D., Torgerson, P. R., Gibb, H. J., Hald, T., Lake R. J., Praet, N. Bellinger, D. C., de Silva, N. R., Gargouri, N., Speybroeck, N.,Cawthorne, A., Mathers, C., Stein, C., Angulo, F. J., and Devleesschauwr B.. (2015) World Health Organization Global Estimates and Regional Comparisons of the Burden of Foodborne Disease in 2010. PLoS Med 12(12), e1001923.

Hebbelstrup Jensen, B., Olsen, K. E., Struve, C., Krogfelt, K. A., and Petersen, A. M. (2014). Epidemiology and clinical manifestations of enteroaggregative *Escherichia coli*. Clinical Microbiology Reviews, 27(3), 614-630.

Heilmann, C. (2011). Adhesion mechanisms of staphylococci. Bacterial Adhesion, 105-123.

Henderson, I. R., Czeczulin, J., Eslava, C., Noriega, F., and Nataro, J. P. (1999). Characterization of Pic, a secreted protease of Shigella flexneri and enteroaggregative *Escherichia coli*. Infection and Immunity, 67(11), 5587-5596.

Hernandes, R. T., Elias, W. P., Vieira, M. A., and Gomes, T. A. (2009). An overview of atypical enteropathogenic *Escherichia coli*. FEMS Microbiology Letters, 297(2), 137-149.

Hicks, S., Candy, D. C., and Phillips, A. D. (1996). Adhesion of enteroaggregative *Escherichia coli* to pediatric intestinal mucosa in vitro. Infection and Immunity, 64(11), 4751-4760.

Hiremath, M. B., and Lava, R. (2020). Study of virulence factors and antibiotic susceptibility pattern of extraintestinal pathogenic *Escherichia coli*. Indian Journal of Microbiology Research. 7(4), 330-334.

Hoiby, N., Bjarnsholt, T., Moser, C., Jensen, P. O., Kolpen, M., Qvist, T. Aanæs, K.; Pressler, T.; Skov, M. and Ciofu, O. (2017). Diagnosis of biofilm infections in cystic fibrosis patients. APMIS, 125(4), 339-343.

Holden, V. I., and Bachman, M. A. (2015). Diverging roles of bacterial siderophores during infection. Metallomics, 7(6), 986-995.

Hunt J.M. (2010). Shiga toxin-producing *Escherichia coli* (STEC). Clinical Laboratory Medicine, 30, 21-45.

Hussain A., Ewers C., Nandanwar N., Guenther S., Jadhav S., Wieler L.H. and Ahmed N. (2012). Multiresistant Uropathogenic *Escherichia coli* from a Region in India Where Urinary Tract Infections Are Endemic: Genotypic and Phenotypic Characteristics of Sequence Type 131 Isolates of the CTX-M-15 Extended-Spectrum-β-Lactamase-Producing Lineage. Antimicrobial Agents and Chemotherapy; 56(12), 6358–6365.

Ibrahim, M. E., Bilal, N. E., and Hamid, M. E. (2012). Increased multi-drug-resistant *Escherichia coli* from hospitals in Khartoum state, Sudan. African Health Sciences, 12(3), 368-375.

Iguchi, A., von Mentzer, A., Kikuchi, T., and Thomson, N. R. (2017). An untypeable enterotoxigenic *Escherichia coli* represents one of the dominant types causing human disease. Microbial Genomics, 3(9), e000121.

Isac, R., Basaca, D. G., Olariu, I. C., Stroescu, R. F., Ardelean, A. M., Steflea, R. M., Gafencu, M., Chirita-Emandi, A., Bagiu, I. C., Horhat, F. G., Valcanescu, D., Ionescu, D. and Doros, G. (2021). Antibiotic resistance patterns of uropathogens causing urinary tract infections in children with congenital anomalies of kidney and urinary tract. Children, 8(7), 585.

Iseri, L., Apan, T. Z., Aksoy, A., Koç, F., Göçmen, J. S., and Nuristani, D. (2011). The prevalence of enterotoxigenic *E. coli* isolated from the stools of children aged 0-10 years with diarrhea in mid-Anatolia region, Turkey. Brazilian Journal of Microbiology, 42, 243-247.

Ishida, H., Ishida, Y., Kurosaka, Y., Otani, T., Sato, K., and Kobayashi, H. (1998). In vitro and in vivo activities of levofloxacin against biofilm-producing Pseudomonas aeruginosa. Antimicrobial Agents and Chemotherapy, 42(7), 1641-1645.

Isidean S.D., Riddle M.S., Savarino S.J. and Porter C.K. (2011). A systematic review of ETEC epidemiology focusing on colonization factor and toxin expression. Vaccine.; 29, 6167-6178.

Jackson, L. A., Benson, P., Neuzil, K. M., Grandjean, M., and Marino, J. L. (2005). Burden of community-onset *Escherichia coli* bacteremia in seniors. Journal of Infectious Diseases, 191(9), 1523-1529.

Jacoby G.A. (2009). AmpC beta-lactamases. Clinical Microbiology Review, 22, 161-182.

Jafari, F., Garcia-Gil, L. J., Salmanzadeh-Ahrabi, S., Shokrzadeh, L., Aslani, M. M., Pourhoseingholi, M. A., Derakhshan, F., and Zali, M. R. (2009). Diagnosis and the prevalence of enteropathogenic bacteria in children less than 5 years of age

with acute diarrhea in Tehran children's hospitals. Journal of Infection, 58(1), 21-27.

Jafari, F., Shokrzadeh, L., Hamidian, M., Salmanzadeh-Ahrabi, S., and Zali, M. R. (2008). Acute diarrhea due to enteropathogenic bacteria in patients at hospitals in Tehran. Japanese Journal of Infectious Diseases, 61(4), 269-73.

Jaggi, N., Chatterjee, N., Singh, V., Giri, S. K., Dwivedi, P., Panwar, R., and Sharma, A. P. (2019). Carbapenem resistance in *Escherichia coli* and Klebsiella pneumoniae among Indian and international patients in North India. Acta Microbiologica et Immunologica Hungarica, 66(3), 367-376.

Jaloob Aljanaby, A. A., and Hashim Alfaham, Q. M. (2017). Phenotypic and molecular characterization of some virulence factors in multidrug resistance *Escherichia coli* isolated from different clinical infections in Iraq. American Journal of Biochemistry and Molecular Biology, 7, 65-78.

Jamal, M., Ahmad, W., Andleeb, S., Jalil, F., Imran, M., Nawaz, M. A., Hussain, T., Ali, M., Rafiq, M., and Kamil, M. A. (2018). Bacterial biofilm and associated infections. Journal of the Chinese Medical Association, 81(1), 7-11.

Jandhyala D.M., Vanguri V., Boll E.J., Lai Y., McCormick B.A. and Leong J.M. (2013). Shiga toxin-producing *Escherichia coli* O104:H4: an emerging pathogen with enhanced virulence. Infectious Disesae Clinics, 27: 631-649.

Jarquin, C., Morales, O., McCracken, J. P., Lopez, M. R., Lopez, B., Reyes, L., Gomez, G.A., Bryan, J.P., Peruski L.F., Parsons, M. B. and Pattabiraman V. (2022). Burden of Diarrheagenic *Escherichia coli* in Santa Rosa, Guatemala in active health-services surveillance during 2008-2009 and 2014-2015. Tropical Medicine and International Health,

available at: https://onlinelibrary.wiley.com/doi/epdf/10.1111/tmi.13735

Jenkins, C., Chart, H., Willshaw, G. A., Cheasty, T., and Smith, H. R. (2006). Genotyping of enteroaggregative *Escherichia coli* and identification of target

genes for the detection of both typical and atypical strains. Diagnostic Microbiology and Infectious Disease, 55(1), 13-19.

Jerse A. E., Yu J., Tall B. D. and Kaper J. .B. (1990). A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells. Proceedings of the National Academy of Sciences of the United States of America, 87(20), 7839–7843.

Joensen, K. G., Tetzschner, A. M., Iguchi, A., Aarestrup, F. M., and Scheutz, F. (2015). Rapid and easy in silico serotyping of *Escherichia coli* isolates by use of whole-genome sequencing data. Journal of Clinical Microbiology, 53(8), 2410-2426.

Johansson Gudjonsdottir, M., Elfvin, A., Hentz, E., Adlerberth, I., Tessin, I., and Trollfors, B. (2019). Changes in incidence and etiology of early-onset neonatal infections 1997–2017–a retrospective cohort study in western Sweden. BMC Pediatrics, 19(1), 1-10.

Johnson A.P. and Woodford N. (2013). Global spread of antibiotic resistance: the example of New Delhi metallo-beta-lactamase (NDM)-mediated carbapenem resistance. Journal of Medical Microbiology, 62, 499-513.

Johnson, J. R., and Russo, T. A. (2002). Extraintestinal pathogenic *Escherichia coli*: “the other bad E coli”. Journal of Laboratory and Clinical Medicine, 139(3), 155-162.

Juhna, T., Birzniece, D., Larsson, S., Zulenkovs, D., Sharipo, A., Azevedo, N. F., Menard-Szczebara, F., Castagnet, S., Feliers, C., and Keevil, C. W. (2007). Detection of *Escherichia coli* in biofilms from pipe samples and coupons in drinking water distribution networks. Applied and Environmental Microbiology, 73(22), 7456-7464.

Kackar, S., Suman, E., and Kotian, M. S. (2017). Bacterial and fungal biofilm formation on contact lenses and their susceptibility to lens care solutions. Indian Journal of Medical Microbiology, 35(1), 80-84.

Kaira, S., and Pai, C. (2018). Study of uropathogenic *Escherichia coli* with special reference to its virulence factors. International Journal Community Medicine Public Health, 1(1), 177-181.

Kammili, N., Rani, M., Styczynski, A., Latha, M., Pavuluri, P. R., Reddy, V., and Alsan, M. (2020). Plasmid-mediated antibiotic resistance among uropathogens in primigravid women—Hyderabad, India. PloS One, 15(5), e0232710.

Kaper JB, Nataro JP, Mobley HL (2004). Pathogenic *Escherichia coli*. Nature Reviews Microbiology, 2, 123-140.

Kaper, J. B., and O'Brien, A. D. (2014). Overview and historical perspectives. Microbiology Spectrum, 2(6), 2-6.

Kar, D., Bandyopadhyay, S., Bhattacharyya, D., Samanta, I., Mahanti, A., Nanda, P. K., Mondal, B., Dandpat, P., Das, A. K., Dutta, T. K., Bandyopadhyay, S., and Singh, R. K. (2015). Molecular and phylogenetic characterization of multidrug-resistant extended spectrum beta-lactamase producing *Escherichia coli* isolated from poultry and cattle in Odisha, India. Infection, Genetics and Evolution, 29, 82-90.

Kara, D., Luppens, S. B., and ten Cate, J. M. (2006). Differences between single-and dual-species biofilms of Streptococcus mutans and Veillonella parvula in growth, acidogenicity and susceptibility to chlorhexidine. European Journal of Oral Sciences, 114(1), 58-63.

Karaiskos, I., and Giamarellou, H. (2014). Multidrug-resistant and extensively drug-resistant Gram-negative pathogens: current and emerging therapeutic approaches. Expert Opinion on Pharmacotherapy, 15(10), 1351-1370.

Karch, H., Denamur, E., Dobrindt, U., Finlay, B. B., Hengge, R., Johannes, L., Ron E.Z., Tonjum, T., Sansonetti P.J. and Vicente, M. (2012). The enemy within us: lessons from the 2011 European *Escherichia coli* O104: H4 outbreak. EMBO Molecular Medicine, 4(9), 841-848.

Karigoudar, R. M., Karigoudar, M. H., Wavare, S. M., and Mangalgi, S. S. (2019). Detection of biofilm among uropathogenic *Escherichia coli* and its correlation with antibiotic resistance pattern. Journal of Laboratory Physicians, 11(01), 017-022.

Karmali, M. A. (1989). Infection by verocytotoxin-producing *Escherichia coli*. Clinical Microbiology Reviews, 2(1), 15-38.

Karmali, M. A., Mascarenhas, M., Shen, S., Ziebell, K., Johnson, S., Reid-Smith, R., Issac-Renton, J., Clark, C., Rahn, K., and Kaper, J. B. (2003). Association of genomic O island 122 of *Escherichia coli* EDL 933 with verocytotoxin-producing *Escherichia coli* seropathotypes that are linked to epidemic and/or serious disease. Journal of Clinical Microbiology, 41(11), 4930-4940.

Karmali, M., Petric, M., Steele, B., and Lim, C. (1983). Sporadic cases of haemolytic-uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. The Lancet, 321(8325), 619-620.

Katongole, P., Nalubega, F., Florence, N. C., Asiimwe, B., and Andia, I. (2020). Biofilm formation, antimicrobial susceptibility and virulence genes of Uropathogenic *Escherichia coli* isolated from clinical isolates in Uganda. BMC Infectious Diseases, 20(1), 1-6.

Katouli, M., Brauner, A., Haghighi, L. K., Kaijser, B., Muratov, V., and Mollby, R. (2005). Virulence characteristics of *Escherichia coli* strains causing acute cystitis in young adults in Iran. Journal of Infection, 50(4), 312-321.

Kauffmann, F. (1947). The serology of the coli group. Journal of Immunology, 57(1), 71-100.

Kauffmann, F. (1944). Zur serologie der coli-gruppe. Acta Pathol. Microbiol. Scand, 21, 20-45. cited from: Fratamico, P. M., DebRoy, C., Liu, Y., Needleman, D. S., Baranzoni, G. M., and Feng, P. (2016). Advances in molecular serotyping and subtyping of *Escherichia coli*. Frontiers in Microbiology, 7, 644.

Kaur, P., Chakraborti, A., and Asea, A. (2010). Enteroaggregative *Escherichia coli*: an emerging enteric food borne pathogen. Interdisciplinary Perspectives on Infectious Diseases, 2010, 254159.

Kausar, Y., Chunchanur, S. K., Nadagir, S. D., Halesh, L. H., and Chandrasekhar, M. R. (2009). Virulence factors, serotypes and antimicrobial susceptibility pattern of *Escherichia coli* in urinary tract infections. Al Ameen Journal of Medical Sciences, 2(1), 47-51.

Kaye, K. S., Gupta, V., Mulgirigama, A., Joshi, A. V., Scangarella-Oman, N. E., Yu, K., Ye, G., and Mitrani-Gold, F. S. (2021). Antimicrobial resistance trends in urine *Escherichia coli* isolates from adult and adolescent females in the United States from 2011 to 2019: rising ESBL strains and impact on patient management. Clinical Infectious Diseases, 73(11), 1992-1999.

Keskimäki, M., Mattila, L., Peltola, H., and Siitonen, A. (2000). The prevalence of diarrheagenic *Escherichia coli* in Finns with or without diarrhea during a round-the-world trip. Journal of Clinical Microbiology, 38(12), 4425-4429.

Khan, A., Singh, P., and Srivastava, A. (2018). Synthesis, nature and utility of universal iron chelator–Siderophore: A review. Microbiological Research, 212, 103-111.

Khan, A., Yamasaki, S., Sato, T., Ramamurthy, T., Pal, A., Datta, S., Chowdhury, N. R., Das, S. C., Sikdar, A., Tsukamoto, T., Bhattacharya, S. K., Takeda, Y., and Nair, G. B. (2002). The prevalence and genetic profiling of virulence determinants of non-O157 Shiga toxin-producing *Escherichia coli* isolated from cattle, beef, and humans, Calcutta, India. Emerging Infectious Diseases. 2002; 8(1), 54-62.

Kim KS (2012). Current concepts on the pathogenesis of *Escherichia coli* meningitis: implications for therapy and prevention. Current Opinion in Infectious Diseases, 25, 273-278.

Kim, J. S., Lee, M. S., and Kim, J. H. (2020). Recent updates on outbreaks of Shiga toxin-producing *Escherichia coli* and its potential reservoirs. Frontiers in Cellular and Infection Microbiology, 10, 273.

King, L. A., Nogareda, F., Weill, F. X., Mariani-Kurkdjian, P., Loukiadis, E., Gault, G., Jaurdan-Dasilva, N., Bingen, E., Mace, M., Thevenot, D., Ong, N., Castor, C., Noel, H., Cauteren, D. V., Charron, M., Vaillant, V., Aldabe, B., Goulet, V., Delmas, G., Couturier, E., Strat, Y. L., Combe, C., Delmas, Y., Terrier, F., Vendrely, B., Rolland, P., and de Valk, H. (2012). Outbreak of Shiga toxin–producing *Escherichia coli* O104: H4 associated with organic fenugreek sprouts, France, June 2011. Clinical Infectious Diseases, 54(11), 1588-1594.

Kleanthous, C., Hemmings, A. M., Moore, G. R., and James, R. (1998). Immunity proteins and their specificity for endonuclease colicins: telling right from wrong in protein–protein recognition. Molecular Microbiology, 28(2), 227-233.

Klein, E. Y., Tseng, K. K., Pant, S., and Laxminarayan, R. (2019). Tracking global trends in the effectiveness of antibiotic therapy using the Drug Resistance Index. BMJ Global Health, 4(2), e001315.

Knetsch, M. L., and Koole, L. H. (2011). New strategies in the development of antimicrobial coatings: the example of increasing usage of silver and silver nanoparticles. Polymers, 3(1), 340-366.

Kobayashi, H., Takami, H., Hirayama, H., Kobata, K., Usami, R., and Horikoshi, K. (1999). Outer membrane changes in a toluene-sensitive mutant of toluene-tolerant Pseudomonas putida IH-2000. Journal of Bacteriology, 181(15), 4493-4498.

Kohler C.D. and Dobrindt U. (2011). What defines extraintestinal pathogenic *Escherichia coli*? International Journal of Medical Microbiology, 301:642-647.

Kosek, M., Bern, C., and Guerrant, R. L. (2003). The global burden of diarrheal disease, as estimated from studies published between 1992 and 2000. Bulletin of the World Health Organization, 81, 197-204.

Kotloff, K. L., Nataro, J. P., Blackwelder, W. C., Nasrin, D., Farag, T. H., Panchalingam, S., Wu, Y., Sow, S. O., Sur, D., Breiman, R. F., Faruque, A. S., Zaidi, A. K., Saha, D., Alonso, P. L., Tamboura, B., Sanogo, D., Onwuchekwa, U., Manna, B., Ramamurthy, T., Kanungo, S., Ochieng, J. B., Omore, R., Oundo, J. O., Hossain, A., Das, S. K., Ahmed, S., Qureshi, S., Quadri, F., Adegbola, R. A., Antonio, M., Hossain. M. J., Akinsola, A., Mandomando, I., Nhampossa, T., Acacio, S., Biswas, K., O'Reilly, C. E., Mintz, E. D., Berkeley, L. Y., Muhsen, K., Sommerfelt, H., Robins-Browne, R. M., and Levine, M. M. (2013). Burden and aetiology of diarrheal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. The Lancet, 382(9888), 209-222.

Krasowska, A., and Sigler, K. (2014). How microorganisms use hydrophobicity and what does this mean for human needs?. Frontiers in Cellular and Infection Microbiology, 4, 112.

Kronvall, G. (2010). Antimicrobial resistance 1979–2009 at Karolinska hospital, Sweden: normalized resistance interpretation during a 30-year follow-up on Staphylococcus aureus and *Escherichia coli* resistance development. APMIS, 118(9), 621-639.

