# Training Report

**Internship Training Report**

**Submitted to: Mr. Shaminder Singh (Assistant Professor)**

**Lovely Professional University, Punjab**

**in partial fulfilment of the requirements**

**For the degree of**

**Master of Science in Clinical Microbiology**

**Submitted by:**
**Neha Sharma**
**(Registration Number: 11401160)**

**SCHOOL OF PHYSIOTHERAPY AND PARAMEDICAL SCIENCES**
**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. Shaminder Singh** (Internal supervisor), Lovely Professional University and **Dr. Sheevani** (External supervisor). This work has not been submitted in part or in full in any other university for any degree or diploma.

Name: Neha Sharma

Date: 06/05/2016

Place: Lovely Professional University, Punjab

# CERTIFICATE

This is to certify that **Ms. Neha Sharma** bearing **Registration Number- 11401160** has completed 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.

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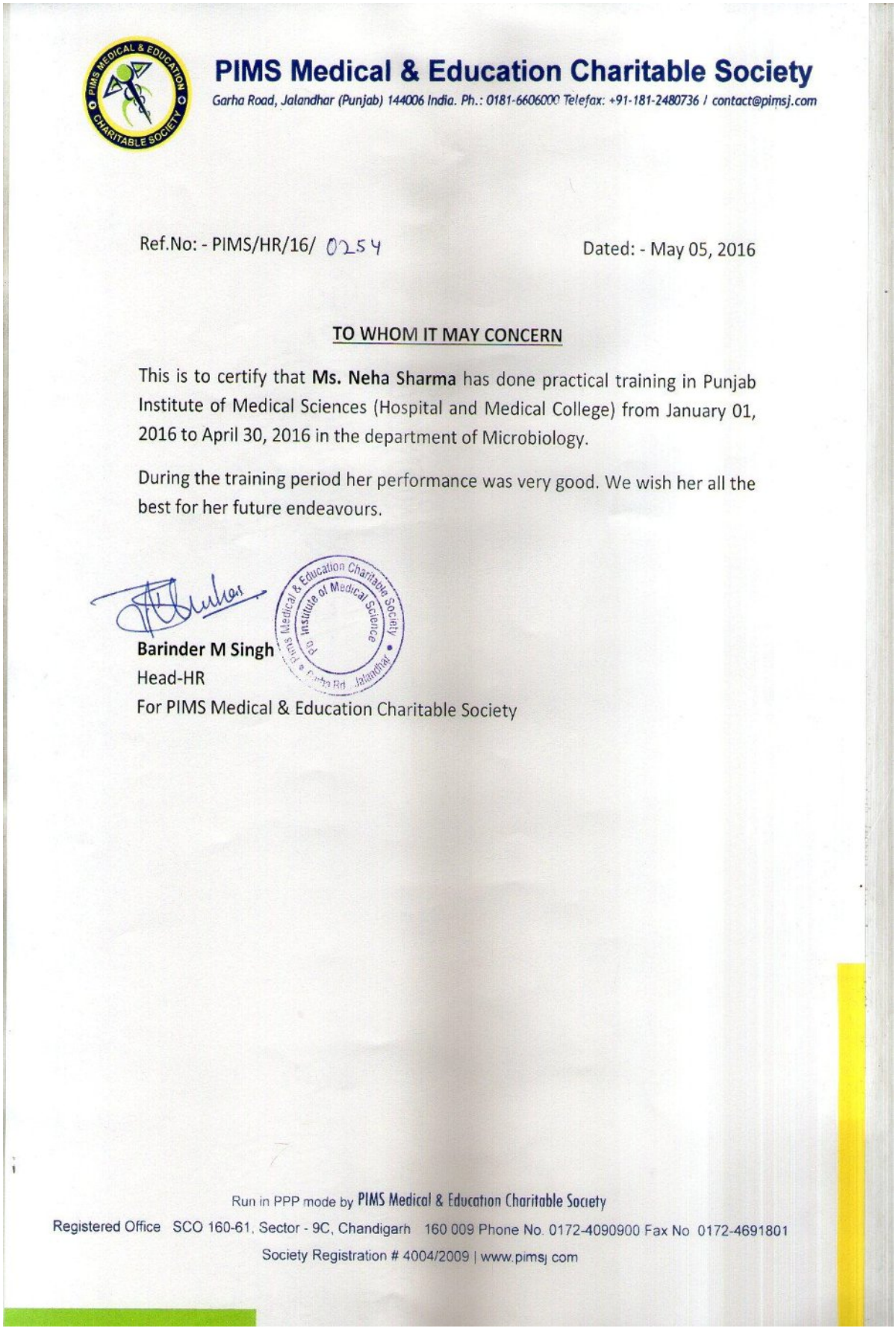

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Ref.No: - PIMS/HR/16/ 0254 Dated: - May 05, 2016

**TO WHOM IT MAY CONCERN**

This is to certify that **Ms. Neha Sharma** 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
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# ABSTRACT

**Background:** *Escherichia coli* (*E.coli*) is a part of normal intestinal flora and are an important reservoir of the antibiotic resistant genes for extended spectrum beta lactamases (ESBLs). These resistant antibiotic genes can be easily spread among Gram negative bacteria (GNB) as these are carried on plasmids. Faecal carriers of ESBL producing *E.coli*in hospitalized patients and in community can be a reservoir for person to person transmission strengthening their dissemination.

**Objective:** To study about the prevalence of ESBL producing *E.coli* in faecal samples of hospitalized patients and normal healthy individuals in an urban hospital of Jalandhar, Punjab.

**Materials and methods:** It is a prospective study carried over a period of four months. *E.coli* isolates were isolated from the commensal gut flora of inpatients admitted in different departments like Medicine, Surgery or Intensive care unit (ICU)along with the normal, healthy inhabitants of the Jalandhar region in Punjab were screened for ESBL production using double disc synergy test and combined disc test.

**Results:** A total of 120 *E.coli* isolates (100 from hospitalized patients and 20 from normal healthy individuals) were isolated. Out of 120*E.coli* isolates; a total of 62 were positive for ESBL production which include 57% from the hospitalized patients and remaining 25% from normal healthy individuals. Prevalence was significantly higher in children of age group 0-10 years (36.80%). Coresistance was observed with Cotrimoxazole (61.40%), Gentamicin (33.33%), and Fluroquinolones (45.62%).

**Conclusions:** The high prevalence of ESBL producing *E.coli* in the gut of both hospitalized patients and healthy individuals may contribute to hospital acquired infections (HAI) as well as community acquired infections (CAI) by these bacteria.

# ACKNOWLEDGEMENT

I bow my head before God, the entire and only source of every knowledge and wisdom endowed to mankind and who blessed me with the ability to do this work.

I would sincerely like to express my cordial gratitude and appreciation to my external supervisor, **Dr. Sheevani**, (Professor), Department of Microbiology, Punjab Institute of Medical Sciences Jalandhar. Without her constant help and vigilant guidance, the completion of this project was not possible. I am really indebted to her for her accommodative attitude, patience and sympathetic behaviour. I express my profound sense of gratitude to my internal supervisor, **Mr. Shaminder Singh** (Assistant Professor), for his keen interest, much needed help and guidance throughout the course of this training. His encouraging attitude reinforced my interest and inspired me throughout the training. I consider myself privileged to have work under him.

I would like to thank **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 valuable suggestions and constant encouragement during the tenure of the project work.

I would also like to thank, **Dr. (Brig) Kailash Chand** (HOD), **Dr. Shashi Chopra** (Professor), **Dr. Gomty Mahajan** (Associate Professor), **Dr. Jaspal Kaur** (Associate professor), **Dr. Shivya Thakur** (Tutor), **Dr. Shweta** (Tutor) and **Dr. Priyanka** (Assistant Professor) for their constant and valuable support for the completion of my work

Words may fall short to covey my gratitude and love to my all friends **Pawanjit Kaur, Jaspreet Kaur, Prabhjot Kaur, Dinobandhu Nandi, Jibandeep Paul, Deepika Manhas, Dipu Sharma, Neeraj Sharma, Rohini Kumari**for their generous help at the time of need.

The support of family is worth mentioning I express my humble obligations to my mother ol**Mrs.Vandana Sharma** for her huge sacrifice, moral support, cooperation, encouragement, patience, tolerance and prayers for my health and success which enabled me to achieve this excellent goal.

Finally, I would like to thank everybody who was important to the successful realization of this project, as well as expressing my apology that I could not mention personally one by one.

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# CHAPTER 1: INTRODUCTION

Over the last few decades there had been a considerable increase in the emergence and spread of antimicrobial resistant enzymes favouring the predominance of antibiotic resistant bacteria which results in morbidity, mortality and increased hospital expenditure(1). The gastrointestinal tract (GIT) plays an important role in development of antibiotic resistant microorganism harbouring the microorganisms as commensal.Antibiotic consumption may lead to alteration in the genome of the microorganisms leading to emergence of resistant microorganisms (2). The resistant microorganisms may then spread into the environment through faeces.

New classes of enzymes conferring resistance to different class of antibiotics have emerged due to antibiotic selection pressure leading to survival of antibiotic resistant bacteria such as Methicilin resistant *Staphylococcus aureus*, Metallo beta lactamases (MBL), Vancomycin resistant *Staphylococcus aureus* (VRSA), Extended spectrum beta lactamases (ESBL) producing bacteria, *Klebsiella pneumonia* carbapenamase producing bacteria (KPC), Vancomycin resistant *Enterococcus* (VRE), Multi drug resistant *Mycobacterium tuberculosis* (MDR-TB) (3). Among them most alarming are the ESBL producing bacteria that have disseminated across the globe since its first description in 1983 (4).

Plasmid borne extended spectrum beta lactamases have evolved as a result of the extensive and exuberant use of the beta lactam antibiotics (5). Beta lactamases production is the most important mechanism of resistance exploited by the GNB.ESBLs are the enzymes capable of hydrolysing penicillins, cephalosporins, first, second, and third generation cephalosporins and monobactams but not cephamycins and carbapenems. These enzymes are inhibited by beta lactamases inhibitors such as clavulanic acid, sulbactam and tazobactam (6).The GIT is the site where development and exchange of antimicrobial resistant genes occur (7,8).*E.coli,* a Gram negative pathogen forms a part of the normal intestinal microflora. These normal intestinal commensal can also contribute to life threatening etiologies like gastrointestinal infections, urinary tract infections (UTI) etc (9).The genes that encode ESBL are located on plasmid so they can be easily transmitted. Moreover these plasmids may often carry resistant genes for other group of antibiotics such as fluroquinolones, trimethoprim-sulfmethaxozole, and aminoglycosides thus limiting the treatment options (10, 11).The normal commensal *E.coli* may carry genes for antimicrobial resistance that can be transferred to the pathogenic

*E.coli* and to other gram negative bacteria. These resistant pathogens appear to be susceptible to antimicrobial agents by routine susceptibility testing and thus treatment of infections caused by these organisms may result in treatment failure posing a huge cause of concern for the clinicians (12).

