Training Report

Internship Training Report

Submitted to: Mr. Harpreet Singh (Lecturer)

Lovely Professional University, Punjab

in partial fulfillment of the requirements

For the degree of

Master of Science in Clinical Microbiology

Submitted by:

Pawanjit Kaur
(Registration Number 11402585)

SCHOOL OF PHYSIOTHERAPY AND PARAMEDICAL SCIENCE
LOVELY PROFESSIONAL UNIVERSITY, PUNJAB, INDIA
May, 2016

DECLARATION

I hereby declare that the work embodied in this internship report was carried by me under the supervision of Mr. Harpreet Singh (Internal supervisor), Lovely Professional University and Dr. Gomty Mahajan (External supervisor). This work has not been submitted in part or in full in any other university for any degree or diploma.

Name: Pawanjit Kaur

Date: 06/05/2016

Place: Lovely Professional University, Punjab

CERTIFICATE

This is to certify that **Ms. Pawanjit Kaur** bearing **Registration Number11402585** has completed his/her Master of Science in Clinical Microbiology internship under our guidance and supervision. This report is record of the candidate own work carried out by him/her under my supervision. I certify that the matter embodied in this report is original and has been not submitted anywhere for the reward of any other degree.

Dr. Gomty Mahajan
Associate professor
Department of Microbiology
Punjab Institute of Medical Sciences, Jalandhar

Mr. Harpreet Singh
Lecturer
Lovely School of Physiotherapy and Paramedical Sciences, Punjab
Lovely Professional University, Punjab

PIMS Medical & Education Charitable Society

Garha Road, Jalandhar (Punjab) 144006 India. Ph.: 0181-6606000 Telefax: +91-181-2480736 / contact@pimsj.com

Ref.No: - PIMS/HR/16/ 0251

Dated: - May 05, 2016

TO WHOM IT MAY CONCERN

This is to certify that **Ms. Pawanjit Kaur** has done practical training in Punjab Institute of Medical Sciences (Hospital and Medical College) from January 01, 2016 to April 30, 2016 in the department of Microbiology.

During the training period her performance was very good. We wish her all the best for her future endeavours.

Barinder M Singh
Head-HR
For PIMS Medical & Education Charitable Society

ABSTRACT

Context: Infections due to Extended Spectrum beta-Lactamases (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* have become an important clinical problem. These organisms are important regarding the infection control by the physicians. Antimicrobial resistance showed by different uropathogens is one of the barricades that might hinder a successful treatment. Detection of Extended Spectrum beta-Lactamase (ESBL) production among uropathogens is an important marker of endemicity.

Aims: The present prospective study was done to identify the trends of uropathogens, to find the prevalence of ESBL isolates and to study the antibiotic resistance profile of the ESBL uropathogenic isolates.

Materials and Methods: This study was conducted in the Department of Microbiology of a teaching tertiary care hospital for 3 months from 10th January 2016 to 10th April 2016. Urine samples were collected from patients whowere clinically suspected to have UTI. After incubation, plates were checked for presence of suspected pathogens. Organisms were identified to species level by conventional methods. The susceptibility to antibiotics was determined by Kirby Bauer method on Muller Hinton agar. Isolates were screened for ESBL production by using disk diffusion of cefotaxime, ceftazidime, ceftriaxone and cefpodoxime placed on inoculated plates containing Muller Hinton agar according to the CLSI recommendations. Phenotypic confirmatory test for ESBL producers was done by combined disc diffusion for all the isolates that were screened positive for the ESBL production following CLSI guidelines. Combined disk diffusion method was also done in this study.

Results:

A total of urine samples from male and female patients visiting the out-patient department (OPD) and in-patient department (IPD) of our hospital were collected. A significantly higher number of IPD and OPD males (56.30% and 28.57%) were found to be culture positive. *Escherichia coli* (79%) were the most frequently isolated uropathogen followed by *Klebsiella*

pneumoniae(6.94%). However, strains of *Escherichia coli* (68.89%) were the highest ESBL producing isolates followed by *Acinetobacter baumanii* (9.63%). ESBL producing isolates were found to be multidrug-resistant.

Conclusion: Our study confirms a global trend toward increased resistance to beta-lactam antibiotics. We emphasize on the formulation of antibiotic policy for a particular geographical area.

ACKNOWLEDGEMENT

I would like to express my heartfelt gratitude to my chief guide **Dr. Gomty Mahajan** (Associate professor) for her indispensible suggestions, help and guidance. Her keenness, meticulous approach and active interest in work made my task simple, easier and fascinating. Her constant guidance, vivid understanding of the subject was chiefly responsible for the successful completion of this project. I consider myself extremely privileged to have worked under her supervision.

I would like to extend my sincere thanks to my internal guide **Mr. Harpreet Singh** (Assistant professor) for his constant moral support throughout the project.

Acknowledgement is also due to **Dr. Monika Gulati** (Dean), Lovely Faculty of Applied Medical Sciences and **Mr. Gurinder Singh** (HOD), Lovely school of Physiotherapy and Paramedical Sciences, Lovely Professional University and all the staff members of Lovely school of Physiotherapy and Paramedical Sciences for their constant encouragement and valuable suggestions during the tenure of the project work.

I would like to express my special gratitude to **Dr. (Brig) Kailash Chand** (HOD) of Microbiology department, **Dr. Shashi Chopra** (Professor), **Dr. Sheevani** (Professor), **Dr. Jaspal Kaur** (Associate professor), **Dr. ShivyaThakur** (Tutor)**,Dr. Shweta** (Tutor) and **Dr. Priyanka** (Assistant professor) for their kind and constant support, guidance and encouragement for completion of my work.

I would like to acknowledge the technical staff of Microbiology Laboratory of Punjab Institute of Medical Sciences (PIMS), Jalandhar, for their support in completing the project.

I would like to express my sincere gratitude to my parents for their kind co-operation, encouragement and magnanimous support throughout the project.

Pawanjit Kaur

11402585

M.Sc. Clinical Microbiology

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF CHARTS

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

During the past few decades, ever-increasing use of antimicrobial agents has led to selective pressure in favour of bacteria that have acquired resistance enzymes (1). This antimicrobial resistance has contributed to the morbidity and mortality in both developing and developed countries (2). Gram negative bacteria or pathogens harbouring ESBLs (Extended Spectrum beta-Lactamases) have caused numerous outbreaks of infection and are becoming an increasing therapeutic problem in various countries. It has found that the incidence of ESBL producing strains among clinical isolates has been steadily increasing over last few years resulting in limitations of therapeutic strategies (3).

There are several micro-organisms which are responsible for urinary tract infection. Urinary tract infection includes the infection of urethra, bladder, ureters and kidneys which comprise whole of urinary tract (4). UTI may be asymptomatic or associated with symptoms. There are mainly ascending routes and haematogenous or descending routes of infection that leads to UTI (5). UTI may result in serious complications such as bacteraemia and sepsis which may often leads to the death of patients. UTI is common in both male and female. Most of UTI has been caused due to the Gram negative bacteria. The common Gram negative bacteria responsible for causing UTI are *Escherichia coli*, *Klebsiella* species, *Proteus* species, *Pseudomonas* species, *Citrobacter* species and some other *Enterobacteriaceae* species. The excessive usage of antibiotics such as beta-lactams, Fluoroquinolones, Aminoglycosides for the development of antimicrobial resistance. The Gram negative bacteria producing beta-lactamase enzyme has the ability to break down beta-lactam ring containing antibiotics such as Penicillins and Cephalosporins and render them ineffective for treatment (6).

The Cephalosporins had been developed in response to the increased prevalence of beta-lactamases in certain organisms and the spread of these beta-lactamases into new hosts. Third generation Cephalosporins were not only effective against many beta-lactamase producing organisms, but has lessened nephrotoxic effects compared to aminoglycosides and polymyxins.

The first report of plasmid-encoded beta-lactamases capable of hydrolysing the extended spectrum Cephalosporins was published in 1983 (7). These new beta-lactamases which had the ability to hydrolyse extended spectrum Cephalosporins and Monobactams were coined Extended Spectrum beta-lactamases (8, 9). ESBLs were first detected in Western Europe in

mid-1980s. Philippon, Labia and Jacoby in the First fundamental review of ESBLs in 1989, noted that ESBLs represented the first example in which beat-lactamase mediated resistance to beta-lactam antibiotic resulted from fundamental changes in the substrate spectra of the enzyme (10). By definition, ESBLs are beta-lactamases capable of hydrolysing or capable of conferring bacterial resistance to penicillins, first, second, third generation Cephalosporins and Aztreonam(but not the Cephamycins or Carbapenems) by hydrolysis of these antibiotics and which are inhibited by beta-lactamase inhibitors such as Clavulanic acid, Sulbactam and Tazobactams (11).

Beta-lactamases are generally classified into two schemes: The Ambler molecular classification Scheme and the Bush-Jacoby-Medieros functional classification system (13-15). According to Ambler scheme, beta-lactamases are divided into four major classes (A to D). This classification scheme is based upon protein homology (amino acid similarity) and not phenotypic characteristics. Classes A, C and D of Ambler classification scheme are serine beta-lactamases while Class B enzymes are metallo-beta-lactamases. The Bush-Jacoby-Medieros classification scheme is based upon the functional similarities of substrate and inhibitor profile of beta-lactamases. The classification scheme is of much more importance in diagnostic laboratory because of the consideration of beta-lactamase inhibitors and beta-lactam substrates that are clinically relevant.

The term ESBL will be taken to mean those beta-lactamases of Bush-Jacoby-Medeiros group 2be and those of group 2d which share most of the fundamental properties of group 2be enzymes (14).

The 2be designates that these enzymes are derived from group 2b beta-lactamases (for instance, TEM-1, TEM-2 and SHV-1), the e of 2be depicts that the beta-lactamases have an extended spectrum. Group 2b enzymes are capable of hydrolysing Penicillins, Ampicillin and to a lesser extent Carbenicillin or Cephalothin (15). TEM-1 is produced by enteric Gram negative bacilli i.e., *Escherichia coli* which is most common plasmid-mediated beta-lactamase resistant to Ampicillin and SHV-1 is produced by majority of *Klebsiella pneumoniae* (16-21). There was a time when virtually ESBL isolates used to be reported from the hospital environment. However, with increasing use of broad spectrum antibiotics in the community setting and increasing number of ESBL positive patients who carry the organism from hospital to the community, more and more reports are emerging about community acquired outbreaks of ESBL infection (14).

CHAPTER 2: REVIEW OF LITERATURE

The era of antibiotics began with the introduction of Penicillin in 1940s which has been recognised as the greatest achievement in therapeutic medicine.

Penicillium was discovered accidentally in 1929 by the British Microbiologist, Alexander Fleming. By the 1940s, penicillin was available for medical use and was successfully used to treat infections in soldiers during World War II. Since then, penicillin has been commonly used to treat a wide range of infections. In 1967 the first penicillin-resistant *Streptococcus pneumoniae* was observed in Australia, and seven years later in the U.S. another case of penicillin-resistant *S. pneumoniae* was observed in a patient with pneumococcal meningitis (22).

Resistance of pathogenic organisms to countenance antibiotics has become a worldwide problem with serious consequences on the treatment of infectious diseases. The heightened use/misuse of antibiotics in human medicine, agriculture and veterinary is primarily contributing to the phenomenon. There is an alarming increase of antibiotic resistance in bacteria that cause either community infections or hospital acquired infections. Of particular interest are the multidrug resistant pathogens, e.g. *Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii* etc. (Alekshun and Levy, 2007).

Beta-lactamases are enzymes produced by bacteria that breakdown (and thus inactivate) a particular class of antibiotics called the beta-lactams. In the early 1940s, through the work of Dr. Florey, Dr. Chain and Dr. Heatley from Oxford University, that penicillin was purified and shown to cure specific bacterial infections. Since that time, many chemical derivatives have been developed from penicillin to combat resistance that has arisen in bacteria. These derivatives commonly referred to as the extended-spectrum beta-lactams, include antibiotics called the cephalosporins, carbapenems and monobactams (23).

The main mechanism of bacterial resistance to the beta-lactam class of antibiotics consists of the production of beta-lactamases, which are hydrolytic enzymes with the ability to inactivate these antibiotics before they reach the penicillin-binding proteins located at the cytoplasmic membrane.

The extended-spectrum beta-lactamases (ESBLs) are classified in the molecular (Ambler) class A and functional (Bush-Jacoby-Medeiros) group 2be; they are characterised by the ability to hydrolyse an oxyimino-beta-lactam at a rate 10% of that for benzyl penicillin along with inhibition by clavulanic acid (24-28).

Among *Enterobacteriaceae*, ESBLs have been found mainly in *Klebsiella* spp. and *E. coli* but have been reported also in other genera, such as *Citrobacter*, *Enterobacter*, *Morganella*, *Proteus*, *Providencia*, *Salmonella*, and *Serratia* (29-33). ESBLs were also found in non-fermentative Gram negative bacteria, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Infections caused by ESBL-positive organisms often involve immunocompromised patients, making it difficult to eradicate these organisms in high-risk wards, such as intensive care units

ESBLs were first described in Germany (1983) and France (1985) among *Klebsiella* species. ESBLs exist in every region of the world and in most genera of *Enterobacteriaceae*. Currently, ESBLs are becoming a major threat for patients in the hospital, long-term care facilities and community. These bacteria have not only caused outbreaks but have become endemic in many hospitals throughout the world (37).

Infections by ESBL producers have been associated with adverse clinical outcomes that have led to increased mortality, prolonged hospitalization and rising medical costs (38).Those adverse outcomes have also been related, at least in part, to a delay in the administration of an effective therapy (39-42). When a significant proportion of Gram-negative isolates in a particular unit are ESBL producers, empirical therapy may change towards use of imipenem, quinolones, or beta-lactam/beta-lactamase inhibitor combinations. In some centres this has been associated with emergence of imipenem resistance in *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, and in ESBL-producing organisms themselves (43, 44).

The Gram-negative bacteria have rapidly expanded resistance to broad-spectrum beta-lactam antibiotics during the past two decades (45). More than 200 types of ESBLs have been found worldwide, most belonging to the *Enterobacteriaceae* family (46). *Escherichia coli* are the bacteria with the ability to produce ESBL enzymes. As a member of *Enterobacteriaceae* family, *E. coli* causes some hospital infections such as sepsis, enteritis, gastroenteritis, neonatal meningitis, and urinary tract infections (47). Excessive usage of antibiotic is also a risk factor for acquisition of an ESBL-producing organism. A strong relationship exists between third-generation cephalosporin use and acquisition of an ESBL-producing strain (48, 49).

The ESBL enzymes were initially recognised in clinical isolates in the 1980s; they were derived mainly from the TEM or SHV types of beta-lactamases, by point mutations in the parent enzymes which did not possess extended-spectrum beta-lactam substrate activity (50). The CTX-M type of ESBLs is becoming increasingly more prevalent, particularly in *E. coli* and *K. pneumoniae* (23, 25).More than 50 enzymes of the latter type have so far been

identified, which can be divided into five main groups on the basis of amino acid changes (CTX-M1, CTX-M2, CTX-M8, CTX-M9 and CTX-M25, respectively) (51). Additional clinically relevant types of ESBLs include mainly the VEB, PER, GES, etc. (23).

Beta-lactamases may be chromosomally encoded and universally present in a species or plasmid mediated. The chromosomal enzymes were believed to have evolved from PBPs with which they show same sequence homology. This was probably a result of the selective pressure exerted by β-lactam–producing soil organisms found in the environment .The first plasmid mediated β-lactamase in Gram negative bacteria TEM-1 was described in the early 1960s (52). It was isolated from a patient named Temoniera in Greece hence designated as TEM (53).

Urinary tract infections (UTIs) are the most frequent community acquired bacterial infections which are associated with significant morbidity and mortality (54, 55). Microorganisms responsible for urinary tract infection (UTI) such as *Escherichia coli* have the ability to produce ESBLs in large quantities. These enzymes are plasmid mediated beta-lactamase in Gram negatives (56, 57). ESBL enzymes are plasmid mediated enzymes capable of hydrolysing and inactivating a wide variety of beta-lactams, including third generation cephalosporins, penicillins and aztreonam. These enzymes are the result of mutations of TEM-1, TEM-2 and SHV-1. All of these beta-lactamase enzymes are commonly found in *Enterobacteriaceae* family (58). These enzymes confer high level resistance to early penicillins and low level resistance to first generation cephalosporins. Excessive use of third generation cephalosporins and aztreonam has believed to be the major cause of the mutations in the enzymes that led to the emergence of ESBLs (59). Their activity against cephamycins and imipenem has not been detected. Because of their greatly extended substrate range these enzymes were called extended spectrum beta-lactamases (60).

The resistance shown by ESBL containing organisms is now a worldwide problem. The introduction of Ceftazidime and Aztreonam shortly after Cefotaxime has accelerated the evolution of ESBLs in hospitals worldwide (61, 62). The emergence of ESBL producers along with multiple resistant isolates possesses a serious problem in hospital setting. The widespread usage of antibiotics coupled with the transmissibility of resistance determinants mediated by plasmids, transposons and gene cassettes in integerons are factors that contribute to the increase in antibiotic resistance in bacterial pathogens (63).

The first ESBL to be identified was found in 1983 but it was in France in 1985 and in the US at the end of the 1980s and the beginning of the 1990s that the initial nosocomial outbreaks occurred (Rice et al., 1990). Soon, thereafter it was discovered that many of the *Klebsiella*

pneumoniae strains that caused nosocomial infections in France in early 1990s were ESBL producers (Sirot et al., 1987).

Several phenotypic tests for detection of ESBL producing organisms have been developed since the 1980s. All methods utilize the two characteristics of ESBLs: reduction of susceptibility to extended spectrum cephalosporins and inhibition by clavulanate. The CLSI (Clinical and Laboratory Standard Institute) recommends screening of *E. coli*, *K. pneumoniae* and *K. oxytoca* (and *Proteus mirabilis*, if clinically relevant such as bacteremic isolates) for potential production of ESBL. The CLSI guidelines recommended following methods of ESBL detection, these consist of the initial screen test and phenotypic confirmatory test (63). Susceptibilities to more than one of cefpodoxime, ceftazidime, ceftriaxone, cefotaxime and aztreonam are evaluated using disk diffusion or broth dilution method in the initial screen test. A decrease in susceptibilities to one or more antibiotics tested may indicate production of ESBLs and warrant performance of the subsequent phenotypic confirmatory tests.

From an international aspect, the use of antibiotics, especially broad-spectrum agents, has been narrowed in Sweden (Cars et al., 2001). Since February 2007, clinical laboratories were required to report all cases involving ESBL-producing *Enterobacteriaceae* strains to the Swedish Institute for Infectious Disease Control, and the number of such cases increased by 100% from 2008 to 2011 (SIIDC, 2012). In recent years, there have also been larger nosocomial outbreaks of clonally ESBL strains: one at a neonatal care unit with ESBL-related mortalities, a large outbreak in Uppsala involving *K. pneumoniae* with CTX-M-15, and in Kristians and caused by a multi resistant CTX-M-15-producing *E. coli* strain (Alsterlund et al., 2009). According to data from the European Antimicrobial Resistance Surveillance System (EARSS), 2.6% of *E. coli* and 1.7% of *K. pneumoniae* strains in Sweden were resistant to third-generation cephalosporins in 2010 (EARSS, 2011).

A study was conducted by Fernandez et al., in 2006, his study showed the emergence of new TEM and SHV enzymes in Europe and distinct epidemic clones have been found, for example *Salmonella* isolates with TEM-52 in Spain and *E. coli* and *K. pneumoniae* isolates with SHV-12 in Italy (Perilli et al., 2011). Isolates with the CTX-M-9 group are common in Spain and strains with the CTX-M-3 enzymes have been described chiefly in Eastern Europe, although clones producing the CTX-M group 1 (including the CTX-M-15 type) are the most widespread throughout Europe (Coque et al., 2008 and Canton et al., 2008). *E. coli* and the CTX-M enzymes are not uncommon in outpatients. Moreover, the resistance exhibited by *K. pneumoniae* has reached a higher level with emergence of carbapenemases such as OXA-48, which was first found in Turkey (Aktas et al., 2008).