Kulkarni, S. R., Peerapur, B. V., and Sailesh, K. S. (2017). Isolation and antibiotic susceptibility pattern of *Escherichia coli* from urinary tract infections in a tertiary care hospital of North Eastern Karnataka. Journal of Natural Science, Biology, and Medicine, 8(2), 176-180.

Kumarasamy K.K., Toleman M.A., Walsh T.R., Bagaria J., Butt F., Balakrishnan R., Chaudhary U., Doumith M., Giske C.G., Irfan S., Krishnan P., Kumar A.V., Maharjan S., Mushtaq S., Noorie T., Paterson D.L., Pearson A., Perry C., Pike R., Rao B., Ray U., Sarma J.B., Sharma M., Sheridan E., Thirunarayan M.A., Turton J., Upadhyay S., Warner M., Welfare W., Livermore D.M. and Woodford N. (2010). Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infectious Diseases, 10, 597-602.

Kurutepe, S., Surucuoglu, S., Sezgin, C., Gazi, H., Gulay, M., and Ozbakkaloglu, B. (2005). Increasing antimicrobial resistance in *Escherichia coli* isolates from community-acquired urinary tract infections during 1998-2003 in Manisa, Turkey. Japanese Journal of Infectious Diseases, 58(3), 159.

Lagerstrom, K. M., and Hadly, E. A. (2021). The under-investigated wild side of *Escherichia coli*: genetic diversity, pathogenicity and antimicrobial resistance in wild animals. Proceedings of the Royal Society B, 288(1948), 20210399.

Lan, R., Alles, M. C., Donohoe, K., Martinez, M. B., and Reeves, P. R. (2004). Molecular evolutionary relationships of enteroinvasive *Escherichia coli* and Shigella spp. Infection and Immunity, 72(9), 5080-5088.

Lanata, C. F., Mendoza, W., and Black, R. E. (2002). Improving diarrhea estimates." Geneva, Switzerland: World Health Organization

Lancaster, L. E., Wintermeyer, W., and Rodnina, M. V. (2007). Colicins and their potential in cancer treatment. Blood Cells, Molecules, and Diseases, 38(1), 15-18.

Lanjewar M., De A.S. and Mathur M.(2010). Diarrheagenic *E. coli* in hospitalized patients: Special reference to Shiga-like toxin producing *Escherichia coli*. Indian Journal of Pathology and Microbiology, 53(1), 75-78.

Lankford, C. E., and Byers, B. R. (1973). Bacterial assimilation of iron. CRC Critical Reviews in Microbiology, 2(3), 273-331.

Lasa Uzcudun, I. (2006). Towards the identification of the common features of bacterial biofilm development. International Microbiology, 2006, 9 (1), 21-28.

Lascols C., Hackel M., Marshall S.H., Hujer A.M., Bouchillon S., Badal R., Hoban D. and Bonomo R.A. (2011). Increasing the prevalence and dissemination of NDM-1 metallo-beta-lactamase in India: data from the SMART study (2009). Journal of Antimicrobial Chemotherapy, 66, 1992-1997.

Lascowski, K. M. S., Guth, B. E. C., Martins, F. H., Rocha, S. P. D., Irino, K., and Pelayo, J. S. (2013). Shiga toxin-producing E scherichia coli in drinking water

supplies of north Parana State, Brazil. Journal of Applied Microbiology, 114(4), 1230-1239.

Laupland K.B., Church D.L., Vidakovich J., Mucenski M. and Pitout J.D. (2008). Community-onset extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli*: importance of international travel. Journal of Infections, 57, 441-448.

Laupland, K. B., and Church, D. L. (2014). Population-based epidemiology and microbiology of community-onset bloodstream infections. Clinical Microbiology Reviews, 27(4), 647-664.

Laxminarayan, R., and Chaudhury, R. R. (2016). Antibiotic resistance in India: drivers and opportunities for action. PLoS medicine, 13(3), e1001974.

Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A. K., Wertheim, H. F., Sumpradit, N., Vlieghe, E., Hara, P. G. L, Gould, I. M., Goossens, H., Greko, C., So, P. A.. D., Bigdeli, M., Tomson, P. G., Woodhouse, W., Ombaka, E., Peralta, P. A. Q., Qamar, F. N., Mir, F., Kariuki, S., Bhutta, P. Z. A., Coates, P. A., Bergstrom, R., Wright, G. D., Brown, E. D. and Cars, P. O. (2013). Antibiotic resistance—the need for global solutions. The Lancet Infectious Diseases, 13(12), 1057-1098.

Lee, D. S., Lee, S. J., and Choe, H. S. (2018). Community-acquired urinary tract infection by *Escherichia coli* in the era of antibiotic resistance. BioMed Research International, 2018. Article ID 7656752

Lee, J. G., Han, D. S., Jo, S. V., Lee, A. R., Park, C. H., Eun, C. S., and Lee, Y. (2019). Characteristics and pathogenic role of adherent-invasive *Escherichia coli* in inflammatory bowel disease: Potential impact on clinical outcomes. PLoS One, 14(4), e0216165.

Lee, J., Oh, C. E., Choi, E. H., and Lee, H. J. (2013). The impact of the increased use of piperacillin/tazobactam on the selection of antibiotic resistance among invasive *Escherichia coli* and Klebsiella pneumoniae isolates. International Journal of Infectious Diseases, 17(8), e638-e643.

Lee, K. K., and Yii, K. C. (1996). A comparison of three methods for assaying hydrophobicity of pathogenic vibrios. Letters in Applied Microbiology, 23(5), 343-346.

Legese, M. H., Weldearegay, G. M., and Asrat, D. (2017). Extended-spectrum beta-lactamase-and carbapenemase-producing Enterobacteriaceae among Ethiopian children. Infection and Drug Resistance, 10, 27-34.

Leotta, G. A., Chinen, I., Epszteyn, S., Miliwebsky, E., Melamed, I. C., Motter, M., Ferrer, M., Marey, E., and Rivas, M. (2005). Validation of a multiplex PCR for detection of Shiga toxin-producing *Escherichia coli*. Revista Argentina de Microbiologia, 37(1), 1-10.

Leriche, V., Briandet, R., and Carpentier, B. (2003). Ecology of mixed biofilms subjected daily to a chlorinated alkaline solution: spatial distribution of bacterial species suggests a protective effect of one species to another. Environmental Microbiology, 5(1), 64-71.

Leverstein-Van Hall M.A., Stuart J.C., Voets G.M., Versteeg D., Tersmette T. and Fluit A.C. (2010). Global spread of New Delhi metallo-beta-lactamase 1. Lancet Infectious Diseases ,10, 830-831.

Levine M.M. (1987). *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohaemorrhagic and enteroadherent. Journal of Infectious Diseases, 115(3), 377-388.

Levine, M. M., and Edelman, R.. (1984). Enteropathogenic *Escherichia coli* of classic serotypes associated with infant diarrhea: epidemiology and pathogenesis. Epidemiological Reviews, 6, 31-51.

Levine, M. M., Ferreccio, C., Prado, V., Cayazzo, M., Abrego, P., Martinez, J., Maggi, L., Baldini, M. M., Martin, W., Maneval, D., Kay, B., Guers, L., Lior, H., Wasserman, S. S., and Nataro, J. P. (1993). Epidemiologic studies of *Escherichia coli* diarrheal infections in a low socioeconomic level peri-urban community in Santiago, Chile. American Journal of Epidemiology, 138(10), 849-869.

Lewis, K. (2005). Persister cells and the riddle of biofilm survival. Biochemistry (Moscow), 70(2), 267-274.

Lievin-Le Moal, V., and Servin, A. L. (2006). The front line of enteric host defense against unwelcome intrusion of harmful microorganisms: mucins, antimicrobial peptides, and microbiota. Clinical Microbiology Reviews, 19(2), 315-337.

Lin, W. P., Huang, Y. S., Wang, J. T., Chen, Y. C., and Chang, S. C. (2019). The prevalence of and risk factor for community-onset third-generation cephalosporin-resistant *Escherichia coli* bacteremia at a medical center in Taiwan. BMC Infectious Diseases, 19(1), 1-11.

Lindemann, P. C., Risberg, K., Wiker, H. G., and Mylvaganam, H. (2012). Aminoglycoside resistance in clinical *Escherichia coli* and Klebsiella pneumoniae isolates from Western Norway. APMIS, 120(6), 495-502.

Lior H. (1996). Classification of *Escherichia coli*. In: Gyles C L, editor. *Escherichia coli* in domestic animals and humans. Wallingford, United Kingdom: CAB International; 1996, 31–72.

Liu, X. W., Sheng, G. P., and Yu, H. Q. (2009). Physicochemical characteristics of microbial granules. Biotechnology Advances, 27(6), 1061-1070.

Liu, Y., Zhu, M., Fu, X., Cai, J., Chen, S., Lin, Y., Jiang, N. Chen, S., and Lin, Z. (2021). *Escherichia coli* Causing Neonatal Meningitis During 2001–2020: A Study in Eastern China. International Journal of General Medicine, 14, 3007-3016.

Ljungh, A., and Wadstrom, T. (1982). Salt aggregation test for measuring cell surface hydrophobicity of urinary *Escherichia coli*. European Journal of Clinical Microbiology, 1(6), 388-393.

Loconsole, D., Giordano, M., Centrone, F., Accogli, M., Casulli, D., De Robertis, A. L., Morea, A., Quarto, M., Parisi, A., Scavia, G., Chironna, M., and Bloody Diarrhea Apulia Working Group. (2020). Epidemiology of Shiga toxin-producing *Escherichia coli* infections in Southern Italy after implementation of symptom-

based surveillance of bloody diarrhea in the pediatric population. International Journal of Environmental Research and Public Health, 17(14), 5137.

Lopez Sastre, J. B., Fernández Colomer, B., Coto Cotallo, G. D., and Ramos Aparicio, A. (2005). Trends in the epidemiology of neonatal sepsis of vertical transmission in the era of group B streptococcal prevention. Acta Paediatrica, 94(4), 451-457.

Lopez-Cerero, L., Navarro, M. D., Bellido, M., Martin-Peña, A., Vinas, L., Cisneros, J. M., Gomes-Langley, S.L., Sanchez-Monteseirin, H., Morales, I., Pascual, A. and Rodríguez-Baño, J. (2014). *Escherichia coli* belonging to the worldwide emerging epidemic clonal group O25b/ST131: risk factors and clinical implications. Journal of Antimicrobial Chemotherapy, 69(3), 809-814.

Louden, B. C., Haarmann, D., and Lynne, A. M. (2011). Use of blue agar CAS assay for siderophore detection. Journal of Microbiology and Biology Education, 12(1), 51-53.

Luna-Gierke, R. E., Griffin, P. M., Gould, L. H., Herman, K., Bopp, C. A., Strockbine, N., and Mody, R. K. (2014). Outbreaks of non-O157 Shiga toxin-producing *Escherichia coli* infection: USA. Epidemiology and Infection, 142(11), 2270-2280.

Ly, M. H., Naïtali-Bouchez, M., Meylheuc, T., Bellon-Fontaine, M. N., Le, T. M., Belin, J. M., and Wache, Y. (2006). Importance of bacterial surface properties to control the stability of emulsions. International Journal of Food Microbiology, 112(1), 26-34.

MacKinnon, M. C., McEwen, S. A., Pearl, D. L., Lyytikäinen, O., Jacobsson, G., Collignon, P., Gregson, D.B., Valiquette, L. and Laupland, K. B. (2021). Increasing incidence and antimicrobial resistance in *Escherichia coli* bloodstream infections: a multinational population-based cohort study. Antimicrobial Resistance and Infection Control, 10(1), 1-10.

Magana, M., Sereti, C., Ioannidis, A., Mitchell, C. A., Ball, A. R., Magiorkinis, E., Chatzipanagiotou, S.; Hamblin, M.R.; Hadjifrangiskou, M.and Tegos, G. P. (2018). Options and limitations in clinical investigation of bacterial biofilms. Clinical Microbiology Reviews, 31(3), e00084-16.

Magill, S. S., Edwards, J. R., Bamberg, W., Beldavs, Z. G., Dumyati, G., Kainer, M. A., Lynfield, R., Maloney, M., McAllister-Hollod, L., Nadle, J., Ray, S.M., Thompson, D. L., Wilson, L.E., and Fridkin, S. K. (2014). Multistate point-the prevalence survey of healthcare-associated infections. New England Journal of Medicine, 370(13), 1198-1208.

Mahalingam, N., Manivannan, B., Khamari, B., Siddaramappa, S., Adak, S., and Bulagonda, E. P. (2018). Detection of antibiotic resistance determinants and their transmissibility among clinically isolated carbapenem-resistant *Escherichia coli* from South India. Medical Principles and Practice, 27(5), 428-435.

Maharjan, G., Khadka, P., Siddhi Shilpakar, G., Chapagain, G., and Dhungana, G. R. (2018). Catheter-Associated Urinary Tract Infection and Obstinate Biofilm Producers. The Canadian journal of infectious diseases and medical microbiology Journal Canadien des maladies Infectieuses et de la Microbiologie Medicale, 2018, 7624857.

Maheshwari, R., Bhutani, N., and Suneja, P. (2019). Screening and characterization of siderophore producing endophytic bacteria from *Cicer arietinum* and *Pisum sativum* plants. Journal of Applied Biology and Biotechnology, 7 (5), 7-14.

Maiya, P. P., Pereira, S. M., Mathan, M., Bhat, P., Albert, M. J., and Baker, S. J. (1977). Aetiology of acute gastroenteritis in infancy and early childhood in southern India. Archives of Disease in Childhood, 52(6), 482-485.

Majowicz, S. E., Scallan, E., Jones-Bitton, A., Sargeant, J. M., Stapleton, J., Angulo, F. J., Yeung, D. H., and Kirk, M. D. (2014). Global incidence of human Shiga toxin-producing *Escherichia coli* infections and deaths: a systematic review and knowledge synthesis. Foodborne Pathogens and Disease, 11(6), 447-455.

Makinen, P. L., and Makinen, K. K. (1994). The Enterococcus faecalis extracellular metalloendopeptidase (EC 3.4. 24.30; coccolysin) inactivates human endothelin at bonds involving hydrophobic amino acid residues. Biochemical and Biophysical Research Communications, 200 (2), 981-985.

Makinen, P. L., Clewell, D. B., An, F., and Makinen, K. K. (1989). Purification and substrate specificity of a strongly hydrophobic extracellular metalloendopeptidase ("gelatinase") from Streptococcus faecalis (strain 0G1-10). Journal of Biological Chemistry, 264(6), 3325-3334.

Maksimova, Y. G. (2014). Microbial biofilms in biotechnological processes. Applied Biochemistry and Microbiology, 50(8), 750-760.

Makvana, S., and Krilov, L. R. (2015). *Escherichia coli* infections. Pediatrics in Review, 36(4), 167-70.

Malchione, M. D., Torres, L. M., Hartley, D. M., Koch, M., and Goodman, J. L. (2019). Carbapenem and colistin resistance in Enterobacteriaceae in Southeast Asia: review and mapping of emerging and overlapping challenges. International Journal of Antimicrobial Agents, 54(4), 381-399.

Malekzadegan, Y., Khashei, R., Ebrahim-Saraie, H. S., and Jahanabadi, Z. (2018). Distribution of virulence genes and their association with antimicrobial resistance among uropathogenic *Escherichia coli* isolates from Iranian patients. BMC Infectious Diseases, 18(1), 1-9.

Malik, S., Rana, J. S., and Nehra, K. (2021). The prevalence and antibiotic susceptibility pattern of uropathogenic *Escherichia coli* strains in Sonipat region of Haryana in India. Biomedical and Biotechnology Research Journal, 5(1), 80-87.

Maloo, A., Fulke, A. B., Mulani, N., Sukumaran, S., and Ram, A. (2017). Pathogenic multiple antimicrobial resistant *Escherichia coli* serotypes in recreational waters of Mumbai, India: a potential public health risk. Environmental Science and Pollution Research, 24(12), 11504-11517.

Manandhar, S., Singh, A., Varma, A., Pandey, S., and Shrivastava, N. (2018). Evaluation of methods to detect in vitro biofilm formation by staphylococcal clinical isolates. BMC Research Notes, 11(1), 1-6.

Mandal, A., Sengupta, A., Kumar, A., Singh, U. K., Jaiswal, A. K., Das, P., and Das, S. (2017). Molecular epidemiology of extended-spectrum β-lactamase–producing *Escherichia coli* pathotypes in diarrheal children from low socioeconomic status communities in Bihar, India: Emergence of the CTX-M type. Infectious Diseases: Research and Treatment, 10, 1178633617739018.

Manolov, D. G. (1959). A New Type of the Genus Shigella-" Shigella 13". Journal of Hygiene, Epidemiology, Microbiology and Immunology, 3(2), 184-90.

Maranhao, H. S., Medeiros, M. C. C., Scaletsky, I. C. A., Fagundes-Neto, U., and Morais, M. B. (2008). The epidemiological and clinical characteristics and nutritional development of infants with acute diarrhea, in north–eastern Brazil. Annals of Tropical Medicine and Parasitology, 102(4), 357-365.

Mare, A. D., Ciurea, C. N., Man, A., Tudor, B., Moldovan, V., Decean, L., and Toma, F. (2021). Enteropathogenic *Escherichia coli*—A Summary of the Literature. Gastroenterology Insights, 12(1), 28-40.

Marier, R., Wells, J., Swanson, R., Callahan, W., and Mehlman, I. (1973). An outbreak of enteropathogenic *Escherichia coli* foodborne disease traced to imported French cheese. The Lancet, 302(7842), 1376-1378.

Marmot M. (2006). Health in an unequal world. The Lancet, 368, 2081–2094.

Marrie, T. J., Nelligan, J., and Costerton, J. W. (1982). A scanning and transmission electron microscopic study of an infected endocardial pacemaker lead. Circulation, 66(6), 1339-1341.

Martinez-Medina, M., Mora, A., Blanco, M., Lopez, C., Alonso, M. P., Bonacorsi, S., Nicolas-Chanoine, M., Darfeuille-Michaud, A., Garcia-Gil, J., and Blanco, J. (2009). Similarity and divergence among adherent-invasive *Escherichia coli* and

extraintestinal pathogenic *E. coli* strains. Journal of Clinical Microbiology, 47(12), 3968-3979.

Martinez-Medina, M., Naves, P., Blanco, J., Aldeguer, X., Blanco, J. E., Blanco, M. Ponte, C.; Soriano, F.; Darfeuille-Michaud, A., and Garcia-Gil, L. J. (2009). Biofilm formation as a novel phenotypic feature of adherent-invasive *Escherichia coli* (AIEC). BMC Microbiology, 9(1), 1-16.

Mathewson, J. J., Salameh, B. M., DuPont, H. L., Jiang, Z. D., Nelson, A. C., Arduino, R., Smith, M.A., and Masozera, N. (1998). HEp-2 cell-adherent *Escherichia coli* and intestinal secretory immune response to human immunodeficiency virus (HIV) in outpatients with HIV-associated diarrhea. Clinical Diagnostic Laboratory Immunology, 5(1), 87-90.

Mathur, T., Singhal, S., Khan, S., Upadhyay, D. J., Fatma, T., and Rattan, A. (2006). Detection of biofilm formation among the clinical isolates of staphylococci: an evaluation of three different screening methods. Indian Journal of Medical Microbiology, 24(1), 25-29.