Recent years have shown a considerable increase in the prevalence of faecal carriage of ESBL producing *E.coli* in both hospital and community setting. Earlier ESBL producing *E.coli* were associated with nosocomial outbreaks but now it have been demonstrated in community isolates as well (13). The most common type of ESBL associated with community acquired *E.coli* infections is the CTX-M type and with the hospital acquired infections are the classical TEM and SHV types (13-14). The drift in the epidemiology of infections due to ESBL producing *E.coli* from hospitals to community have been studied in various parts of the world implicating wide dissemination of the resistant pathogens both in hospitals as well as community (13-18).

In order to direct appropriate use of antimicrobial agents for treatment of infections caused by ESBL producing *E.coli*, prior screening and confirmation of ESBL production by standardized methods as per CLSI (Clinical and Laboratory Standard Institute) guidelines is mandatory as routine susceptibility testing may falsely report these resistant pathogens as sensitive (19).

To the best of our knowledge very few studies are carried related to prevalence of faecal carriage of ESBL producing *E.coli* in hospitals and community settingsinPunjab. So the present study was undertaken to fill up the gap in our knowledge regarding the prevalence of faecal carriage of ESBL producing *E.coli* in hospitalized and healthy individuals in the Jalandhar region of Punjab.

# CHAPTER 2:REVIEW OF LITERATURE

The beginning of antibiotic era with the discovery of penicillin by Alexander Fleming in the year 1928 is marked by the consecutive development of antimicrobial resistance among different pathogens. Alexander Fleming in 1928 observed the growth of a mould of the genus *Penicillum* inhibiting the growth of bacteria in close vicinity of the mould. He identified that the mould produces an antibacterial substance called penicillin. Even before the introduction of penicillin for the therapeutic use, a bacterial enzyme pencillinase was discovered by Abraham and Chain in 1940 (20).Only after few years of introduction of penicillin for clinical use, penicillin resistant beta lactamases were reported in various Gram positive and Gram negative bacteria (2). These beta lactams were thought to break down the beta lactam ring of the antibiotic resulting in ineffective antibiotic.The first plasmid-mediated beta-lactamase in gram-negative bacteria was TEM-1 which was described in the early 1960s (21).

To counter the effect of beta lactamases, broad spectrum cephalosporin were introduced and they remained the first line of treatment for over 20 years. But soon after the availability of these antibiotics for clinical use, enzymes resistant to broad spectrum cephalosporins emerged known as ESBL. In 1983, the first report of bacteria producing ESBL was published (5, 22).TEM was named so as it was first found in *E.coli* isolated from the blood of a patient named Temoneira from Greece (5).Many new antibiotics were developed to treat infections to resist the action of beta lactamases. But with the discovery of each new antibiotic, a new class of beta lactamases have emerged such as the use of broad spectrum cephalosporins have lead the development of broad spectrum beta lactamases (TEM-1, SHV-1) which have emerged as a result of single nucleotide mutation in the TEM or SHV beta lactamases (6). Widespread use of third generation cephalosporins and aztreonam is believed to be the predominant cause of mutations in the earlier discovered broad spectrum beta lactamases (TEM, SHV) and has led to the development of ESBL. The term ESBL was first used by Phillippon, so that they can be differentiated from the broad spectrum beta lactamases (23).By the end of 20$^{th}$ century many studies have been published studying the recovery of ESBLs from various clinical isolates and in different parts of the world (24-26).

Human GIT harbours a large number of bacteria and *E.coli* forms one of the largest groups, so there is an important role played by the gut in the acquisition and transmission of resistant pathogens (7, 27).*E.coli* is a GNB belonging to the family Enterobactericiae and colonizes the

gastrointestinal tract of the human forming the most abundant facultative anaerobe of the human intestinal microflora. Though *E.coli* is found as a commensal microorganismin the intestine of human, there are variants of *E.coli* that are pathogenic as well. Pathogenic strains can be classified as intestinal pathogens causing diarrhoea and extra intestinal pathogens causing a variety of other infections including urinary tract infections, meningitis and septicaemia (28).The heightened use of antibiotics results in killing of the sensitive strain of *E.coli* and selecting a more resistant ESBL producing *E.coli* to survive and grow rapidly to increase in number. These resistant bacteria may then spread to the other person through environment contaminated with faeces of these persons. Poor hand hygiene and close contact with the cattle harbouring the resistant *E.coli* can be an important contributing factors for the spread of ESBL producing *E.coli* from a common source or from person to person (7).

## 2.1 Classification of ESBLs

The Ambler molecular classification and the Bush-Jacoby-Medeiros functional classification are the two most commonly used classification systems for β-lactamases (29).Ambler scheme divides β-lactamases into four major classes (A to D). The basis of this classification scheme rests upon protein homology (amino acid similarity) and not phenotypic characteristics. In the Ambler classification scheme, β-lactamases of classes A, C and D are serine β-lactamases. In contrast, the class B enzymes are Metallo beta lactamases. With the exception of OXA-type enzymes (which are class D enzymes), the ESBLs are of molecular class A (5).The majority of ESBLs identified in clinical isolates to date, have been SHV or TEM types, which have evolved from narrow-spectrum β-lactamases such as TEM-1, -2 and SHV-1. The majority of ESBLs identified in clinical isolates to date, have been SHV or TEM types, which have evolved from narrow-spectrum β-lactamases such as TEM-1, -2 and SHV-1. The 2be designation shows that these enzymes are derived from group 2b beta-lactamases (for example, TEM-1, TEM-2 and SHV-1); the ‘e’ of 2be denotes that the beta-lactamases have an extended spectrum. The ESBLs derived from TEM-1, TEM-2 or SHV-1 differs from their progenitors by as few as one amino acid. This has resulted in a profound change in the enzymatic activity of the ESBLs, so that they can now hydrolyze the third-generation cephalosporins or aztreonam (hence the extension of spectrum compared to the parent enzymes (5).

## 2.2 Epidemiology of ESBL producing *E.coli*

Antimicrobial resistance is rapidly spreading across the globe and entails a significant threat to public health. Antibiotic resistance increases the morbidity, mortality and costs of treating infectious diseases (31, 32).The gut plays a vital role in the development of antibiotic resistance, and the emergence of resistant microorganisms in the gut may be related to ingestion or antibiotic-induced alterations in microorganisms. The resistant organisms then contaminate the environment (7, 32).Asymptomatic faecal carriage of ESBL-producing bacteria had been reported from several countries and continents with wide differences in carriage rates between geographic areas. Over the years prevalence of ESBL producing *E.coli* has increased enormously. Various studies have documented the prevalence and susceptibility of ESBL producing *E.coli* and each one had reported quite different ESBL rates. As mentioned earlier also, ESBLs were first described in 1983 from Germany and England. The prevalence of ESBL-producing *Klebsiella* species in Europe varied from country to country (33). Various surveys conducted across the region had demonstrated the geographical variation. In a survey of laboratories in the Netherlands less than 1% of *E. coli* and *K. pneumoniae* strains possessed ESBL (34).While in an another study conducted in France and Italy, Ceftazidime resistance was observed in as many as 40% of strains of *K.pneumoniae*(35).The first ESBL-producing organisms were first reported in the US in 1988 (36).The prevalence of ESBL production amongst *Enterobacteriaceae* in the US ranges from 0 to 25% in different regions (6).

A study conducted in Vietnam observed a prevalence of 87.4 % of GNB from various clinical specimens out of a total 350 isolates. Of these GNB isolates, 88.9% were *Enterobacteriaceae*, of which 14.7% were ESBL positive (37). A study conducted by Ko et al., at South Korea, documented 22.4% of *K. pneumoniae* isolates and 10.2% of *E. coli* isolates as ESBL producers (38). In a study from India the percentage of ESBL-positive isolates was found to be elevated, with 23.1% of isolates being ESBL positive (39). Of the isolates from India, 48.4% of isolates were *E. coli* and 51.6% were *K. pneumoniae*(39). ESBL prevalence of 30 to 60% of from intensive care units in Brazil, Colombia, and Venezuela had been reported (40-43).Moreover several studies had been conducted to test the prevalence faecal carriage of ESBL producing Gram negative bacteria as these carriers can form an important reservoirs for the transmission of HAI and CAI caused by these bacteria. In a study conducted at South Africa byMahomed et al., out of 300

samples analysed, *E.coli* isolates were obtained from 97 stool samples and *K.pneumoniae* from 12 patients only. ESBL production was positive only in 3 *E.coli* isolates (3.1percent) and, 11 isolates out of 12 *K.pneumoniae* were ESBL positive. The combined faecal carriage of ESBL producing *E.coli* and *K.pneumoniae* was 4.7 percent (14/300) (44).Reuland et al. in a study determined the rate of faecal carriage of ESBL producing *Enterobacteriaceae* in the Dutch community in Netherlands. Out of the total 720 samples, 73 of them were tested positive for ESBL producing *Enterobacteriaceae,* among which the predominant was *E.coli* (45).In contrast, rates reported in Europe never exceeded 10%, with the exception of a recent report of 11.6% observed in 2011 among patients upon admission to a geriatric unit in Belgium. Community carriage in Africa has been studied very poorly. Reported rates appear to be quite high, from 10.0% in Senegal (46)to 30.9% in Niger (47).Thus, the reported prevalence of carriage of ESBL-producing bacteria may be influenced by characteristics of the population under study such as the geographic area, previous use of antibiotics, healthcare environment.

The following table also describes various studies conducted across the world in different years to study the prevalence of ESBL producing *E.coli* and other Gram negative bacteria, the associated risk factors.