In a recent study based on the Tigecycline Evaluation and Surveillance Trial (TEST) global surveillance database, the rate of ESBL production was highest among the *K. pneumoniae* isolates collected in Latin America, followed by Asia/Pacific Rim, Europe, and North America (44.0%, 22.4%, 13.3% and 7.5%, respectively) (64). The same ranking order between the different geographical regions was observed regarding the prevalence of ESBLs among the *E. coli* isolates, although the corresponding rates were lower (13.5%, 12.0%, 7.6%, and 2.2%, respectively) (64).

Detailed data derived from the TEST database regarding the prevalence of ESBL production among *Enterobacteriaceae* isolates in Europe have recently been presented (65). According to these data that refer to 22 European countries for the period of 2004 to 2007, the rate of ESBL production among 515 *K. pneumoniae* isolates and 794 *E. coli* isolates was 15.5% and 9.8%, respectively. Marked differences were observed in the country-specific data; the highest rate of ESBL production was noted in Greece, while the lowest was noted in Denmark. Relevant data collected by the European Antimicrobial Resistance Surveillance System (EARSS Annual Report 2007).

Another investigation was conducted at a tertiary hospital in Nigeria, among the overall ESBL producing isolates, 35% being community origin and 65% from hospitals. In this investigation, ESBL isolates showed high resistance to tetracycline, gentamicin, ceftriaxone, cefuroxime, ciprofloxacin and Augmentin (Amoxicillin and clavulanic acid combination). (Ruth et al., 2011).

A study conducted at the National Public health laboratory (NPHL), Kathmandu, Nepal reported that 31.57% of *E. coli* were confirmed as Extended Spectrum beta-lactamase producers, these isolates further exhibited co-resistance to several antibiotics (Thakur et al., 2013).

In another research conducted at a tertiary hospital in Mwanza, Tanzania, the overall prevalence of ESBLs in all Gram-negative bacteria (377 clinical isolates) was 29%. The ESBL prevalence was 64% in *K. pneumoniae* but 24% in *E. coli* (Mshana et al., 2009).

In a study of inpatients in Saudi Arabia in 2008, Tawfik and colleagues found that 26% of *K. pneumoniae* isolates produced ESBLs, the majority of which were SHV-12 and TEM-1 enzymes, and 36% were CTX-M-15 (Tawfik et al., 2011). Another investigation conducted in the same country in 2004–2005 showed that 10% of clinical urinary *E. coli* isolates from inpatients and 4% of such isolates from outpatients were ESBL producers (Khanfar et al., 2009).

The extent of the ecological disaster related to ESBL-producing *Enterobacteriaceae* in parts of Asia and the Indian subcontinent, and the number of reports of very high frequency of such bacteria in those regions continues to rise. It is likely that some of the successful ESBL-producing clones originate from Asia. Deficient sewage routines (the ‘‘Delhi belly’’) and poor quality of drinking water, in combination with a lack of control over prescription and sales of antibiotics, are probably major factors that have promoted the development of resistance.

According to the SENTRY surveillance program there have been rapid increase in ESBL-producing *K. pneumoniae* (up to 60%) and *E. coli* (13–35%) in different parts of China, with a predominance of the CTX-M-14 and CTX-M-3 enzymes (Hawkey, 2008; Hirakata et al., 2005). It has been found that 66% of third generation cephalosporin resistant *E. coli* and *K. pneumoniae* from three medical centres in India harboured the CTX-M-15 type of ESBL, which was also the only CTX-M enzyme found (Ensor et al., 2006), and an investigation of 10 other centres in that country showed that rates of ESBL-producing *Enterobacteriaceae* reached 70% (Mathai et al., 2002).

Recently ESBL production was observed in 48% of *E. coli*, 44% of *K. pneumoniae* and 50% of *P. aeruginosa* isolates in a tertiary hospital in Patiala, Punjab (Rupinder et al., 2013). In other recent studies, authors observed ESBL rates of 46% and 50% in out and in-patients, respectively (Sankar et al., 2012), and Nasa and co-workers detected ESBL production in almost 80% of clinical isolates (Nasa et al., 2012). Investigations from India and Pakistan show an alarming and rapid increase in the prevalence of *Enterobacteriaceae* with NDM-1 with prevalence rate from 6.9% in a hospital in Varanasi, India, to 18.5% in Rawalpindi, Pakistan (Perry et al., 2011) and perhaps the spread of these enzyme could be even more rapid than the spread of the CTX-M enzymes.

Sensitivity testing showed a multidrug resistance in ESBL producing *E. coli* and *K. pneumoniae*. Maximum resistance was recorded in *E. coli* (ESBL) as cefotaxime (98.9%), Ceftazidime (96.7%) and Cefuroxime (93.4%) while minimum resistance was seen with Imipenem (0.8%), fosfomycin (1.2%) and Nitrofurantoin as well Piperacillin/Tazobactam (2.2%) each. The ESBL producing *Klebsiella* showed maximum resistance to ceftazidime (100%), cefotaxime (89%), and Cefuroxime (84%) while minimum resistance was seen with imipenem (4%), Nitrofurantoin and Piperacillin/Tazobactam (8%) (Majda et al., 2013).

Although there are some differences between countries, the highest prevalence of ESBL-producing *K. pneumoniae* in the world is seen primarily in Latin America, data from 33 centres in Latin America collected over the period 2004–2007 within the Tigecycline

Evaluation and Surveillance Trial (TEST) showed ESBLs in 36.7% of *K. pneumoniae* isolates and in 20.8% of 932 *E. coli* isolates (Rossi et al., 2008).

A large variety of different types of SHV have also been described. One extensive outbreak of Enterobacteriaceae producing the CTX-M-14 enzyme occurred in Calgary, Canada (Pitout et al., 2005).

In a large study performed in 2001, it was demonstrated that about 5.3% of *E. coli* in the United States harbored ESBLs (Winokur et al., 2001), and an investigation conducted in 2009 showed that 9% of *E. coli* isolates at a cancer centre in Texas were ESBL producers (Bhusal et al., 2011)

Sanchez et al., investigated data obtained from The Surveillance Network (TSN) concerning in vitro antimicrobial resistance in US outpatients between 2000 and 2010, and their results showed that resistance to ceftriaxone rose from 0.2% to 2.3% and resistance to cefuroxime increased from 1.5% to 5%, but the bacterial isolates in focus were not tested for ESBLs (Sanchez et al., 2012).

There is considerable geographical difference in ESBLs in European countries. Within countries, hospital to hospital variability in occurrence may also be marked (66). A large study from more than 100 European intensive care units (ICU) found that the prevalence of ESBLs in *Klebsiellae* ranged from as low as 3% in Sweden to as high as 34% in Portugal (67). In Turkey, a survey of *Klebsiella* species from ICUs from eight hospitals showed that 58% of 193 isolates harboured ESBLs (68). Moland and colleagues have shown that ESBL producing isolates were found in 75% of 24 medical centres in the United States (69). ESBLs have also been documented in Israel, Saudi Arabia, and a variety of North African countries (70-72). From China, the figures of ESBL producers vary between 2540% (73%). National surveys have indicated the presence of ESBLs in 58% of *E. coli* isolates from Japan, Korea, Malaysia and Singapore but 1224% of isolates from Thailand, Taiwan, Philippines and Indonesia (74).

In India, the prevalence rate varies in different institutions from 28 to 84% (75). A study from Coimbatore, Tamil Nadu, showed the presence of ESBLs to be 40% while from Nagpur this figure was 50% in urinary isolates (76, 77). Another comparatively recent study in 2005, from New Delhi, showed 68.78 % of the strains of gram negative bacteria to be ESBL producers (78).

CHAPTER 3: AIMS AND OBJECTIVES

The present three month prospective study was done to

i. Identify the trends of uropathogens: To isolate and identify prevalent Gram negative pathogens in IPD & OPD patients.
ii. To find the prevalence of ESBL isolates.
iii. To study the antibiotic resistance profile of the ESBL uropathogenic isolates.

CHAPTER 4: MATERIALS AND METHODS

The study was conducted in Department of Microbiology of Punjab Institute of Medical Sciences (PIMS) Jalandhar. Samples were collected from In-Patient Department (IPD) and Out-Patient Departments (OPD). Samples were collected from the Departments of Gynaecology, Surgery, Medicine, Emergency, Paediatrics, Intensive Care Unit and NICU etc. Total 1209 samples were collected and processed from January to April 2016.

4.1 Study Design and Data Collection

A total of 1209 urine samples were processed in 3 months. Demographic data for each patient was collected viz. name, age, gender, In-Patient Department/Out-Patient Department, date of submission of samples in hospital. The pathogens were identified based upon the standard bacteriological procedures including routine microscopy, Gram's Staining, Colony characteristics on Blood Agar, Mac Conkey Agar media and Biochemical reactions followed by Antibiotic sensitivity testing.

4.2 Sample Collection

Urine samples were collected and transported to Clinical Microbiology Laboratory. Mid-stream Urine samples were collected in wide mouthed sterile container aseptically. For routine culture, Catheter or Cystoscopic Specimens, Mid-stream urine specimens and supra-pubic aspirates were accepted. The sample after collection was sent to the microbiology laboratory immediately. Unique ID bearing all the information of the particular patient was sent along with the sample.

4.3 Sample Processing

4.3.1 Microscopy:

Received Urine samples were processed by performing Wet-mount preparation.

Wet-Mount preparation:

i. Clean and grease free slide was taken.
ii. A drop of urine sample was placed on clean and grease free slide.
iii. Coverslip was placed over it and examined the slide, first under low-power objective (10X).
iv. Then under high power objective (40X) for the presence of pus cells, RBCs, WBCs, epithelial cells, yeast cells, bacteria, parasites and crystals.

Reference Values for components of Urine

Components	Reference Values
RBCs/HPF	Rare
WBCs/HPF	0-4
Epithelial cells/HPF	Occasional (may be higher in female)
Casts/LPF	Occasional hyaline
Bacteria	negative
Mucus	negative to 2+
Crystals	types present vary with pH (Crystals such as cysteine, leucine, Tyrosine and cholesterol are abnormal).

4.4 Culture methods:

Samples were inoculated on following media:

1. Mac Conkey Agar
2. Blood Agar

4.4.1 Mac Conkey Agar: (HIMEDIA™ M081B)

MacConkey Agar is Selective, Differential and an Indicator Medium.

Selective Medium: MacConkey Agar is a Selective Medium. The selective action of this medium is attributed to bile salts, which are inhibitory to most species of gram-positive bacteria and promotes the growth of Gram negative bacteria.

Differential Medium: Gram negative bacteria usually grow well on the medium and are differentiated by their ability to ferment lactose. Lactose fermenting strains grow as red or pink. Lactose non-fermenting strains are colourless and transparent and typically do not alter appearance of the medium.

Indicator Medium: It is also called indicator medium as it contains a pH indicator neutral red, which changes its color in acidic conditions (pH falls below 6.8). Organisms which ferment lactose produce acid as end-product and this result in acidic condition required by the indicator to turn pink.

Table 1: Composition of Mac Conkey Agar

Ingredients	**Grams/Litre**
Peptone	17
Lactose	10
Sodium taurocholate	5
Neutral red	0.03
Agar	13.5

Final pH: 7.1±0.2 at 25^{o} Celsius

Media Preparation:

i. 49.53 grams of powdered MacConkey Agar was dissolved in 1000 ml distilled water. Heat to boiling to dissolve the medium completely.
ii. Sterilized by autoclaving at 15 lbs. pressure (121°C) for 15 minutes.
iii. Cool to 45-50°C. Mixed well before pouring into sterile Petri plates.

Procedure of Inoculation: (Streak method)

1. After wearing mask and gloves, an inoculating loop was sterilized by placing it over flame till the loop turned red hot.
2. Specimen was taken by using 0.001ml calibrated loop and primary inoculum was made.
3. Loop was sterilized and primary streak was made from primary inoculum.
4. Loop was again sterilized and secondary streak was made.
5. With the help of sterilized loop, tertiary streak was made.
6. The plate was incubated for 24 hours at 37 degree Celsius.

Interpretation of Results

Lactose fermenter bacterial colonies appeared: Pink

Non-Lactose fermenter bacterial colonies appeared: Pale.

Colony characteristics

Escherichia coli: pink to red, non-mucoid colonies.

Klebsiella species: pink, mucoid colonies.

Proteus species: pale conies, 2-3mm in size, fishy odour and show characteristic swarming growth.

Pseudomonas species: pale yellow colonies, fluorescent growth.

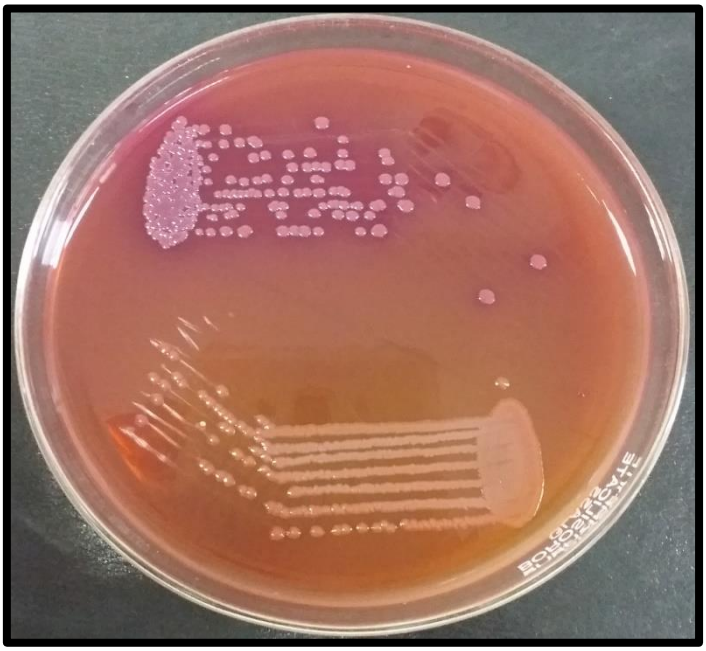


Figure 1: Lactose fermenting and non-lactose fermenting colonies on Mac Conkey Agar.

4.4.2 Blood Agar: (HIMEDIA™ M073)

Blood Agar is a general purpose enriched medium often used to grow fastidious organisms and to differentiate bacteria based on their haemolytic properties. Blood Agar is not a consistently defined medium. The term Blood Agar generally refers to an enriched base medium to which defibrinated mammalian blood has been added. Blood Agar is a bright red, opaque medium. There is variety of complex nutrients found in blood that supports the most fastidious bacteria. Such Media are said to be complex (incapable of being chemically recreated) and enriched (containing an uncommonly rich array of nutrients). Bacteria growing on blood Agar can be classified in part on what they do to the red blood cells incorporated into medium. Some bacteria produce hemolysin (hemo = blood, lysin = to split), enzymes that destroy Red blood cells.

Types of hemolysis:

i. Alpha-hemolysis
ii. Beta-hemolysis
iii. Gamma-hemolysis

Table 2: Composition of Blood Agar base

Ingredients	**Grams/Litre**
Proteose peptone	15
Liver Extract	2.5
Yeast Extract	5
Sodium chloride	5
Agar	15

Final pH (at 25°C) 7.4±0.2

Media Preparation

i. 40 grams of powdered Blood Agar base was dissolved in 1000 ml distilled water. Heat to boiling to dissolve the medium completely.
ii. Sterilized by autoclaving at 15 lbs. pressure (121°C) for 15 minutes.
iii. Cooled to 50°C and aseptically added 5% v/v sterile defibrinated blood. Mixed well and poured into sterile Petri plates.

Procedure of Inoculation

1. After wearing mask and gloves, an inoculating loop was sterilized by placing it over flame till the loop turned red hot.
2. Specimen was taken by using 0.001ml calibrated loop and primary inoculum was made.
3. Loop was sterilized and primary streak was made from primary inoculum.
4. Loop was again sterilized and secondary streak was made.
5. With the help of sterilized loop, tertiary streak was made.
6. The plate was incubated for 24 hours at 37 degree Celsius.

Colony characteristics

Escherichia coli: large, creamy white, non-mucoid colonies.

Klebsiella species: large, creamy white, mucoid colonies.

Proteus species: small colonies, fishy odour, swarming growth.

Pseudomonas species: beta-hemolytic colonies.

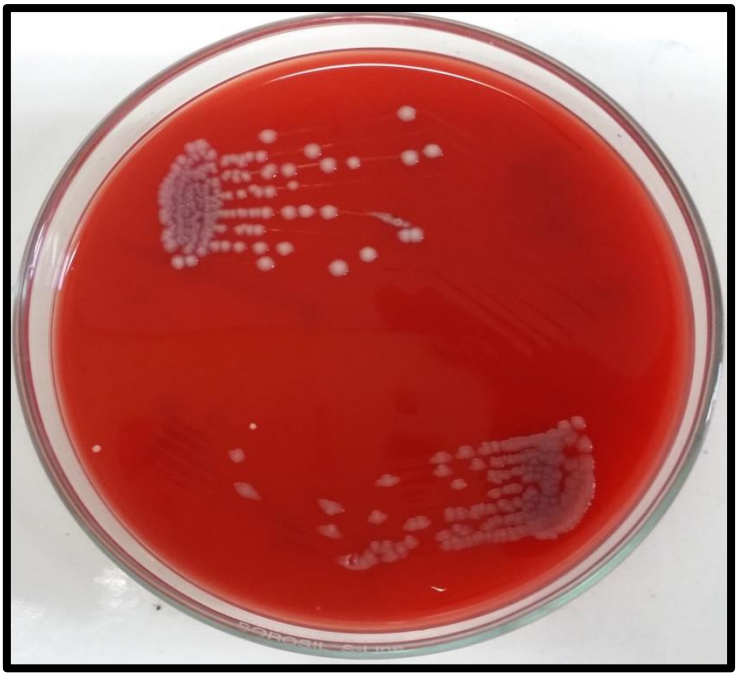


Figure 2: Growth of *Escherichia coli* and *Klebsiella pneumoniae* on Blood Agar

4.5 Gram's staining

Gram staining was performed to identify the organisms obtained on culture media after the incubation. It is a type of staining used to differentiate bacteria of various groups (Gram positive and Gram negative).

Reagents required:

i. Crystal Violet : Primary Stain
ii. Gram's Iodine : Mordant
iii. Acetone : Decolorizer
iv. Safranin : Counter-stain

Preparation of smear

i. Clean and dry glass slide was taken. Smear was prepared by rubbing swab on the center of slide.
ii. In case of fluid materials one loop full of fluid material was taken with inoculating loop and spread evenly on the center of the slide about 2-2.5 cm in diameter.
iii. Then slide was allowed to air dry.
iv. After this smear was heat fixed by gently passing it over flame for 4-5 time.

Procedure

i. Slide was placed on rack so that smear face upwards.
ii. Crystal violet was poured over the smear for 1 min.
iii. Gently washed with tap water.
iv. Covered with Gram's iodine and allowed to stand for 1 min.
v. Gently washed with tap water.
vi. Alcohol was added drop wise on the tilted slide having smear till the purple color came out.
vii. Gently washed the slide with tap water.
viii. Smear was air dried and observed under oil-immersion objective.

Gram positive organisms appeared – Purple

Gram negative organisms appeared - Pink.