Matsushita, S., Yamada, S., Kai, A., and Kudoh, Y. (1993). Invasive strains of *Escherichia coli* belonging to serotype O121: NM. Journal of Clinical Microbiology, 31(11), 3034-3035.

McDade, J. J., and Weaver, R. H. (1959). Rapid methods for the detection of gelatin hydrolysis. Journal of Bacteriology, 77(1), 60–64.

McEwen, S. A., and Collignon, P. J. (2018). Antimicrobial resistance: a one health perspective. Microbiology Spectrum, 6(2), 6-2.

McGough, S. F., MacFadden, D. R., Hattab, M. W., Mølbak, K., and Santillana, M. (2020). Rates of increase of antibiotic resistance and ambient temperature in Europe: a cross-national analysis of 28 countries between 2000 and 2016. Eurosurveillance, 25(45), 1900414.

McKellar, Q. A., Sanchez Bruni, S. F., and Jones, D. G. (2004). Pharmacokinetic/pharmacodynamic relationships of antimicrobial drugs used in

veterinary medicine. Journal of Veterinary Pharmacology and Therapeutics, 27(6), 503-514.

McRose, D. L., Seyedsayamdost, M. R., and Morel, F. M. (2018). Multiple siderophores: bug or feature? Journal of Biological Inorganic Chemistry, 23(7), 983-993.

Mehrishi, P., Faujdar, S. S., Kumar, S., Solanki, S., and Sharma, A. (2019). Antibiotic susceptibility profile of uropathogens in rural population of Himachal Pradesh, India: Where We are heading?. Biomedical and Biotechnology Research Journal, 3(3), 171-175.

Melton-Celsa, A. R. (2014). Shiga toxin (Stx) classification, structure, and function. Microbiology Spectrum, 2(4), 2-4.

Mermel, L. A., Allon, M., Bouza, E., Craven, D. E., Flynn, P., O'Grady, N. P., Raad, I. I., Rijinders, B. J. A., Sheretz, and Warren, D. K. (2009). Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 Update by the Infectious Diseases Society of America. Clinical Infectious Diseases, 49(1), 1-45.

Micenkova, L., Bosak, J., Kucera, J., Hrala, M., Dolejsova, T., Sedo, O., Linke, D., Fiser, R., and Smajs, D. (2019). Colicin Z, a structurally and functionally novel colicin type that selectively kills enteroinvasive *Escherichia coli* and Shigella strains. Scientific Reports, 9 (1), 1-12.

Micenkova, L., Bosak, J., Vrba, M., Sevcikova, A., and Smajs, D. (2016). Human extraintestinal pathogenic *Escherichia coli* strains differ in the prevalence of virulence factors, phylogroups, and bacteriocin determinants. BMC Microbiology, 16 (1), 1-8.

Micenkova, L., Staudova, B., Bosak, J., Mikalova, L., Littnerova, S., Vrba, M., Sevcikova, A., Woznicova, V., and Smajs, D. (2014). Bacteriocin-encoding genes and ExPEC virulence determinants are associated in human fecal *Escherichia coli* strains. BMC Microbiology, 14 (1), 1-9.

Michelacci, V., Prosseda, G., Maugliani, A., Tozzoli, R., Sanchez, S., Herrera-Leon, S., Dallman, T., Jenkins, C., Caprioli, A., and Morabito, S. (2016). Characterization of an emergent clone of enteroinvasive *Escherichia coli* circulating in Europe. Clinical Microbiology and Infection, 22(3), 287-e11-287.e19.

Miethke, M., and Marahiel, M. A. (2007). Siderophore-based iron acquisition and pathogen control. Microbiology and Molecular Biology Reviews, 71(3), 413-451.

Minato, Y., Dawadi, S., Kordus, S. L., Sivanandam, A., Aldrich, C. C., and Baughn, A. D. (2018). Mutual potentiation drives synergy between trimethoprim and sulfamethoxazole. Nature Communications, 9(1), 1-7.

Mirani, Z. A., Fatima, A., Urooj, S., Aziz, M., Khan, M. N., and Abbas, T. (2018). Relationship of cell surface hydrophobicity with biofilm formation and growth rate: A study on Pseudomonas aeruginosa, Staphylococcus aureus, and *Escherichia coli*. Iranian Journal of Basic Medical Sciences, 21(7), 760.

Mishra, M. P., Sarangi, R., and Padhy, R. N. (2016). The prevalence of multidrug-resistant uropathogenic bacteria in pediatric patients of a tertiary care hospital in eastern India. Journal of Infection and Public Health, 9(3), 308-314.

Mittal, S., Sharma, M., and Chaudhary, U. (2014). Study of virulence factors of uropathogenic *Escherichia coli* and its antibiotic susceptibility pattern. Indian Journal of Pathology and Microbiology, 57(1), 61-64.

Mittal, S., Sharma, M., and Chaudhary, U. (2015). Biofilm and multidrug resistance in uropathogenic *Escherichia coli*. Pathogens and Global Health, 109(1), 26-29.

Modgil, V., Mahindroo, J., Narayan, C., Kalia, M., Yousuf, M., Shahi, V., Koundal, M., Chaudhary, P., Jain, R., Sandha, K. S., Tanwar, S., Gupta, P., and Taneja, N. (2020). Comparative analysis of virulence determinants, phylogroups, and antibiotic susceptibility patterns of typical versus atypical Enteroaggregative *E. coli* in India. PLoS Neglected Tropical Diseases, 14(11), e0008769.

Mohammadzadeh, M., Goudarzi, H., Dabiri, H., and Fallah, F. (2015). Distribution of Enterotoxigenic *Escherichia coli* among *E. coli* isolates from diarrheal samples referred to educational hospitals in Tehran-Iran. Novelty in Biomedicine, 3(3), 144-147.

Mohanty, M., Kar, P. K., Rout, B., and Behera, T. R. (2021). Diarrhea and Associated Clinical Features in Different Pathotypes of Diarrheagenic *E. coli* Isolated in Children: A Case-Control Study in a Tertiary Care Hospital. Journal of Communicable Diseases, 53(3), 250-258.

Montgomerie, J. Z., Bindereif, A., Neilands, J. B., Kalmanson, G. M., and Guze, L. B. (1984). Association of hydroxamate siderophore (aerobactin) with *Escherichia coli* isolated from patients with bacteremia. Infection and Immunity, 46(3), 835-838.

Moreira, J. M., Simões, M., Melo, L. F., and Mergulhao, F. J. (2015). *Escherichia coli* adhesion to surfaces–a thermodynamic assessment. Colloid and Polymer Science, 293(1), 177-185.

Moseley, S. L., Echeverria, P., Seriwatana, J., Tirapat, C., Chaicumpa, W., Sakuldaipeara, T., and Falkow, S. (1982). Identification of enterotoxigenic *Escherichia coli* by colony hybridization using three enterotoxin gene probes. Journal of Infectious Diseases, 145(6), 863-869.

Moulin-Schouleur, M., Répérant, M., Laurent, S., Brée, A., Mignon-Grasteau, S., Germon, P., Rasschaert, D., and Schouler, C. (2007). Extraintestinal pathogenic *Escherichia coli* strains of avian and human origin: link between phylogenetic relationships and common virulence patterns. Journal of Clinical Microbiology, 45(10), 3366-3376.

Moulin-Schouleur, M., Schouler, C., Tailliez, P., Kao, M. R., Bree, A., Germon, P., Oswald, E., Mainil, J., Blanco, M., and Blanco, J. (2006). Common virulence factors and genetic relationships between O18: K1: H7 *Escherichia coli* isolates of human and avian origin. Journal of Clinical Microbiology, 44(10), 3484-3492.

Mukherjee, M., Basu, S., Mukherjee, S. K., and MajuMder, M. (2013). Multidrug-resistance and extended spectrum beta-lactamase production in uropathogenic *E. coli* which were isolated from hospitalized patients in Kolkata, India. Journal of Clinical and Diagnostic Research: JCDR, 7(3), 449-453.

Muller, D., Benz, I., Liebchen, A., Gallitz, I., Karch, H., and Schmidt, M. A. (2009). Comparative analysis of the locus of enterocyte effacement and its flanking regions. Infection and Immunity, 77(8), 3501-3513.

Muni, S., Dey, S., Krishan Nandan, K., Paul Biswas, P., and Sen, A. (2014). A bacteriological study among patients below five years of age suffering from diarrhea and gastroenteritis with special reference to the responsible serotypes of *Escherichia coli* at a tertiary healthcare hospital in Eastern Bihar. Journal of Evolution of Medical and Dental Scinces, 3, 12592-12601.

Murray, C. J., Aravkin, A. Y., Zheng, P., Abbafati, C., Abbas, K. M., Abbasi-Kangevari, M., ... and Borzouei, S. (2020). Global burden of 87 risk factors in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. The Lancet, 396(10258), 1223-1249.

Musale V., Kale S., Raorane A., Doijad S. and Barbuddhe S. (2014). Pevalance of enterotoxic *E. coli* in raw vegetables and sprouts sold in markets of goa. Indian Journal of Microbiology Research,1(1), 46-51.

Mythreyi, N., Rathina Kumar, S., Sriram, K., Pavithra, M., and Asit, R. G. (2011). Salt Aggregation Test and Hemagglutination Assay for Understanding Bacterial Adherence. Journal of Pharmacy Research, 4(11), 4055-4056.

Nagoba, B., and Vedpathak, D. (2011). Medical applications of siderophores. European Journal of General Medicine, 8(3), 229-235.

Nair, G. B., Ramamurthy, T., Bhattacharya, M. K., Krishnan, T., Ganguly, S., Saha, D. R., Rajendran, K., Manna, B., Ghosh, M., Okamoto, K., and Takeda, Y. (2010). Emerging trends in the etiology of enteric pathogens as evidenced from an

active surveillance of hospitalized diarrheal patients in Kolkata, India. Gut Pathogens, 2(1), 1-13.

Nataloni, M., Pergolini, M., Rescigno, G., and Mocchegiani, R. (2010). Prosthetic valve endocarditis. Journal of Cardiovascular Medicine, 11(12), 869-883.

Natarajan, M., Kumar, D., Mandal, J., Biswal, N., and Stephen, S. (2018). A study of virulence and antimicrobial resistance pattern in diarrheagenic *Escherichia coli* isolated from diarrheal stool specimens from children and adults in a tertiary hospital, Puducherry, India. Journal of Health, Population and Nutrition, 37(1), 1-11.

Nataro J.P.and Kaper J.B. (1998). Diarrheagenic *Escherichia coli*. Clinical Microbiology Review, 11(1), 142-201.

Nataro, J. P., Baldini, M. M., Kaper, J. B., Black, R. E., Bravo, N., and Levine, M. M. (1985). Detection of an adherence factor of enteropathogenic *Escherichia coli* with a DNA probe. Journal of Infectious Diseases, 152(3), 560-565.

Nataro, J. P., Kaper, J. B., Robins-Browne, R. O. Y., Prado, V., Vial, P., and Levine, M. M. (1987). Patterns of adherence of diarrheagenic *Escherichia coli* to HEp-2 cells. The Pediatric Infectious Disease Journal, 6(9), 829-831.

Nataro, J. P., Mai, V., Johnson, J., Blackwelder, W. C., Heimer, R., Tirrell, S., Edberg, S. C., Braden, C. R., Glenn Morris, J., Jr, and Hirshon, J. M. (2006). Diarrheagenic *Escherichia coli* infection in Baltimore, Maryland, and New Haven, Connecticut. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America, 43(4), 402–407.

National Action Plan on Antimicrobial resistance India (2017). Available at: https://ncdc.gov.in/WriteReadData/linkimages/AMR/File645.pdf

Navarro, A., Eslava, C., Hernandez, U., Navarro-Henze, J. L., Aviles, M., Garcia-de la Torre, G., and Cravioto, A. (2003). Antibody responses to *Escherichia coli* O157 and other lipopolysaccharides in healthy children and adults. Clinical and Vaccine Immunology, 10(5), 797-801.

Navarro-Garcia, F., and Elias, W. P. (2011). Autotransporters and virulence of enteroaggregative *E. coli*. Gut Microbes, 2(1), 13-24.

Neilands, J. B. (1981). Iron absorption and transport in microorganisms. Annual Review of Nutrition, 1(1), 27-46.

Neter, E., Westphal, O., Lüderitz, O., Gino, R. M., and Gorzynski, E. A. (1955). Demonstration of Antibodies against Enteropathogenic *Escherichia coli* in Sera of Children of Various Ages. Pediatrics, 16(6), 801-808.

Newitt, S., MacGregor, V., Robbins, V., Bayliss, L., Chattaway, M. A., Dallman, T., Ready, D., Aird, H., Puleston, R., and Hawker, J. (2016). Two linked enteroinvasive *Escherichia coli* outbreaks, Nottingham, UK, June 2014. Emerging Infectious Diseases, 22(7), 1178-1184.

Nguyen T.V , Le P.V., Le C.H. and Weintraub A. (2005). Antibiotic Resistance in Diarrheagenic *Escherichia coli* and *Shigella* Strains Isolated from Children in Hanoi, Vietnam. Antimicrobial Agents and Chemotherapy, 49(2), 816-819.

Nichols, W. W., Dorrington, S. M., Slack, M. P., and Walmsley, H. L. (1988). Inhibition of tobramycin diffusion by binding to alginate. Antimicrobial Agents and Chemotherapy, 32(4), 518-523.

Nicolle L.E. (2013). Urinary tract infection. Critical Care Clinic, 29, 699-715.

Nicolle L.E. (2014). Urinary tract infections in special populations: diabetes, renal transplant, HIV infection, and spinal cord injury. Infectious Disease Clinics, 28, 91-104.

Nielubowicz, G. R., and Mobley, H. L. (2010). Host–pathogen interactions in urinary tract infection. Nature Reviews Urology, 7(8), 430-441.

Niranjan V. and Malini A. (2014). Antimicrobial resistance pattern in *Escherichia coli* causing urinary tract infection among inpatients. Indian Journal of Medical Research,139 (6), 945–948.

Niyas, F. M., and Gopinath, P. (2018). Detection of hemolysin and gelatinase in uropathological *Escherichia coli*. Research Journal of Pharmacy and Technology, 11(5), 1734-1736.

Noie Oskouie, A., Hasani, A., Ahangarzadeh Rezaee, M., Soroush Bar Haghi, M. H., Hasani, A., and Soltani, E. (2019). A relationship between O-serotype, antibiotic susceptibility and biofilm formation in Uropathogenic *Escherichia coli*. Microbial Drug Resistance, 25(6), 951-958.

Noor, S., and Gopinath, P. (2018). Detection of Carbapenemase Resistance among Clinical Isolates of *Escherichia coli*. Research Journal of Pharmacy and Technology, 11(2), 486-488.

Ny, S., Edquist, P., Dumpis, U., Grondahl-Yli-Hannuksela, K., Hermes, J., Kling, A. M., Kligeberg, A., Kozlov, R., Kallman, O., Lis, D. O., Pomorska-Wesolowska, M., Saule, M., Wisell, K. T., Vuopio, J., Paligin, I., and NoDRS UTI Study Group, N. U. (2019). Antimicrobial resistance of *Escherichia coli* isolates from outpatient urinary tract infections in women in six European countries including Russia. Journal of Global Antimicrobial Resistance, 17, 25-34.

Nyholm, O., Halkilahti, J., Wiklund, G., Okeke, U., Paulin, L., Auvinen, P., Haukka, K., and Siitonen, A. (2015). Comparative genomics and characterization of hybrid Shigatoxigenic and enterotoxigenic *Escherichia coli* (STEC/ETEC) strains. PLoS One, 10(8), e0135936.

O'Connor, R., O'Doherty, J., O'Regan, A., and Dunne, C. (2018). Antibiotic use for acute respiratory tract infections (ARTI) in primary care; what factors affect prescribing and why is it important? A narrative review. Irish Journal of Medical Science (1971), 187(4), 969-986.

O'Sullivan J., Bolton D. J., Duffy G., Baylis C., Tozzoli R., Wasteson Y.and Lofdahl S.(2007). Methods for Detection and Molecular Characterisation of Pathogenic *Escherichia coli*. CO-ORDINATION ACTION FOOD-CT-2006-036256. Pathogenic *Escherichia coli* Network.

Obuekwe, C. O., Al-Jadi, Z. K., and Al-Saleh, E. S. (2009). Hydrocarbon degradation in relation to cell-surface hydrophobicity among bacterial hydrocarbon degraders from petroleum-contaminated Kuwait desert environment. International Biodeterioration and Biodegradation, 63(3), 273-279.

Ochman, H., and Selander, R. K. (1984). Standard reference strains of *Escherichia coli* from natural populations. Journal of Bacteriology, 157(2), 690-693.

Ochoa, T. J., Barletta, F., Contreras, C., and Mercado, E. (2008). New insights into the epidemiology of enteropathogenic *Escherichia coli* infection. Transactions of the Royal Society of Tropical Medicine and Hygiene, 102(9), 852-856.

Okhuysen, P. C., and DuPont, H. L. (2010). Enteroaggregative *Escherichia coli* (EAEC): A Cause of Acute and Persistent Diarrhea of Worldwide Importance. Journal of Infectious Diseases, 202 (4), 503–505.

Okike, I. O., Johnson, A. P., Henderson, K. L., Blackburn, R. M., Muller-Pebody, B., Ladhani, S. N., Anthony, M., Ninis, N., Heath, P. T., and neoMen Study Group (2014). Incidence, etiology, and outcome of bacterial meningitis in infants aged <90 days in the United Kingdom and Republic of Ireland: prospective, enhanced, national population-based surveillance. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America, 59(10), e150–e157.

Omolajaiye, S. A., Afolabi, K. O., and Iweriebor, B. C. (2020). Pathotyping and antibiotic resistance profiling of *Escherichia coli* isolates from children with acute diarrhea in Amatole district municipality of Eastern Cape, South Africa. BioMed Research International, 2020.

Ori, E. L., Takagi, E. H., Andrade, T. S., Miguel, B. T., Cergole-Novella, M. C., Guth, B. E. C., Hernandes, R. T., Dias, R. C. B., Pinheiro, S. R. S., Camargo, C. H., Romero, E. C., and Dos Santos, L. F. (2019). Diarrheagenic *Escherichia coli* and Escherichia albertii in Brazil: pathotypes and serotypes over a 6-year period of surveillance. Epidemiology and Infection, 147, 1-9.

Orskov I., Orskov F., Jann B. and Jann K. (1977). Serology, Chemistry and Genetics of O and K antigens of *Escherichia coli*. Bateriological Reviews; 41 (3), 667-710.

Orskov, F., and Orskov, I. (1984). 2 Serotyping of *Escherichia coli*. Methods in Microbiology, 14, 43-112.

Orskov, F., and Orskov, I. (1992). *Escherichia coli* serotyping and disease in man and animals. Canadian Journal of Microbiology, 38(7), 699-704.

Orskov, I., Wachsmuth, I. K., Taylor, D. N., Echeverria, P., Rowe, B., Sakazaki, R., and Orskov, F. (1991). Two new *Escherichia coli* O groups: O172 from» Shiga-like» toxin II-producing strains (EHEC) and O173 from enteroinvasive *E. coli* (EIEC). APMIS, 99(1-6), 30-32.

Otaiwi, M. S., Tarrad, J. K., and AL-Yasari, H. F. (2019). The prevalence of *E. coli* serotypes among diarrheal patients in Hilla City, Iraq. Research Journal of Pharmacy and Technology, 12(7), 3347-3349.