**Table 1:** Worldwide distribution of ESBL producing *Enterobacteriaceae*

| **Study group** | **Year of study** | **Study area** | **Clinical isolates** | **ESBL prevalence (%)** | **Risk factors** |
|---|---|---|---|---|---|
| Blom et al. (48) | 2016 | Sweden | *E.coli* | 10 | Foreign travel |
| Asir J. et al. (49) | 2015 | India | *E.coli* | 21 | Invasive devices like urinary catheters |
| Mahomed et al. (44) | 2014 | South Africa | *E.coli* and *K.pneumoniae* | 4.7 | Contaminated food and water |
| Shakya et al. (50) | 2013 | India | *E.coli* | 9 | Higher socioeconomic status |
| Reuland et al. (45) | 2012 | Netherland | *Enterobacteriaceae* | 10.1 | Foreign travel |
| Wickramasinghe et al. (51) | 2012 | UK | *E.coli* | 11.3 | Foreign travel |
| Woerther et al. (52) | 2011 | Niger | *Enterobacteriaceae* | 31 | Transmission from hospitals |
| Peirano et al. (53) | 2011 | Canada | *E.coli* | 14 | Foreign travel |
| Andriatahina et al. (54) | 2011 | Madagascar | *Enterobacteriaceae* | 10.1 | Hospital acquisition |

## 2.3 Risk factors

Numerous studies have used a case-control design with which to assess risk factors for colonization and infection with ESBL-producing organisms (55, 56, and 49)**.**The prevalence of ESBLs among clinical isolates varies between countries and from institution to institution. Several studies have already revealed various risk factors associated with colonisation and infection with ESBL-producing organisms (57). Prolonged hospital stay and patients on medical devices like urinary catheters, central venous lines, etc., have been associated with infections by these organisms (58, 59).

## 2.4 Treatment

In low-endemic countries such as Sweden, treatment of infections with ESBL producing bacteria is often delayed due to the use of empirical antibiotics with a "narrower" spectrum. Another problem when treating patients with these infections is that the plasmids carrying the ESBL gene often have additional mechanisms that give rise to co-resistance to many other antibiotics (60).Carbapenems are considered the first choice for treatment of patients infected with ESBL-producing Enterobacteriaceae, especially in cases involving severe septicaemia or septic shock (11).The available data are too limited to support therapy with Tigecycline. Treatment with a beta-lactam/beta-lactamase inhibitor (e.g. Piperacilin-tazobactam) might be used as a Carbapenem-sparing regimen when the susceptibility results are known. However, the emergence of CTX-M-15-producing bacteria also frequently leads to production of OXA-1- beta-lactamase, which is worrisome and renders the beta-lactam/beta-lactamase inhibitor ineffective (61).

# CHAPTER 3: AIMS AND OBJECTIVES

The aims and objectives of this thesis are:

1. To investigate the prevalence of faecal carriage of ESBL producing *E.coli* in hospitalized patients and normal healthy individuals to gain the understanding of the epidemiology of the resistant strains.
2. To study the antibiogram of ESBL producing *E.coli.*
3. To access the development of multi drug resistance in ESBL producing *E.coli.*
4. To compare the prevalence of faecal carriage of ESBL producing *E.coli* in healthy individuals and hospitalized patients.

# CHAPTER4: MATERIALS AND METHODS

The present study was undertaken in the Department of Microbiology at Punjab Institute of Medical Sciences, Jalandhar. It was a prospective study carried over a period of four months from 1st January 2016 to 30th April 2016.

## 4.1 Clinical isolates

### 4.1.1 Test group

A total of hundred clinical isolates of *E.coli* from the faecal samples received from patients admitted in various clinical departments of the Punjab Institute of Medical Sciences, Jalandhar formed the test group of this study.

### 4.1.2 Control group

Twenty clinical isolates of *E.coli* obtained from the stool samples of normal healthy individuals from the community formed the control group of this study.

*Detailed information regarding the age, gender and clinical history was noted for both the groups. Specific precautions were taken with the control group that the individuals were negative for any existing illness and were not on any antibiotic treatment since the last 30 days and they did not visit any hospital for any purpose during past one month period.

## 4.2 Materials used

The materials used in the present study are given in the following table. All the glassware used in this project was purchased from Thermo Fisher Scientific India Pvt. Ltd., Mumbai, India. The media, antimicrobial agents, chemicals used were obtained from HiMedia™ Laboratories Pvt. Ltd. Mumbai, India.

**Table 2:**List of materials used in project.

| S.No. | Material used | Type | Purpose |
|---|---|---|---|
| 1 | Media | Mac-Conkey agar | Culture/Isolation |
| | | Peptone broth | Susceptibility testing |
| | | Mueller Hinton agar | Susceptibility testing |
| 2. | Biochemical test media | Simmon's citrate agar | Citrate test |

| | | | |
|---|---|---|---|
| | | Christenson's urease agar | Urease test |
| | | Glucose peptone phosphate broth | Methyl red test |
| | | Andrade's peptone water | Sugar fermentation test |
| | | Motility agar | Motility test |
| 3 | Biochemical test (reagents and indicators) | 3% Hydrogen peroxide | Catalase test |
| | | Kovac's reagent | Indole test |
| | | Methyl red indicator | Methyl red test |
| | | Phenol red indicator | Triple sugar iron test and urease test |
| | | Andrade indicator | Sugar fermentation test |
| 4 | Staining reagents | Crystal violet | Gram's stain (Primary stain) |
| | | Gram's iodine | Gram's stain (Mordant) |
| | | Acetone | Gram's stain ( Decolourizer) |
| | | Safranin | Gram's stain (Counter stain) |
| 5 | Glassware | Conical flasks | Media preparation |
| | | Beakers | Media preparation |
| | | Measuring cylinder | Media preparation |
| | | Petri plates | Media preparation |
| | | Glass slides | Catalase test, Gram's staining |
| | | Test tubes | Susceptibility testing, Biochemical test |
| 6 | Instruments and Equipments | Weighing balance | Media preparation |
| | | Compound Microscope | Observation of |
| | | Bacteriological Incubator | Incubation |
| | | Bio safety hood | Inoculation into various culture media and biochemical test media, Smear preparation, Subculture |

### 4.2.1 Sterilisation

Glassware used in the project was washed with 2% soap solution followed by washing with distilled water. They were then dried in hot air oven and wrapped in butter paper and plugged using cotton plug and sterilized in autoclave for 15 minutes at 15 per square inch (psi) pressure at 121degree Celsius (°C). Andrade's peptone water base medium used to study fermentation reactions was sterilized by autoclaving at 15 psi pressure (121°C) for 15 minutes. The sterile sugar solutions are added after sterilisation to the Andrade's peptone water medium. The urease agar medium was sterilized by autoclaving at 10 psi pressure (115°C) for 20 minutes. Mac Conkey agar used to culture *E.coli* was sterilized by autoclaving at 15 psi pressure (121°C). Mueller Hinton agar used for susceptibility testing was sterilized by autoclaving at 15 psi pressure (121°C).

## 4.3 Methods for isolation and identification of *E.coli* isolates

### 4.3.1 Specimen collection

Stool sample was collected by the patients themselves in a wide open mouthed sterile container according to the instructions by trained and experienced physician. Approximately 5 gram of stool sample was collected. The containers were properly labelled with the patient's name, age, and sex and identification number. The specimens were then transported to the laboratory as quickly as possible.

### 4.3.2 Isolation and identification of *E.coli* isolates

*E.coli* were isolated by culture and then identified by its colony morphology, staining character, motility, and other relevant biochemical tests as per conventional standard methods. The methods followed for the identification of the *E.coli* isolateswere in accordance with the Bergey's manual of systematic bacteriology (62).

## A. Culture

**Media Used:** Mac-Conkey Agar

## Media Inoculation

All the stool samples received were inoculated on Mac-Conkey agar by surface streak plate technique. The plates were then incubated at $37^0$ C ±2°C for 24-48 hours.

## Colony characteristics

On Mac Conkey agar, *E.coli* showed circular, 2-4 mm in diameter, convex, smooth colonies with entire margin, pink in color due to lactose fermentation, opaque colonies and can be easily emulsifiable in normal saline.

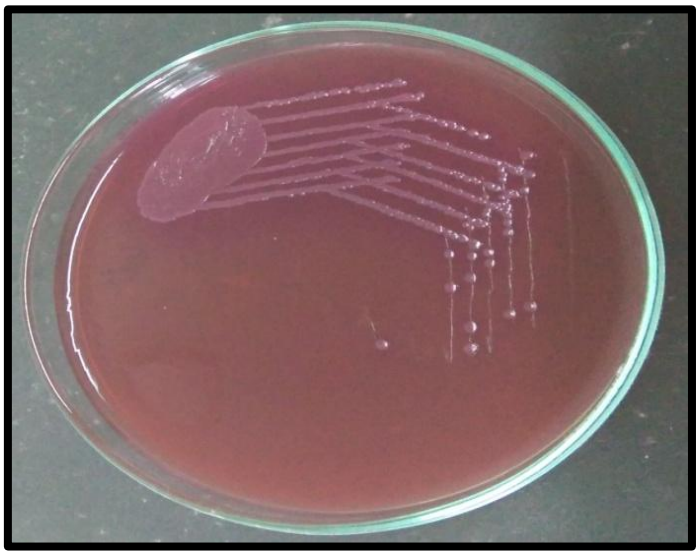

**Figure 1:** Growth of *E.coli* on Mac Conkey agar

## B. Gram staining of *E.coli*

Smear prepared from the colonies obtained on the Mac Conkey agar were stained by Gram staining after heat fixing.

## Procedure

- The smear was flooded initially with primary stain, crystal violet for 1 minute and then washed under tap water.
- Grams iodine solution, the mordant was flooded and let to stay for 1 minute, followed by washing with acetone and tap water.
- Finally, safranin, the counter stain was added in decolourization step for 1 minute followed by final washing under tap water.
- After air drying, the slide was observed under light microscope at 100X magnification after the final rinsing and air-drying.

## Interpretation

*E.coli* appears as Gram negative, non sporing bacilli on a Gram stained smear.

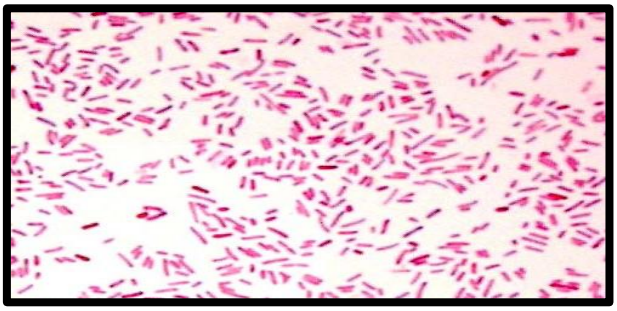

**Figure 2:** Observation of *E.coli* using Gram stained smear (Gram negative bacilli)

## C. Biochemical test

Following set of biochemical tests are performed for the identification of *E.coli.*

## 1. Catalase test

This test is used to identify the bacteria producing the enzyme catalase which breaks hydrogen peroxide ($H_2O_2$) resulting in the formation of water and oxygen causing the formation of bubbles. A well grown colony of the test organism is picked with the help of cover slip and placed onto 3-4 drops of 3% $H_2O_2$on a clean glass slide. Immediate formation of bubbles indicated a positive Catalase test.