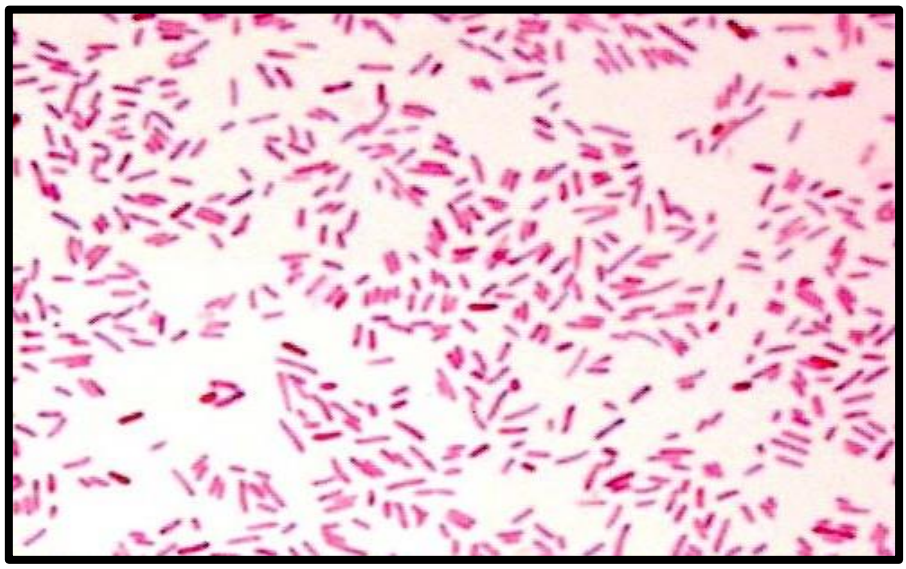


Figure 3: Gram's staining of Gram negative bacilli

4.6 BIOCHEMICAL TESTS

After performing Gram stain, identification of different bacteria was done by performing biochemical tests. Following biochemical tests were performed for the identification of bacteria:

4.6.1 Indole test

Principle

Some bacteria can produce indole from amino acid tryptophan using the enzyme typtophanase. Production of indole is detected using Kovac's reagent (p-dimethylaminobenzaldehyde, hydrochloric acid and Iso-amylalcohol). Indole reacts with the aldehyde in the reagent to give a red color. An alcoholic layer concentrates the red color as a ring at the top.

Procedure

i. 2-4 isolated bacterial colonies were picked from the Mac Conkey Agar plate with the help of straight wire and inoculated in peptone water tube having tryptophan.
ii. Tube was incubated at 37 degree Celsius for overnight.
iii. Few drops of Kovac's reagent were added.
iv. Formation of a red colored ring at the top was taken as positive.

Interpretation of result

Positive: formation of red colored ring at the top. Examples: *Escherichia coli, Proteus vulgaris, Klebsiella oxytoca* etc.

Negative: formation of yellow colored ring at the top. Examples: *Klebsiella pneumoniae, Proteus mirabilis.*

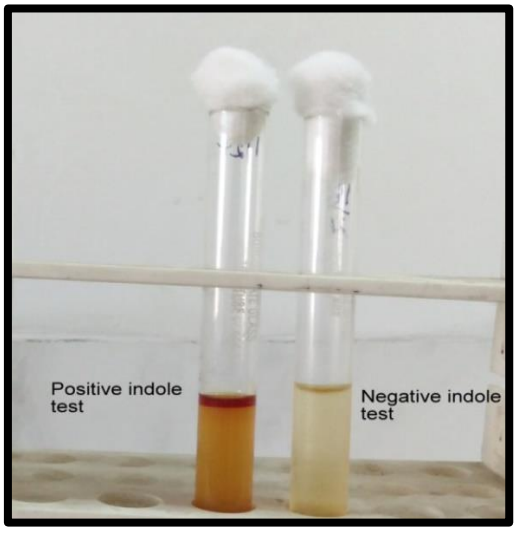


Figure 4: Interpretation of Indole test

4.6.2 Methyl red (MR) test

Principle

This is to detect the ability of an organism to produce and maintain stable acid end products from glucose fermentation. Some bacteria produce large amounts of acids from glucose fermentation that they overcome the buffering action of the system. Methyl Red is a pH indicator, which remains red in color at a pH of 4.4 or less.

Procedure

i. 2-4 isolated bacterial colonies were picked from MacConkey Agar with the help of straight wire and inoculated in Glucose Phosphate Broth (contains glucose and phosphate buffer) tube.
ii. Tube was incubated at 37 degree Celsius for 24-48 hours.
iii. 5 drops of Methyl red were added to detect the acid production.

Interpretation of results

Positive: Development of Red color.

Example: *Escherichia coli*

Negative: Development of yellow color.

Example: *Klebsiella pneumoniae*

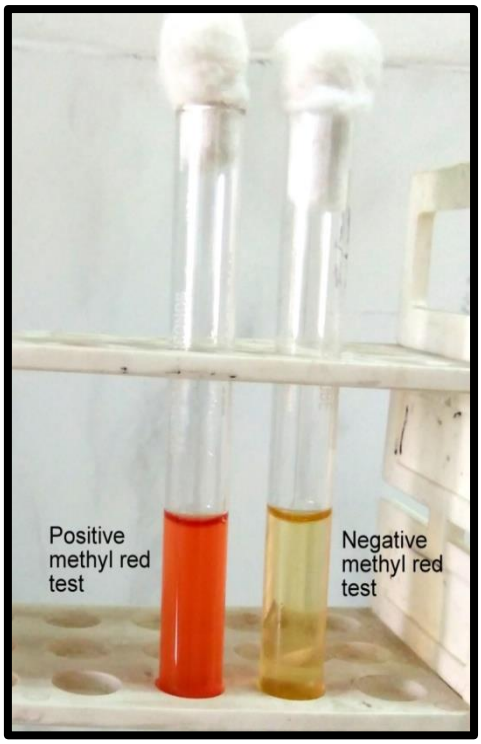


Figure 5: Interpretation of Methyl red test.

4.6.3 Citrate utilization test

Principle

This test detects the ability of an organism to utilize citrate as the sole source of carbon and energy. Bacteria are inoculated on a medium containing sodium citrate and a pH indicator bromothymol blue. The medium also contains inorganic ammonium salts, which is utilized as sole source of nitrogen. Utilization of citrate involves the enzyme citritase, which breaks down citrate to oxaloacetate and acetate. Oxaloacetate is further broken down to pyruvate and Carbon dioxide. Production of Sodium carbonate as well as ammonia from utilization of sodium citrate and ammonium salt respectively results in alkaline pH. This results in change of medium's color from green to blue.

Procedure

i. 2-4 isolated bacterial colonies were picked with the help of straight wire and inoculated into slope of Simmon's Citrate Agar.

ii. Incubated at 37 degree Celsius for overnight.

iii. After 24 hours change in color was observed.

Interpretation of results

Positive: Blue color and visible growth on Simmon's Citrate Agar medium. Examples: *Klebsiella pneumoniae, Citrobacter* species, *Acinetobacter baumanii* etc. Negative: Green color and no visible growth on Simmon's Citrate Agar medium. Example: *Escherichia coli*

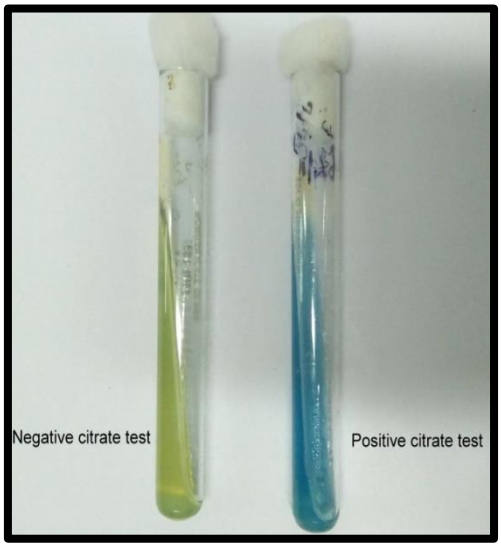


Figure 6: Interpretation of Citrate utilization test.

4.6.4 Sugar fermentation test

Principle

This test is used to differentiate bacteria on the basis of carbohydrate fermentation abilities. The ability of an organism to ferment a specific carbohydrate (Glucose, Sucrose, Lactose and Mannitol) added in basal medium (peptone broth containing Andrade's reagent) results in production of acid or acid and gas. Fermentation reactions are detected by the color change of a pH indicator as acid products are formed. A color change only occurs when enough acid products have been produced by fermentation of the carbohydrate to lower the pH to 6.8 or less. Another by-product of fermentation is the production of gas, which may be hydrogen or carbon dioxide. Durham's tube is added to the fermentation broth, the presence of a gas bubble at the top of the tube is another indication that fermentation of the carbohydrate has taken place.

Procedure

i. A few colonies were picked and inoculated in each of the sugar medium or carbohydrate broth, present in test tubes :
    a) Glucose broth having green cap
    b) Sucrose broth having sky blue cap
    c) Mannitol broth having move cap
    d) Lactose broth having red cap

ii. Then the tubes were incubated at 37 degree Celsius for 18-24 hours.

iii. After 24 hours tubes were observed for the production of acid or acid and gas.

Interpretation of results

Acid but no gas: pink color but no bubble formation at the top of the duhram's tube.

Acid and gas: pink color and bubble formation at the top of the duhram's tube.

No acid and no gas: no change in color of medium and no bubble formation.

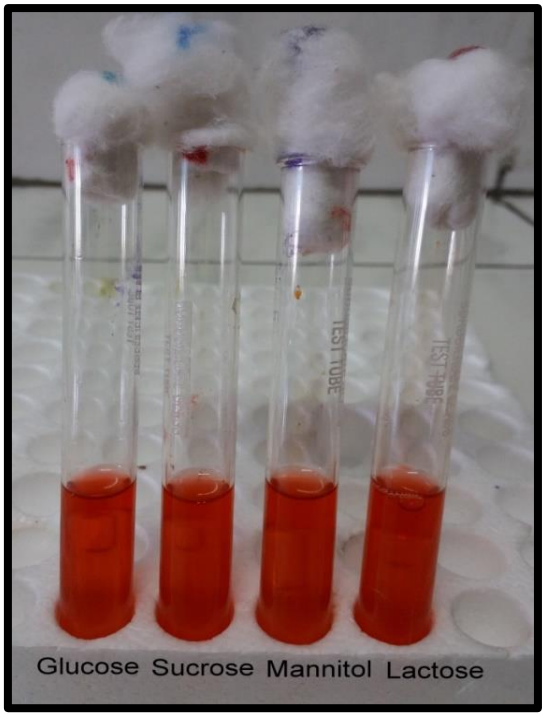


Figure 7: Interpretation of Sugar fermentation test.

4.6.5 Catalase test

Principle Catalase is the enzyme that breaks hydrogen peroxide (H_2O_2) into Water (H_2O) and Oxygen (O_2).

Procedure

i. A drop of 3% hydrogen peroxide was placed on a clean and grease free slide.

ii. Then with the help of sterilized wooden applicator stick or coverslip a colony was picked and immersed in 3% hydrogen peroxide solution.

iii. Observed the slide for immediate formation of bubbles indicating oxygen production.

Interpretation of results

Positive: Immediate and sustained production of bubbles, indicating the presence of catalase.

Example: members of *Enterobacteriaceae*, *Pseudomonas* species.

Negative: No bubble formation.

Figure 8: Interpretation of Catalase positive test.

4.6.6 Oxidase test

This test is generally performed to differentiate between *Enterobacteriaceae* (oxidase negative) and *Pseudomonadaceae* (oxidase positive).

Principle

The oxidase test is used to identify bacteria that produce cytochrome c oxidase, an enzyme of the bacterial electron transport chain. When present, the Cytochrome c oxidase oxidizes the reagent (tetramethyl-p-phenylenediamine dihydrochloride) to (Indophenols) purple color end product. When the enzyme is not present, the reagent remains reduced and is colorless.

Procedure

i. The filter paper disc (already prepared by soaking in oxidase reagent) was taken.

ii. 5-7 colonies were picked up from culture plate with glass rod and placed it on a piece of filter paper disk.

iii. Observed the reaction within 30 seconds.

Interpretation of results

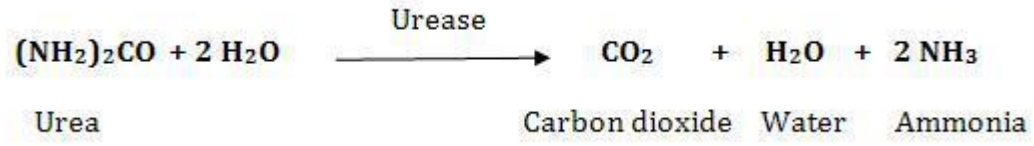
Positive: blue-purple color within 10 seconds. Example*: Pseudomonas* species. Negative: no change in color. Example: members of *Enterobacteriaceae* family, *Acinetobacter* species.

Figure 9: Interpretation of Oxidase test.

4.6.7 Urease test

Principle

Urease is an enzyme that splits urea into ammonia, carbon-dioxide and water. The ammonia combines with carbon dioxide and water to form ammonium carbonate which turns the medium alkaline, turning the indicator phenol red from its original orange yellow colour to bright pink colour after incubation of 18-24 hours.



Procedure:

i. A test tube containing Christensen's Urea agar medium slant was taken.
ii. An isolated *Escherichia coli, Citrobacter* bacterial colony was picked and inoculated with the help of straight wire.
iii. Then the tube was incubated at 37 degree Celsius for 18-24 hours.
iv. After 24 hours of incubation tube was observed.

Interpretation of results:

Positive reaction: Development of magenta to bright pink color.

Example: *Klebsiella* species, *Proteus* species.

Negative reaction: No change in color. Examples: *Escherichia coli* species.

Figure 10: Interpretation of Urease test.

4.6.8 Phenylalanine deaminase (PPA) test

Principle

Bacteria produce enzyme phenylalanine deaminase that can metabolize amino acid: phenylalanine. This enzyme act on phenylalanine and leaves phenylpyruvic acid which can be detected by adding an oxidizing agent such as ferric chloride to the incubated tube. If the acid is present, a green color can be detected. The green color fades, so the test should be read immediately.

Procedure

i. An isolated bacterial colony was inoculated on phenylalanine agar slant in test tube.
ii. Tube was incubated at 37 degree Celsius for 18-24 hours.
iii. After incubation, few drops of ferric chloride were added to phenylalanine agar slant with sterile dropper.
iv. Allowed the tube to stand for 1 minute. Observed the color of slant.

Interpretation of result

Positive reaction: Development of light to dark green color within 1-5 minutes after applying ferric chloride reagent. Example: *Proteus* species.

Negative reaction: yellow color.

Figure 11: Interpretation of Phenylalanine deaminase test.

4.6.9 Triple sugar iron (TSI) test

The purpose of this test is to determine the fermentation of lactose or sucrose and H_2S production.

Principle

TSI contains three sugars – glucose (1%), sucrose (10%), and lactose (10%). Phenol Red is indicator that turns yellow at an acid pH resulting from fermentation. If an organism ferments glucose only, the reaction will be K/A, or alkaline slant over acid butt. This is because the fermentation takes place in the butt, which results in enough acid to lower the pH here and turn the butt yellow. However, in the slant, aerobic oxidation or respiration takes place and not enough acid is produced to lower the pH, so it remains red. If sucrose or lactose is fermented, the reaction will be A/A (acid slant over acid butt). Because of the high concentration of these sugars, enough acid is produced by fermentation in the butt to lower the pH of both the butt and the slant, turning both yellow. Gas production, sometimes resulting from fermentation, can also be detected as actually splitting or pushing the agar upwards. Sodium thiosulfate and ferrous ammonium sulfate present in the medium detects the production of hydrogen sulfide and is indicated by black color in the butt of the tube.

Procedure

i. An isolated colony was picked with sterile straight wire. First stabbed in butt and then streaked along the surface (zig-zag to cover surface) of slant.
ii. Then incubated at 37 degree Celsius for 24 hours.
iii. After incubation the carbohydrate fermentation, gas production and H_2S production in TSI agar tube were observed.

Interpretation of results

a) If the medium in the slant becomes red, but the medium in the butt becomes yellow with or without gas production. This indicates that the organism only ferments glucose. Example: *Salmonella* species.
b) If the medium in the slant becomes yellow and the medium in the butt also becomes yellow. This indicates the fermentation of glucose, lactose and/or sucrose. Example: *Escherichia coli, Klebsiella* species.
c) If the medium in the slant and butt becomes red. This indicates that the tested organism is a non-fermenter.
d) H_2S production results in the blackening of the medium in the butt of the tube.

e) CO_2 production is indicated by splitting of Agar in two or more sections.

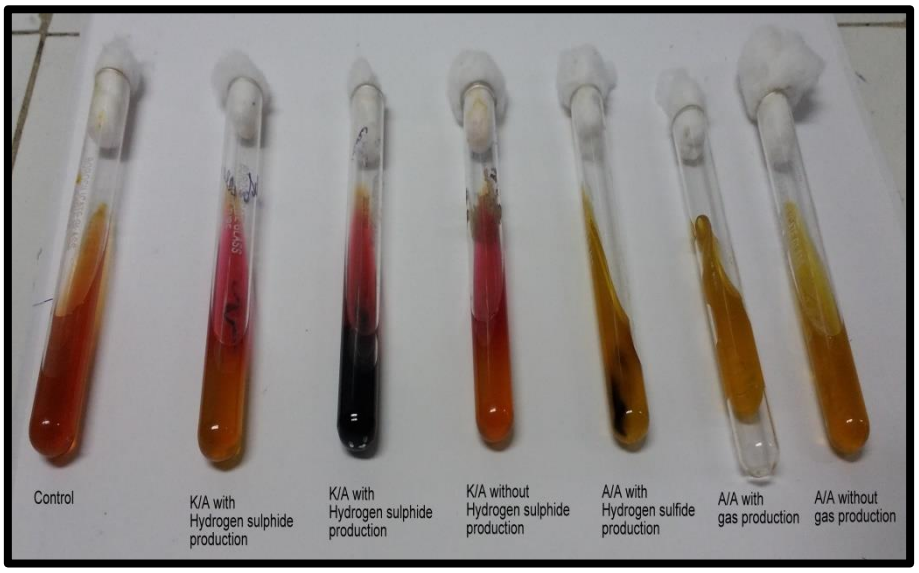


Figure 12: Interpretation of Triple sugar iron (TSI) test.

4.6.10 Motility test

Principle

This test is performed to differentiate between motile and non-motile bacteria. Motile bacteria move with structures called flagella. In semi-solid agar media, motile bacteria 'swarm' and give a diffuse spreading growth that is easily recognized by the naked eye.

Procedure

i. A tube of Motility Agar medium was taken. An isolated bacterial colony was picked by using sterile straight wire.
ii. Inoculated the bacterial colony in Motility Agar medium by stab inoculation.
iii. Then the tube was incubated at 37 degree Celsius for 18-24 hours.
iv. After this tube was observed by holding it up against the light source.

Interpretation of results

Positive: Cloudy, diffuse growth. Examples*: Escherichia coli, Citrobacter* species, *Proteus* species etc.

Negative: Well-defined growth along the stab. Example: *Klebsiella* species.

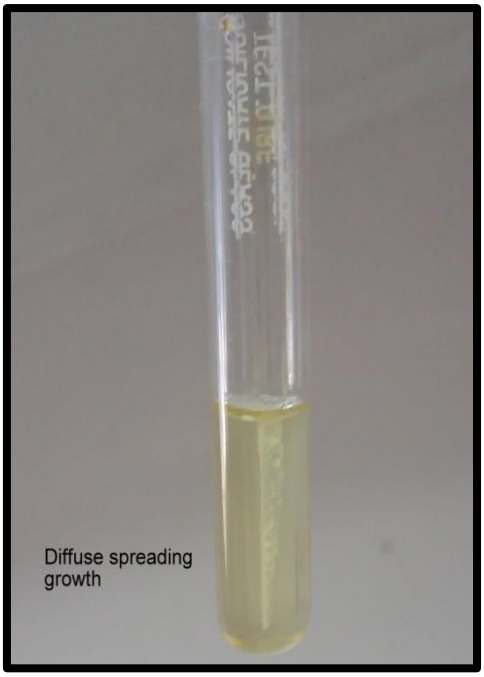


Figure 13: Interpretation of Motility test.

4.7 Antimicrobial susceptibility testing (AST)

Antimicrobial susceptibility testing of all isolates was performed on Mueller Hinton Agar by standard Kirby Bauer disk diffusion method.