Ozerol, I. H., Bayraktar, M. R., Iseri, L., Otlu, B., and Durmaz, R. (2005). The prevalence and molecular typing of enterotoxigenic *Escherichia coli* strains isolated from diarrheic stools in Malatya, Turkey. The new Microbiologica, 28(3), 237-243.

Page A.V. and Liles W.C. (2013). Enterohemorrhagic *Escherichia coli* Infections and the Hemolytic-Uremic Syndrome. Medical Clinics, 97, 681-695.

Page, M. G. (2019). The role of iron and siderophores in infection, and the development of siderophore antibiotics. Clinical Infectious Diseases, 69(Supplement_7), S529-S537.

Panda, P. S., Chaudhary, U., and Dube, S. K. (2016). Comparison of four different methods for detection of biofilm formation by uropathogens. Indian Journal of Pathology and Microbiology, 59(2), 177.

Paralikar, P., Ingle, A. P., Tiwari, V., Golinska, P., Dahm, H., and Rai, M. (2019). Evaluation of antibacterial efficacy of sulfur nanoparticles alone and in

combination with antibiotics against multidrug-resistant uropathogenic bacteria. Journal of Environmental Science and Health, Part A, 54(5), 381-390.

Park, S. Y., Kim, K. M., Lee, J. H., Seo, S. J., and Lee, I. H. (2007). Extracellular gelatinase of Enterococcus faecalis destroys a defense system in insect hemolymph and human serum. Infection and Immunity, 75(4), 1861-1869.

Park, S. Y., Shin, Y. P., Kim, C. H., Park, H. J., Seong, Y. S., Kim, B. S., Seo, S. J., and Lee, I. H. (2008). Immune evasion of Enterococcus faecalis by an extracellular gelatinase that cleaves C3 and iC3b. The Journal of Immunology, 181(9), 6328-6336.

Parreira, V. R., Arns, C. W., and Yano, T. (1998). Virulence factors of avian *Escherichia coli* associated with swollen head syndrome. Avian Pathology, 27(2), 148-154.

Parvez, S. A., and Rahman, D. (2018). Virulence Factors of Uropathogenic *E. coli*. .In: Microbiology of Urinary Tract Infections-Microbial Agents and Predisposing Factors, Published by IntechOpen, 7-21.

Patwardhan, V., Kumar, D., Goel, V., and Singh, S. (2017). Changing the prevalence and antibiotic drug resistance pattern of pathogens seen in community-acquired pediatric urinary tract infections at a tertiary care hospital of North India. Journal of Laboratory Physicians, 9(04), 264-268.

Paul, D., Anto, N., Bhardwaj, M., Prendiville, A., Elangovan, R., Bachmann, T. T., Chandra, D.D. and Bhattacharjee, A. (2021). Antimicrobial resistance in patients with suspected urinary tract infections in primary care in Assam, India. JAC-Antimicrobial Resistance, 3(4), dlab164.

Pavlickova, S., Klancnik, A., Dolezalova, M., Mozina, S. S., and Holko, I. (2017). Antibiotic resistance, virulence factors and biofilm formation ability in *Escherichia coli* strains isolated from chicken meat and wildlife in the Czech Republic. Journal of Environmental Science and Health, part b, 52(8), 570-576.

Pawlowski S.W., Warren C.A. and Guerrant R. (2009). Diagnosis and treatment of acute or persistent diarrhea. Gastroenterology, 136:1874-1886.

Payne, S. M. (1993). Iron acquisition in microbial pathogenesis. Trends in Microbiology, 1(2), 66-69.

Peirano G.and Pitout J.D. (2010). Molecular epidemiology of *Escherichia coli* producing CTX-M beta-lactamases: the worldwide emergence of clone ST131 O25:H4. International Journal of Antimicrobial Agents, 35, 316-321.

Pereira, A. L., Silva, T. N., Gomes, A. C.M.M., Araújo, A. C. G., and Giugliano, L. G. (2010). Diarrhea-associated biofilm formed by enteroaggregative *Escherichia coli* and aggregative Citrobacter freundii: a consortium mediated by putative F pili. BMC Microbiology, 10(1), 1-18.

Perez-Llarena, F. J., and Bou, G. (2009). β-Lactamase inhibitors: the story so far. Current Medicinal Chemistry, 16(28), 3740-3765.

Petkovsek, Z., Zgur-Bertok, D., and Erjavec, M. S. (2012). Colicin insensitivity correlates with a higher prevalence of extraintestinal virulence factors among *Escherichia coli* isolates from skin and soft-tissue infections. Journal of Medical Microbiology, 61(6), 762-765.

Petrova, Olga E., and Karin Sauer. "Escaping the biofilm in more than one way: desorption, detachment or dispersion." Current Opinion in Microbiology 30 (2016), 67-78.

Pi, H., Jones, S. A., Mercer, L. E., Meador, J. P., Caughron, J. E., Jordan, L., Newton, S. M., Conway, T., and Klebba, P. E. (2012). Role of catecholate siderophores in gram-negative bacterial colonization of the mouse gut. PLoS One, 7(11), e50020.

Piljic, D., Piljic, D., Ahmetagic, S., Ljuca, F., and Porobic Jahic, H. (2010). Clinical and laboratory characteristics of acute community-acquired urinary tract infections in adult hospitalized patients. Bosnian Journal of Basic Medical Sciences, 10(1), 49–53.

Pitout J.D. (2008). Multiresistant Enterobacteriaceae: new threat of an old problem. Expert Review of Anti-Infective Therapy, 6, 657-669.

Pitout J.D. (2010). Infections with extended-spectrum beta-lactamase-producing enterobacteriaceae: changing epidemiology and drug treatment choices. Drugs, 70, 313-333.

Pitout J.D. (2012). Extraintestinal pathogenic *Escherichia coli*: an update on antimicrobial resistance, laboratory diagnosis and treatment. Expert Review of Anti-Infective Therapy, 10, 1165-1176.

Pitout J.D. and Laupland K.B. (2008). Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. Lancet Infectious Diseases, 8, 159-166.

Pitout J.D., Nordmann P., Laupland K.B. and Poirel L. (2005). Emergence of Enterobacteriaceae producing extended-spectrum beta-lactamases (ESBLs) in the community. Journal of Antimicrobial Chemotherapy, 56, 52-59.

Platts-Mills, J. A., Liu, J., Rogawski, E. T., Kabir, F., Lertsethtakarn, P., Siguas, M., ... and Mota, F. S. (2018). Use of quantitative molecular diagnostic methods to assess the aetiology, burden, and clinical characteristics of diarrhea in children in low-resource settings: a reanalysis of the MAL-ED cohort study. The Lancet Global Health, 6(12), e1309-e1318.

Pluschke, G., Mayden, J., Achtman, M., and Levine, R. P. (1983). Role of the capsule and the O antigen in resistance of O18: K1 *Escherichia coli* to complement-mediated killing. Infection and Immunity, 42(3), 907-913.

Poitrineau P., Forestier C., Meyer M., Jallat C., Rich C., Malpuech G. and De Champs C. (1995). Retrospective case-control study of diffusely adhering Escherichia coli and clinical features in children with diarrhea. Journal of Clinical Microbiology, 33. 1961–1962.

Poolman, J. T., and Wacker, M. (2016). Extraintestinal pathogenic *Escherichia coli*, a common human pathogen: challenges for vaccine development and progress in the field. The Journal of Infectious Diseases, 213(1), 6-13.

Prabhakar, P. K. (2020). Bacterial Siderophores and Their Potential Applications: A Review. Current Molecular Pharmacology, 13(4), 295-305.

Pralhad, Awati B, Ramesh BK, Patil NA, Kumar P, Jaganath Rao B, Vinay, P. T., Mallinath, K. C., Kharate, A., Suryakanth, P., and Revappayya, M. (2018). Occurrence of *Escherichia coli* O157: H7 in Faecal Sample of Sheep and Goats in North East Karnataka. International Journal of Current Microbiology and Applied. Sciences, 7(12), 242-250.

Prasad, A. K., Lyngdoh, W. V., Devi, T. S., and Durairaj, E. (2022). Presence of Resistant DEC Strains in a Tertiary Healthcare Center in North East India in Children under 18 Years. Journal of Laboratory Physicians. 10.1055/s-0042-1742421.

Prasada, S., Bhat, A., Bhat, S., Mulki, S. S., and Tulasidas, S. (2019). Changing antibiotic susceptibility pattern in uropathogenic *Escherichia coli* over a period of 5 years in a tertiary care center. Infection and Drug Resistance, 12, 1439-1443.

Priya, S., Kalaivani, R., and Seetha, K.S. (2015). Virulence factors, antimicrobial resistance and beta-lactamase production among extra intestinal E. coli isolates from various clinical samples. Global Journal for Research Analysis, 4 (5), 74-77.

Pullanhi, U., Khan, S., Vinod, V., Mohan, K., and Kumar, A. (2019). Outcome of acute urinary tract infections caused by uropathogenic *Escherichia coli* with phenotypically demonstrable virulence factors. Annals of African Medicine, 18(3), 138- 142.

Purwar, S., Bhattacharya, D., Metgud, S. C., Kumar, D., Chitambar, S. D., and Roy, S. (2016). A cross-sectional study on aetiology of diarrheal disease, India. Indian Journal of Medical Microbiology, 34(3), 375-379.

Purwar, S., Roy, S., and Metgud, S. (2016). Non-O157: H7 Shiga toxin producing diarrheagenic *Escherichia coli* (STEC) in Southern India: a tinderbox for starting epidemic. Journal of Clinical and Diagnostic Research: JCDR, 10(10), DC11.

Qadri, F., Svennerholm, A. M., Faruque, A. S. G., and Sack, R. B. (2005). Enterotoxigenic *Escherichia coli* in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. Clinical Microbiology Reviews, 18(3), 465-483.

Rafaque, Z., Abid, N., Liaqat, N., Afridi, P., Siddique, S., Masood, S., Kanwal, S., and Dasti, J. I. (2020). In-vitro Investigation of Antibiotics Efficacy Against Uropathogenic *Escherichia coli* Biofilms and Antibiotic Induced Biofilm Formation at Sub-Minimum Inhibitory Concentration of Ciprofloxacin. Infection and Drug Resistance, 13, 2801.

Raghavan, P. R., Roy, S., Thamizhmani, R., and Sugunan, A. P. (2017). Diarrheagenic *Escherichia coli* infections among the children of Andaman Islands with special reference to pathotype distribution and clinical profile. Journal of Epidemiology and Global Health, 7(4), 305-308.

Rajendran, P., Ajjampur, S. S. R., Chidambaram, D., Chandrabose, G., Thangaraj, B., Sarkar, R., Samuel, P., Rajan, D. P., and Kang, G. (2010). Pathotypes of diarrheagenic *Escherichia coli* in children attending a tertiary care hospital in South India. Diagnostic Microbiology and Infectious Disease, 68(2), 117-122.

Rajendran, P., Rajan, D. P., Kang, G., and Thorpe, C. M. (2009). Shiga toxin-producing *Escherichia coli* infection in South India. Journal of Medical Microbiology, 58(11), 1525-1526.

Ramirez, M. S., and Tolmasky, M. E. (2017). Amikacin: uses, resistance, and prospects for inhibition. Molecules, 22(12), 2267.

Ranjini, C. Y., Kasukurthi, L. R., Madhumati, B., and Rajendran, R. (2015). The prevalence of multidrug resistance and extended spectrum beta-lactamases among

uropathogenic *Escherichia coli* isolates in a tertiary care hospital in South India: An alarming trend. Community Acquired Infection, 2(1), 19-24.

Rasheed, M. U., Thajuddin, N., Ahamed, P., Teklemariam, Z., and Jamil, K. (2014). Antimicrobial drug resistance in strains of *Escherichia coli* isolated from food sources. Revista do Instituto de Medicina Tropical de Sao Paulo, 56, 341-346.

Rath, S., and Padhy, R. N. (2015). Surveillance of acute community acquired urinary tract bacterial infections. Journal of Acute Disease, 4(3), 186-195.

Rathnakar, U. P., Sharma, N. K., Garg, R., Unnikrishnan, B., and Krishnahn, G. (2012). A Study on the Sale of Antimicrobial Agents without Prescriptions in Pharmacies in an Urban Area in South India. Journal of Clinical and Diagnostic Research, 6(6).

Ravikant, Kumar, P., Ranotkar, S., Zutshi, S., Lahkar, M., Phukan, C., and Saikia, K. K. (2016). The prevalence and identification of extended spectrum β-lactamases (ESBL) in *Escherichia coli* isolated from a tertiary care hospital in North-East India. Indian Journal of Experimental Biology. , 54, 108–114.

Ray, J., Paul, R., Haldar, A., and Mondol, S. (2015). A study on antibiotic resistance pattern of *Escherichia coli* isolated from urine specimens in Eastern India. International Journal of Meical. Sciences and Public Health, 4, 1670-1674.

Recacha, E., Machuca, J., Diaz-Diaz, S., Garcia-Duque, A., Ramos-Guelfo, M., Docobo-Perez, F., Blazquez, J., Pascual, A., and Rodriguez-Martinez, J. M. (2019). Suppression of the SOS response modifies spatiotemporal evolution, post-antibiotic effect, bacterial fitness and biofilm formation in quinolone-resistant *Escherichia coli*. Journal of Antimicrobial Chemotherapy, 74(1), 66-73.

Reissbrodt, R., and Rabsch, W. (1988). Further differentiation of Enterobacteriaceae by means of siderophore-pattern analysis. Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene. Series A: Medical Microbiology, Infectious Diseases, Virology, Parasitology, 268(3), 306-317.

Rendueles, O., Beloin, C., Latour-Lambert, P., and Ghigo, J. M. (2014). A new biofilm-associated colicin with increased efficiency against biofilm bacteria. The ISME Journal, 8(6), 1275-1288.

Renner, L. D., and Weibel, D. B. (2011). Physicochemical regulation of biofilm formation. MRS Bulletin, 36(5), 347-355.

Rezatofighi, S. E., Mirzarazi, M., and Salehi, M. (2021). Virulence genes and phylogenetic groups of uropathogenic *Escherichia coli* isolates from patients with urinary tract infection and uninfected control subjects: a case-control study. BMC Infectious Diseases, 21(1), 1-11.

Riley, L. W., Remis, R. S., Helgerson, S. D., McGee, H. B., Wells, J. G., Davis, B. R., Hebert, R. J., Olcott, E. S., Johnson, L. M., Hargrett, N. T., Blake, P. A., and Cohen, M. L. (1983). Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. New England Journal of Medicine, 308(12), 681-685.

Rizzo, K., Horwich-Scholefield, S., and Epson, E. (2019). Carbapenem and cephalosporin resistance among Enterobacteriaceae in healthcare-associated infections, California, USA. Emerging Infectious Diseases, 25(7), 1389-1393.

Robins-Browne, R. M., Bordun, A. M., Tauschek, M., Bennett-Wood, V. R., Russell, J., Oppedisano, F., Lister, N. A., Bettelheim, K. A., Fairley, C. K., Sinclair, M. I., and Hellard, M. E. (2004). *Escherichia coli* and community-acquired gastroenteritis, Melbourne, Australia. Emerging Infectious Diseases, 10(10), 1797–1805.

Robinson, A. E., Heffernan, J. R., and Henderson, J. P. (2018). The iron hand of uropathogenic *Escherichia coli*: the role of transition metal control in virulence. Future Microbiology, 13(07), 745-756.

Rogers, B. A., Sidjabat, H. E., and Paterson, D. L. (2011). *Escherichia coli* O25b-ST131: a pandemic, multiresistant, community-associated strain. Journal of Antimicrobial Chemotherapy, 66(1), 1-14.

Romero, R., Oyarzun, E.., Mazor, M., Sirtori, M. , Hobbins, J. C., and Bracken, M. (1989). Meta-analysis of the relationship between asymptomatic bacteriuria and preterm delivery/low birth weight. Obstetrics and Gynecology, 73(4), 576-582.

Romling, U., and Balsalobre, C. (2012). Biofilm infections, their resilience to therapy and innovative treatment strategies. Journal of Internal Medicine, 272(6), 541-561.

Rosenberg, M., and Kjelleberg, S. (1986). Hydrophobic interactions: role in bacterial adhesion. In: Advances in Microbial Ecology, 9, 353-393. Springer Boston, M.A.

Roy, S., Datta, S., Das, P., Gaind, R., Pal, T., Tapader, R., Mukherjee, S., and Basu, S. (2015). Insight into neonatal septicaemic *Escherichia coli* from India with respect to phylogroups, serotypes, virulence,extended-spectrum-β-lactamases and association of ST131 clonal group. Epidemiology and Infection, 143(15), 3266-3276.

Ruchi, T., Sujata, B., and Anuradha, D. (2015). Comparison of phenotypic methods for the detection of biofilm production in uro-pathogens in a tertiary care hospital in India. International Journal of Current Microbiology and Applied Sciences, 4(9), 840-49.

Ruiz-Rodriguez, M., Sánchez-Martínez, Y., Suárez-Cadena, F. C., and García-Ramírez, J. C. (2021). The prevalence and characterization of urinary tract infection in socially vulnerable pregnant women in Bucaramanga, Colombia. Revista de la Facultad de Medicina, 69(2). e77949

Russo, T. A., and Johnson, J. R. (2000). Proposal for a new inclusive designation for extraintestinal pathogenic isolates of *Escherichia coli*: ExPEC. The Journal of Infectious Diseases, 181(5), 1753-1754.

Russo, T. A., and Johnson, J. R. (2003). Medical and economic impact of extraintestinal infections due to *Escherichia coli*: focus on an increasingly important endemic problem. Microbes and Infection, 5(5), 449-456.

Sabir, N., Ikram, A., Zaman, G., Satti, L., Gardezi, A., Ahmed, A., and Ahmed, P. (2017). Bacterial biofilm-based catheter-associated urinary tract infections: Causative pathogens and antibiotic resistance. American Journal of Infection Control, 45(10), 1101-1105.

Saikia, T., Das, J. K., Barkataky, D., and Hazarika, N. K. (2016). Detection of virulence markers in uropathogenic *Escherichia coli*, their serotypes and antibiotic sensitivity patterns. Interational Journal of Current Microbiology and Applied Sciences, 5, 784-792.

Saka, H. K., Dabo, N. T., Muhammad, B., García-Soto, S., Ugarte-Ruiz, M., and Alvarez, J. (2019). Diarrheagenic *Escherichia coli* pathotypes from children younger than 5 years in Kano State, Nigeria. Frontiers in Public Health, 7, 348.

Sakazaki, R., Tamura, K., and Saito, M. (1967). Enteropathogenic *Escherichia coli* associated with diarrhea in children and adults. Japanese Journal of Medical Science and Biology, 20(5), 387-399.

Saleem, M., and Daniel, B.. (2017). Detection of Siderophore production in Uropathogenic Escherichia. coli in patients with Type 2 Diabetes Mellitus. International Journal of Medical Microbiology and Tropical Diseases, 3 (4), 176-177.

Salvadori, M., and Bertoni, E. (2013). Update on hemolytic uremic syndrome: diagnostic and therapeutic recommendations. World Journal of Nephrology, 2(3), 56.

San, T., Moe, I., Ashley, E. A., and San, N. (2021). High burden of infections caused by ESBL-producing MDR *Escherichia coli* in paediatric patients, Yangon, Myanmar. JAC-Antimicrobial Rresistance, 3(1), dlab011.