**Figure 3:** Interpretation of catalase test results for *E.coli*

*E.coli* gives a positive catalase test.

**2. Indole production test:** Pure culture of the bacteria was grown in the peptone broth for 24-48 hours. Kovac's reagent (Paradimethylamino- benzaldehyde was dissolved in a solution containing 25ml concentrated hydrochloric acid and 75ml isoamyl alcohol) was

added to peptone broth culture incubated at $37^0$C for 24 hours. The formation of a cherry red colour on the top layer of isoamyl alcohol indicates the production of indole by the test culture.

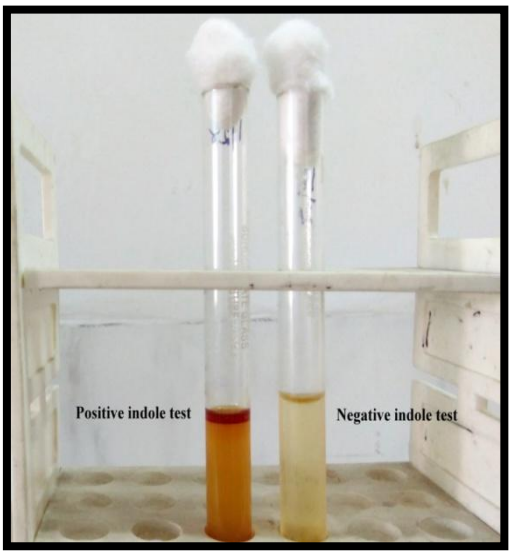


**Figure 4:** Interpretation of indole production test for *E.coli* (Positive indole test).

**3. Methyl red test:** A loop-full of culture was inoculated into the glucose peptone broth and incubated at $37^0$ C ±2°C for 24-48 hours. The test was run in duplicates and an un-inoculated control was run along the test. After incubation, 0.2ml of methyl red indicator was added into each tube and observed for red coloration. Development of red colour in the culture show positive result and absence of red coloration indicates negative result.

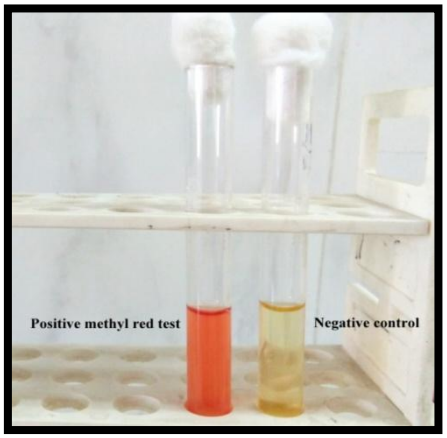


**Figure 5:** Development of red colour by *E.coli* in methyl red test

**4. Motility test:** Bacterial motility is observed macroscopically by a diffuse zone of growth spreading from the line of inoculation into the motility agar medium. A loop-full of

overnight grown culture was stabbed onto motility agar tube and incubated at $37^0$ C for 24-48 hours. Motile culture show diffuse growth and non-motile culture show growth only along the line of inoculation.

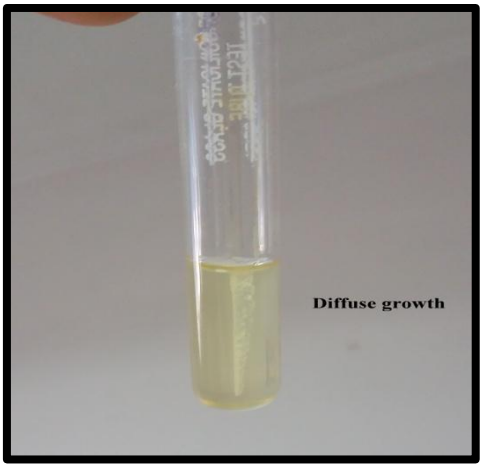


**Figure 6:** Detection of motility of *E.coli* using motility test

*E.coli* gives positive motility test as it is a motile bacterium.

**5. Citrate utilisation test:** Simmon's citrate agar slant was inoculated with the test culture by the streak method followed by incubation at 30±2ºC for 48 hours. After incubation the slants were observed for growth and also for color change from green to blue.

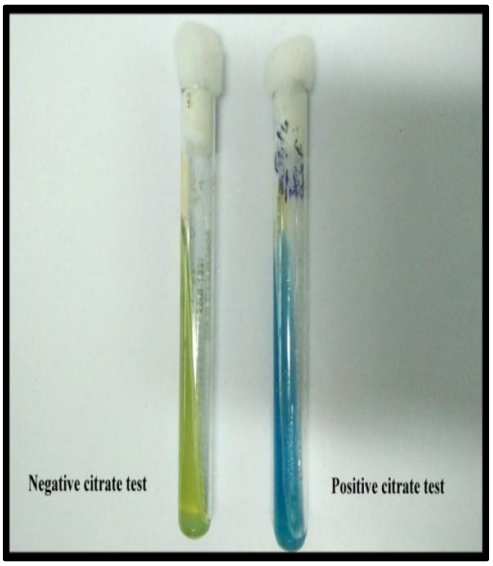


**Figure 7:** Interpretation of citrate test results for *E.coli* isolates (negative)

**6. Urease test:** A loop-full of the culture was inoculated into Urea agar slants. An un-inoculated slant was also maintained. These tubes were then incubated at 30+2ºC for 48 hours. After incubation, the slants were observed for colour change from yellow to pink.

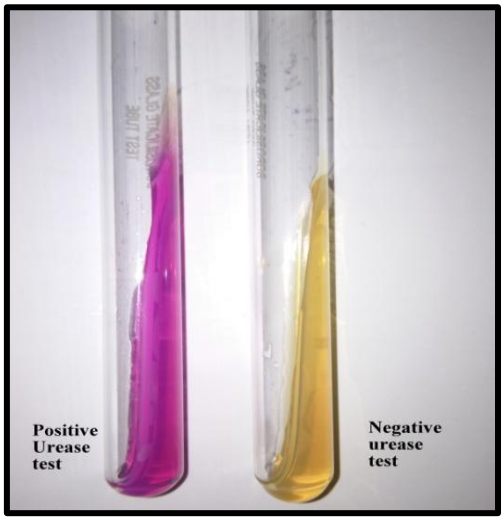


**Figure 8:** Urease test results (*E.coli* gives negative urease test).

**7. Sugar fermentation test:** This test is used to check the sugar fermentation property of the test isolate. The sugars used are glucose, lactose, lactose, sucrose and mannitol. Fermentation is indicated by change in the colour of the Andrade's indicator to pink due to production of an acid and gas production is seen by formation of bubbles in the Durham's tube.

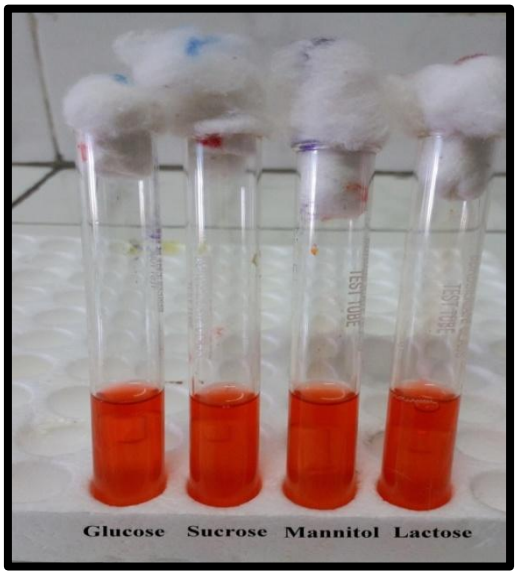


**Figure 9:** Fermentation of carbohydrates by *E.coli* is indicated by pink colour and gas production is seen in the form of bubbles in Durham's tube

**8. Triple sugar iron agar (TSI) test:** This is a multi- principle test used to test the fermentation of sugars, gas production and hydrogen sulphide production. A loopful of bacterial culture was inoculated on to the triple sugar iron agar tube having both slant and

butt. The tubes were then examined for the production of acid (yellow slant or butt) and gas (displacement of the medium) and hydrogen peroxide production (blackening of the medium).

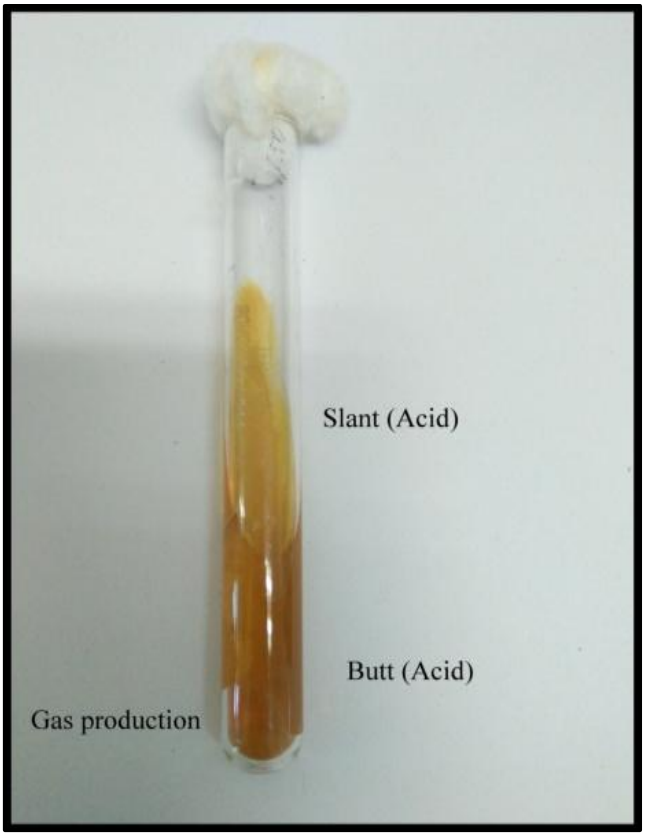


**Figure 10:** Analyzing the activity of *E.coli* using TSI

*E.coli* gives production of acid in both slant and butt with production of gas.

## 4.4 Susceptibility testing for *E.coli*

Routine susceptibility was determined using the disk diffusion method on Mueller-Hinton agar and commercially available antibiotic discs as per guidelines issued by the **Clinical and Laboratory Standard Institute (CLSI)** published in the document M100-S24 (2011) (63).