4.7.1 Mueller Hinton Agar (MHA): (HIMEDIA™ M173)

Mueller Hinton Agar is a microbiological growth medium used for antibiotic susceptibility testing. This Agar medium is recommended for the disk diffusion method of antibiotic susceptibility testing. Mueller Hinton Agar is recommended for the diffusion of antimicrobial agents impregnated on paper disc through an agar gel as described in CLSI Approved Standard.

Table 3: Composition of Mueller Hinton Agar

Ingredients	**Grams/Litre**
Beef, infusion from	300
Casein acid hydrolysate	17.5
Starch	1.5
Agar	17

Final pH (at 25°C) 7.3±0.1

Media Preparation

i. 38 grams powdered Mueller Hinton Agar was in 1000 ml distilled water. Heated to boiling to dissolve the medium completely.
ii. Sterilized by autoclaving at 15 lbs. pressure (121°C) for 15 minutes. Mixed well before pouring.

Procedure

i. First the growth was passed in peptone water and incubated for 2-4 hours at 37 degree Celsius.
ii. A sterilized cotton swab was dipped in this inoculated peptone water.
iii. Squeeze the cotton swab on the walls of the test tube and lawn culture made on whole MHA plate.
iv. By using a sterilized forceps antibiotic discs were placed on MHA Agar.
v. Plates were incubated for 24 hours at 37 degree Celsius.

The diameter of zone of inhibition of growth was interpreted as recommended by CLSI (Clinical and Laboratory Standards Institute) guidelines. Antibiotics disks used for sensitivity testing were Nitrofurantoin (300 mcg), Norfloxacin (10 mcg),Ciprofloxacin (5 mcg), Amikacin (30 mcg), Netillin (30 mcg), Gentamicin (10 mcg), Meropenem (10 mcg), Imipenem (10 mcg), Polymyxin-B (300 Units), Cephadroxil (30 mcg), Cefuroxime (30 mcg), Ceftriaxone (30 mcg), Cefpodoxime (10 mcg), Cefixime (5 mcg), Cefoperazone (75 mcg), Ceftazidime (30 mcg), Cefepime (30 mcg), Cefoxitin (30 mcg), Cefotaxime (30 mcg), Piperacillin/Tazobactam (100/10 mcg), Amoxycillin/Clavulanic acid (50/10 mcg). All the antibiotic discs were procured from HIMEDIA, Mumbai.

Table 4: Zone Size Interpretative Chart (Gram-negative Urine isolates):

S.No.	Antimicrobial Agent	Symbol	Disc Content (mcg)	Sensitive (mm or more)	Intermediate (mm)	Resistant (mm or less)
1.	Amikacin	AK	30	17	15-16	14
2.	Cefuroxime	CXM	30	18	15-17	14
3.	Ceftriaxone	CTR	30	23	20-22	19
4.	Cefotaxime	CTX	30	26	23-25	22
5.	Ceftazidime	CAZ	30	21	18-20	17
6.	Cefoperazone	CPZ	75	21	16-20	15
7.	Cefpodoxime	CPD	10	21	18-20	17
8.	Cefixime	CFM	5	19	16-18	15
9.	Cefepime	CPM	30	18	15-17	14
10.	Cefoxitin	CX	30	18	15-17	14
11.	Ciprofloxacin	CIP	5	21	16-20	15
12.	Norfloxacin	NX	10	17	13-16	12
13.	Gentamicin	GEN	10	15	13-14	12
14.	Netillin	NET	30	15	13-14	12
15.	Imipenem	IPM	10	23	20-22	19
16.	Meropenem	MRP	10	23	20-22	19
17.	Nitrofurantoin	NIT	300	17	15-16	14
18.	Amoxicillin/ Clavulanic acid	AMC	50/10	18	14-17	13
19.	Piperacillin/ Tazobactam	PIT	100/10	21	18-20	17
20.	Polymyxin-B *(P. aeruginosa)*	PB	300	12	-	11
21.	Colistin *(P. aeruginosa)*	CL	10	11	-	10

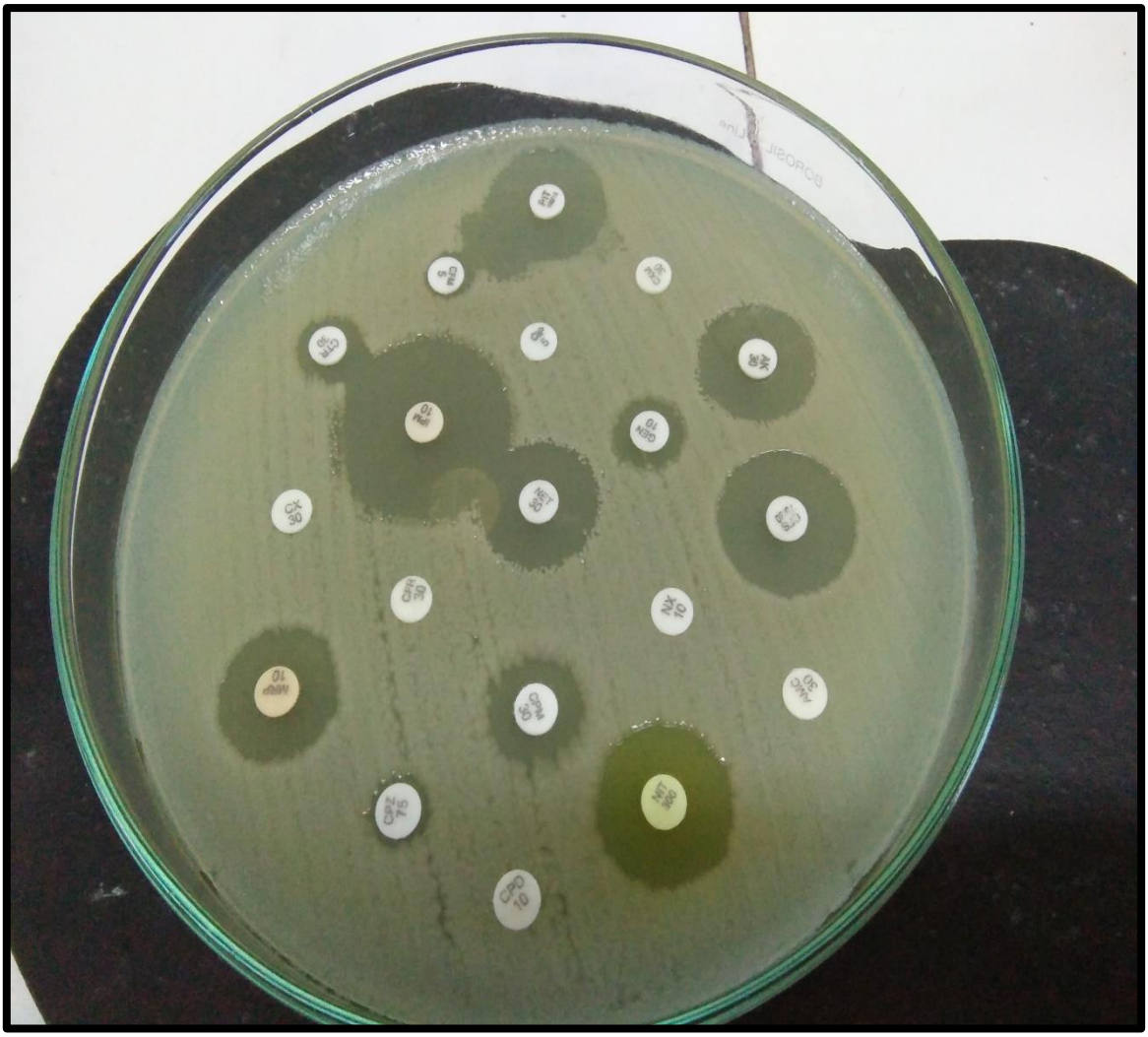


Figure 14: Antimicrobial susceptibility pattern on Muller Hinton Agar plate.

4.8 Detection of Extended Spectrum beta lactamase (ESBL) producers

4.8.1 Screening of ESBL producers

The screening test for ESBL producers was done according to the criteria recommended by Clinical and Laboratory Standards Institute (CLSI) guidelines. On Mueller Hinton Agar, the zone size of less than or equal to 27mm for cefotaxime (30mcg), zone size less than or equal to 22mm for ceftazidime (30mcg), zone size of less than or equal to 25mm for ceftriaxone (30mcg) and zone size of less than or equal to 17mm for cefpodoxime indicated that the strain probably produced ESBL.

Table 5: Standard Zone sizes of antibiotics for ESBL screening as per CLSI guidelines

Antibiotics	**Strength (mcg)**	**Zone size (mm)**
Cefotaxime	30	≤ 27
Ceftazidime	30	≤ 22
Ceftriaxone	30	≤ 25
Cefpodoxime	10	≤ 17

4.8.2 Phenotypic confirmatory test for ESBL production

4.8.2.1 Cephalosporin/Clavulanate combination disks:

Ceftazidime (30 µg) disks with and without clavulanate (10 µg) for phenotypic confirmation of the presence of ESBLs in Gram negative isolates were used. According to CLSI recommendation the disk tests were performed with confluent growth on Mueller Hinton Agar. A difference of ≥5 mm between the zone diameters of the cephalosporin disks and their respective Cephalosporin/ Clavulanate disks was taken as phenotypic confirmation of ESBL production.

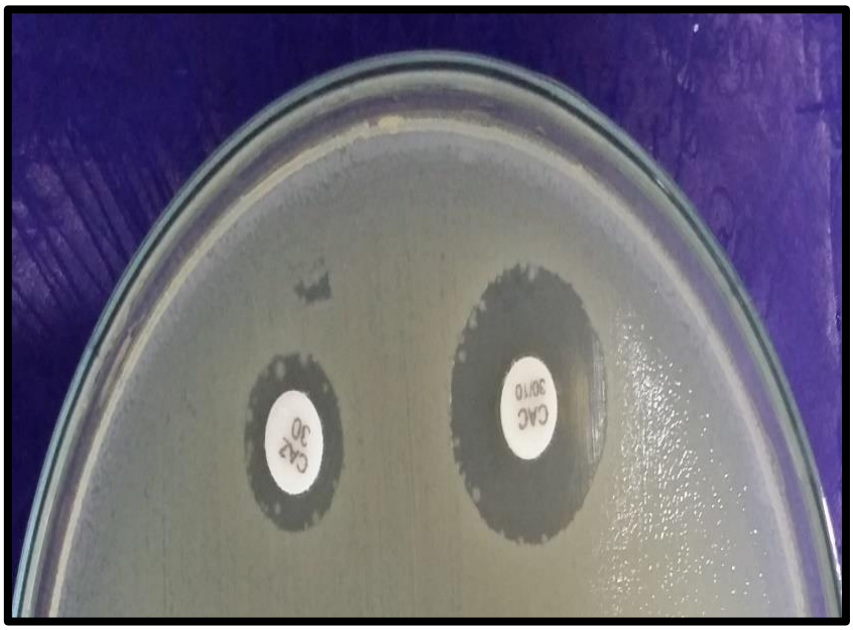


Figure 15: Combined disk test result.

Other method used for detection of ESBL producers was Double-Disk Synergy Test.

4.8.2.2 Double-Disk Synergy Test (DDST

This is simple and reliable method for detection of ESBL production. The disc that contains oxyimino β lactam i.e., Ceftriaxone (30µg) is placed 30mm apart (centre - centre) from Piperacillin/Tazobactam disk (100/10µg) or Cefoperazone is placed apart from Cefoperazone/Sulbactam, clear extension of the edge of the inhibition zone towards or Piperacillin/Tazobactam or Cefoperazone-Sulbactam disc is interpreted as positive ESBL production.

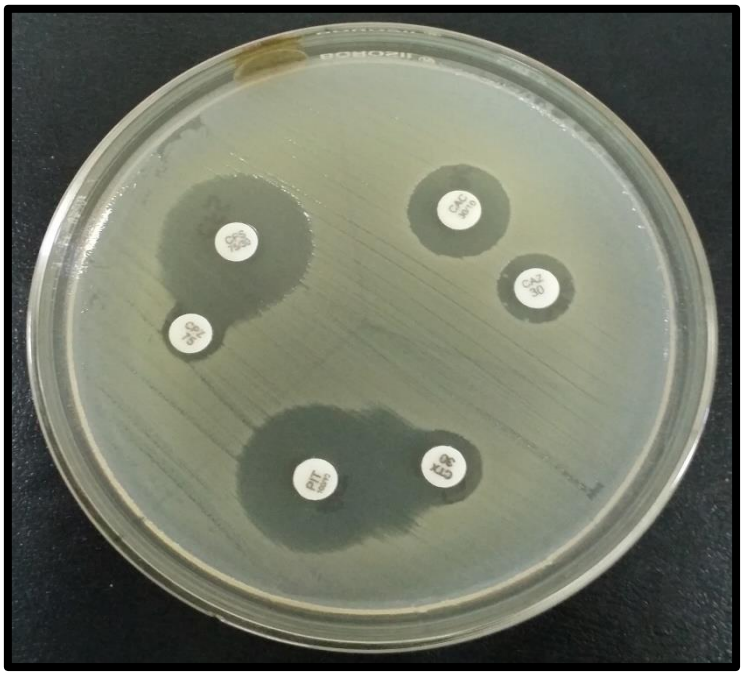


Figure 16: Double disk test result.

CHAPTER 5: RESULTS

The study was conducted in Punjab Institute of Medical Sciences (PIMS), Jalandhar. During three consecutive months (January 2016 to April 2016) of study, a total of 1209 urine specimens were submitted to Microbiology laboratory for culture. Out of these, 360 (29.78%) urine specimens showed positive growth for Gram negative bacteria while other 849 (70.22%) urine specimens showed negative growth, Gram positive isolates, Candida or contamination(others) . This is shown in **Table 6, Chart 1.**

Table 6: Incidence of Gram negative bacterial isolates obtained in Urine samples

	Number of Urine samples (N)	**Percentage (%)**
Gram negative isolates	360	29.78%
No growth / others	849	70.22%
Total	**1209**	

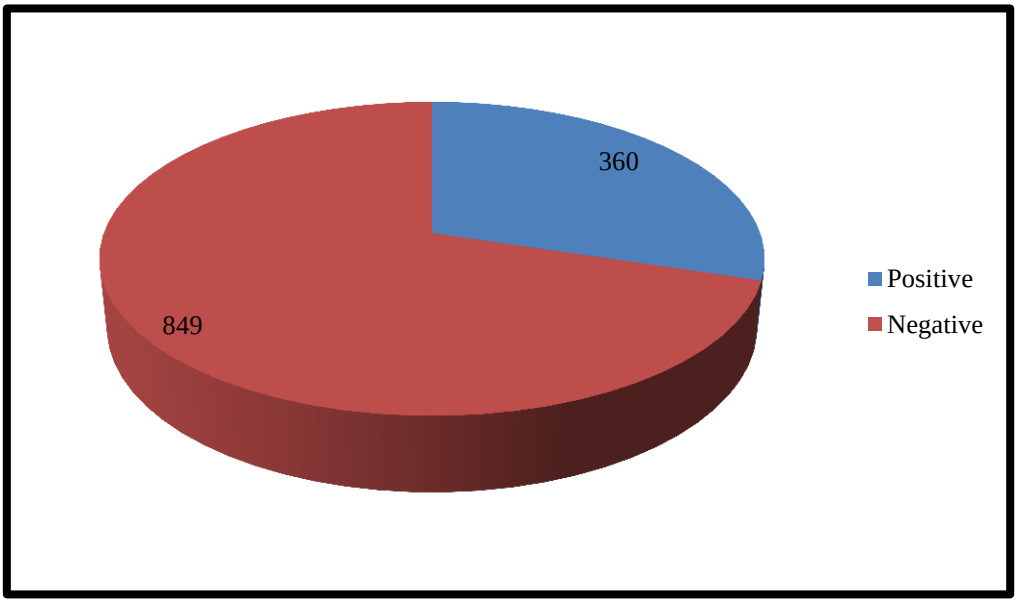


Chart 1: Incidence of positive bacterial growth obtained in urine samples.

Out of these 360 Gram negative isolates, there were 240 isolates from In-patient Department (IPD) and 120 isolates from Out-patient Department that met the criteria for positive growth. This is shown in **Table 7, Chart 2.**

Table 7: Prevalence of isolates obtained in IPD and OPD

Departments	**Number of Isolates (N)**
In-patient department (IPD)	240
Out-patient department (OPD)	120
Total	**360**

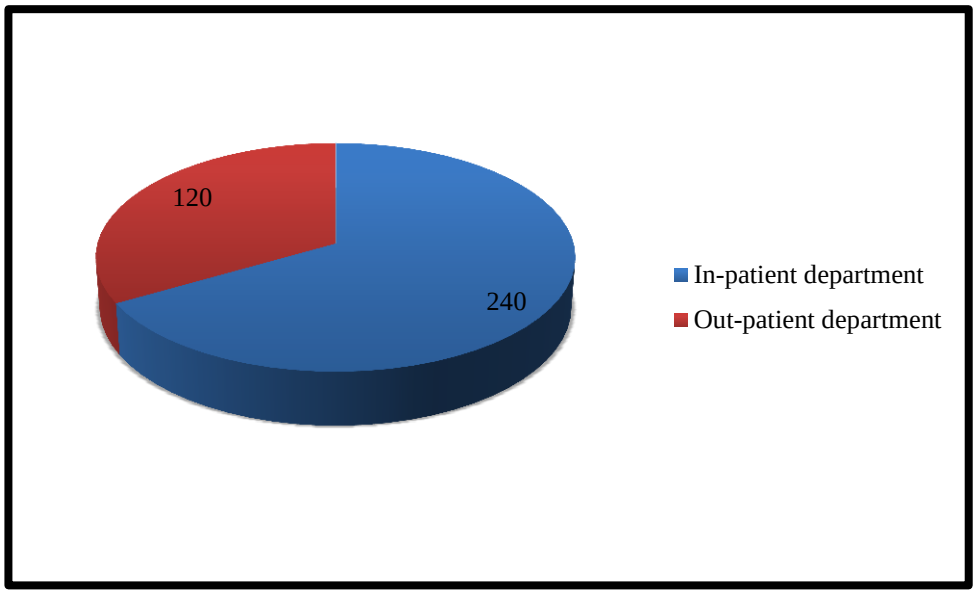


Chart 2: Distribution of Gram negative isolates in IPD and OPD

Among the isolated 360 pathogens, prevalence of Gram negative bacteria revealed that *Escherichia coli* (IPD = 50%, OPD = 29.44%) was the most prevalent uropathogen followed by *Klebsiella* species (IPD = 5.83% , OPD =1.11%), *Acinetobacter* species(IPD = 4.16%, OPD = 0%), *Proteus* species (IPD = 3.33%, OPD =1.11%), *Pseudomonas aeruginosa* (IPD = 2.5% , OPD = 0.83%) and *Citrobacter* species (IPD = 0.83%, OPD =0.83%). This is shown in **Table 8, Chart 3.1 and Chart 3.2.**

Table 8: Prevalence of Gram negative bacteria in IPD and OPD patients

Gram negative Bacterial isolates	**Isolates in IPD N**	**Isolates in OPD N**	**Total**
Escherichia coli	180	106	286
Klebsiella species	21	4	25
Proteus species	12	4	16
Pseudomonas aeruginosa	9	3	12
Acinetobacter species	15	0	15
Citrobacter species	3	3	6
Total	**240**	**120**	**360**