Sanchez, C. J., Mende, K., Beckius, M. L., Akers, K. S., Romano, D. R., Wenke, J. C., and Murray, C. K. (2013). Biofilm formation by clinical isolates and the implications in chronic infections. BMC Infectious Diseases, 13(1), 1-12.

Sanchez, G. V., Baird, A. M. G., Karlowsky, J. A., Master, R. N., and Bordon, J. M. (2014). Nitrofurantoin retains antimicrobial activity against multidrug-resistant urinary *Escherichia coli* from US outpatients. Journal of Antimicrobial Chemotherapy, 69(12), 3259-3262.

Sandhu, K. S., Clarke, R. C., McFadden, K., Brouwer, A., Louie, M., Wilson, J., Lior, H., and Gyles, C. L. (1996). The prevalence of the eaeA gene in verotoxigenic *Escherichia coli* strains from dairy cattle in Southwest Ontario. Epidemiology and Infection, 116(1), 1-7.

Sanin-Ramirez, D., Calle-Meneses, C., Jaramillo-Mesa, C., Nieto-Restrepo, J. A., Marín-Pineda, D. M., and Campo-Campo, M. N. (2019). Etiological the prevalence of urinary tract infections in symptomatic pregnant women in a high complexity hospital in Medellín, Colombia, 2013-2015. Revista Colombiana de Obstetricia y Ginecología, 70(4), 243-252.

Sansonetti, P. J., Kopecko, D. J., and Formal, S. B. (1982). Involvement of a plasmid in the invasive ability of Shigella flexneri. Infection and Immunity, 35(3), 852-860.

Santos, A. P. A., Watanabe, E., and Andrade, D. D. (2011). Biofilm on artificial pacemaker: fiction or reality? Arquivos Brasileiros de Cardiologia, 97, e113-e120.

Sarowska, J., Futoma-Koloch, B., Jama-Kmiecik, A., Frej-Madrzak, M., Ksiazczyk, M., Bugla-Ploskonska, G., and Choroszy-Krol, I. (2019). Virulence factors, the prevalence and potential transmission of extraintestinal pathogenic *Escherichia coli* isolated from different sources: recent reports. Gut Pathogens, 11(1), 1-16.

Scaletsky, I. C., Aranda, K. R., Souza, T. B., and Silva, N. P. (2010). Adherence factors in atypical enteropathogenic *Escherichia coli* strains expressing the localized adherence-like pattern in HEp-2 cells. Journal of Clinical Microbiology, 48(1), 302-306.

Scaletsky, I. C., Silva, M. L., and Trabulsi, L. R. (1984). Distinctive patterns of adherence of enteropathogenic *Escherichia coli* to HeLa cells. Infection and Immunity, 45(2), 534-536.

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., Jones, J. L., and Griffin, P. M. (2011). Foodborne illness acquired in the United States—major pathogens. Emerging Infectious Diseases, 17(1), 7-15.

Scheutz, F., Cheasty, T., Woodward, D., and Smith, H. R. (2004). Designation of O174 and O175 to temporary O groups OX3 and OX7, and six new *E. coli* O groups that include Verocytotoxin-producing *E. coli* (VTEC): O176, O177, O178, O179, O180 and O181. APMIS, 112(9), 569-84.

Schlackow, I., Stoesser, N., Walker, A. S., Crook, D. W., Peto, T. E., and Wyllie, D. H. (2012). Increasing incidence of *Escherichia coli* bacteraemia is driven by an increase in antibiotic-resistant isolates: electronic database study in Oxfordshire 1999–2011. Journal of Antimicrobial Chemotherapy, 67(6), 1514-1524.

Schmidt H., Karch H. and Beutin L. (1994). The large-sized plasmids of enterohemorrhagic *Escherichia coli* O157 strains encode hemolysins which are presumably members of the *E. coli* α-hemolysin family. FEMS Microbiol Lett, 117, 189–196.

Schmidt, H., Beutin, L., and Karch, H. (1995). Molecular analysis of the plasmid-encoded hemolysin of *Escherichia coli* O157: H7 strain EDL 933. Infection and Immunity, 63(3), 1055-1061.

Schmidt, H., Knop, C., Franke, S., Aleksic, S., Heesemann, J., and Karch, H. (1995). Development of PCR for screening of enteroaggregative *Escherichia coli*. Journal of Clinical Microbiology, 33(3), 701-705.

Schmidtchen, A., Frick, I. M., Andersson, E., Tapper, H., and Bjorck, L. (2002). Proteinases of common pathogenic bacteria degrade and inactivate the antibacterial peptide LL-37. Molecular Microbiology, 46(1), 157-168.

Schrag, S. J., and Stoll, B. J. (2006). Early-onset neonatal sepsis in the era of widespread intrapartum chemoprophylaxis. The Pediatric Infectious Disease Journal, 25(10), 939-940.

Schrettl, M., Ibrahim-Granet, O., Droin, S., Huerre, M., Latge, J. P., and Haas, H. (2010). The crucial role of the Aspergillus fumigatus siderophore system in interaction with alveolar macrophages. Microbes and Infection, 12(12-13), 1035-1041.

Schwab, F., Gastmeier, P., and Meyer, E. (2014). The warmer the weather, the more gram-negative bacteria-impact of temperature on clinical isolates in intensive care units. PloS One, 9(3), e91105.

Schwaber M.J. and Carmeli Y. (2007). Mortality and delay in effective therapy associated with extended-spectrum beta-lactamase production in Enterobacteriaceae bacteraemia: a systematic review and meta-analysis. Journal of Antimicrobial Chemotherapy, 60:913-920.

Schwyn, B., and Neilands, J. B. (1987). Universal chemical assay for the detection and determination of siderophores. Analytical Biochemistry, 160(1), 47-56.

Searle, L. J., Meric, G., Porcelli, I., Sheppard, S. K., and Lucchini, S. (2015). Variation in siderophore biosynthetic gene distribution and production across environmental and faecal populations of *Escherichia coli*. PloS One, 10(3), e0117906.

Sehgal R., Kumar Y. and Kumar S. (2008). The prevalence and geographical distribution of *Escherichia coli* O157 in India: a 10-year survey. Transactions of the Royal Society of Tropical Medicine and Hygiene, 102(4), 380-383.

Servin, A. L. (2005). Pathogenesis of Afa/Dr diffusely adhering *Escherichia coli*. Clinical Microbiology Reviews, 18(2), 264-292.

Shah, C., Baral, R., Bartaula, B., and Shrestha, L. B. (2019). Virulence factors of uropathogenic *Escherichia coli* (UPEC) and correlation with antimicrobial resistance. BMC Microbiology, 19(1), 1-6.

Shah, M. K., Aziz, S. A., Zakaria, Z., Lin, L. C., and Goni, M. D. (2018). A Review on pathogenic *Escherichia coli* in Malaysia Adv. Anim. Vetrinary Sciences, 6(2), 95-107.

Shahid, M., Malik, A., Akram, M., Agrawal, L. M., Khan, A. U., and Agrawal, M. (2008). Prevalent phenotypes and antibiotic resistance in *Escherichia coli* and Klebsiella pneumoniae at an Indian tertiary care hospital: plasmid-mediated cefoxitin resistance. International Journal of Infectious Diseases, 12(3), 256-264.

Sharma, A. K., Dhasmana, N., Dubey, N., Kumar, N., Gangwal, A., Gupta, M., and Singh, Y. (2017). Bacterial virulence factors: secreted for survival. Indian Journal of Microbiology, 57(1), 1-10.

Sharma, G., Sharma, S., Sharma, P., Chandola, D., Dang, S., Gupta, S., and Gabrani, R. (2016). *Escherichia coli* biofilm: development and therapeutic strategies. Journal of Applied Microbiology, 121(2), 309-319.

Sharma, M., Pathak, S., and Srivastava, P. (2013). The prevalence and antibiogram of Extended Spectrum β-Lactamase (ESBL) producing Gram negative bacilli and further molecular characterization of ESBL-producing *Escherichia coli* and Klebsiella spp. Journal of Clinical and Diagnostic Research: JCDR, 7(10), 2173-2177.

Sharma, S., Kaur, N., Malhotra, S., Madan, P., Ahmad, W., and Hans, C. (2016). Serotyping and antimicrobial susceptibility pattern of *Escherichia coli* isolates from urinary tract infections in pediatric population in a tertiary care hospital. Journal of Pathogens, 2016, 2548517

Shepherd A.K. and Pottinger P.S. (2013). Management of urinary tract infections in the era of increasing antimicrobial resistance. Med Clin North Am, 97, 737-757.

Shetty, V. A., Kumar, S. H., Shetty, A. K., Karunasagar, I., and Karunasagar, I. (2012). The prevalence and characterization of diarrheagenic *Escherichia coli* isolated from adults and children in Mangalore, India. Journal of Laboratory Physicians, 4(01), 024-029.

Sheu, C. C., Chang, Y. T., Lin, S. Y., Chen, Y. H., and Hsueh, P. R. (2019). Infections caused by carbapenem-resistant Enterobacteriaceae: an update on therapeutic options. Frontiers in Microbiology, 10, 80.

Shin, S. H., Lim, Y., Lee, S. E., Yang, N. W., and Rhee, J. H. (2001). CAS agar diffusion assay for the measurement of siderophores in biological fluids. Journal of Microbiological Methods, 44(1), 89-95.

Shrestha, L. B., Bhattarai, N. R., and Khanal, B. (2018). Comparative evaluation of methods for the detection of biofilm formation in coagulase-negative staphylococci and correlation with antibiogram. Infection and Drug Resistance, 11, 607.

Shrivastava, A. K., Kumar, S., Mohakud, N. K., Suar, M., and Sahu, P. S. (2017). Multiple etiologies of infectious diarrhea and concurrent infections in a pediatric outpatient-based screening study in Odisha, India. Gut Pathogens, 9(1), 1-12.

Shruthi, N. (2012). Phenotypic study of virulence factors in *Escherichia coli* isolated from antenatal cases, catheterized patients, and faecal flora. Journal of Clinical and Diagnostic Research: JCDR, 6(10), 1699.

Silva, I., Andrade, S., Almeida, S., Barbosa, K., Bispo, M., Silva, J., Goncalves, V., Rodrigues, M., Pribul, B., Rodrigues, D., Fialho, A., Assis, R., and Cabral, C. (2020). *E. coli* O157: H7 outbreak and hemolytic uremic syndrome in a day care center in Brazil. International Journal of Infectious Diseases, 101, 137.

Silva, R. M., Toledo, M. R., and Trabulsi, L. R. (1980). Biochemical and cultural characteristics of invasive *Escherichia coli*. Journal of Clinical Microbiology, 11(5), 441-444.

Singh, P., Metgud, S. C., Roy, S., and Purwar, S. (2019). Evolution of diarrheagenic *Escherichia coli* pathotypes in India. Journal of Laboratory Physicians, 11(04), 346-351.

Singh, S. K., Seema, K., and Gupta, M. (2016). Detection of AmpC β-lactamase and adherence factors in uropathogenic *Escherichia coli* isolated from aged patients. Microbial Pathogenesis, 100, 293-298.

Singh, S., Singh, M., Kumari, P., and Rana, R. S. (2015). Role of diarrheagenic *Escherichia coli* in children< 5 years of age hospitalised for acute/persistent diarrhea at a tertiary care hospital in Lucknow. Journal of The Academy of Clinical Microbiologists, 17(1), 19-24.

Singh, T., Das, S., Ramachandran, V. G., Shah, D., Saha, R., Dar, S. A., and Rai, A. (2017). Typical and atypical enteropathogenic *Escherichia coli* in diarrhea and their role as carrier in children under five. Indian Journal of Medical Research, 145(4), 551-557.

Singh, T., Singh, P. K., Dar, S. A., Haque, S., Akhter, N., and Das, S. (2019). Changing paradigm of antibiotic resistance amongst *Escherichia coli* isolates in Indian pediatric population. PloS One, 14(4), e0213850.

Singha, P., Maurya, A. P., and Dhar, D. (2015). Sulphonamide resistance in clinical isolates of *Escherichia coli* and their association with class I integron: A study from India. Archives of Clinical Microbiology, 6, 1-5.

Singleton, D. R., Fidel Jr, P. L., Wozniak, K. L., and Hazen, K. C. (2005). Contribution of cell surface hydrophobicity protein 1 (Csh1p) to virulence of hydrophobic Candida albicans serotype A cells. FEMS Microbiology Letters, 244(2), 373-377.

Sixma, T. K., Kalk, K. H., van Zanten, B. A., Dauter, Z., Kingma, J., Witholt, B., and Hol, W. G. (1993). Refined structure of *Escherichia coli* heat-labile enterotoxin, a close relative of cholera toxin. Journal of Molecular Biology, 230(3), 890-918.

Smajs, D., Micenkova, L., Smarda, J., Vrba, M., Sevcikova, A., Valisova, Z., and Woznicova, V. (2010). Bacteriocin synthesis in uropathogenic and commensal *Escherichia coli*: colicin E1 is a potential virulence factor. BMC Microbiology, 10(1), 1-10.

Small, P. L., and Falkow, S. (1988). Identification of regions on a 230-kilobase plasmid from enteroinvasive *Escherichia coli* that are required for entry into HEp-2 cells. Infection and Immunity, 56(1), 225-229.

Smith A.C. and Hussey M.A. (2005). Gram staining protocols. Americal society for microbiology. Available at: https://asm.org/getattachment/5c95a063-326b-4b2f-98ce-001de9a5ece3/gram-stain-protocol-2886.pdf

Snelling, A. M., Macfarlane-Smith, L. R., Fletcher, J. N., and Okeke, I. N. (2009). The commonly-used DNA probe for diffusely-adherent *Escherichia coli* cross-reacts with a subset of enteroaggregative *E. coli*. BMC Microbiology, 9, 269.

Sokurenko, E. V., Chesnokova, V., Doyle, R. J., and Hasty, D. L. (1997). Diversity of the *Escherichia coli* type 1 fimbrial lectin: differential binding to mannosides and uroepithelial cells. Journal of Biological Chemistry, 272(28), 17880-17886.

Somogyi-Ganss, E., Chambers, M. S., Lewin, J. S., Tarrand, J. J., and Hutcheson, K. A. (2017). Biofilm on the tracheoesophageal voice prosthesis: considerations for oral decontamination. European Archives of Oto-Rhino-Laryngology, 274(1), 405-413.

Spellberg, B., and Doi, Y. (2015). Editor's choice: the rise of fluoroquinolone-resistant *Escherichia coli* in the community: scarier than we thought. The Journal of Infectious Diseases, 212(12), 1853-1855.

Srivastava, S., and Bhargava, A. (2016). Biofilms and human health. Biotechnology Letters, 38(1), 1-22.

Stenutz R., Weintraub A., Widmalm G. (2006). The structures of *Escherichia coli* O-polysaccharide antigens. FEMS Microbiology Reviews, 30, 382-403.

Stepanovic, S., Vuković, D., Hola, V., Bonaventura, G. D., Djukić, S., Ćirković, I., and Ruzicka, F. (2007). Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS, 115(8), 891-899.

Stoll, B. J., Hansen, N. I., Sánchez, P. J., Faix, R. G., Poindexter, B. B., Van Meurs, K. P., Bizarro, M. J., Goldberg, R. N., Frantz, I. D., Hale, E. C., Shankaran, S., Kennedy, K., Carlo, W. A., Watterberg, K. L., Bell, E. F., Walsh, M. C., Schibler, K., Laptook, A. R., Shane , A. L., Schrag, S. J., DAS. A., and Higgins, R. D. (2011). Early onset neonatal sepsis: the burden of group B Streptococcal and *E. coli* disease continues. Pediatrics, 127(5), 817-826.

Sudershan R.V, Kumar R.N., Kulkarni B., Kashinath, L., Bhaskar V., and Polasa K. (2014). *E. coli* pathotypes and their antibiotic resistance in young children with diarrhea in Hyderabad, India. International Journal of Current Microbiology and Applied Sciences, 3(9), 647-654.

Suganya, D., Kanimozhi, K., Panneerselvam, A., and Dhanapaul, K. (2016). Molecular Characterization of Diarrheagenic *Escherichia coli* from Tiruchirappalli District, Tamil Nadu, India. International Journal of Current Microbiology and Applied Sciences, 5(3), 478-84.

Sukumaran, T. S., and Kumar, A. M. (2017). Antimicrobial resistance among uropathogenic bacteria in Rural Kerala, India. International Journal of Current Microbiology and Applied Sciences, 6(9), 2287-2296.

Schulz, S., Stephan, A., Hahn, S., Bortesi, L., Jarczowski, F., Bettmann, U., Paschke, A., Tuse, D., Stahl, C. H., Giritch, A., and Gleba, Y. (2015). Broad and efficient control of major foodborne pathogenic strains of *Escherichia coli* by mixtures of plant-produced colicins. Proceedings of the National Academy of Sciences, 112(40), E5454-E5460.

Suman, E., Jose, J., Varghese, S., and Kotian, M. S. (2007). Study of biofilm production in *Escherichia coli* causing urinary tract infection. Indian Journal of Medical Microbiology, 25 (3), 305-306.

Surya, S. G., Vysakh, P. R., Kandasamy, S., Gnanaprakash, K., Deshpande, S. A., and Bhaskara Prabhu, N. (2014). The prevalence and molecular detection of diarrheagenic *Escherichia coli* in pediatric age group attending a tertiary care

hospital in Coimbatore, Tamil Nadu. International Journal of Research in Health Sciences, 2 (4), 973-978.

Tamura, K., Sakazaki, R., Murase, M., and Kosako, Y. (1996). Serotyping and categorisation of *Escherichia coli* strains isolated between 1958 and 1992 from diarrheal diseases in Asia. Journal of Medical Microbiology, 45(5), 353-358.

Tan, M., Lau, J., and Guglielmo, B. J. (2014). Ethanol locks in the prevention and treatment of catheter-related bloodstream infections. Annals of Pharmacotherapy, 48(5), 607-615.

Tayal, R., Baveja, S., and De, A. (2015). Analysis of biofilm formation and antibiotic susceptibility pattern of uropathogens in patients admitted in a tertiary care hospital in India. International Journal of Health and Allied Sciences, 4(4), 247-247.

Taylor, J., Wilkins, M. P., and Payne, J. M. (1961). Relation of rabbit gut reaction to enteropathogenic *Escherichia coli*. British Journal of Experimental Pathology, 42(1), 43.

Temkin, E., Fallach, N., Almagor, J., Gladstone, B. P., Tacconelli, E., Carmeli, Y., and DRIVE-AB Consortium. (2018). Estimating the number of infections caused by antibiotic-resistant *Escherichia coli* and Klebsiella pneumoniae in 2014: a modelling study. The Lancet Global Health, 6(9), e969-e979.

Tenaillon O., Skurnik D., Picard B. and Denamur E. (2010). The population genetics of commensal *Escherichia coli*. Nature Reviews Microbioloy, 8, 207-217.

Tenke, P., Koves, B., Nagy, K., Hultgren, S. J., Mendling, W., Wullt, B., Grabe, M.; Wagenlehner, F.M.E.; Cek, M.; Pickard, R.; Botto, H.; Naber, K.G.and Johansen, T. E. B. (2012). Update on biofilm infections in the urinary tract. World Journal of Urology, 30(1), 51-57.

Terlizzi, M. E., Gribaudo, G., and Maffei, M. E. (2017). UroPathogenic *Escherichia coli* (UPEC) infections: virulence factors, bladder responses, antibiotic, and non-antibiotic antimicrobial strategies. Frontiers in Microbiology, 8, 1566.