**Table 3:** List of antimicrobials used in the project

| **Antimicrobial agent** | **Symbol** | **Disc content (mcg)** |
|---|---|---|
| Cefoperazone | CPZ | 75 |
| Ceftazidime | CAZ | 30 |
| Ceftriaxone | CTR | 30 |
| Cefpodoxime | CPD | 10 |
| Ceftazidime + Clavulanic acid | CAC | 30/10 |
| Piperacilin +Tazobactum | PIT | 100/10 |
| Cefepime | CPM | 50 |
| Cefoxitin | CX | 30 |
| Ofloxacin | OF | 5 |
| Gentamycin | GEN | 120 |
| Co-Trimoxazole (Trimethoprim-Sulphamethoxazole) | COT | 25 (1.25/ 23.75) |
| Doxycycline | DO | 30 |

**Quality control:** Disc from each batch was standardized using the reference strain of *E.coli* ATCC (American type culture collection) 25922 and zone of inhibition were compared with standard values as mentioned in the CLSI guidelines.

## 4.4.1. Standardization of the inoculum

With a sterile loop 3-4 colonies are transferred to sterile peptone broth tube containing 5 millilitres of peptone broth and standardization with 0.5 Mc Farland standards by adding more organisms or more peptone broth, the solution approximately corresponds to $1.5 \times 10^8$ microorganisms per ml.

## 4.4.2. Inoculation of the test plate and disc placement

Before use, the Mueller Hinton plates were dried in the incubator at $37^0$C for 10-15 minutes. Within 15 minutes after sterilization of inoculums, a sterile cotton swab was immersed into the bacterial suspension. The excess broth was removed by retaining the swab with firm pressure against the inner side of the tube above the fluid level. The swab was then streaked evenly on the surface of the plate by rotating the plate approximately $60^0$ each time to get a

uniform distribution of the inoculums. The inoculum was allowed to dry for 10-15 minutes at room temperature keeping the lid closed. The disc were then placed on the inoculums surface by a sterile forceps 15mm away from the edge of the Petri dish and having 20 mm gap between the discs. The plates were then incubated at $37^0$C for 24 hours.

### 4.4.3 Reading of the sensitivity test

After overnight incubation each plate was examined and the diameter of the complete zone of inhibition was measured in millimetres with the help of a scale placed under the surface of the Petri dish. Zone of the inhibition were measured in two directions of right angles to each other through the centre of the disc and the average of the two discs was taken and compared with the standard to report the sensitivity of an antimicrobial agent as Sensitive, Intermediate, or Resistant.

### 4.4.4 Interpretation of the Susceptibility testing for *E.coli*

**Table 4:** Interpretation of antimicrobial susceptibility testing for *E.coli*

| **Antimicrobial agent** | **Sensitive (mm)** | **Intermediate (mm)** | **Resistant (mm)** |
|---|---|---|---|
| Cefpodoxime | 21 | 18-20 | 17 |
| Ceftazidime | 21 | 18-20 | 17 |
| Cefotaxime | 26 | 23-25 | 22 |
| Ceftriaxone | 23 | 20-22 | 19 |
| Cefepime | 18 | 15-17 | 14 |
| Cefoxitin | 18 | 15-17 | 14 |
| Cefoperazone | 21 | 16-20 | 15 |
| Ofloxacin | 16 | 13-15 | 12 |
| Gentamicin | 15 | 13-14 | 12 |
| Piperacilin-Tazobactam | 21 | 18-20 | 17 |
| Ceftazidime-Clavulanic acid | 21 | 8-20 | 17 |
| Doxycycline Hydrochloride | 14 | 11-13 | 10 |
| Co-Trimoxazole (Trimethoprim-Sulphamethoxazole) | 16 | 11-15 | 10 |

## 4.5 Methods for the detection of ESBL production in *E.coli* isolates

### 4.5.1 Screening method for testing ESBL production

Screening method for detection of ESBL production is based on measuring the zone of inhibition. *E.coli* isolates may be regarded as positive for screening test for ESBL production under the following conditions:

**Table 5:** Interpretation of screening method for ESBL production for *E.coli*

| **Antibiotic (mcg)** | **Diameters of zone of inhibition(mm)** |
|---|---|
| Cefpodoxime, 10 | ≤ 17 |
| Ceftazidime, 30 | ≤ 22 |
| Cefotaxime, 30 | ≤ 27 |
| Ceftriaxone, 30 | ≤ 25 |

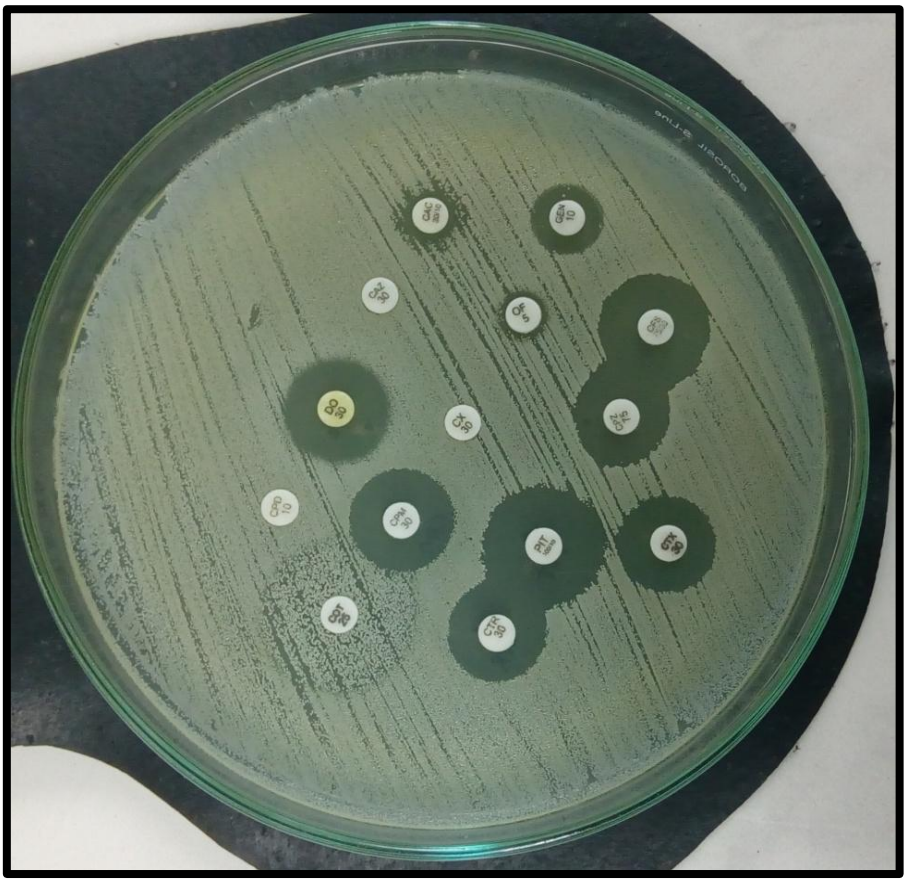

**Figure 11:** Antimicrobial susceptibility results of ESBL positive *E.coli* isolates using screening method

The isolates showing positive screening test are then tested further by confirmatory methods for ESBL detection.

## 4.5.2 Phenotypic confirmatory methods for the detection of ESBL production

### 4.5.2.1 Double disc synergy test:

Standardized inoculums of the test isolate were swabbed on the surface of a Mueller Hinton agar plate. A combination disc such as Ceftazidime and Clavulanic acid (30/10mcg), Piperacilin and Tazobactam disc (100/10 mcg), was placed at the centre surface of the plate. Disc containing 30 mcg of Ceftazidime, Cefotaxime, Ceftriaxone, and 10 mcg of Cefpodoxime were placed at a distance of 1.8 cm away from the central disk.
An extension in the zone of inhibition around the peripheral disc towards the centrally placed Ceftazidime/clavulanic acid disc indicated ESBL production.

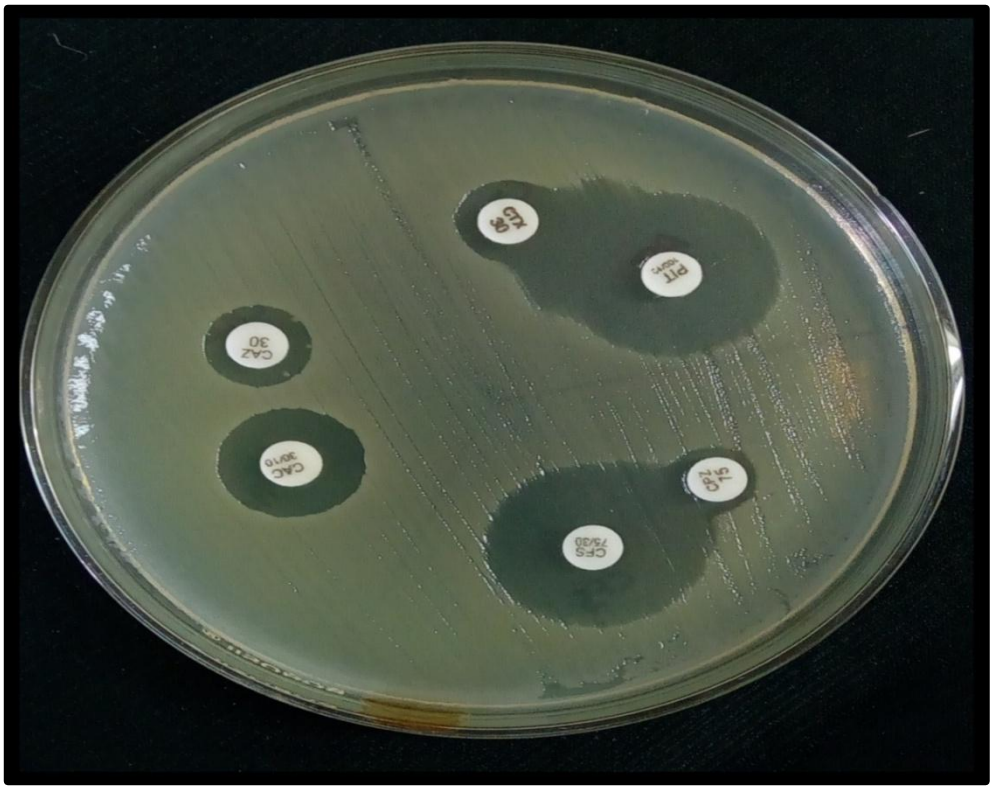


**Figure 12:** Double disc synergy test: Confirming ESBL production using a cephalosporin inhibitor combination disc and third generation cephalosporins disc.