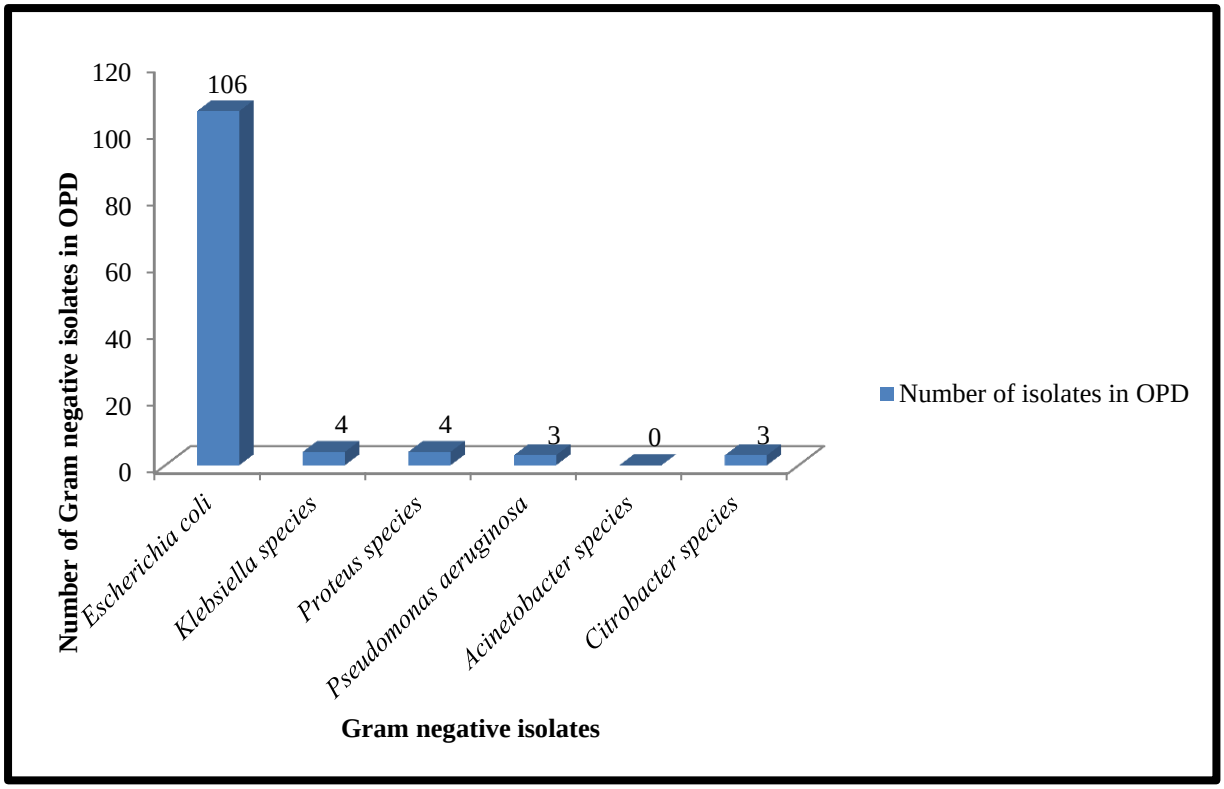


Chart 3.1: Prevalence of Gram negative isolates in OPD

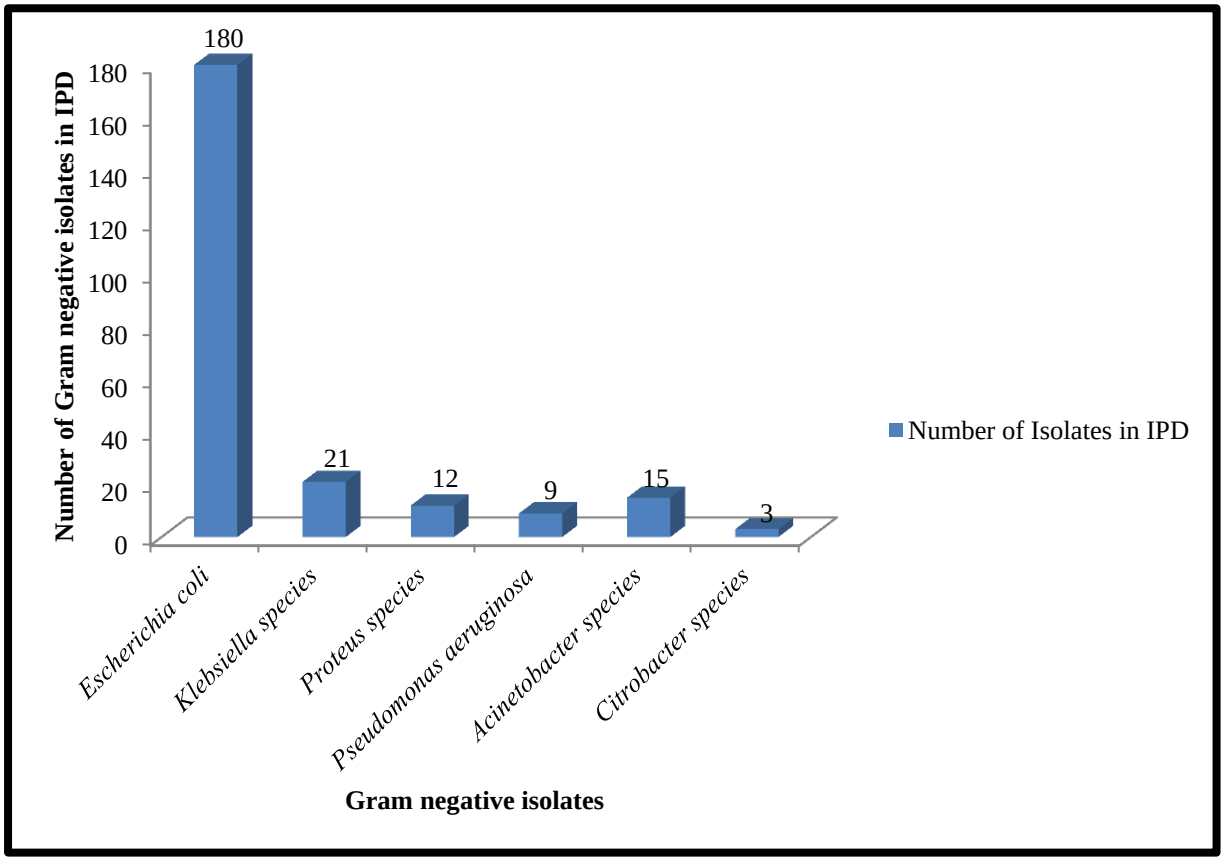


Chart 3.2: Prevalence of Gram negative isolates in IPD.

In case of IPD Urine samples, most of the positive cultures were isolated from patients of age group >45 years followed by 18-35 years of age group. While in case of OPD urine samples, most of the positive cultures were isolated from patients of 18-35 years followed by >45 years. This is shown in **Table 9, Chart 4.1 and Chart 4.2**

Table 9: Age-wise distribution of isolates in In-Patient and Out-Patient Departments

Age Groups	**Isolates in IPD (N)**	**Isolates in OPD (N)**	**Total**
< 18 years	9	15	24
18-35 years	75	51	126
35-45 years	42	24	66
>45 years	114	30	144
Total	**240**	**120**	**360**

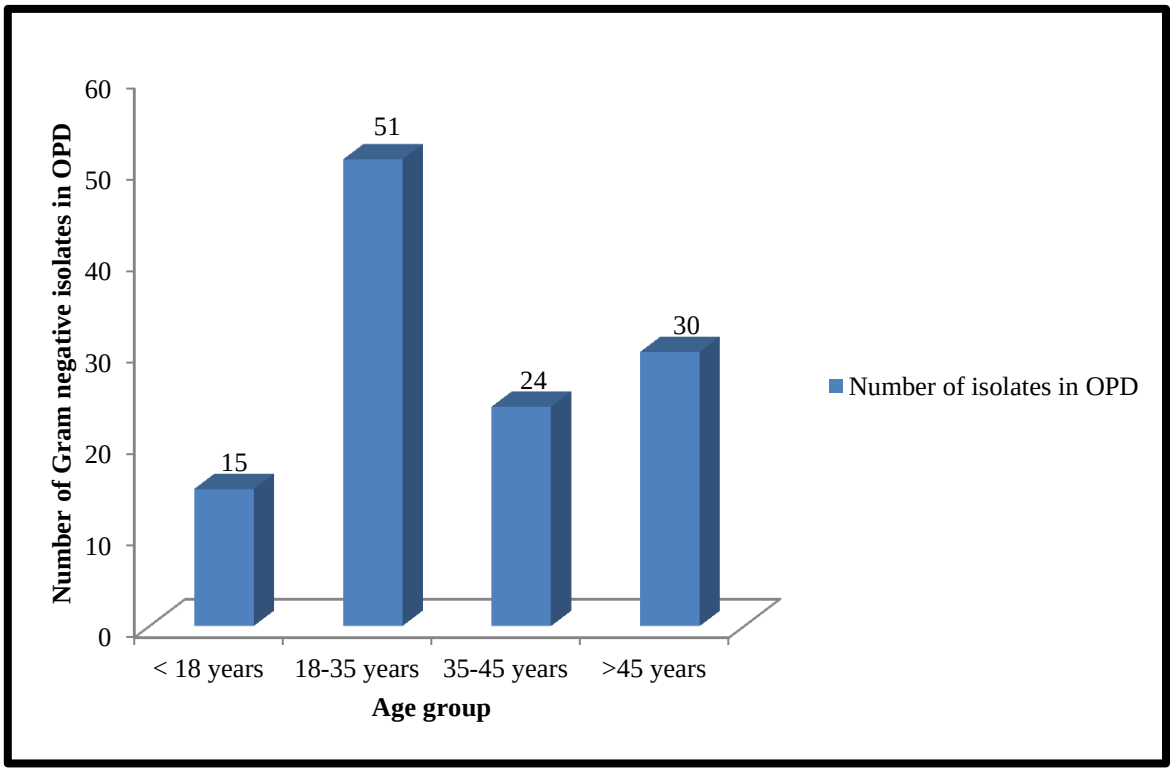


Chart 4.1: Age-wise distribution of isolates in OPD

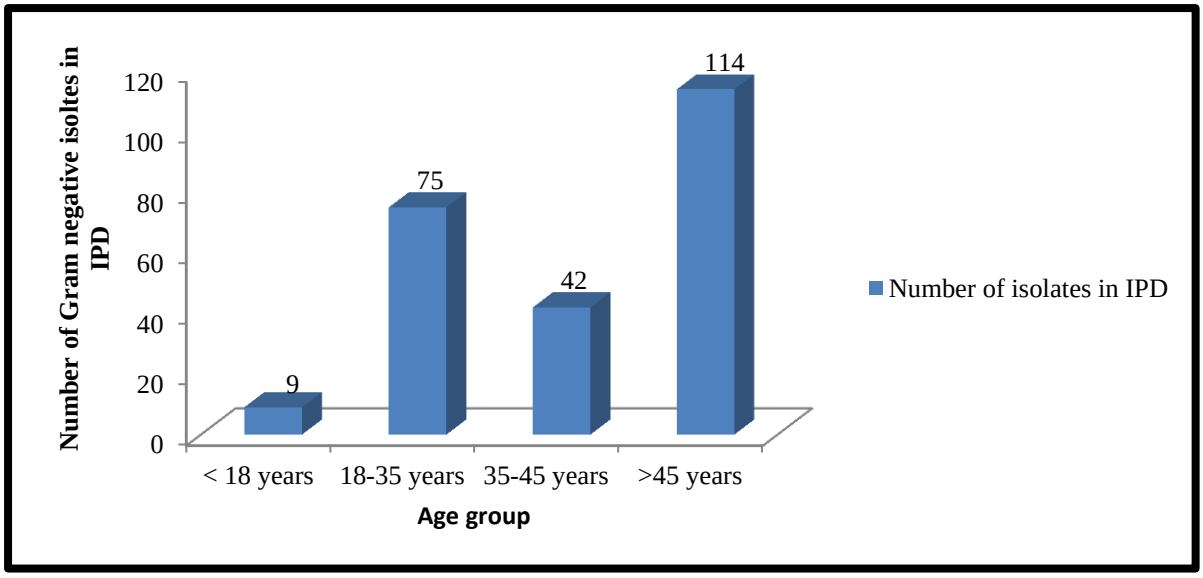


Chart 4.2: Age-wise distribution of isolates in IPD

Out of 360 positive isolates, maximum (180 isolates) were from Gynaecology ward gave positive results followed by 68 isolates from Medicine ward and the least number of isolates (10) from Orthopaedics ward gave positive results. This is shown in **Table 10, Chart 5.1 and Chart 5.2**

Table 10: Ward-wise distribution of isolates

Wards	**Number of Isolates (N)**	**Percentage (%)**
Medicine	68	18
Gynaecology	180	50
ICU	54	15
Orthopaedics	10	2.7
NICU + Paediatric	12	3.33
Emergency	36	10
Total	**360**	

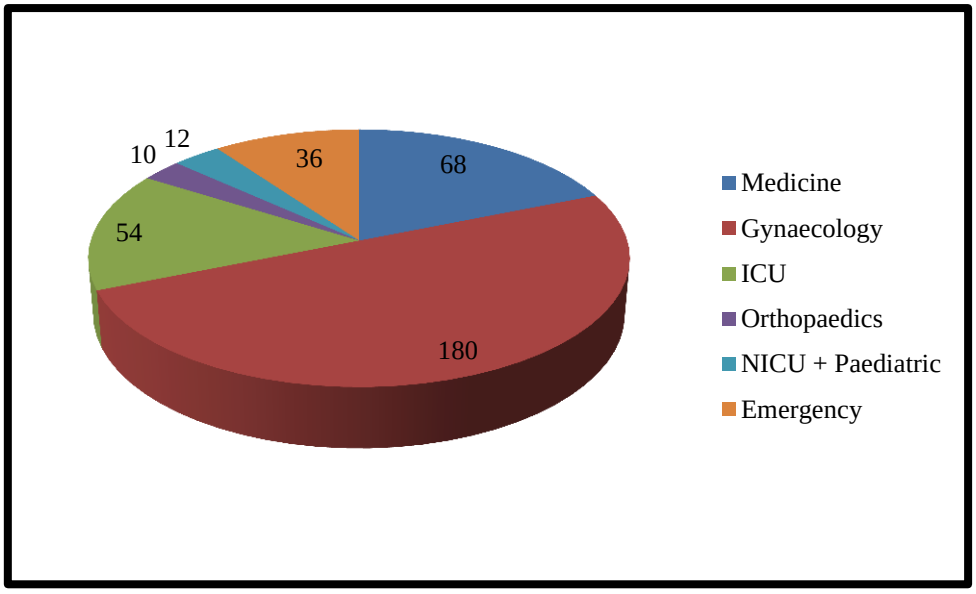


Chart 5.1: Ward-wise distribution of isolates

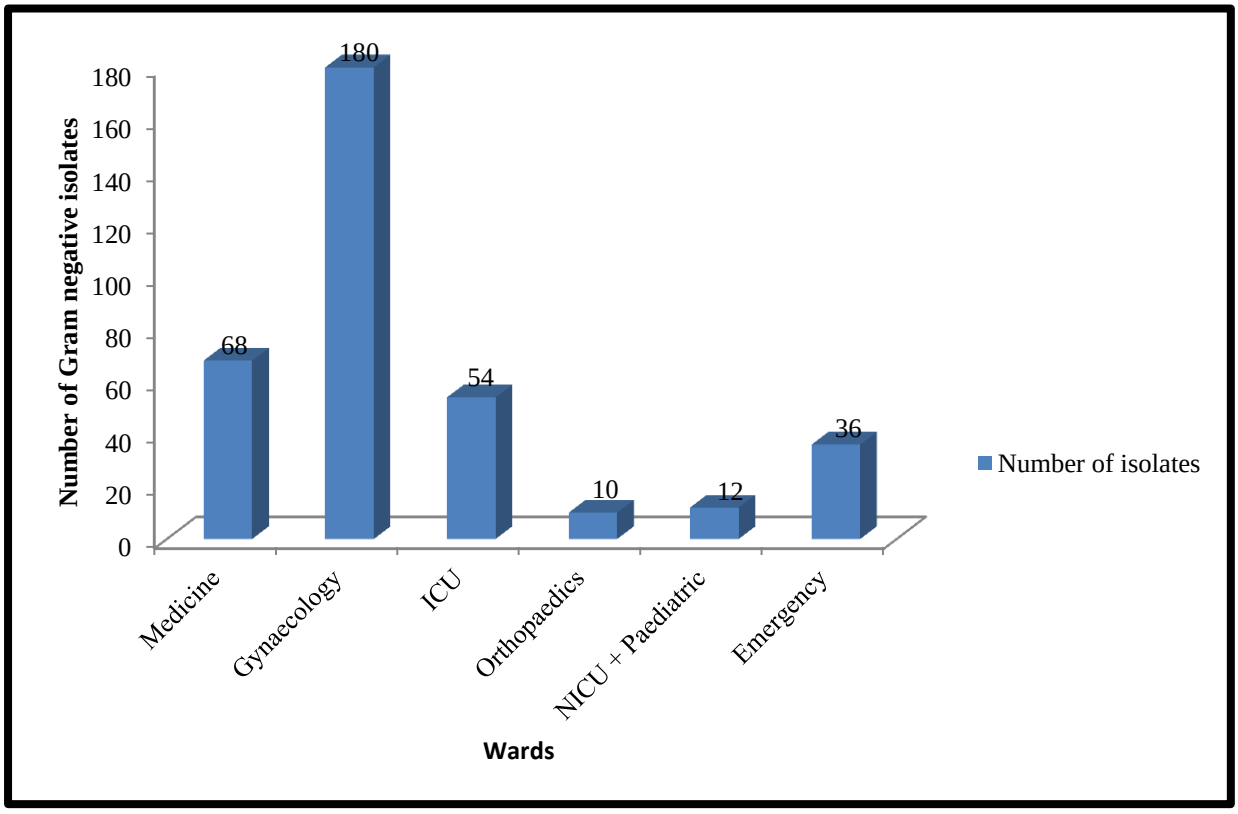


Chart 5.2: Ward-wise distribution of isolates

ESBL positive isolates

Out of 360 Gram negative bacterial isolates, 135(37.5%) isolates were found to be ESBL producers while other 225 (62.5%) were non-ESBL producers. This is shown in **Table 11, Chart 6.**

Table 11: Prevalence of ESBL producing isolates

	Number of isolates (N)	**Percentage (%)**
ESBL producers	135	37.5
Non-ESBL producers	225	62.5
Total	**360**	

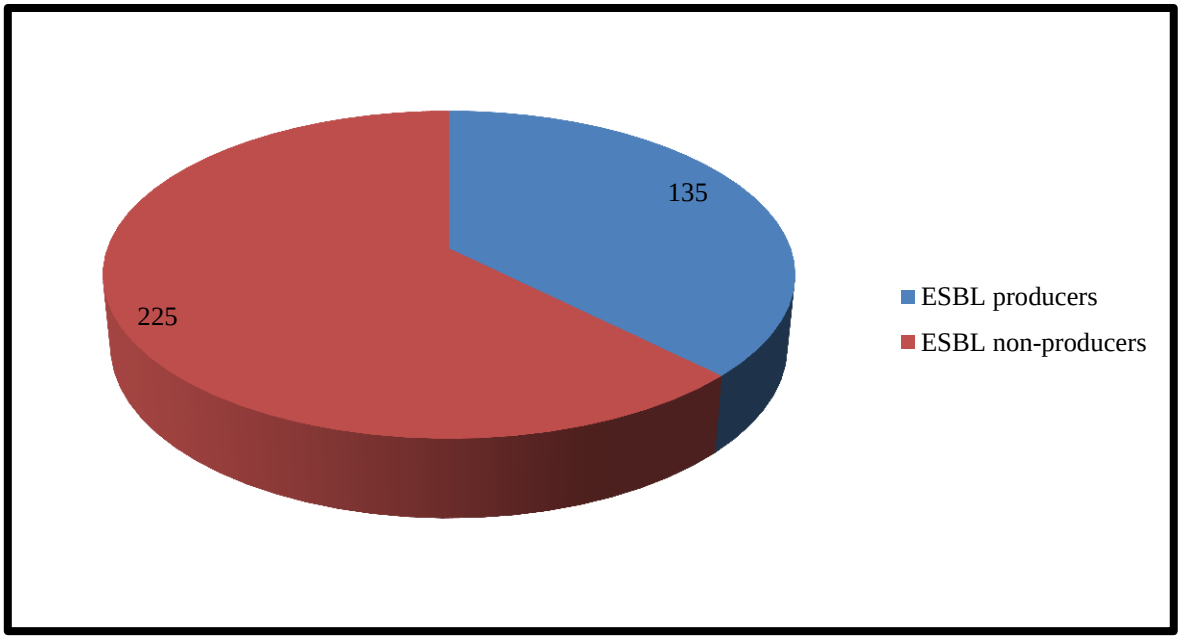


Chart 6: Prevalence of ESBL producing isolates

There were 117 (86.66%) isolates from male patients that gave ESBL positive results while 18 (13.33%) isolates from female patients were found to be ESBL producers. This is shown in **Table 12, Chart 7.**