Tevyashova, A. N. (2021). Recent Trends in Synthesis of Chloramphenicol New Derivatives. Antibiotics, 10(4), 370.

Thakur, N., Jain, S., Changotra, H., Shrivastava, R., Kumar, Y., Grover, N., and Vashistt, J. (2018). Molecular characterization of diarrheagenic *Escherichia coli* pathotypes: Association of virulent genes, serogroups, and antibiotic resistance among moderate-to-severe diarrhea patients. Journal of Clinical Laboratory Analysis, 32(5), e22388.

Thakur, R., Kumar, Y., Singh, V., Gupta, N., Vaish, V. B., and Gupta, S. (2016). Serogroup distribution, antibiogram patterns and the prevalence of ESBL production in *Escherichia coli*. Indian Journal of Medical Research, 143(4), 521-524.

The Drugs and Cosmetic Act (2016). The drugs and cosmetic act rules. The drugs and cosmetic acts 1940. available at: https://cdsco.gov.in/opencms/export/sites/CDSCO_WEB/Pdf-documents/acts_rules/2016DrugsandCosmeticsAct1940Rules1945.pdf

Thomas, V. C., Hiromasa, Y., Harms, N., Thurlow, L., Tomich, J., and Hancock, L. E. (2009). A fratricidal mechanism is responsible for eDNA release and contributes to biofilm development of Enterococcus faecalis. Molecular Microbiology, 72(4), 1022-1036.

Thurlow, L. R., Thomas, V. C., Narayanan, S., Olson, S., Fleming, S. D., and Hancock, L. E. (2010). Gelatinase contributes to the pathogenesis of endocarditis caused by Enterococcus faecalis. Infection and Immunity, 78(11), 4936-4943.

Tomar, P., Ranjan, K. P., Ranjan, N., Jain, S. B., and Bansal, H. (2018). Uropathogenic *Escherichia coli*: Correlation between virulence factors and serotypes. Asian Pacific Journal of Health Sciences, 5(2), 1-4.

Torres, A. G. (2017). *Escherichia coli* diseases in Latin America-a 'One Health' multidisciplinary approach. Pathogens Disease. 75 (2), ftx012.

Torretta, S., Drago, L., Marchisio, P., Ibba, T., and Pignataro, L. (2019). Role of biofilms in children with chronic adenoiditis and middle ear disease. Journal of Clinical Medicine, 8(5), 671.

Torti, J. F., Cuervo, P., Nardello, A., and Pizarro, M. (2021). Epidemiology and Characterization of Shiga Toxin-Producing *Escherichia coli* of Hemolytic Uremic Syndrome in Argentina. Cureus, 13(8), e17213.

Tozzoli, R., and Scheutz, F. (2014). Diarrheagenic *Escherichia coli* infections in humans. In: Pathogenic *Escherichia coli*, Molecular and Cellular Microbiology, Caister Academic Press, 1-18.

Trabulsi, L. R., Keller, R., and Gomes, T. A. T. (2002). Typical and Atypical Enteropathogenic *Escherichia coli*. Emerging Infectious Diseases, 8(5), 508-513.

Tran, P. L., Lowry, N., Campbell, T., Reid, T. W., Webster, D. R., Tobin, E., Aslani, A., Mosley, T., Dertien, J., Colmer-Hamood, J. A., and Hamood, A. N. (2012). An organoselenium compound inhibits Staphylococcus aureus biofilms on hemodialysis catheters in vivo. Antimicrobial Agents and Chemotherapy, 56(2), 972-978.

Tribedi, P., and Sil, A. K. (2014). Cell surface hydrophobicity: a key component in the degradation of polyethylene succinate by P seudomonas sp. AKS 2. Journal of Applied Microbiology, 116(2), 295-303.

Triveni, A. G., Kumar, M. S., Manjunath, C., Shivannavar, C. T., and Gaddad, S. M. (2018). Biofilm formation by clinically isolated Staphylococcus aureus from India. The Journal of Infection in Developing Countries, 12(12), 1062-1066.

Tshitshi, L., Manganyi, M. C., Montso, P. K., Mbewe, M., and Ateba, C. N. (2020). Extended Spectrum Beta-Lactamase-Resistant Determinants among Carbapenem-Resistant Enterobacteriaceae from Beef Cattle in the North West Province, South Africa: A Critical Assessment of Their Possible Public Health Implications. Antibiotics, 9(11), 820.

Tumbarello M., Sanguinetti M., Montuori E., Trecarichi E.M., Posteraro B.and Fiori B. (2007). Predictors of mortality in patients with bloodstream infections caused by extended-spectrum-beta-lactamase-producing Enterobacteriaceae: importance of inadequate initial antimicrobial treatment. Antimicrobial Agents and Chemotherapy, 51, 1987-1994.

Tuomanen, E., Cozens, R., Tosch, W., Zak, O., and Tomasz, A. (1986). The rate of killing of *Escherichia coli* byβ-lactam antibiotics is strictly proportional to the rate of bacterial growth. Microbiology, 132(5), 1297-1304.

Unhanand, M., Mustafa, M. M., McCracken Jr, G. H., and Nelson, J. D. (1993). Gram-negative enteric bacillary meningitis: a twenty-one-year experience. The Journal of Pediatrics, 122(1), 15-21.

Vachvanichsanong, P., McNeil, E. B., and Dissaneewate, P. (2021). Extended-spectrum beta-lactamase *Escherichia coli* and Klebsiella pneumoniae urinary tract infections. Epidemiology and Infection, 149, E12.

Vagarali, M. A., Karadesai, S. G., Patil, C. S., Metgud, S. C., and Mutnal, M. B. (2008). Haemagglutination and siderophore production as the urovirulence markers of uropathogenic *Escherichia coli*. Indian Journal of Medical Microbiology, 26(1), 68-70.

Vagrali, M., A. (2009). Siderophore production by uropathogenic *Escherichia coli*. Indian Journal of Pathology and Microbiology. 52, 126–127.

Vaish, R., Pradeep, M. S. S., Setty, C. R., and Kandi, V. (2016). Evaluation of virulence factors and antibiotic sensitivity pattern of *Escherichia coli* isolated from extraintestinal infections. Cureus, 8(5), e605.

van de Sande-Bruinsma, N., Grundmann, H., Verloo, D., Tiemersma, E., Monen, J., Goossens, H., Ferech, M., European Antimicrobial Resistance Surveillance System Group, and European Surveillance of Antimicrobial Consumption Project Group (2008). Antimicrobial drug use and resistance in Europe. Emerging Infectious Diseases, 14(11), 1722–1730.

Van den Beld, M. J. C., and Reubsaet, F. A. G. (2012). Differentiation between Shigella, enteroinvasive *Escherichia coli* (EIEC) and noninvasive *Escherichia coli*. European Journal of Clinical Microbiology and Infectious Diseases, 31(6), 899-904.

van den Hoogen, A., Gerards, L. J., Verboon-Maciolek, M. A., Fleer, A., and Krediet, T. G. (2010). Long-term trends in the epidemiology of neonatal sepsis and antibiotic susceptibility of causative agents. Neonatology, 97(1), 22–28.

Van Loosdrecht, M. C., Norde, W., Lyklema, J., and Zehnder, A. J. (1990). Hydrophobic and electrostatic parameters in bacterial adhesion. Aquatic Sciences, 52(1), 103-114.

Vardakas, K. Z., Tansarli, G. S., Rafailidis, P. I., and Falagas, M. E. (2012). Carbapenems versus alternative antibiotics for the treatment of bacteraemia due to Enterobacteriaceae producing extended-spectrum β-lactamases: a systematic review and meta-analysis. Journal of Antimicrobial Chemotherapy, 67(12), 2793-2803.

Vargas, M., Gascon, J., De Anta, M. T. J., and Vila, J. (1999). The prevalence of Shigella enterotoxins 1 and 2 among Shigella strains isolated from patients with traveler's diarrhea. Journal of Clinical Microbiology, 37(11), 3608-3611.

Varkey, D. R., Balaji, V., and Abraham, J. (2014). Molecular characterisation of extended spectrum beta lactamase producing strains from blood sample. International Journal of Pharmacy and Pharmaceutical Sciences, 6(3), 276-278.

Varshney, K. R., and Dimri, S. (2021). Antibiotic sensitivity pattern of bacterial isolates recovered from clinical samples at tertiary care hospital in western UP, India. International Journal of Health and Clinical Research, 4(9), 1-8.

Vasanthi, R., Karthikeyan, D., and Jeya, M. (2014). Study of biofilm production and antimicrobial resistance pattern of the bacterial isolates from invasive devices. International Journal of Research in Health Sciences, 31, 274-81.

Vasser, M., Barkley, J., Miller, A., Gee, E., Purcell, K., Schroeder, M. N., Basle C., and Neil, K. P. (2021). Notes from the Field: Multistate Outbreak of *Escherichia coli* O26 Infections Linked to Raw Flour—United States, 2019. Morbidity and Mortality Weekly Report, 70(16), 600-601.

Vejborg, R. M., and Klemm, P. (2009). Cellular chain formation in *Escherichia coli* biofilms. Microbiology, 155(5), 1407-1417.

Verma, S., Kumar, M., Kashyap, S., Singh, M., and Venkatesh, V. (2013). Current scenario of *Escherichia coli* and its serotype ‘‘O157: H7’’in Indian subcontinent. International Journal of Innovative Research in Science, Engineering and Technology, 2, 2641-2644.

Vijayan, C., Ajaykumar, V. J., Bhattacharya, A., and Bhanurekka, V. (2017). Detection of enterohaemorrhagic *E. coli* O157: H7 from beef and chevon sold in and around Puducherry. Journal of Entomology and Zoology Studies, 5(6), 1395-1403.

Vila, J., Saez-Lopez, E., Johnson, J. R., Romling, U., Dobrindt, U., Canton, R., Giske, C. G., Naas, T., Caratolli, A., Martinez-Medina, M., Bosch, J., Retamar, P., Ridriguez-Bano, J., Baquero, F., and Soto, S. M. (2016). *Escherichia coli*: an old friend with new tidings. FEMS Microbiology Reviews, 40(4), 437-463.

Vila, J., Vargas, M., Casals, C., Urassa, H., Mshinda, H., Schellemberg, D., and Gascon, J. (1999). Antimicrobial resistance of diarrheagenic *Escherichia coli* isolated from children under the age of 5 years from Ifakara, Tanzania. Antimicrobial Agents and Chemotherapy, 43(12), 3022-3024.

Viswanathan, R., Singh, A. K., Ghosh, C., Dasgupta, S., Mukherjee, S., and Basu, S. (2012). Profile of neonatal septicaemia at a district-level sick newborn care unit. Journal of Health, Population, and Nutrition, 30(1), 41-48.

Voeroes, S., Redey, B., and Csizmazia, F. (1964). Antigenic Structure of a New Enteropathogenic *E. coli* Strain. Acta Microbiologica, 11(2), 125-9.

Vranic, S. M., and Uzunovic, A. (2016). Antimicrobial resistance of *Escherichia coli* strains isolated from urine at outpatient population: A single laboratory experience. Materia Socio-medica, 28(2), 121-124.

Walia, K., Madhumathi, J., Veeraraghavan, B., Chakrabarti, A., Kapil, A., Ray, P., Singh, H., Sistla, S., and Ohri, V. C. (2019). Establishing antimicrobial resistance surveillance and research network in India: journey so far. Indian Journal of Medical Research, 149(2), 164-179.

Waltner-Toews, R. I., Paterson, D. L., Qureshi, Z. A., Sidjabat, H. E., Adams-Haduch, J. M., Shutt, K. A., Jones, M., Tian, C., Pasculle, A. W., and Doi, Y. (2011). Clinical characteristics of bloodstream infections due to ampicillin-sulbactam-resistant, non-extended-spectrum-β-lactamase-producing *Escherichia coli* and the role of TEM-1 hyperproduction. Antimicrobial Agents and Chemotherapy, 55(2), 495-501.

Wang, F., Yang, Q., Kase, J. A., Meng, J., Clotilde, L. M., Lin, A., and Ge, B. (2013). Current trends in detecting non-O157 Shiga toxin–producing *Escherichia coli* in food. Foodborne Pathogens and Disease, 10(8), 665-677.

Wang, R., Bono, J. L., Kalchayanand, N., Shackelford, S., and Harhay, D. M. (2012). Biofilm formation by Shiga toxin–producing *Escherichia coli* O157: H7 and Non-O157 strains and their tolerance to sanitizers commonly used in the food processing environment. Journal of Food Protection, 75(8), 1418-1428.

Wanger, A. R., Murray, B. E., Echeverria, P., Mathewson, J. J., and DuPont, H. L. (1988). Enteroinvasive *Escherichia coli* in travelers with diarrhea. The Journal of Infectious Diseases, 158(3), 640-642.

Wani, S. A., Nabi, A., Fayaz, I., Ahmad, I., Nishikawa, Y., Qureshi, K., Khan, M. A., and Chowdhary, J. (2006). Investigation of diarrhoeic faecal samples for enterotoxigenic, Shiga toxin-producing and typical or atypical enteropathogenic *Escherichia coli* in Kashmir, India. FEMS Microbiology Letters, 261(2), 238-244.

Wanke, C. A., Schorling, J. B., Barrett, L. J., Desouza, M. A., and Guerrant, R. L. (1991). Potential role of adherence traits of *Escherichia coli* in persistent diarrhea in an urban Brazilian slum. The Pediatric Infectious Disease Journal, 10(10), 746-751.

Wasinski, B. (2019). Extra-intestinal pathogenic *Escherichia coli*–threat connected with food-borne infections. Annals of Agricultural and Environmental Medicine, 26(4), 532-537.

Waters, C. M., Antiporta, M. H., Murray, B. E., and Dunny, G. M. (2003). Role of the Enterococcus faecalis GelE protease in determination of cellular chain length, supernatant pheromone levels, and degradation of fibrin and misfolded surface proteins. Journal of Bacteriology, 185(12), 3613-3623.

Watt, S., Lanotte, P., Mereghetti, L., Moulin-Schouleur, M., Picard, B., and Quentin, R. (2003). *Escherichia coli* strains from pregnant women and neonates: intraspecies genetic distribution and the prevalence of virulence factors. Journal of Clinical Microbiology, 41(5), 1929-1935.

Watts, R. E., Totsika, M., Challinor, V. L., Mabbett, A. N., Ulett, G. C., De Voss, J. J., and Schembri, M. A. (2012). Contribution of siderophore systems to growth and urinary tract colonization of asymptomatic bacteriuria *Escherichia coli*. Infection and Immunity, 80(1), 333-344.

Weintraub, A. (2007). Enteroaggregative *Escherichia coli*: epidemiology, virulence and detection. Journal of Medical Microbiology, 56(1), 4-8.

Wells, J. G., Davis, B. R., Wachsmuth, I. K., Riley, L. W., Remis, R. S., Sokolow, R., and Morris, G. K. (1983). Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. Journal of Clinical Microbiology, 18(3), 512-520.

Whitchurch, C. B., Tolker-Nielsen, T., Ragas, P. C., and Mattick, J. S. (2002). Extracellular DNA required for bacterial biofilm formation. Science, 295(5559), 1487-1487.

Whittam T. S., Wolfe M. L., Wachsmuth I. K., Orskov F., Orskov I. and Wilson . R A.(1993) Clonal relationships among *Escherichia coli* strains that cause hemorrhagic colitis and infantile diarrhea. Infection and Immunity, 61, 1619–1629.

WHO (2017). Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. Available at: https://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf

WHO (2021), WHO model list of essential medicines. Available at: https://www.who.int/groups/expert-committee-on-selection-and-use-of-essential-medicines/essential-medicines-lists

WHO. (2009). Community-based surveillance of antimicrobial use and resistance in resource-constrained settings: report on five pilot projects (No. WHO/EMP/MAR/2009.2). World Health Organization.

WHO-DBT Govt. of India. 2019. Indian priority pathogen list. To guide research, discovery and development of new antibiotics in India. Available at: https://dbtindia.gov.in/sites/default/files/IPPL_final.pdf

Williamson, D. A., Lim, A., Wiles, S., Roberts, S. A., and Freeman, J. T. (2013). Population-based incidence and comparative demographics of community-associated and healthcare-associated *Escherichia coli* bloodstream infection in Auckland, New Zealand, 2005–2011. BMC Infectious Diseases, 13(1), 1-8.

Wolf, M. K. (1997). Occurrence, distribution, and associations of O and H serogroups, colonization factor antigens, and toxins of enterotoxigenic *Escherichia coli*. Clinical Microbiology Reviews, 10(4), 569-584.

Woodworth, K. R., Walters, M. S., Weiner, L. M., Edwards, J., Brown, A. C., Huang, J. Y., Malik, S., Slayton, R. B., Paul, P., Capers, C., Kainer, M. A., Wilde, N., Shugart, A., Mahon, G., Kallen, A. J., Patel, J., McDonald, L. C., Srinivasan, A., Craig, M., and Cardo, D. M. (2018). Vital signs: containment of novel multidrug-

resistant organisms and resistance mechanisms—United States, 2006–2017. Morbidity and Mortality Weekly Report, 67(13), 396.

Wu, C. Y., Peng, Y. Z., Wang, R. D., and Zhou, Y. X. (2012). Understanding the granulation process of activated sludge in a biological phosphorus removal sequencing batch reactor. Chemosphere, 86(8), 767-773.

Wu, H., Moser, C., Wang, H. Z., Hoiby, N., and Song, Z. J. (2015). Strategies for combating bacterial biofilm infections. International Journal of Oral Science, 7(1), 1-7.

Xu, J., Xu, Y., Wang, H., Guo, C., Qiu, H., He, Y., Zhang, Y., Li, X., and Meng, W. (2015). Occurrence of antibiotics and antibiotic resistance genes in a sewage treatment plant and its effluent-receiving river. Chemosphere, 119, 1379-1385.

Yang, S. C., Lin, C. H., Aljuffali, I. A., and Fang, J. Y. (2017). Current pathogenic *Escherichia coli* foodborne outbreak cases and therapy development. Archives of Microbiology, 199(6), 811-825.

Yang, S. C., Lin, C. H., Sung, C. T., and Fang, J. Y. (2014). Antibacterial activities of bacteriocins: application in foods and pharmaceuticals. Frontiers in Microbiology, 5, 241.

Zhang, Y., Liao, Y. T., Sun, X., and Wu, V. C. (2020). Is Shiga toxin-producing *Escherichia coli* O45 no longer a food safety threat? The danger is still out there. Microorganisms, 8(5), 782.

Zhou, K., Tao, Y., Han, L., Ni, Y., and Sun, J. (2019). Piperacillin-tazobactam (TZP) resistance in *Escherichia coli* due to hyperproduction of TEM-1 β-lactamase mediated by the promoter Pa/Pb. Frontiers in Microbiology, 10, 833.

Zhou, S. X., Wang, L. P., Liu, M. Y., Zhang, H. Y., Lu, Q. B., Shi, L. S., Ren, X., Wang, Y., Lin, S., Zhang, C., Geng M., Zhang X., Zhu Y., Li, Z., Fang, L., Liu, W., and Yang, W. Z. (2021). Characteristics of diarrheagenic *Escherichia coli* among patients with acute diarrhea in China, 2009–2018. Journal of Infection, 83(4), 424-432.

Zimmerli, W., and Sendi, P. (2017). Orthopaedic biofilm infections. APMIS, 125(4), 353-364.