### 4.5.2.2 Combined disc test (Inhibitor potentiated disc test)

Cephalosporins disc (Cefotaxime 30 mcg, Ceftazidime 30 mcg, Cefpodoxime 30 mcg) with and without clavulanic acid, 10 mcg were placed on the Mueller Hinton agar incorporated with the test organism. An increase in the inhibition zone diameter of greater than or equal to5mm in cephalosporins disc combined with clavulanic acid, compared to cephalosporins alone, indicated ESBL production.

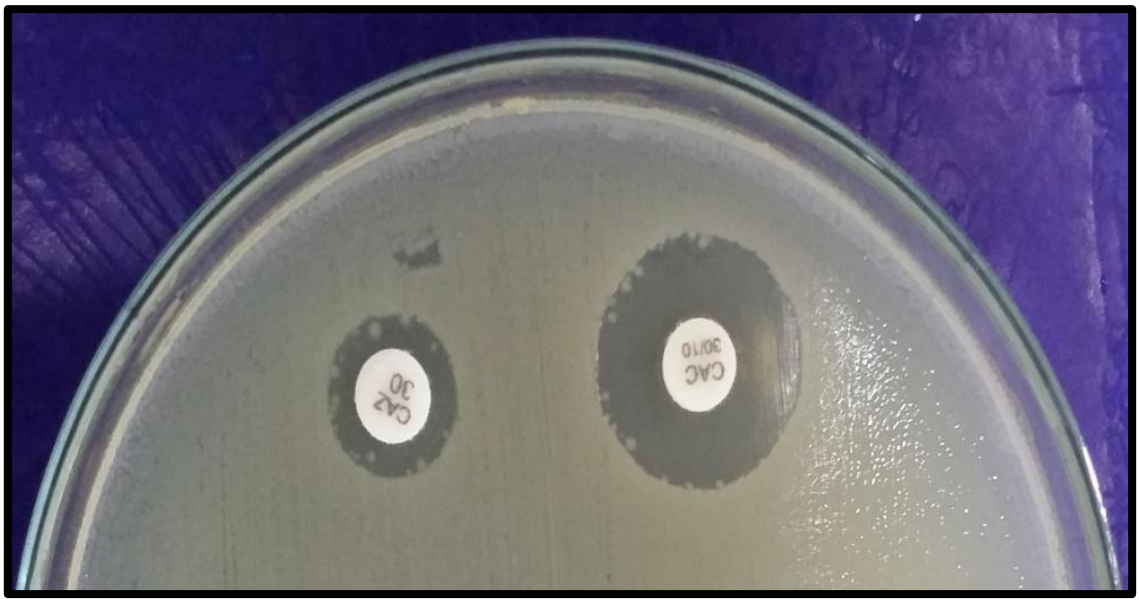


**Figure 13:** Confirmation of ESBL production using combined disc test

# CHAPTER 5: RESULTS

The present study was conducted on a total of 120 clinical isolates of *E.coli* which were isolated from stool samples received at the Department of Microbiology in Punjab Institute of Medical Sciences, Jalandhar for bacteriological processing.

## 5.1 Age distribution of test and control group

Out of a 100 patients studied in the test group (0-70 years), maximum 28 were in the age group 0-10 years followed by 22 in 11-20 years. There were only 07 (7%) patients in the age group 41-50 years. A total 0f 20 healthy individuals were included in the control group and out of the total 20 individuals, maximum, 5 (25%) were in the age group 0-10 years. There were 4 individuals (20%) in the age groups 11-20 years and 21-30 years, 3 (15%) individuals in the age group 41-50 years, and 2 (10%) each in the age groups 31-40 years and 51-60.

**Table 6:** Age wise distribution of the test and control group

| **Age group** | **Number of patients in the test group (N=100) (%)** | **Number of person in the control group (N=20) (%)** |
|---|---|---|
| 0-10 | 28 (28.00) | 5 (25.00) |
| 11-20 | 22 (22.00) | 4 (20.00) |
| 21-30 | 11 (11.00 ) | 4 (20.00) |
| 31-40 | 09 (09.00) | 2 (10.00) |
| 41-50 | 07 (07.00) | 3 (15.00) |
| 51-60 | 15 (15.00) | 2 (10.00) |
| 61-70 | 08 (08.00) | 0 (0.00) |
| **TOTAL** | **100** | **20** |

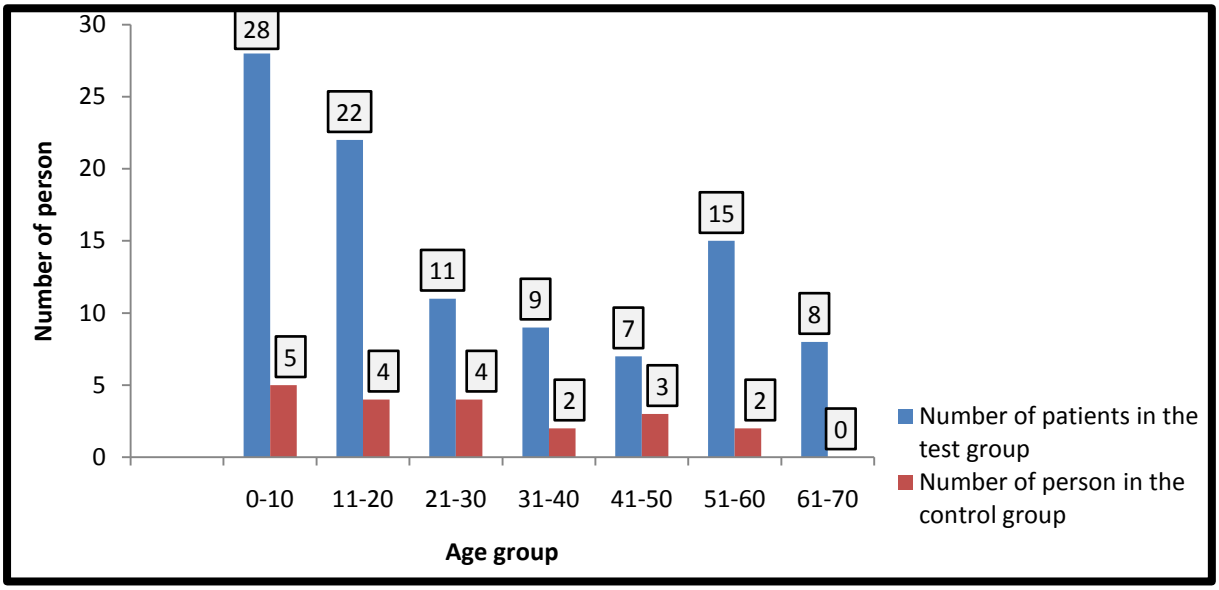


**Chart 1:** Age wise distribution of the test group and control group

## 5.2 Distribution of test and control group as per gender

In the test group, 58 (58%) out of 100 patients were male whereas 42 (42%) were females while in the control group, 12 (60%) out of 20 were males while 8 (40%) out of 20 were females.

**Table 7:** Gender wise distribution of test and control group

| **Gender** | **Test Group (N=100) (%)** | **Control group (N=20) (%)** | **Total (%)** |
|---|---|---|---|
| Male | 58 (58.00) | 12 (60.00) | 70 (58.33) |
| Female | 42 (42.00) | 08 (40.00) | 50 (41.66) |
| **Total** | **100** | **20** | **120** |

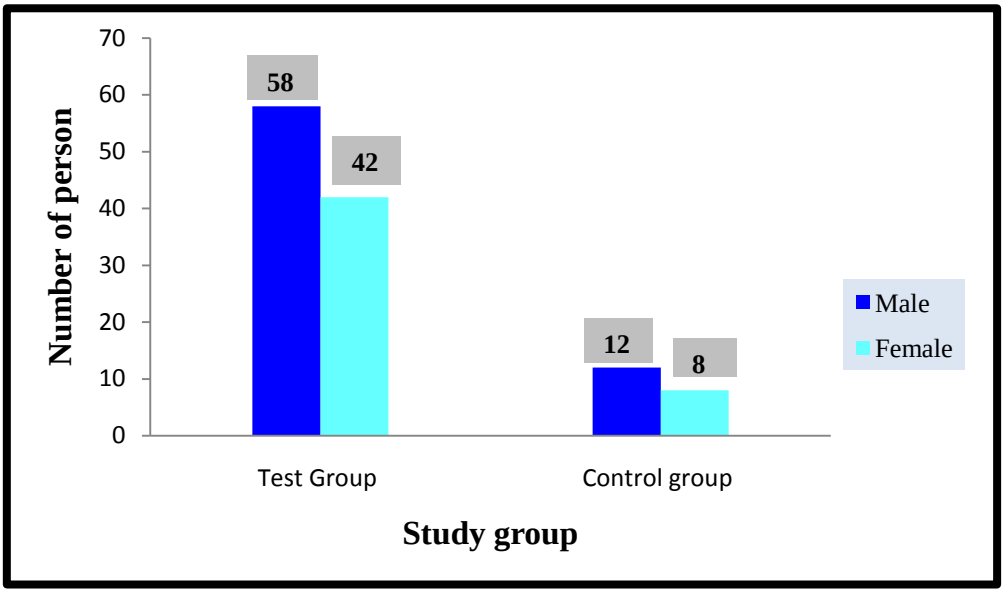


**Chart 2:** Gender wise distribution of the test group and the control group

## 5.3 Distribution of test group in relation to the wards

Out of 100 samples received during the course of the project, maximum numbers of the samples were received of patients admitted in various medical wards of the hospital, (52/100), followed by patients admitted in Intensive Care Units, (30/100). Least number of samples was received from the intensive care unit of the hospital.