Table 12: Gender-wise distribution of ESBL positive isolates

Gender	**ESBL positive isolates (N)**	**Percentage (%)**
Male	117	86.66
Female	18	13.33
Total	**135**	

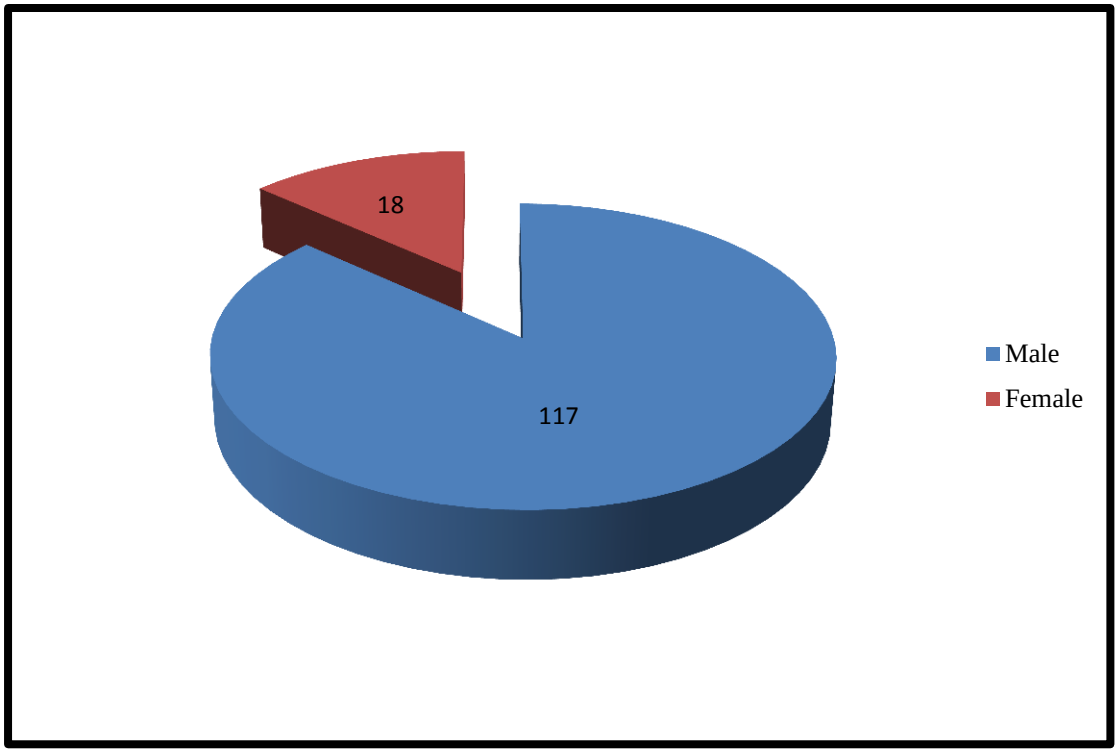


Chart 7: Gender-wise distribution of ESBL positive isolates

There were 95(70.37%) isolates from IPD and 40 (29.62%) isolates from OPD that gave ESBL positive results. This is shown in **Table13, Chart 8.**

Table 13: Distribution of ESBL positive isolates in IPD and OPD

Departments	**ESBL positive isolates (N)**	**Percentage (%)**
IPD	95	70.37
OPD	40	29.63
Total	**135**	

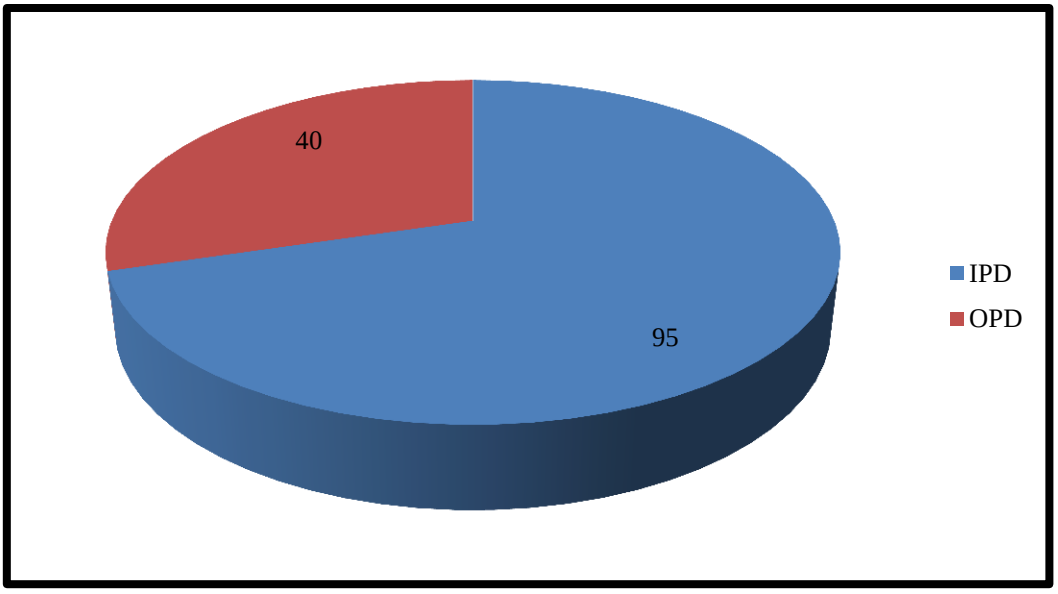


Chart 8: Distribution of ESBL positive isolates in IPD and OPD

Among the135 ESBL producers, Occurrence of ESBL positive *Escherichia coli* was maximum in both In-patient Department (59) and Out-patient department (34) followed by ESBL positive *Acinetobacter* species among IPD patients were 13, ESBL positive *Proteus* species were 9 among IPD patients and 3 were found in patients of OPD . There was no ESBL positive *Citrobacter* species found both among patients of IPD and OPD. This is shown in **Table 14, Chart 9.1 and Chart 9.2**

Table 14: Occurrence of ESBL positive entities in IPD and OPD

Bacterial isolates	**ESBL positive isolates in IPD (N)**	**ESBL positive isolates in OPD (N)**	**Total**
Escherichia coli	59	34	93
Klebsiella species	9	0	9
Proteus species	9	3	12
Pseudomonas aeruginosa	5	3	8
Acinetobacter species	13	0	13
Citrobacter species	0	0	0
Total	**95**	**40**	**135**

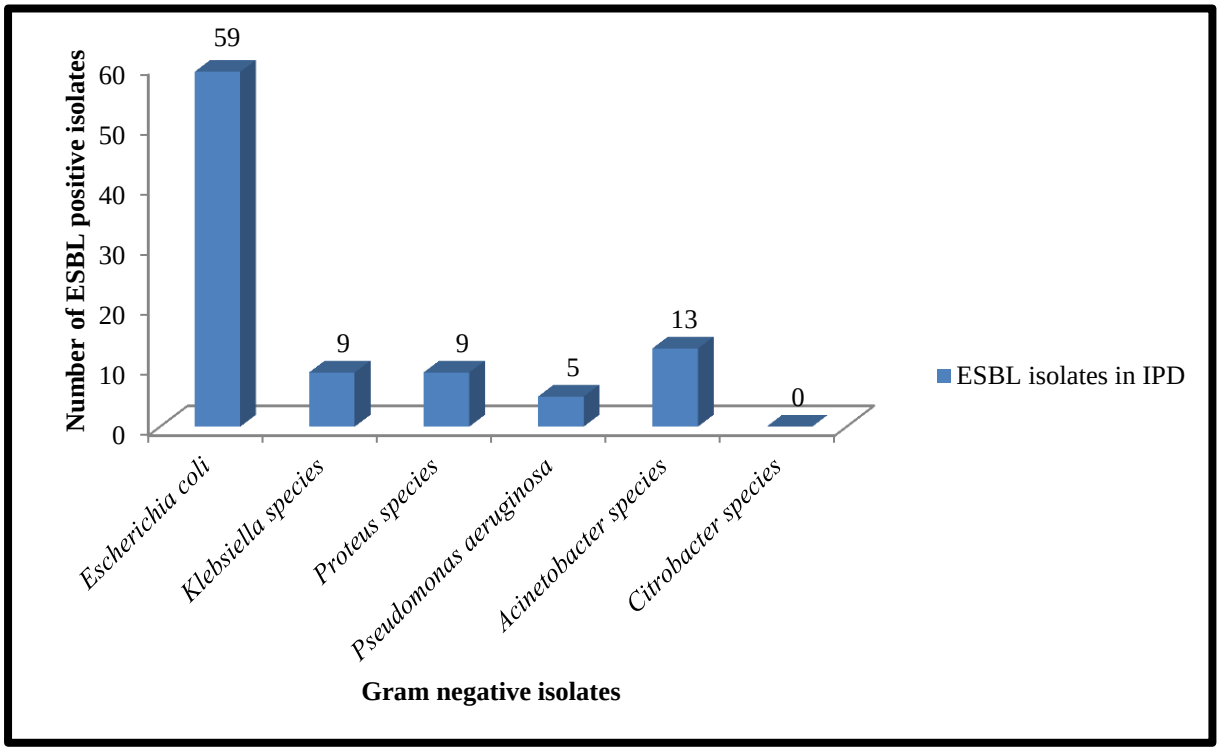


Chart 9.1: Occurrence of ESBL positive entities in In-Patient Department (IPD)

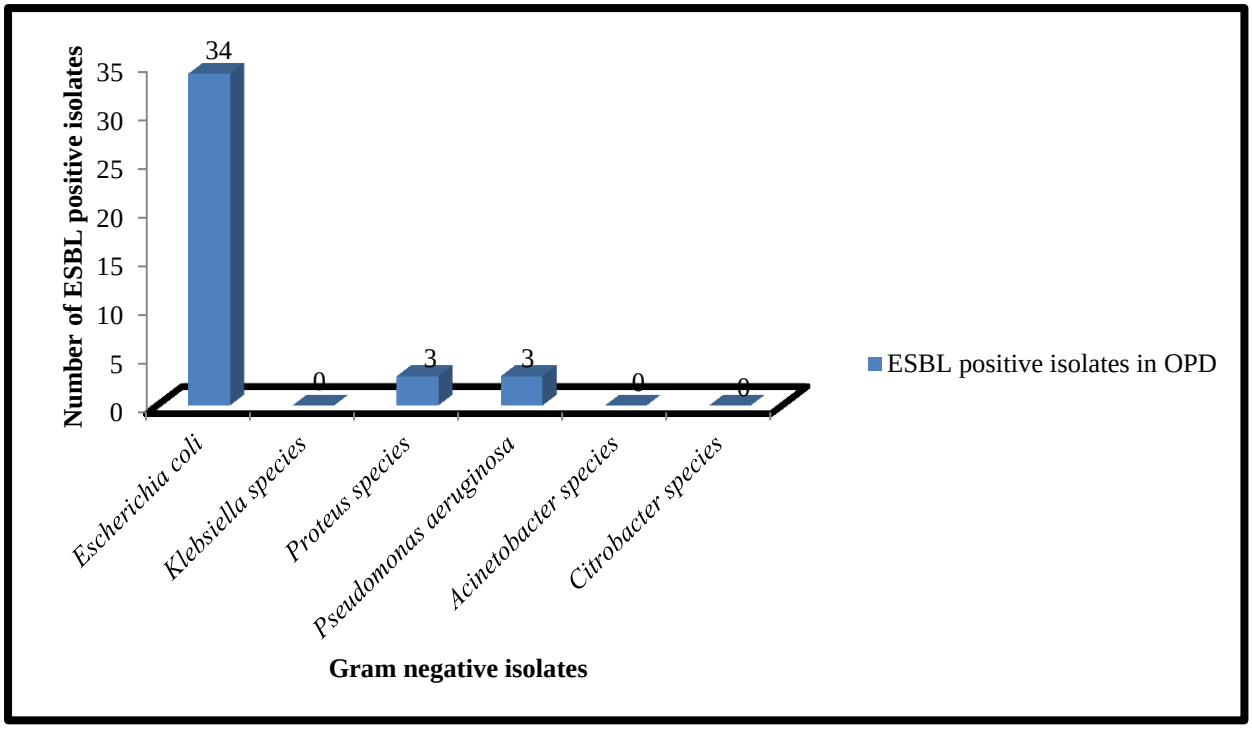


Chart 9.2: Occurrence of ESBL positive entities in Out-Patient Department (OPD)

Out of 135 ESBL positive isolates, maximum 76 (56.29%) isolates from Gynaecology ward were ESBL positive followed by 24 (17.77%) ESBL positive isolates were from ICU ward and there was no ESBL positive isolate found in both NICU and Paediatrics wards. This is shown in **Table 15, Chart 10.**

Table 15: Ward-wise distribution of ESBL positive isolates

Wards	**ESBL positive isolates (N)**	**Percentage (%)**
Medicine	18	13.33
Gynaecology	76	56.29
Surgery	0	0
ICU	24	17.77
Orthopaedics	3	2.22
NICU + Paediatrics	0	0
Emergency	12	8.88
Total	**135**	

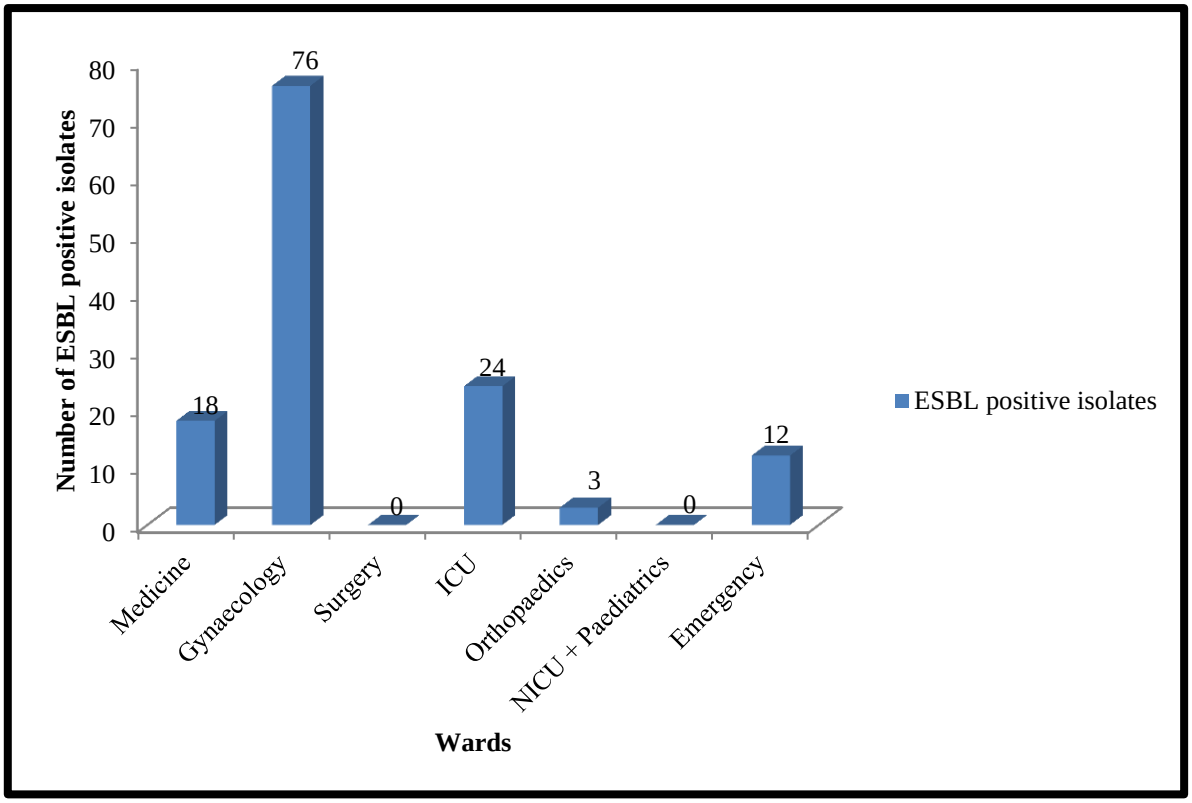


Chart 10: Ward-wise distribution of ESBL positive isolates

The commonest organism isolated was *Escherichia coli* that showed highest resistance to Amoxycillin/ Clavulanic acid (86.01%) followed by Norfloxacin (84.96%). *Escherichia coli* showed Antimicrobial resistance 18.88% to Nitrofurantoin, 84.96% to Norfloxacin, 82.86% to Ciprofloxacin, 24.12% to Amikacin, 45.10% to Gentamicin, 20.97% to Netillin, 46.15% to Meropenem, 8.38% to Imipenem, 17.83% to Polymyxin B, 61.88% to Cefadroxil, 72.37% to Cefuroxime, 74.47% to Ceftriaxone, 82.86% to Cefpodoxime, 79.72% to Cefixime, 76.57% to Cefoperazone, 67.13% to Cefepime, 46.15% to Cefoxitin, 47.20% to Cefotaxime, 25.17% to Piperacillin/ Tazobactam, 86.01% to Amoxicillin/ Clavulanic acid, 20.97% to Ceftazidime/ Clavulanic acid, 32.51% to Cefoperazone/ Sulbactam and 20.97% to Colistin.