Appendix 1

List of major media and reagents

Material	Manufacturer
Antibiotic discs	Tulip diagnostic Ltd.
Anti-O *E. coli* antisera	National Salmonella and Escherichia Center
Catalase reagent	SD Fine Chem Ltd.
Chrome azurol-S	HiMedia Pvt Ltd.
Congo Red	Loba Chemie Pvt. Ltd.
Diarrheagenic *E. coli* detection Kit	HiMedia Pvt Ltd.
ESBL Kit	HiMedia Pvt Ltd.
Extra-pure gelatin	HiMedia Pvt Ltd.
Glucose	HiMedia Pvt Ltd.
Glucose phosphate medium	HiMedia Pvt Ltd.
Lactose	Loba Chemie Pvt. Ltd.
Luria Bertani Broth	SRL Pvt. Ltd.
McConkey agar	Microxpres Pvt. Ltd.
Muller Hinton agar	HiMedia Pvt Ltd.
Nutrient agar	Microxpres Pvt. Ltd.
Nutrient broth	HiMedia Pvt Ltd.
ONPG Discs	HiMedia Pvt Ltd.
Oxidase reagent	HiMedia Pvt Ltd.
Peptone Water	Micromaster Pvt. Ltd.
Simmon Citrate Medium	HiMedia Pvt Ltd.
Sucrose	HiMedia Pvt Ltd.
Triple Sugar Iron Medium	Merck Pvt. Ltd.
Trypticase soy broth	Titan Biotech Ltd.
Urea medium base	Micromaster Pvt. Ltd.
Urea solution	HiMedia Pvt Ltd.

Piprazine-N,N-bis (2-ethansulfonic acid) (PIPES)	HiMedia Pvt Ltd.
N-Cetyl -N,N,N -trimethylammonium bromide (HDTMA)	HiMedia Pvt Ltd.
Brain Heart Infusion Agar	Difco Laboratories
Agarose	HiMedia Pvt Ltd.
Ethidium bromide	HiMedia Pvt Ltd.
Formalin	Gen Chem Pvt. Ltd.
Casamino acid	Difco
Cetrimide	HiMedia Pvt Ltd.
α-naphthol	HiMedia Pvt Ltd.
Ferric chloride	HiMedia Pvt Ltd.
MR reagent	HiMedia Pvt. Ltd.
Nitrate broth	HiMedia Pvt. Ltd.

Appendix 2

List of Equipment used

Equipment	Model No.	Make
Analytical balance	-	Eagle
Autoclave	GTA-001	Genist
Biosafety Cabinet	OL-114-A	Ocean Life Sciences
Electrophoresis unit	GNA-100	Pharmacia
ELISA Reader	Sunrise	Tecan
Hot air oven	-	New India Ltd.
Incubator	DL 2267	Scientific electronics
Laminar air flow	1554-ST	Klenzaid
Microscope	PZB-22	Quasmo
Orbital shaking Incubator	OLSC-109	Ocean Life Sciences
PCR Cycler	5333 45234	Eppendorf
pH meter	CP-901	Century
UV Trans-illuminator	BioVis	Expert Vision
Vortex mixer	-	Arihant International

Appendix 3

Composition of Media and reagents

Media:

Composition per L:

Ingredients	Quantity (gm/l)
1. Nutrient broth:	
Peptone	5.00gm
Beef Extract	1.50 gm
Yeast Extract	1.50 gm
Sodium Chloride	5.00 gm
pH= 7.4±0.2	
2. Nutrient agar	
Nutrient broth	1 L
Agar	15.00 gm
pH = 7.4±0.2	
3. Peptone water:	
Peptone	10.00 gm
Sodium Chloride	5.00 gm
Distilled water	1 L
pH = 7.4	
4. Glucose phosphate medium:	
Peptone	7.00 gm
Dipotassium Hydrogen Phosphate	5.00 gm
Glucose	5.00 gm
Distilled Water	1 L
pH = 6.9±0.2	

5. MacConkey agar:

Peptone	20.00 gm
Sodium Taurocholate	5.00 gm
Agar	20.00 gm
Neutral Red	0.4 gm
Lactose	15.00gm
Distilled Water	1 L

pH = 7.1±0.2

6. Fermentation media:

Sugar (Glucose/lactose/sucrose)	1.00g/100ml

7. Simmon's citrate media:

Sodium Chloride	5.00 gm
Magnesium Sulphate	0.20 gm
Ammonium Dihydrogen Phosphate	1.00 gm
Dipotassium phosphate	1.00 gm
Sodium Citrate	2.00gm
Agar	15.00gm
Bromothymol blue	0.08gm
Distilled water	1 L

pH = 6.8 ± 0.2

8. Triple sugar iron medium:

Peptone	20.00 gm
Yeast extract	3.00 gm
Beef extract	3.00 gm
Glucose	1.00 gm

Lactose	10.00 gm
Sucrose	10.00 gm
Ferrous Ammonium Sulphate	0.20 gm
Sodium Chloride	5.00 gm
Sodium Thiosulphate	0.30gm
Agar	15.00gm
Phenol Red	0.025 gm
Distilled water	1 L

pH = 7.4 ± 0.2

9. Christenson's urease medium:

Ureas Base:

Peptone	1.00 gm
Agar	15.00 gm
Distilled water	1 L
Autoclaved at 121^0C for 15 minutes.	

Urea solution:

Sodium Chloride	5.00 gm
Potassium Dihydrogen Phosphate	2.00 gm
Phenol Red	1.20 gm
Glucose	1.00 gm
Urea	20.00 gm

pH = 7.4 ± 0.2

10. Nitrate Reduction medium:

Peptic digest of animal tissue	5.00 gm
Meat Extract	3.00 gm
Potassium Nitrate	1.00 gm
Sodium Chloride	30.00 gm
Distilled water	1 L

pH = 7.0 ± 0.2

11. Mueller Hinton agar:

Meat Infusion Solids	2.00 gm
Casein Acid Hydrolysate	17.50 gm
Starch	1.50 gm
Agar	17.00 gm
Distilled water	1 L

pH= 7.3±0.1

12. Mueller Hinton broth:

Meat Infusion Solids	300.00 gm
Casein Acid Hydrolysate	17.50 gm
Starch	1.50 gm
Distilled water	1 L

pH= 7.3±0.1

13. Luria Bertani broth:

Tryptone	10.00 gm
Yeast Extract	5.00 gm
Sodium Chloride	10.00 gm
Distilled water	1 L

pH= 7.5

14. Congo Red Agar:

Brain heart infusion broth	37.00 gm
Sucrose	50.00 gm
Agar	10.00 gm
Congo Red indicator	0.8 g
Distilled water	1 L

pH= 7.5

15. Chrome Azurol S Agar:

Blue Dye:

Solution 1 (CAS Solution):	
CAS	0.06 gm
WFI	50 ml
Solution 2 (FeCl2 Solution):	
$FeCl_2.6H_2O$	0.0027 g
10mM HCl	10 ml
Solution 3 (HDTMA Solution):	
HDTMA	0.073g
WFI	40 ml
Final blue dye mixture:	
Solution 1	1 ml
Solution 2	9 ml
Solution 3	40 ml

Mixture Solution:

Minimal Medium 9 (MM9) salt solution stock :	
Potassium dihydrogen phosphate (KH_2PO_4)	15.00 g
Sodium hydroxide (NaOH)	25.00 g
Ammonium chloride (NH_4Cl)	50.00 g
WFI	500 ml
20% Glucose stock:	
Glucose	20.00 g
WFI	100 ml
Sodium hydroxide solution:	
Sodium hydroxide (NaOH)	25.00 g
WFI	150 ml
Casamino acid solution:	
Casamino acid	3.0g

WFI	27 ml

Filter sterilized using a 0.2um syringe filter.

Final CAS agar Media composition:

MM9	100 ml
Piprazine-N,N-bis (2-ethansulfonic acid) PIPES	32.24 g
Casino acid	30 ml
Glucose (20%)	10 ml
Blue dye	100 ml
Agar	15.00 g
WFI	750 ml

16. Fiss Minimum Medium:

Solution 1 (L-asparagine solution):

Dipotassium hydrogen phosphate (K_2HPO_4)	5.00 gm
L-asparagine	5.00 gm
WFI	954 ml

pH 6.8

Solution 2 (50% glucose):

Glucose	10.00 g
WFI	20 ml

Filter sterilized using a 0.22µ syringe filter

Solution 3 (0.005% $ZnCl_2$):

$ZnCl_2$	0.005g
WFI	100 ml

Filter sterilized using a 0.22µ syringe filter

Solution 4 (0.001% $MnSO_4$):

Manganese sulphate	0.001 g
WFI	100 ml

Filter sterilized using a 0.22µ syringe filter

Solution 5 (0.4%$MgSO_4$):

Magnesium sulphate	0.04 g
WFI	10 ml

Filter sterilized using a 0.22µ syringe filter

Final MM media (mixed aseptically):

Solution 1	954 ml
Solution 2	9.94 ml
Solution 3	9.94 ml
Solution 4	9.94 ml
Solution 5	9.94 ml

Solutions and reagents:

a. 0.5 M EDTA solution:

Disodium EDTA	186.1 gm
NaOH	50.00 gm
Distilled water	To make 1L

pH= 8.0

b. 1 M tris HCl solution:

Tris base	121 gm
Concentrated HCl	42 ml
Distilled water	To make 1 L

pH= 8.0

c. mM EDTA solution (pH=8.0):

0.5 M EDTA	0.2 ml
Distilled water	100 ml

d. 10 mM Tris-HCl solution (pH=8.0):

1 M Tris-HCl	1.0 ml
Distilled water	99 ml

e. TBE buffer (10X):

Tris base	1.0 ml
Boric acid	99 ml
0.5 M EDTA	
Distilled water	To make 1 L

pH= 8.0

f. TE buffer :

10 mM Tris-HCL (pH=8.0)

1 mM EDTA (pH=8.0)

g. 2% agarose gel:

Agarose	2.00 gm
TBE Buffer	100 ml

h**. Ethidium bromide:**

Stock solution:

Ethidium Bromide	10.00 mg
Distilled water	10 ml

Working solution:

Stock solution	100 µl
Distilled water	100 ml

i**. Kovac's reagent for indole:**

p-dimethylaminobenzaldehyde	10.00 gm
Isoamyl alcohol	50 ml
Conc. HCl	50 ml

j**. Methyl red reagent:**

Methyl red	0.1 gm
Ethanol	300 ml

Distilled Water	200 ml

k**. Voges proskauer reagent:**

Solution A:	
Potassium Hydroxide	40.00 gm
Distilled water	1000 ml
Solution B:	
α-naphthol	5 ml
Absolute alcohol	95 ml

l**. Catalase reagent:**

Hydrogen peroxide	3 ml
Distilled Water	97 ml

m**. Oxidase reagent:**

Tetramethyl-p-phenylene-diaminedihydrochloride	1.00 gm
Distilled Water	10 ml

n**. Nitrate reduction reagent:**

NR solution I:	
Sulphanilic acid	8.00 gm
5N Acetic acid	1000 ml
NR solution II:	
N,N-dimethyl-1-naphthyamine	5 ml
5N Acetic acid	1000 ml

o**. Crystal violet:**

Methyl violet	10.00 gm
Absolute alcohol	100 ml
Distilled Water	1000 ml

p**. Gram's iodine:**

Iodine	10.00 gm
Potassium Chloride	20.00 gm
Distilled Water	1000 ml

q**. Decolorizer:**

Absolute ethyl alcohol	70 ml
Distilled Water	30 ml

r**. Dilute carbol fuschin:**

Carbol fuschin conc.	1 ml
Distilled Water	9 ml

Appendix -4

List of Antimicrobials used

Antimicrobial agent	**Concentration** (µg/disc)
Amoxyclav	30
Ampicillin	10
Amikacin	30
Chloramphenicol	30
Ciprofloxacin	5
Co-trimoxazole	25
Cefepime	30
Ceftriaxone	30
Cefotaxime	30
Cefuroxime sodium	30
Gentamicin	10
Imipenem	10
Kanamycin	30
Meropenem	10
Nalidixic acid	30
Nitrofurantoin	300
Norfloxacin	10
Piperacillin-Tazobactam	100/10
Trimethoprim	5
Ceftazidime	30

Appendix -5

List of DEC pathotypes primers used

Diarrheagenic E. coli Detection Kit (multiplex): Kit No. MBPCR039-Himedia Pvt. Ltd, India was used containing the following primers:

Primer	Marker for pathotype	Size
eae	EPEC	482bp
bfpA	EPEC	300bp
Elt	ETEC	322bp
Est	ETEC	170bp
hlyA	EHEC	534bp
CVD432	EAEC	194bp

Appendix -6

Abbreviations

A. baumannii	Acinetobacter baumannii
AA	Aggregative adherence
aEPEC	*Atypical* Enteropathogenic *Escherichia coli*
AIEC	Adherent invasive *Escherichia coli*
AMR	Antimicrobial resistance
APEC	Avian pathogenic *Escherichia coli*
AST	Antibiotic Susceptibility Testing
ATP	Adenosine tri-phosphate
Bp	Base pair
C. defficile	Clostridium defficile
CAS	Chrome Azurol S agar
CD	Crohn's Disease
CDC	Centre for disease control
CDCP	Centre for Disease Control and Prevention
CEACAM-6	carcino-embryonic antigen-related cell adhesion molecule-6
CFs	Colonization factors
CLSI	Clinical laboratory standard institute
CNS	Central nervous system
COVID-19	Corona virus disease-2019
CR	Carbapenem resistant
CRA	Congo red agar
CSF	cerebro spinal fluid
CSH	Cell surface hydrophobicity
CT	Cholera enterotoxin
DA	Diffuse adherence
DAEC	Diffusely adherent *Escherichia coli*

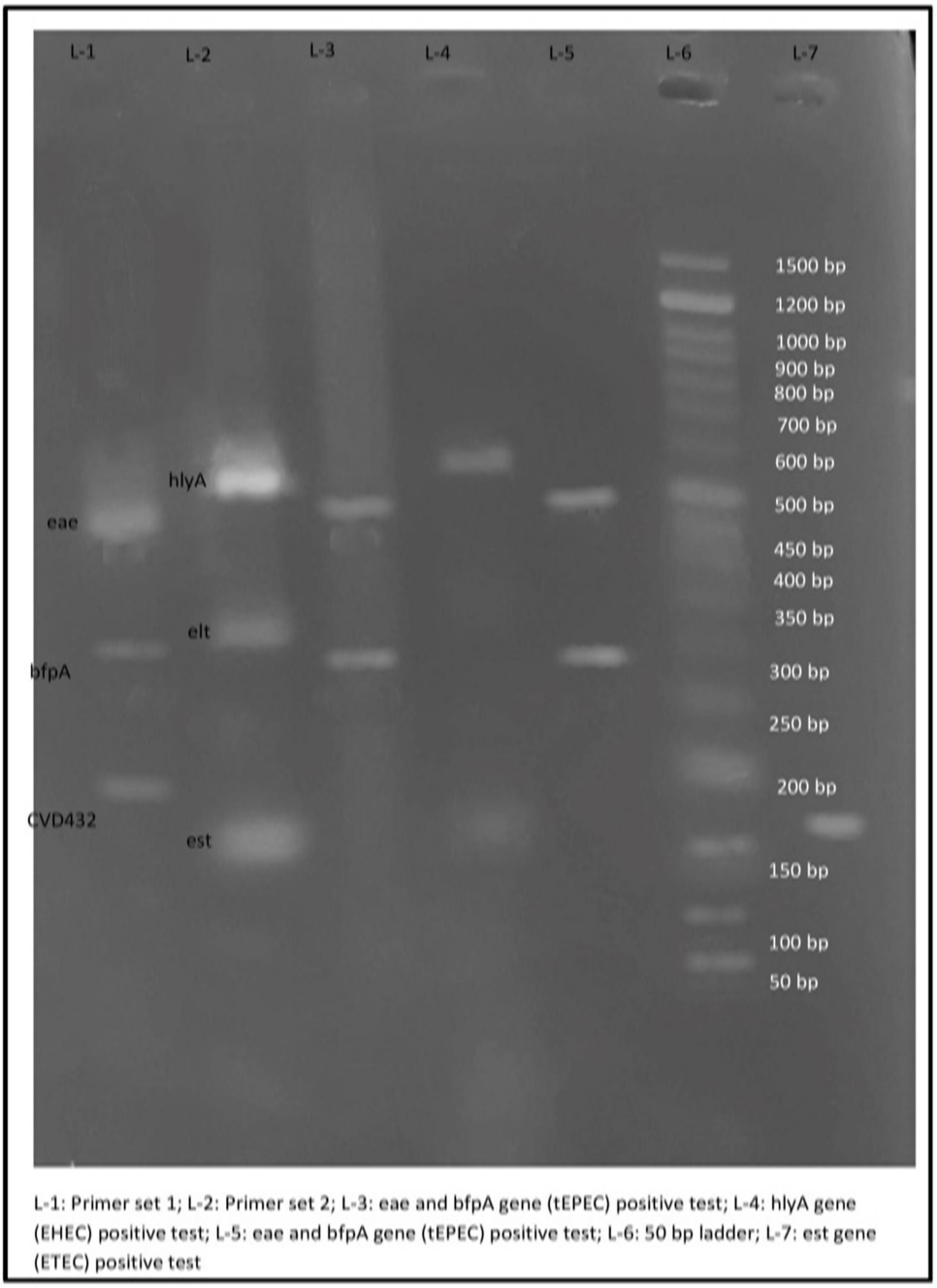
DBT	Department of biotechnology
DEC	Diarrhea-genic *E. coli*
DEPs	Diarrheagenic *E.coli* pathotypes
DNA	Deoxyribose nucleic acid
DRI	Drug resistance index
E. coli	*Escherichia coli*
E. faecalis	Enterococcus faecalis
EAEC	Enteroadherent *Escherichia coli*
EAF	*E. coli* adherence factor
ECDC	European centre for disease control
EHEC	Enterohaemorrhagic *Escherichia coli*
EIEC	Enteroinvessive *Escherichia coli*
ELISA	enzyme-linked immunosorbent assay
EPEC	Enteropathogenic *Escherichia coli*
EPS	exo-polysaccharide
ESBL	Extended spectrum β-lactamase
ETEC	Enterotoxigenic *Escherichia coli*
ExPEC	Extra-intestinal pathogenic *Escherichia coli*
FAO	Food and agriculture Organization
FUR	Ferric uptake regulation
GI	Gastro Intestinal
GPIU	Global the prevalence of infections in urology
HC	hemorrhagic colitis
HIV	human immunodeficiency virus
HlyA	Enterohemorrhagic *Escherichia coli* haemolysin
HUS	hemolytic uremic syndrome
IAP	Intra amniotic prophylaxis
IBD	inflammatory bowel disease
IbeA	invasion of brain endothelium
IMViC	Indole, Methyl red, Voges Praskauer, Citrate
IPEC	Intestinal pathogenic *Escherichia coli*