**Table 8:** Ward wise distribution of test group

| **Wards** | **Number of patients (%)** |
|---|---|
| Surgical | 18 (18) |
| Medical | 52 (52) |
| Intensive Care Unit | 30 (30) |
| **Total** | **100** |

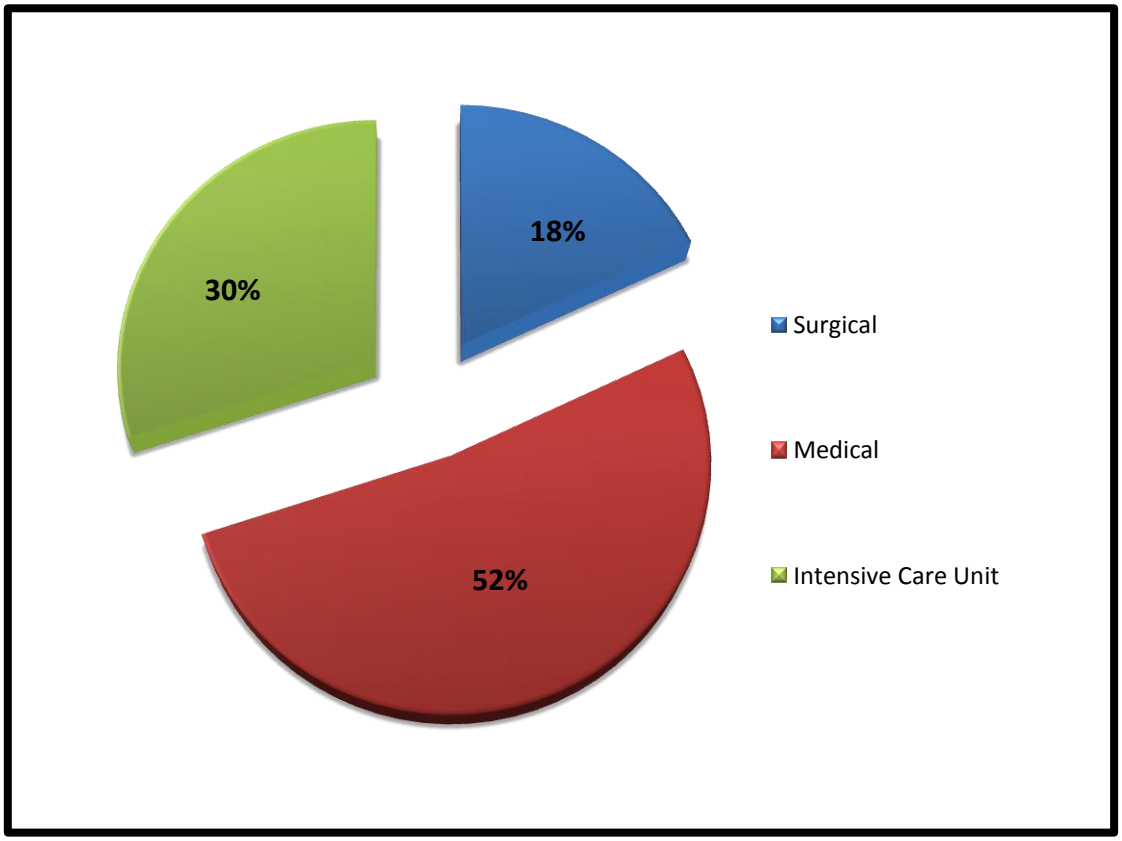


**Chart 3:** Ward wise distribution of the test isolates (N=100)

## 5.4 Antimicrobial susceptibility pattern of test group strains

An average of 45% of the test isolates were sensitive to the third generation cephalosporins with Ceftriaxone showing maximum sensitivity (48%) followed by Ceftazidime (47%). Minimum sensitivity among third generation cephalosporins was shown by Cefpodoxime (43%). 72% of the isolates were sensitive to the combination drugs Piperacilin + tazobactam and Ceftazidime + Clavulanic acid. Cefepime has shown sensitivity to 64% of the test isolates while 60% of the test isolates were sensitive to the Cefoxitin. Multiresistance was observed in most of the isolates. 59% showed sensitivity to Gentamicin, followed by 51% sensitivity to fluroquinolones (Ofloxacin). Multiresistance was also observed in trimethoprim sulfamethoxozole drug, Cotrimoxazole which showed sensitivity to 48% of the test isolates and Doxycycline showed sensitivity to 52% of the test isolates.

**Table 9:** Antimicrobial susceptibility pattern of the test group isolates

| **Antimicrobial agent** | **Number of sensitive *E.coli* isolates (n=100)** | **Percentage of sensitive *E.coli* isolates (%)** | **Number of resistant *E.coli* isolates (n=100)** | **Percentage of Resistant *E.coli* isolates (%)** |
|---|---|---|---|---|
| Cefoperazone | 44 | 44.00 | 56 | 56.00 |
| Ceftazidime | 47 | 47.00 | 53 | 53.00 |
| Ceftriaxone | 48 | 48.00 | 52 | 52.00 |
| Cefpodoxime | 43 | 43.00 | 57 | 57.00 |
| Ceftazidime + Clavulanic acid | 72 | 61.00 | 39 | 39.00 |
| Piperacilin + Tazobactum | 72 | 72.00 | 28 | 28.00 |
| Cefepime | 64 | 64.00 | 36 | 36.00 |
| Cefoxitin | 60 | 60.00 | 40 | 40.00 |
| Ofloxacin | 51 | 51.00 | 49 | 49.00 |
| Gentamicin | 59 | 59.00 | 41 | 41.00 |
| Cotrimoxazole | 48 | 48.00 | 52 | 52.00 |
| Doxycycline | 52 | 52.00 | 48 | 48.00 |

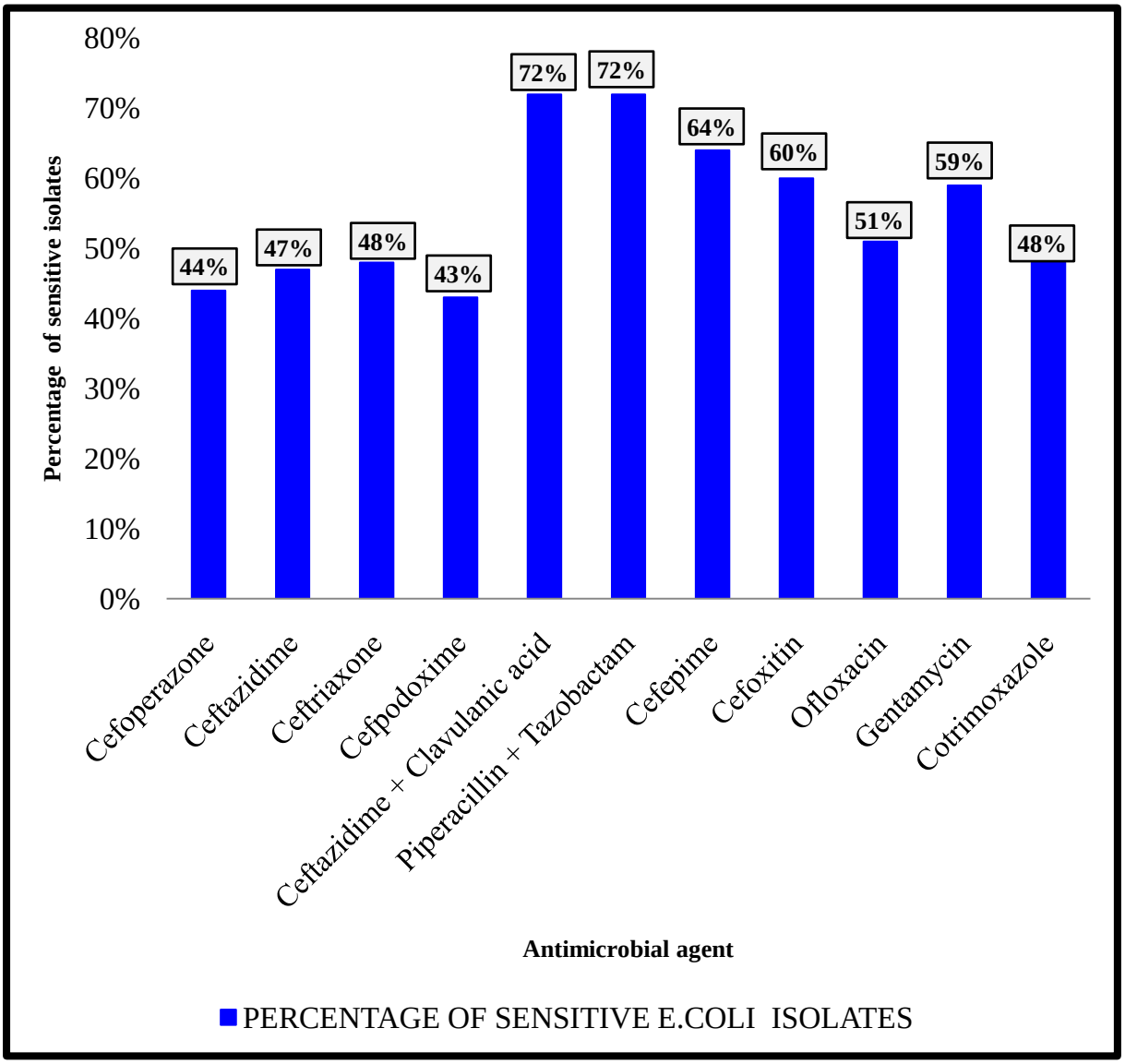


**Chart 4:** Antibiogram of the test group isolates (N=100)

## 5.5 Antibiogram of control group isolates

More than sixty percent of the control group isolates have shown susceptibility to the third generation cephalosporins. Maximum sensitivity was seen in Ceftazidime (75%), followed by Cefoperazone (70%) and Ceftriaxone (65%). Among the combination drugs, Piperacilin Tazobactam showed maximum sensitivity (90%), followed by Ceftazidime Clavulanic acid (85%). Multiresistance was not as common as the test group isolates. More than eighty percent of the isolates were sensitive to most of the drugs such as Cefepime (90%), Cefoxitin, Ofloxacin and Cotrimoxazole (85%). 70% of the control group isolates were sensitive to Gentamicin.