Table 16: Antimicrobial resistance among various Gram-negative isolates

Antibiotics	***Escherichia coli* (N=286)**	***Klebsiella species* (N=25)**	***Proteus species* (N=16)**	***Acinetobacter species* (N=15)**	***Pseudomonas aeruginosa* (N=12)**	***Citrobacter species* (N=6)**
Nitrofurantoin	54 (18.88%)	9 (36%)	6 (37%)	15 (100%)	9 (75%)	6 (100%)
Norfloxacin	243 (84.96%)	18 (72%)	12 (75%)	9 (60%)	9 (75%)	6 (100%)
Ciprofloxacin	237 (82.86%)	15 (60%)	6 (37%)	9 (60%)	9 (75%)	6 (100%)
Amikacin	69 (24.12%)	3 (12%)	3 (18.75%)	12 (80%)	9 (75%)	6 (100%)
Gentamicin	129 (45.10%)	6 (24%)	0 (0%)	12 (80%)	9 (75%)	3 (50%)
Netillin	60 (20.97%)	6 (24%)	9 (56.25%)	9 (60%)	9 (75%)	3 (50%)
Meropenem	132 (46.15%)	15 (60%)	9 (56.25%)	9 (60%)	6 (50%)	3 (50%)
Imipenem	24 (8.39%)	9 (36%)	0 (0%)	9 (60%)	6 (50%)	3 (50%)
Polymyxin-B	51(17.83%)	9 (36%)	6 (37%)	0 (0%)	0 (0%)	0 (0%)
Cefadroxil	177 (61.88%)	12 (48%)	6 (37%)	3 (20%)	6 (50%)	3 (50%)
Cefuroxime	207 (72.37%)	18 (72%)	9 (56.25%)	3 (20%)	9 (75%)	6 (100%)
Ceftriaxone	213 (74.47%)	18 (72%)	9 (56.25%)	12 (80%)	9 (75%)	3 (50%)

Cefpodoxime	237 (82.86%)	21 (84%)	9 (56.25%)	12 (80%)	12 (100%)	6 (100%)
Cefixime	228 (79.72%)	18 (72%)	12 (75%)	15 (100%)	12 (100%)	6 (100%)
Cefoperazone	219 (76.57%)	15 (60%)	9 (56.25%)	12 (80%)	9 (75%)	6 (100%)
Ceftazidime	147 (51.39%)	9 (36%)	0 (0%)	6 (40%)	9 (75%)	0 (0%)
Cefepime	192 (67.13%)	12 (48%)	12 (75%)	15 (100%)	9 (75%)	3 (50%)
Cefoxitin	132 (46.15%)	18 (72%)	12 (75%)	12 (80%)	9 (75%)	6 (100%)
Cefotaxime	135 (47.20%)	18 (72%)	9 (56.25%)	12 (80%)	9 (75%)	6 (100%)
Piperacillin/ Tazobactam	72 (25.17%)	6 (24%)	0 (0%)	9 (60%)	9 (75%)	6 (100%)
Amoxicillin/ Clavulanic acid	246 (86.01%)	18 (72%)	12 (75%)	15 (100%)	9 (75%)	6 (100%)
Ceftazidime/ Clavulanic acid	60 (20.97%)	6 (24%)	0 (0%)	6 (40%)	12 (100%)	0 (0%)
Cefoperazone/ Sulbactam	93 (32.51%)	15 (60%)	0 (0%)	9 (60%)	9 (75%)	6 (100%)
Colistin	60 (20.97%)	3 (12%)	6 (37%)	0 (0%)	0 (0%)	0 (0%)

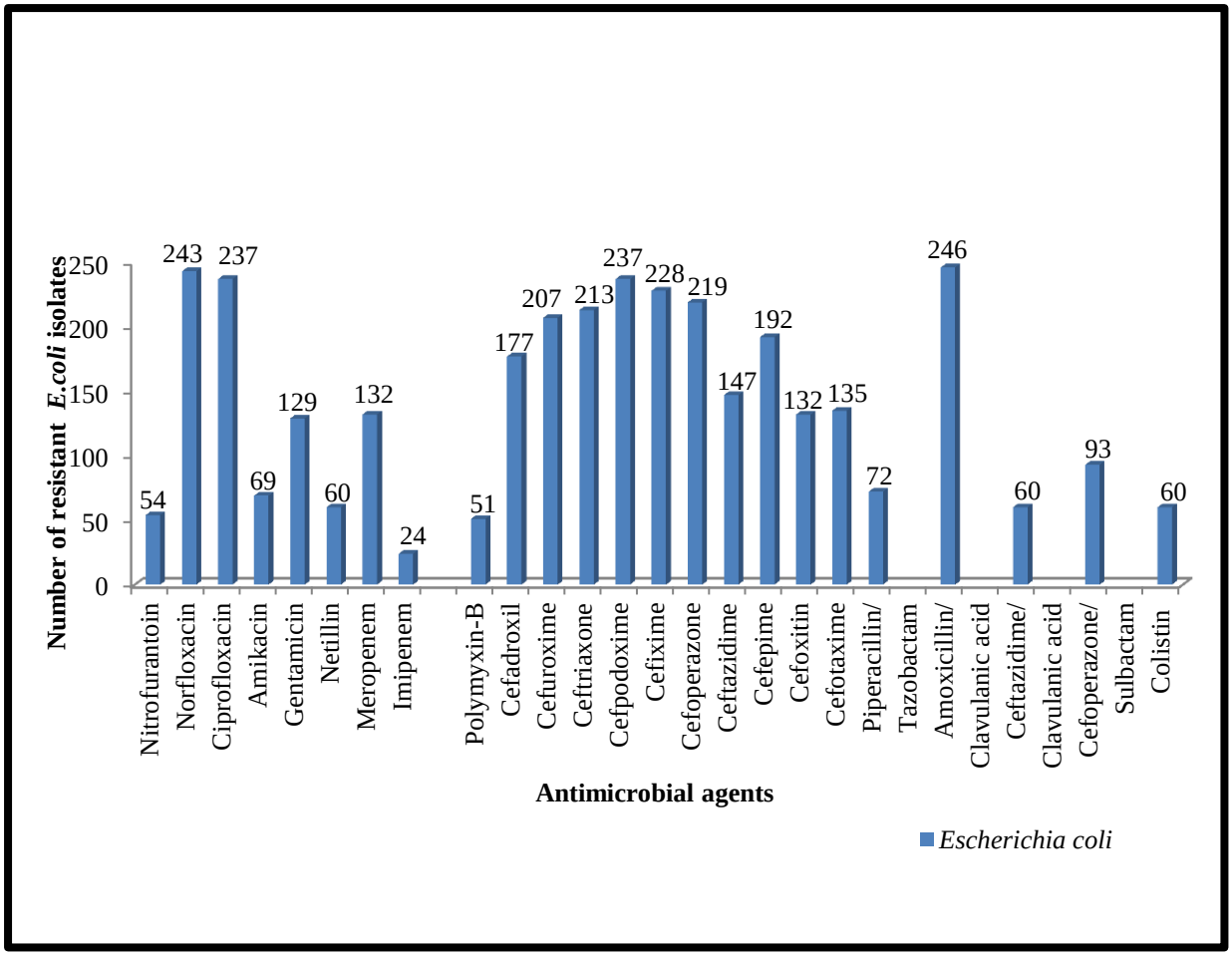


Chart 11: Antimicrobial resistance among *Escherichia coli*

CHAPTER 6: DISCUSSION

The main objective of this study was to access the prevalence and antimicrobial susceptibility pattern of Gram negative ESBL producers in Urine samples collected from In-patients and Out-patients visiting Punjab Institute of Medical Sciences , Jalandhar.

In this study, out of 360 Gram negative isolates, 240 (66.7%) were isolated from In-Patient Department and 120 (33.33%) isolated from Out-Patient Department. More In-Patient department samples were found to be positive as compared to Out-patient department samples. This correlates with the study conducted by Bajpai et al., their study showed more IPD samples 194 (42.1%) were found to be positive and 63 (30%) OPD samples (79). Study conducted by Kumar et al., showed out of 75 urine isolates, 41 were obtained from IPD and 34 were isolated from OPD.

In this study, types of Gram negative bacterial isolates identified both in OPD and IPD were *Escherichia coli* (79.44%), *Klebsiella* species (6.9%), *Proteus* species (4.44%), *Pseudomonas aeruginosa* (3.33%) and *Acinetobacter baumanii* (9.63%). These were major Gram negative bacteria in our study originated from urine samples and cause Urinary tract infection. A study conducted by Bajpai et al., showed 120 isolates of Escherichia coli (55.3%) followed by 50 (23%) *Klebsiella pneumoniae* and 26 isolates of *Pseudomonas aeruginosa* (16.5%) (79).

Out of 360 positive bacterial isolates, 135 (37.5%) isolates were found to be ESBL producers while other 225 (62.5%) were non-ESBL producers. 117 (86.66%) isolates were from male patients that were ESBL producers, while 18 (13.33%) isolates were found to be ESBL producers in females.

Bajpai et al., conducted a study in which 50 (41.6%) *Escherichia coli* were ESBL positive followed by *Pseudomonas aeruginosa* (36.1%) and *Klebsiella pneumoniae* (26%) (79).

Our study showed that *Escherichia coli* as the most frequent ESBL positive uropathogen followed by *Acinetobacter baumanii* (9.63%), *Proteus* species (8.89%), *Klebsiella pneumoniae* (6.67%), *Pseudomonas aeruginosa* (5.92%).

Islam M B et al., conducted a study in which 82.1% *Escherichia coli* were ESBL producers followed by 17.9% *Klebsiella pneumoniae* (80).

However, another study conducted by Dechen C Tsering et al., their study showed the prevalence of ESBL as 34.03%. *Escherichia coli* (26.15%), *Klebsiella pneumoniae* (54.14%),

Pseudomonas aeruginosa (32.61%), *Proteus mirabilis* (42.86%) were found to be ESBL positive.

Sonavane et al, their study also showed that *Escherichia coli* (41.3%) were the commonest isolate that caused UTI (81).

In study from GMC Chandigarh, *Escherichia coli* were the commonest isolate followed by *Klebsiella* species, *Pseudomonas* species and *Acinetobacter* species.

A study conducted by Babypadmini S, Appalaraju B on Extended spectrum beta-lactamases in urinary isolates *of Escherichia coli* and *Klebsiella pneumoniae* in a tertiary care hospital showed 41% of *Escherichia coli* and 40% of *Klebsiella pneumoniae* were ESBL producers (82).

However study conducted in USA for *Escherichia coli* (2.2%) and *Klebsiella pneumoniae* (6.6%), Canada (2.7%/ 6.2%) showed very low rate of isolation incidence as compared to this study (83,84). Much higher (58%) prevalence of ESBL producers in urinary isolates of Gram negative bacilli was observed in India by Mathur et al (85).

Over the last decade, several studies assessed the occurrence of ESBL among *Enterobacteriaceae* recovered from hospitalized patients. A survey conducted in Brooklyn showed that ESBLs were produced by 17.2% of bacteria (*Klebsiella pneumoniae*, *Proteus mirabilis* and *Escherichia coli*) (86).

The results of study conducted by Spanu et al., showed prevalence of ESBL producers in *Klebsiella pneumoniae* was 20%, 16.3% for *Proteus mirabilis* and 1.2% for *Escherichia coli* (87).

Another study conducted by Aruna K in Mumbai, Revealed 34.87% uropathogens were ESBL producers. This was due to the hot and humid climate prevalent in Mumbai; incidences of UTIs are constantly on the rise. Their study showed 49.32% *Escherichia coli* ESBL producers (88). Statistics have shown that ESBL producing *Escherichia coli* are found to be the highest in India (60%) followed by Hong Kong (48%) and Singapore (33%) (Hsueh et al., 2011).

Study conducted by Singh S et al, showed the presence of ESBL producers among clinical isolates was 36.36% (89).

In a study based on the Tigecycline Evaluation and Surveillance Trail (TEST) global surveillance database, the rate of ESBL production was highest among the *Klebsiella pneumoniae* isolates collected in Latin America, followed by Asia/ Pacific Rim, Europe and North America (44.0%, 22.4%, 13.3% and 7.5%). 22 European countries for the period of 2004 to 2007, the rate of ESBL production among *Klebsiella pneumoniae* isolate was 15.5% and *Escherichia coli* was 9.8% (EARSS Annual Report 2007).

In this study, the commonest organism isolated was *Escherichia coli* that showed highest resistance to Amoxycillin/ Clavulanic acid (86.01%) followed by Norfloxacin (84.96%). *Escherichia coli* showed Antimicrobial resistance 18.88% to Nitrofurantoin, 84.96% to Norfloxacin, 82.86% to Ciprofloxacin, 24.12% to Amikacin, 45.10% to Gentamicin, 20.97% to Netillin, 46.15% to Meropenem, 8.38% to Imipenem, 17.83% to Polymyxin B, 61.88% to Cefadroxil, 72.37% to Cefuroxime, 74.47% to Ceftriaxone, 82.86% to Cefpodoxime, 79.72% to Cefixime, 76.57% to Cefoperazone, 67.13% to Cefepime, 46.15% to Cefoxitin, 47.20% to Cefotaxime, 25.17% to Piperacillin/ Tazobactam, 86.01% to Amoxicillin/ Clavulanic acid, 20.97% to Ceftazidime/ Clavulanic acid, 32.51% to Cefoperazone/ Sulbactam and 20.97% to Colistin.

Similar results were found in a study conducted by Neelam Taneja et al. They reported ESBL producing *Escherichia coli* with a high degree resistance to Piperacillin/ Tazobactam and Amoxicillin/ Clavulanic acid to be 93.1% and 93.4% (90).

Akram et al and Padmini et al also reported 100% susceptibility of urinary isolates of *E. coli* to imipenem (91, 92). Menon et al in their study reported almost similar results of susceptibility for Imipenem, Piperacillin/Tazobactam, Cefoperazone/ Sulbactam, and Ceftazidime/Clavulanate with slight variation (93). Similar susceptibility patterns were also observed in studies conducted outside India. Kibret et al showed a high resistance to Amoxicillin (86.0%) and Tetracycline (72.6%) but a significantly high degree of susceptibility to Nitrofurantoin (96.4%), Norfloxacin (90.6%), and Gentamicin (79.6%) (94).

ESBL producers may have spread through communities, especially those with poor hygienic and sanitation conditions, through faecal contamination of soil and water, since most patients with ESBL producers may have had their gastrointestinal tracts colonized for a long period of time by these organisms as was reported by Paterson and Bonomo (2005) (95). In vitro susceptibility studies of ESBL producing *E. coli* isolated from urine showed that drug resistance was higher in ESBL producers than non-ESBL producers. Analysis of

antimicrobial susceptibility pattern of ESBL producing *E. coli* isolates demonstrated high susceptibility rates to imipenem (100%), β-lactam/β-lactamase inhibitor combination drugs such as Piperacillin/Tazobactam (80.48, 70%), Cefoperazone/Sulbactam (70.73, 80%), Ceftazidime/Clavulanate (70.73, 70%), Amoxicillin/Clavulanic acid (68.29, 70%), and Aminoglycosides such as Amikacin (73.17, 60%) and Gentamicin (68.29, 60%) from urine. High resistance rates were observed to Penicillins such as Ampicillin and Piperacillin, third and fourth generation Cephalosporins and Fluoroquinolones. Norfloxacin and Nitrofurantoin have good susceptibility against ESBL producing *E. coli* isolated from urine. So these drugs are recommended for the treatment of infections caused by ESBL producing *E. coli.*

A study conducted by Ponnusamykonar Poovendran et al., showed that the antibiotic resistance pattern of ESBL producing Escherichia coli isolates have showed maximum resistance to Amoxicillin/ Clavulanic acid (95%), Gentamicin (86%), Tetracycline (83%) and Piperacillin/ Tazobactam (81%). Both ESBL producers and non- ESBL producers were resistant to Amoxicillin/ Clavulanic acid and Gentamicin (96).

Antibiotic resistance showed by different uropathogens is one of the hurdle or obstruction that might hinder or successful treatment. Widespread usage of antibiotics result in development of antibiotic resistance. An individual is at a higher risk of being infected by ESBL producing uropathogens if one can exposed to antibiotics for a longer duration of time, go through serious illness, undergo instrumentation or catheterization or is a resident of nursing home an institute which frequently use third generation cephalosporins (97).

The sensitivity pattern of micro-organisms to various antibiotics varies one time and among different geographical locations.

CHAPTER 7: CONCLUSIONS

The rapidity of the development and spread of resistance is a complex process that is influenced by selective pressure, pre-existence of resistance genes and use of infection control measures. ESBLs are an example of the increasing number and diversity of enzymes that inactivate β-lactam type antibacterial. The combination of these enzymes with other resistance traits gives strong testimony as to the resilience of microbes and their ability to adapt to their environment. The incidence of infections caused by beta-lactam-resistant organisms due to the production of various enzymes has increased in recent years. Detection of ESBL production is of paramount importance both in hospital and community isolates. Infection-control practitioners and clinicians need the clinical laboratory to rapidly identify and characterize different types of resistant bacteria. This in turn is required to minimize the spread of these bacteria and help to select appropriate antibiotics. This is particularly true for ESBL-producing bacteria. The epidemiology of ESBL-producing bacteria is becoming more complex with increasingly blurred boundaries between hospitals and the community. The acquisition of efficient mobile elements has accelerated the transfer of various antibiotic resistance genes. Probably, a ‘‘super bug’’, resistant to relatively all licensed antibiotics, may rise in the future. Constant and careful worldwide surveillance for multidrug-resistant bacteria is urgently warranted. Our ability to successfully treat infections due to these increasingly resistant organisms demands a multifactorial approach combining continued research and development of novel classes of antibacterial, more prudent use of existing agents and an increased emphasis on more effective infection control measures.

CHAPTER 8: BIBLIOGRAPHY

1. Levy SB, Marshall B (2004) Antibacterial resistance worldwide: causes, challenges and responses. Nat Med 10:S122-129.
2. Aggarwal R, Chaudhary U, Sikka R. Detection of Extended Spectrum beta-lactamase production Among Uropathogens. Journal of Laboratory Physicians/ Jan-Jun 2009/ Vol-1/ Issue0-1:7-10.
3. Podschun R Cellmann U. *Klebsiell*a spp. As Nosocomial pathogens: epidemiology, taxonomy, typing methods and pathogenicity factor: Clinical Microbiol Rev. 1998; 11(4); 589-603.
4. Antimicrobial resistance in pathogens causing Urinary tract infections in a rural community of Odisha, India. Journal of Family and Community Medicine. 2013; 20(1).
5. Infection detection blog spot.in/2011/06/ Urinary tract-infections. Html.accessed on February 8th, 2016.
6. Alipourfard I, Nili NY. Antibiogram of ESBL producing *E. coli* and *K. pneumoniae* isolated from Hospital samples Bangladesh J med Microbiol. 2010; 04(01):32-6.
7. Knothe, H., P. Shah, V. Krcmery, M. Antal, and S. Mitsuhashi. 1983. Transferable resistance tocefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. Infection 11:315317.[PubMed]
8. Bush, K., C. Macalintal, B. A. Rasmussen, V. J. Lee, and Y. Yang. 1993. Kinetic interactions of tazobactam with beta-lactamases from all major structural classes. Antimicrob. Agents Chemother. 37:851858.
9. Sirot, D., J. Sirot, R. Labia, A. Morand, P. Courvalin, A. Darfeuille Michaud, R. Perroux, and R. Cluzel. 1987. Transferable resistance to third generation cephalosporins in clinical isolates of *Klebsiella pneumoniae*: identification of CTX1,a novel beta-lactamase. J. Antimicrob. Chemother. 20:323334.
10. Philippon, A., R. Labia, and G. Jacoby. 1989. Extended-spectrum beta-lactamases .Antimicrob. Agents Chemother. 33:11311136.
11. Bradford Pa. "Extended-spectrum beta-lactamases in the 21st century: Characterization, epidemiology and detection of this imp. resistance threat." Clin Microbiol Rev. 2001; 14(48):933-51.

12. Girand-Morin C, Fosse TA. Seven year survey of *Klebsiella pneumoniae* producing YEM-24 extended-spectrum-lactamase in Nice University Hospital (1994-2000) J. Hosp. Infect. 2003; 54:25-31.
13. Ambler, R. P., A. F. Coulson, J. M. Frere, J. M. Ghuysen, B. Joris, M. Forsman, R. C. Levesque, G. Tiraby, and S. G. Waley. 1991. A standard numbering scheme for the class A beta-lactamases. Biochem. J.276:269270.
14. Bush, K., G. A. Jacoby, and A. A. Medeiros. 1995. A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrob. Agents Chemother. 39:12111233.
15. Rasmussen, B. A., and K. Bush. 1997. Carbapenem hydrolysing beta-lactamases. Antimicrob. Agents Chemother. 41:223232.
16. Livermore, D. M. 1995. Beta-lactamases in laboratory and clinical resistance. Clin. Microbiol. Rev. 8:557584.
17. Kartali, G., E. Tzelepi, S. Pournaras, C. Kontopoulou, F. Kontos, D. Sofianou, A. N. Maniatis, and A. Tsakris. 2002. Outbreak of infections caused by *Enterobacter cloacae* producing the integrin associated beta-lactamase IBC1 in a neonatal intensive care unit of a Greek hospital. Antimicrob. Agents Chemother. 46:15771580.
18. Du Bois, S. K., M. S. Marriott, and S. G. Amyes. 1995. TEM and SHV derived Extended-spectrum beta-lactamases: relationship between selection, structure and function. J. Antimicrob. Chemother. 35:722.
19. Philippon, A., R. Labia, and G. Jacoby. 1989. Extended-spectrum beta-lactamases. Antimicrob. Agents Chemother. 33:11311136.
20. Sirot, D., J. Sirot, R. Labia, A. Morand, P. Courvalin, A. Darfeuille Michaud, R. Perroux, and R. Cluzel. 1987. Transferable resistance to third generation cephalosporins in clinical isolates of *Klebsiella pneumoniae*: identification of CTX1, a novel beta-lactamase J. Antimicrob. Chemother. 20:323334.
21. Brun Buisson, C., P. Legrand, A. Philippon, F. Montravers, M. Ansquer, and J. Duval. 1987. Transferable enzymatic resistance to third generation cephalosporins during nosocomial outbreak of multi-resistant *Klebsiella pneumoniae*. Lancet ii: 302306.
22. Review of literature: Doern, G.V. et al., 2001. Antimicrobial resistance among clinical isolates of *Streptococcus pneumoniae* in the United States during 1999–2000, including a comparison of resistance rates since 1994–1995. *Antimicrobial Agents and Chemotherapy* 45(6)1721–1729.