IQR	Inter quartile range
K. pneumoniae	Klebsiella pneumoniae
Kb	Killo base pair
Kd	Killo Dalton
LA	localized adherence
LAL	localized-like
LB	Luria bertani medium
LPS	Lipopolysachharides
LT	Labile toxin
MDR	Multiple drug resistance
MR	Methyl red
NAG	N-acetyl glucosamine
NAM	N-acetyl muramic acid
NAP-AMR	National Action Plan on Anti-Microbial Resistance
NBM	neonatal bacterial meningitis
NMEC	Neonatal meningitis *Escherichia coli*
NR	Nitrate reduction
OD	Optical density
OMPs	Outer membrane proteins
ONPG	Ortho-nitrophenyl-β-D-galactopyranoside
P. aeruginosa	Pseudomonas aeruginosa
P. mirabilis	Proteus mirabilis
PAI	pathogenicity islands
PCR	Polymerase chain reaction
RNA	Ribose nucleic acid
S. aureus	Staphylococcus aureus
S. entiritidis	Salmonella entiritidis
S. epidermidis	Staphylococcus epidermidis
S. typhimurium	Salmonella typhimurium
SAT	Salt aggregation test
Spp	Species

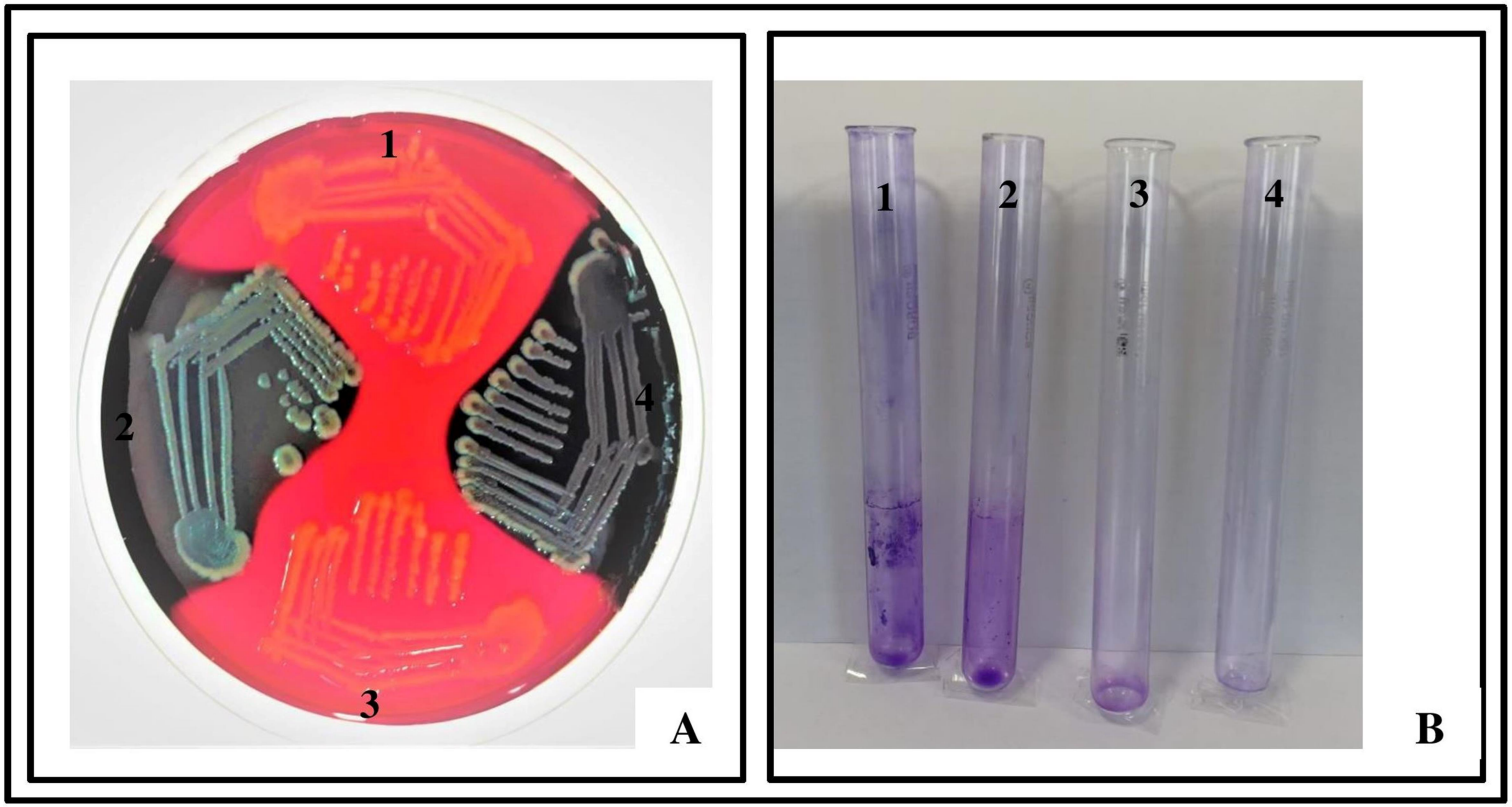
ST	Stable toxin
STEC	Shiga toxin producing *Escherichia coli*
Stx	Shiga like toxin
TCP	Tissue culture plate method
tEPEC	*Typical* Enteropathogenic *Escherichia coli*
TM	Tube Adherence Method
TNF-α	Tissue necrosis factor- α
TSB	Trypticase soy broth
TSI	Triple sugar iron
UPEC	Uropathogenic *Escherichia coli*
UT	Untypeable
UTI	Urinary tract infection
VAT	Vacuolating autotransporter toxins
VP	Voges Praskauer
VTEC	Vero toxin producing *Escherichia coli*
WHO	World Health Organization

Appendix-7

Photographs



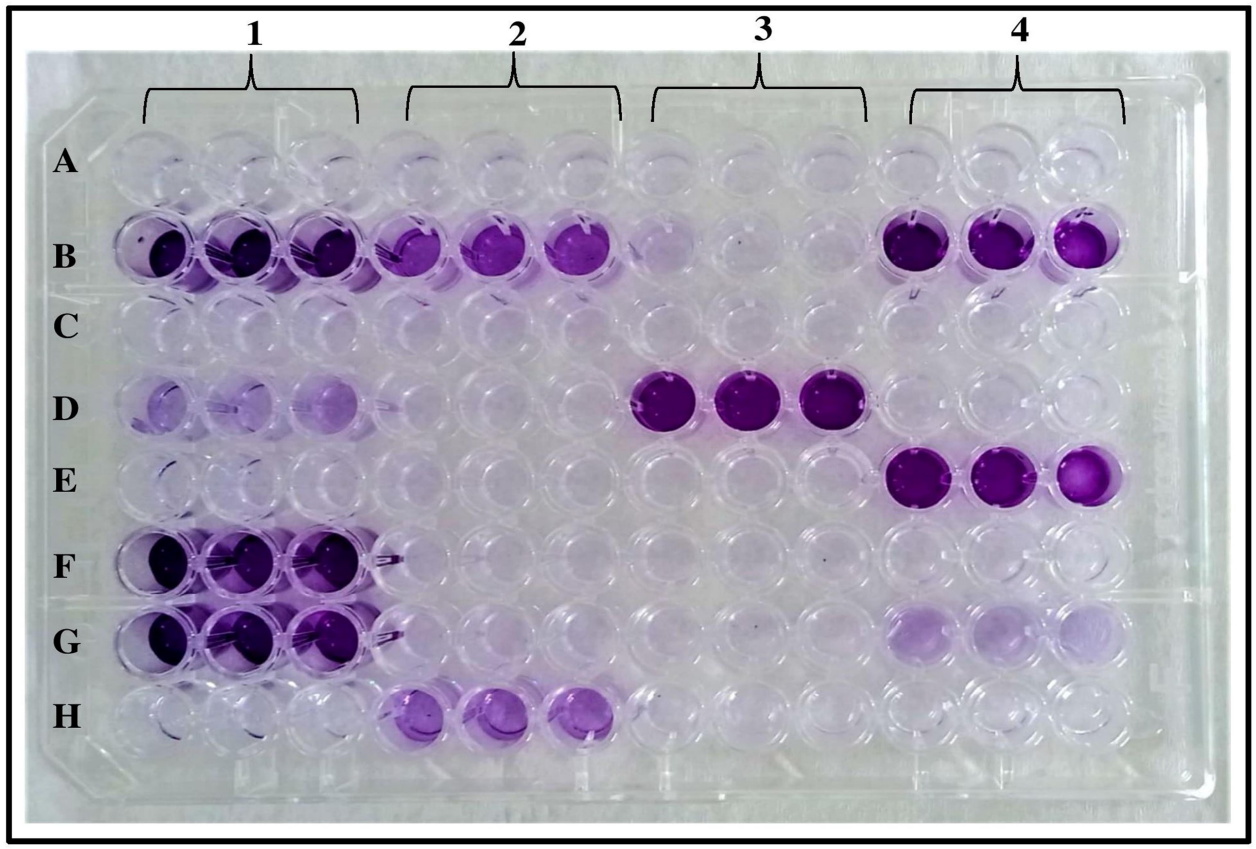
P-A7.1: Detection of DEC pathotype genetic markers using multiplex PCR



P 7.2: Biofilm detection by Congo red agar and Tube methods

A: Biofilm Detection on Congo red agar; A1- Negative control, A2- Positive Control, A3- Negative test, A4- Positive test

B: Biofilm Detection by Tube Method; B1- Positive control, A2- Positive Test, A3- Negative Control, A4- Negative test

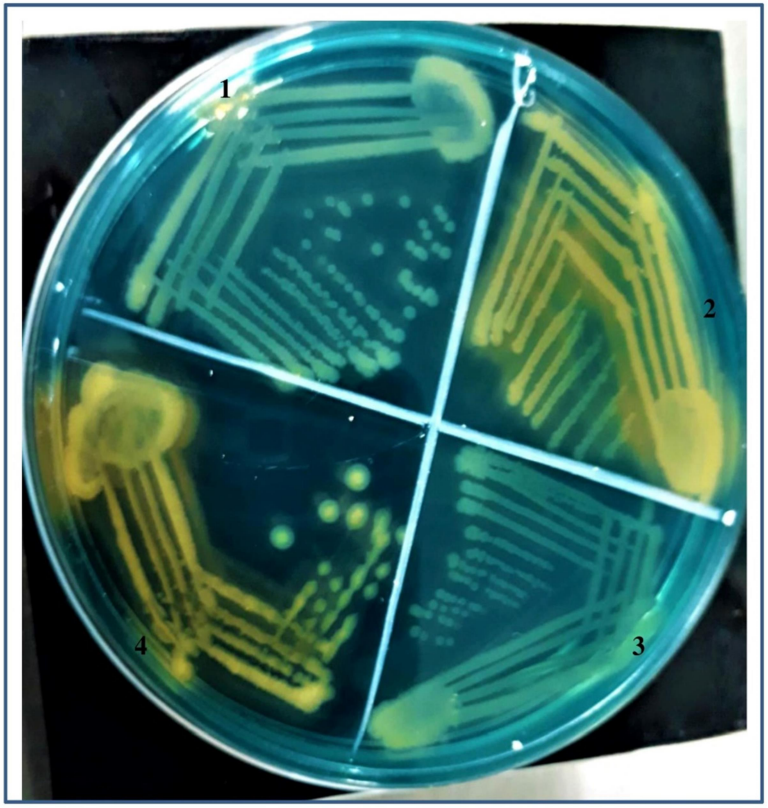


P 7.3: Biofilm Detection by Tissue Culture Plate method

Here:

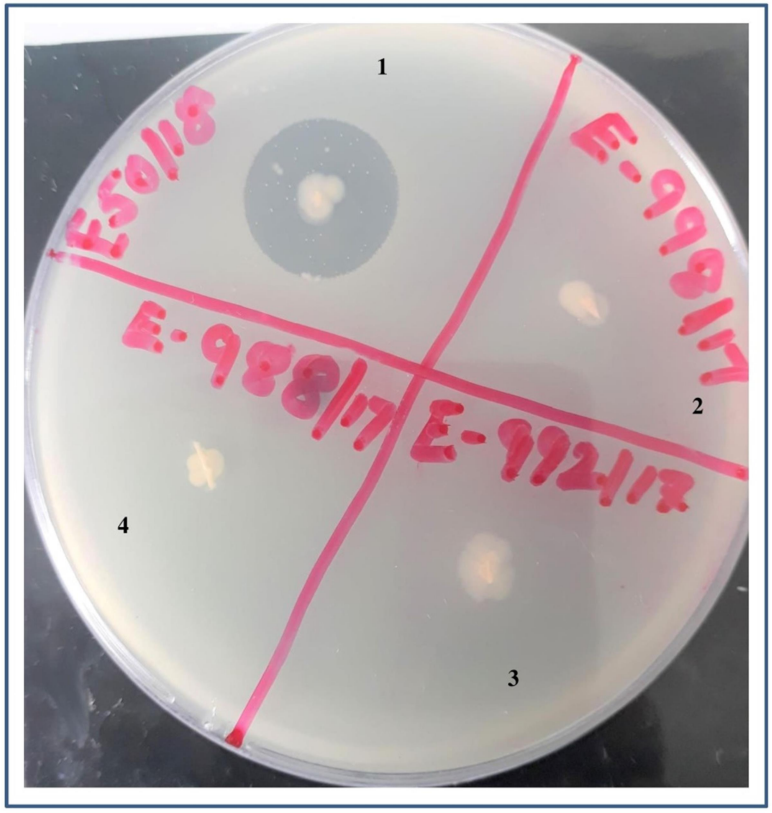
A-1: Negative Control B-1: Positive Control C-1 to H-4: Test samples

F-1, G-1, D-3, B-4 and E-4: Strong positive tests B-2 and H-2: Moderately positive tests



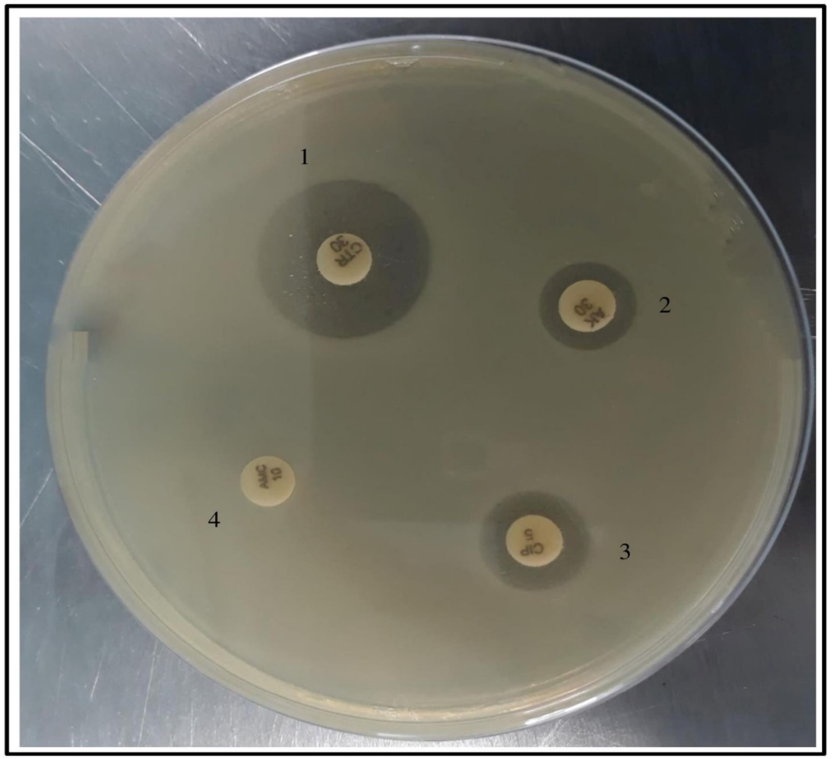
P 7.4: Screening for siderophores production on CAS agar

Here; 1 & 3 - Negative tests, 2 & 4- Postive tests tests



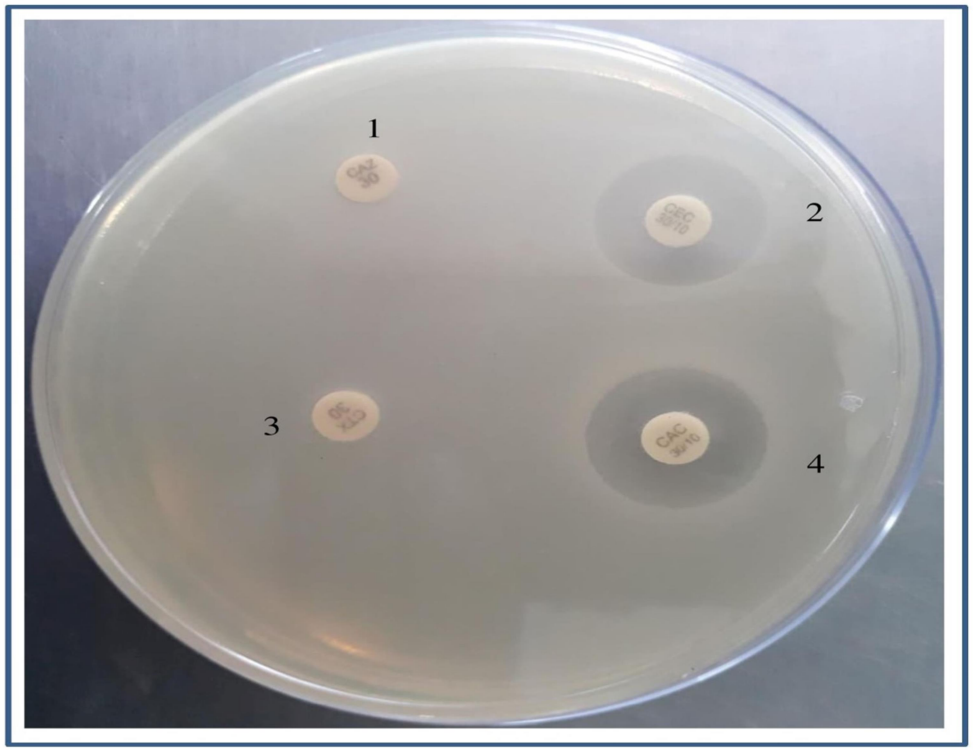
P 7.5: Colicin detection using soft agar overlay technique

Here; 1 - Colicin positive test; and 2, 3 & 4 - Colicin negative tests



P 7.6: Antibiotic Susceptibility Test by Kirby-baur Disk Diffusion Assay

1- CTR: Ceftriaxone- Sensitive, 2-AK: Amikacin- resistant, 3-CIP-Ciprofloxaci-Resistant, 4-AMC: Amoxyclav- Resistant



P 7.7: ESBL-production detection by confirmatory phenotypic assay

2-CAZ: Ceftazidime 30µg 4-CAC: Ceftazidime 30µg+ clauvulanic acid 10 µg

3-CTX: Cefotaxime 30µg 2-CEC: Cefotaxime 30µg+ clauvulanic acid 10 µg

List of Publications from the Thesis Work

S NO.	TITLE OF PAPER WITH AUTHOR NAMES	NAME OF JOURNAL	PUBLISHED DATE	ISSN NO/ VOL NO, ISSUE NO	Indexed in
1.	**Title**: Selection and characterization of siderophores of pathogenic Escherichia coli: Intestinal and extra-intestinal isolates. **Authors**: Gulshan Kumar, Yashwant Kumar, Gaurav Kumar, Ajay Kumar Tahlan	Open Agriculture	April, 2021	Volume:6	Scopus indexed
2.	**Title**: Sero-characterization of intestinal and extra-intestinal *Escherichia coli* (*E. coli*) isolates from different geographical locations in India. **Authors**: Gulshan Kumar, Yashwant Kumar, Gaurav Kumar, Ajay Kumar Tahlan	Research Journal of Pharmacy and Technology	November, 2022	Volume 15, issue 11.	Scopus indexed