23. Jacoby GA, Munoz-Price LS. The new beta-lactamases. N Engl J Med 2005; 352:380e391.
24. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother 1995; 39:1211e1233.
25. Ambler RP, Meadway RJ. Chemical structure of bacterial penicillinases. Nature 1969; 222:24e26.
26. Jones CH, Tuckman M, Keeney D, Ruzin A, Bradford PA. Characterization and sequence analysis of extended spectrum beta-lactamase encoding genes from *Escherichia coli, Klebsiella pneumoniae* and *Proteus mirabilis* isolates collected during tigecycline phase 3 clinical trials. Antimicrob Agents Chemother 2009; 53:465e475.
27. Livermore DM, Canton R, Gniadkowski M, et al. CTX-M: changing the face of ESBLs in Europe. J Antimicrob Chemother 2007; 59:165e174.
28. Bonnet R. Growing group of extended-spectrum beta-lactamases: the CTX-M enzymes. Antimicrob Agents Chemother 2004; 48:1e14.
29. Bush, K., G. A. Jacoby, and A. A. Medeiros**.** 1995. A functional classification scheme for beta-lactamases and its correlation with molecular structures. Antimicrob. Agents Chemother. **39:**1211–1233.
30. Coudron, P. E., E. S. Molland, and C. C. Sanders**.** 1997. Occurrence and detection of extended-spectrum _-lactamases in members of the family *Enterobacteriaceae* at a Veterans Medical Center.
31. Franceschini, N., M. Perilli, B. Segatore, D. Setacci, G. Amicosante, A. Mazzariol, and G. Cornaglia**.** 1998. Ceftazidime and aztreonam resistance in *Providencia stuartii*: characterization of a natural TEM-derived extended spectrum beta-lactamase, TEM-60. Antimicrob. Agents Chemother.
32. Jacoby, G. A., and A. A. Medeiros**.** 1991. More extended-spectrum beta-lactamases. Antimicrob. Agents Chemother. **35:**1697–1704.
33. Tzelepi, E., G. Panagiota, D. Sofianou, V. Loukova, A. Kemerglou, and A. Tsakris**.** 2000. Detection of extended-spectrum _-lactamases in clinical isolates of *Enterobacter cloacae* and *Enterobacter aerogenes*. J. Clin. Microbiol. **38:**542–546.
34. Babini, G., and D. M. Livermore. 2000. Antimicrobial resistance amongst *Klebsiella* spp. from intensive care units in southern and western Europe in 1997–1998. J. Antimicrob. Chemother. **45:**183–189.

35. Kaye, K. S., H. S. Fraimow, and E. Abrutyn. 2000. Pathogens resistant to antimicrobial agents. Epidemiology, molecular mechanisms, and clinical management. Infect. Dis. Clin. North Am. **14:**293–319.
36. Luzzaro, F., M. Perilli, R. Migliavacca, G. Lombardi, P. Micheletti, A. Agodi,S. Stefani, G. Amicosante, and L. Pagani**.** 1998. Repeated epidemics caused by extended-spectrum beta-lactamase-producing *Serratia marcescens* strains. Eur. J. Clin. Microbiol. Infect. Dis. **17:**629–636.
37. Perez F, Endimiani A, Hujer KM, Bonomo RA. The continuing challenge of ESBLs. Curr Opin Pharmacol 2007; *7*: 459-69.
38. Schwaber MJ, Navon-Venezia S, Kaye KS, Ben-Ami R, Schwartz D, Carmeli Y. Clinical and economic impact of bacteraemia with extended-spectrum-beta-lactamase-producing *Enterobacteriaceae*. *Antimicrob Agents Chemother*, 2006; 50: 1257–1262.
39. Anderson DJ, Engemann JJ, Harrell LJ, Carmeli Y, Reller LB, Kaye KS. Predictors of mortality in patients with bloodstream infection due to ceftazidime-resistant *Klebsiella pneumoniae*. *Antimicrob Agents Chemother*, 2006; 50: 1715–1720.
40. Hyle EP, Lipworth AD, Zaoutis TE, Nachamkin I, Bilker WB, Lautenbach E. Impact of inadequate initial antimicrobial therapy on mortality in infections due to extended-spectrum beta-lactamase-producing *Enterobacteriaceae*: variability by site of infection. Arch Intern Med, 2005; 165: 1375–1380.
41. Pena C, Gudiol C, Calatayud L, Tubau F, Dominguez MA, Pujol M, Ariza J, Gudiol F. Infections due to *Escherichia coli* producing extended-spectrum beta-lactamase among hospitalised patients: factors influencing mortality. J Hosp Infect, 2008; 68: 116–122.
42. Tumbarello M, Sanguinetti M, Montuori E, Trecarichi EM, Posteraro B, Fiori B, Citton R, D'Inzeo T, Fadda G, Cauda R, Spanu T. Predictors of mortality in patients with bloodstream infections caused by extended-spectrum-beta-lactamase-producing *Enterobacteriaceae:* importance of inadequate initial antimicrobial treatment. Antimicrob Agents Chemother, 2007; 51: 1987–1994.
43. Meyer KS, Urban C, Eagan JA, Berger BJ, Rahal JJ. Nosocomial outbreak of *Klebsiella* infection resistant to late generation cephalosporins. Ann Intern Med 1993; 119: 353-8.

44. Rahal, JJ, Urban C, Horn D, Freeman K, Segal-Maurer S, Maurer J, *et al*. Class restriction of cephalosporin use to control total cephalosporin resistance in nosocomial *Klebsiella*. *JAMA* 1998; *280*: 1233-7.
45. H Kurokawa; N Shibata; Y Doi; K Shibayama; K Kamachi; T Yagi; A Yoshichika. Antimicrob Agents Chemother, **2003**, 47, 2981-2983.
46. G Liu; BD Ling; Y Zeng; L Lin; YE Xie; J Lei. *Japan Infect Dis*, **2008**, 61, 286-289.
47. F Shahcheraghi; H Noveiri; S Nasiri. *Iran J Med Microbiol*, **2008**, 3, 1-8.
48. Paterson DA, Bonomo RA. Extended-spectrum b-lactamases: a clinical update. Clin Microbiol Rev 2005; 18: 657-86.
49. Lautenbach E, Patel JB, Bilker WB, Edelstein PH, Fishman NO**.** Extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors for infection and impact of resistance on outcomes. Clin Infect Dis 2001; 3: 1162-71.
50. Jacoby GA, Munoz-Price LS. The new beta-lactamases. N Engl J Med 2005; 352:380e391.
51. Wayne PA. Performance standards for antimicrobial susceptibility testing; 18th informational supplement. Clin Lab Standards Inst. 2008. pp. M100–S18.
52. Bradford PA. Extended spectrum beta-lactamases in the 21 century: Characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev. 2001;14:933–51.
53. Datta N and Kontomichalou P. 1965. Penicillinase synthesis controlled by infectious R factor in *Enterobacteriaceae*. *Nature* 208: 239-244.
54. Paterson, A., 2004. Urinary tract infection: an update on imaging strategies. Eur Radiol., pp: 89-100.
55. Polwichai, P., S. Dejsirilert, S. Panpetch, P. Sawanpanyalert, N. Aswapokee and P. Mootsikapun, 2009. Antimicrobial resistance of *Escherichia coli* isolated from urine in Thailand from 2000 to 2005. J. Med. Assoc. Thai., 92: 59-67.
56. Bal, S. beta-lactamase mediated resistance in hospital acquired UTIs Hospital Today. 2000: 5: 96-101.
57. Ringertz S., Olsson-Liljequit B., Kahlmeter G., Kronvall G. (1997). Antimicrobial susceptibility testing in Sweden II. Species-related zone diameter breakpoints to avoid interpretive errors and guard against unrecognised evolution of resistance. Scand J. Infect, Dis. (suppl.), 105, 8-12.
58. Threlfall E.J., Fisher I.S.T., Ward L., Tschape H. and Gerner-Smidt P. (1999). Harmonization of antibiotic susceptibility testing for *Salmonella*: Results of a study

by 18 national reference laboratories within the European Union-funded Enter-Net group. Micro. Drug Resist., 5, 195-199.

59. Babypadmini S, Appalaraju B. Extended spectrum beta-lactamases n urinary isolates of *Escherichia coli* and *Klebsiella pneumoniae*-prevalence and susceptibility pattern in a tertiary care hospital. Indian J. Med Microbiol. 2004; 22:172-4.
60. Jorgensen, J. H., and Turnidge J. D. , 2007. Susceptibility test methods: dilution and disk methods, p. 1152-1172. In Murray P. R., Baron E.J., Jorgensen, J.H., Landry M.L., and Pfaller M.A., Manual of clinical microbiology, 9th ed. ASM Press, Washington, D.C.
61. Sahm DF, Thornsberry C, Mayfield DC, Jones ME, Karlowsky JA. 2001. Multidrug-resistant urinary tract isolates of Escherichia coli prevalence and patient demographics sulfamethoxasole on recurrent bacteriuria and bacterial persistence in mice infected with uropathogenic Escherichia coli, Infect Immun. 70: 7042-49.
62. Water worth P.M., Quantitative methods for bacterial sensitivity testing. Laboratory methods in antimicrobial chemotherapy. Reeves D.S., Philips I., Williams J.D., Wise R. Churchill Livingstone, Baltimore. 1978; P: 31-41.
63. Clinical and Laboratory Standards Institute. 2005 guidelines by CLSI NCCLS. CLSI informational supplement. Approved standard M100-S15 Wayne, PA; 2000; 565.
64. Reinert RR, Low DE, Rossi F, Zhang X,Wattal C, Dowzicky MJ. Antimicrobial susceptibility among organisms from the Asia/Pacific Rim, Europe and Latin and North America collected as part of TEST and the in vitro activity of tigecycline. J Antimicrob Chemother 2007; 60:1018e1029.
65. Hackel M, Badal R, Bouchillon S, et al. Extended-spectrum beta-lactamase production in Europe. In: Abstracts of the 18th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Barcelona, Spain, 2008.
66. Babini GS, Livermore DM. Antimicrobial resistance amongst *Klebsiella* spp. collected from intensive care units in Southern and Western Europe in 19971998. J Antimicrob Chemother. 2000;45:183–9.
67. Hanberger H, Garcia Rodriguez JA, Gobernado M, Goossens H, Nilsson LE, Struelens MJ. Antibiotic susceptibility among aerobic gram negative bacilli in intensive care units in 5 European countries. French and Portuguese ICU Study Groups. JAMA. 1999;281:67–71.
68. Günseren F, Mamikoğlu L, Oztürk S, Yücesoy M, Biberoğlu K, Yuluğ N, et al. A surveillance study of antimicrobial resistance of gram negative bacteria isolated from

intensive care units in eight hospitals in Turkey. J Antimicrob Chemother. 1999;43:373–8.

69. Moland ES, Black JA, Ourada J, Reisbig MD, Hanson ND, Thomson KS. Occurrence of newer beta-lactamases in Klebsiella pneumoniae isolates from 24 US hospitals. Antimicrob Agents Chemother. 2002;46:3837– 42.
70. Borer A, Gilad J, Menashe G, Peled N, Riesenberg K, Schlaeffer F. Extended spectrum beta-lactamase producing Enterobacteriaceae strains in community acquired bacteremia in Southern Israel. Med Sci Monit. 2002;8:CR44–7.
71. E Karsh T, Tawfik AF, A Shammary F, A Salah S, Kambal AM, Shibl A. Antimicrobial resistance and prevalence of extended spectrum β-lactamase among clinical isolates of gram negative bacteria in Riyadh. J Chemother. 1995;7:509–14.
72. AitMhand R, Soukri A, Moustaoui N, Amarouch H, ElMdaghri N, Sirot D, et al. Plasmid mediated TEM-3 extended-spectrum beta-lactamase production in *Salmonella* Typhimurium in Casablanca. J Antimicrob Chemother. 2002;49:169–72.
73. Yu Y, Zhou W, Chen Y, Ding Y, Ma Y. Epidemiological and antibiotic resistant study on extended-spectrum beta-lactamase producing Escherichia coli and *Klebsiella pneumoniae* in Zhejiang Province. Chin Med J (Engl) 2002;115:1479–82.
74. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: A clinical update. Clin Microbiol Rev. 2005;18:657–86.
75. Das A, Ray P, Garg R, Kaur B. Proceedings of the Silver Jubilee Conference. New Delhi: All India Institute of Medical Sciences; 2001. Extended spectrum beta-lactamase production in Gram negative bacterial isolates from cases of septicemia.
76. Babypadmini S, Appalaraju B. Extended spectrum β-lactamases in urinary isolates of *Escherichia coli* and *Klebsiella pneumoniae* prevalence and susceptibility pattern in a tertiary care hospital. Indian J Med Microbiol. 2004; 22:172–4.
77. Tankhiwale SS, Jalgaonkar SV, Ahamad S, Hassani U. Evaluation of extended spectrum beta lactamase in urinary isolates. Indian J Med Res. 2004;120:553–6.
78. Mohanty S, Singhal R, Sood S, Dhawan B, Das BK, Kapil A. Comparative in vitro activity of beta- lactam/beta-lactamase inhibitor combinations against Gram negative bacteria. Indian J Med Res. 2005; 122:425–8.
79. Bajpai T, Pandey M, Varma M, Bhatambare GS. Prevalence of extended spectrum beta-lactamase producing uropathogens and their antibiotic resistance profile in patients visiting a tertiary care hospital in central India: Implications on empiric therapy. Indian J Pathol Microbiol 2014; 57:407-12.

80. Islam MB , Jhora ST , Yusuf MA , Sattar AFMA , Shahidullah SK , Rahman NMW et al ; Frequency and Antimicrobial Sensitivity Pattern of Extended Spectrum β-Lactamases Producing *Escherichia coli* and *Klebsiella pneumoniae* Isolated from urine at a Tertiary Care Hospital; J Shaheed Suhrawardy Med Coll, 2012;4(1):22-25.
81. A Sonavane, M Mathur, D Turbadkar, V Baradkar; Antimicrobial Susceptibility Pattern in Urinary Bacterial Isolates; Bombay Hospital Journal, Vol. 50, No. 2, 2008.
82. Padmini BS, Appalaraju B. Extended spectrum β lactamases in urinary isolates of *E. coli* and *K. pneumoniae*—prevalence and susceptibility pattern in a tertiary care hospital. Indian J Med Microbiol. 2004; 22(3):172–4.
83. Jones RN, Kugler KC, Pfaller MA, Winokur PL. Characteristics of pathogens causing urinary tract infections in hospitals in North America: Results from the SENTRY antimicrobial surveillance program, 1997 SENTRY Surveillance Group. Diagn Microbiol Infect Dis 1999; 35:5563.
84. Sumeeta K, Neelam T, Meera S. Extended spectrum beta-lactamase mediated resistance in urinary tract isolates of family *Enterobacteriaceae*. Indian J Med Res 2002; 116:145149.
85. Purva M, Arti K, Bimal D, Benu D. Prevalence of extended spectrum beta-lactamase producing Gram negative bacteria in a tertiary care Hospital. Indian J Med Res 2002; 115:153157.
86. Saurina, G., J. M. Quale, V. M. Manikal, E. Oydna, and D. Landman. 2000. Antimicrobial resistance in *Enterobacteriaceae* in Brooklyn, NY: epidemiology and relation to antibiotic usage patterns. J. Antimicrob. Chemother. 45**:**895–898.
87. Spanu T, Luzzaro F, Perilli M, et al. Occurrence of Extended-Spectrum β-Lactamases in Members of the Family *Enterobacteriaceae* in Italy: Implications for Resistance to β-Lactams and Other Antimicrobial Drugs. Antimicrobial Agents and Chemotherapy. 2002; 46(1):196-202. doi:10.1128/AAC.46.1.196-202.2002.
88. K. Aruna, T. Mobashshera; Prevalence of extended spectrum beta-lactamase production among Uropathogens in South Mumbai and its antibiogram pattern; EXCLI Journal 2012;11:363-372 – ISSN 1611-2156.
89. Singh S., Kumar S., Sandhu R., Devi R. and Yadav A.K; Prevalence of drug resistance in ESBL producing *Escherichia coli* causing UTI in rural tertiary care hospital from Harayana, India; International Journal of Basic and Applied Medical Sciences 2015 Vol. 5 (3).

90. Neelam Taneja, Pooja Rao, Jitender Arora and Ashok Kumar, 2008. Occurrence of ESBL and Amp C beta- lactamases and susceptibility to newer antimicrobial agents in complicated UTI. Indian J. Med. Res., 127: 85-88.

91. Akram M, Shahid M, Khan AU. Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in the JNMC Hospital Aligarh, India. Ann Clin Microbiol Antimicrob. 2007; 6:4.
92. Padmini BS, Appalaraju B. Extended spectrum β lactamases in urinary isolates of *E. coli* and *K. pneumoniae*—prevalence and susceptibility pattern in a tertiary care hospital. Indian J Med Microbiol. 2004; 22(3):172–4.
93. Menon T, Bindu D, Kumar CP, Nalini S, Thirunarayan MA. Comparison of double disc and three dimensional methods to screen for ESBL producers in a tertiary care hospital. *Indian J Med Microbiol.* 2006; 24(2):117–120.
94. Kibret M, Abera B. Antimicrobial susceptibility of *E. coli* from clinical sources in northeast Ethiopia. Afr Health Sci. 2011; 11(suppl 1):S40–S45.
95. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. *Clin Microbiol Rev.* 2005; 18:657–686.
96. Ponnusamykonar Poovendran, Natarajan Vidhya and Sevanan Murugan; Antimicrobial Susceptibility Pattern of ESBL and Non-ESBL Producing Uropathogenic Escherichia coli (UPEC) and Their Correlation with Biofilm Formation; Intl. J. Microbiol. Res., 4 (1): 56-63, 2013.
97. Chaudhary U, Aggarwal R. Extended spectrum-lactamases (ESBL)-An emerging threat to clinical therapeutics. Indian J Med Microbiol 2004; 22:75-80.

CHAPTER 9: APPENDIX

9.1 LIST OF ABBREVIATIONS

AST:	Antimicrobial Susceptibility testing
CLSI:	Clinical and Laboratory Standards Institute
CO_2:	Carbon-dioxide
CTX:	Cefotaxime
DDST:	Double disk synergy test
EARSS:	European Antimicrobial Resistance Surveillance System
E. coli:	*Escherichia coli*
ESBL:	Extended Spectrum beta-Lactamase
H_2O_2:	Hydrogen-peroxide
H_2O:	Water
IPD:	In-patient department
K. pneumoniae:	*Klebsiella pneumoniae*
K. oxytoca:	*Klebsiella oxytoca*
mcg:	micro-gram
MHA:	Mueller Hinton Agar
MIC:	Minimum inhibitory concentration
mm:	mill-metre
MR:	Methyl red
NCCLS:	National Committee for Clinical Laboratory Standards
NH_3:	Ammonia
$(NH_2)_2$ CO:	Urea
O_2:	Oxygen
OPD:	Out-patient department
pH:	power of hydrogen
PPA:	Phenylalanine deaminase

SHV: Sulfhydryl Variable

SIIDC: Swedish Institute for Infectious Diseases

TEM: Temoniera

TEST: Tigecycline Evaluation and Surveillance Trial

TSN: The Surveillance Network

UTI: Urinary tract infection

U.S: United States