**Table 10:** Antimicrobial susceptibility pattern of the control group isolates

| **Antimicrobial agent** | **Number of sensitive *E.coli* isolates (N=20)** | **Percentage of sensitive *E.coli* isolates (%)** | **Number of resistant *E.coli* isolates (N=20)** | **Percentage of Resistant *E.coli* isolates (%)** |
|---|---|---|---|---|
| Cefoperazone | 14 | 70.00 | 06 | 30.00 |
| Ceftazidime | 15 | 75.00 | 05 | 25.00 |
| Ceftriaxone | 13 | 65.00 | 07 | 35.00 |
| Cefpodoxime | 12 | 60.00 | 08 | 40.00 |
| Ceftazidime + Clavulanic acid | 17 | 85.00 | 03 | 15.00 |
| Piperacilin +Tazobactum | 18 | 90.00 | 02 | 10.00 |
| Cefepime | 18 | 90.00 | 02 | 10.00 |
| Cefoxitin | 17 | 85.00 | 03 | 15.00 |
| Ofloxacin | 17 | 85.00 | 03 | 15.00 |
| Gentamycin | 14 | 70.00 | 06 | 30.00 |
| Cotrimoxazole | 17 | 85.00 | 03 | 15.00 |
| Doxycycline | 16 | 80.00 | 04 | 20.00 |

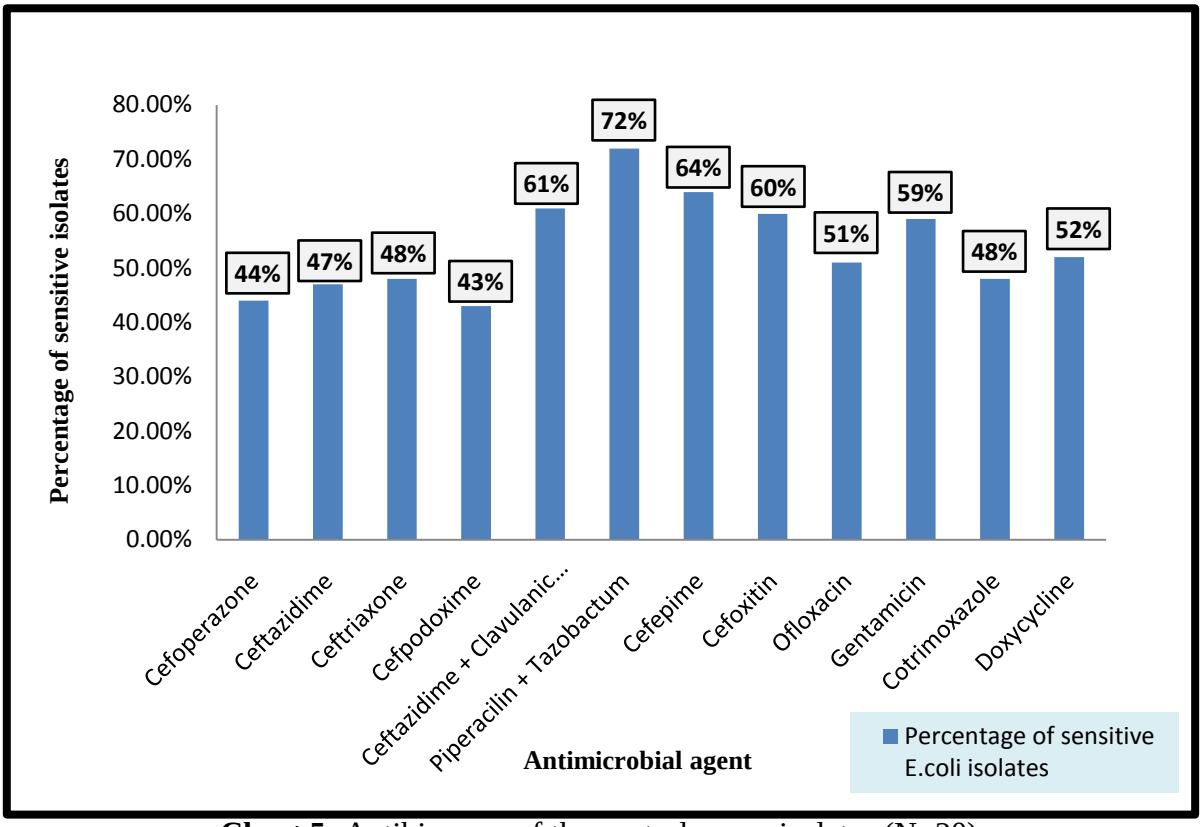


**Chart 5:** Antibiogram of the control group isolates (N=20)

## 5.6 Prevalence of ESBL producing *E.coli* in test group and control group

Out of 100 isolates in the test group, 57 (57%) were found to be positive for ESBL production while 43 (43%) were negative for ESBL production whereas in the control group, out of 20 *E.coli* isolates, only 5 (25%) had shown positive results for ESBL production.

**Table 11:** Prevalence of ESBL positive isolates in test and control group

| | **Test group (%)** | **Control group (%)** | **TOTAL (%)** |
|---|---|---|---|
| **ESBL positive** | 57 (57) | 5 (25) | 62 (51.66) |
| **ESBL negative** | 43 (43) | 15 (75) | 58 (48.33) |
| **Total** | **100** | **20** | **120** |

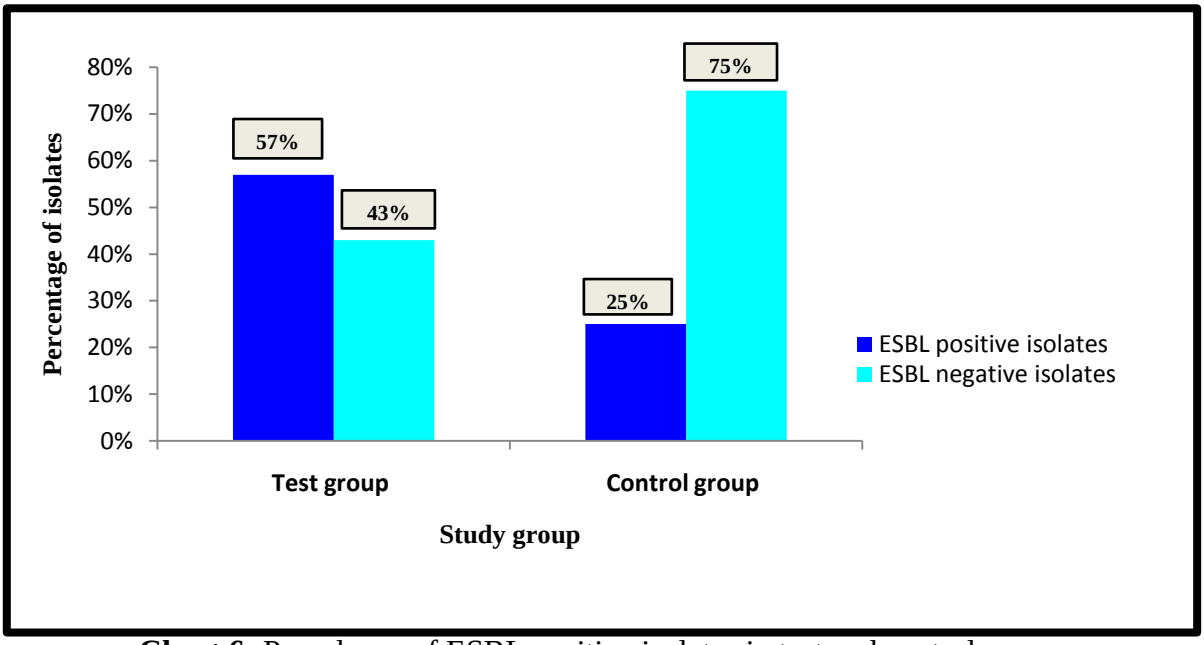


**Chart 6:** Prevalence of ESBL positive isolates in test and control group

## 5.7 Age wise distribution of ESBL positive isolates

Out of 57 ESBL positive isolates in the age group 0-70 years, maximum 21 were in the age group 0-10 years followed by 14 (24.56%) in the age group 11-20 years. Age group 51-60 years have also shown a considerable number, 8 (14.03%) of ESBL positive *E.coli* isolates. The least numbers of ESBL positive E.coli isolates were obtained from patients in the 41-50 years of age group (1.75%).

**Table 12:** Age wise distribution ofESBL positive isolate

| **Age group (Years)** | **Test group (%)** | **Control group (%)** |
|---|---|---|
| 0-10 | 21 (36.80) | 02 (40.00) |
| 11-20 | 14 (24.56) | 01 (20.00) |
| 21-30 | 06 (10.52) | 01 (20.00) |
| 31-40 | 04 (07.01) | 00 (00.00) |
| 41-50 | 01 (01.75) | 00 (00.00) |
| 51-60 | 08 (14.03) | 01 (20.00) |
| 61-70 | 03 (05.26) | 00 (00.00) |
| **TOTAL** | **57 (57.00)** | **05 (25.00)** |

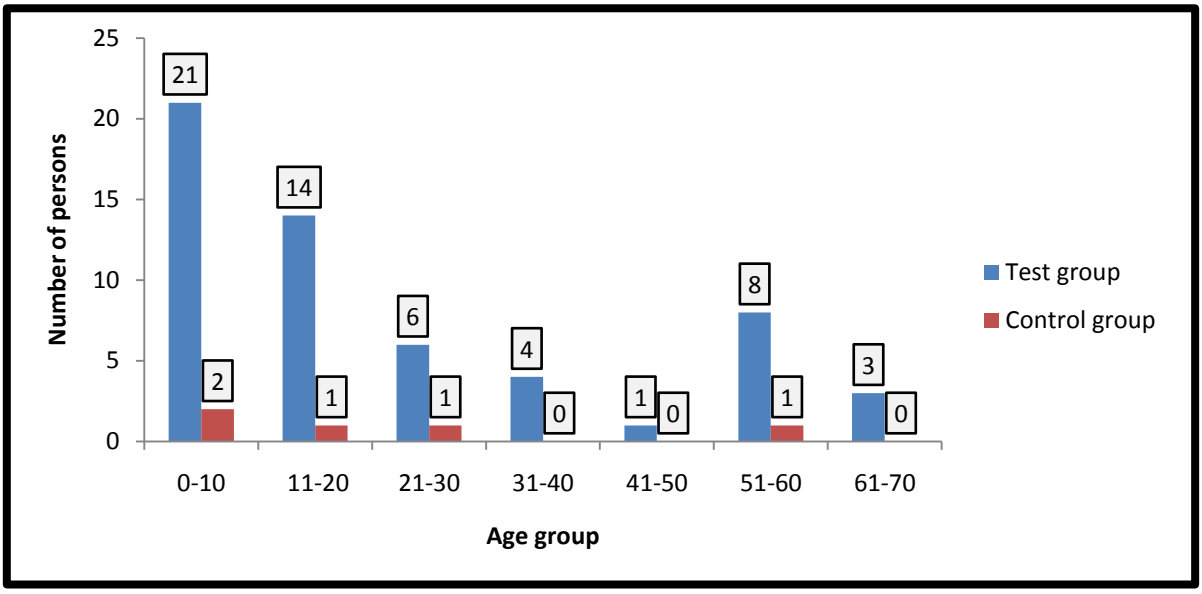


**Chart 7:** Age wise distribution of ESBL positive isolates (N=57)

## 5.8 Ward wise distribution of ESBL positive isolates

Out of 57 ESBL positive *E.coli* isolates, maximum 34 (59.64%) were from the various medical wards followed by 17 (29.82%) ESBL positive *E.coli* isolates from the Intensive care Units. The least numbers of ESBL positive *E.coli* isolates, 6/57 (10.52%) were isolated from the stool samples of the patients admitted in surgical wards of the hospital.

**Table 13:** Ward wise distribution of ESBL positive isolates

| **Wards** | **Number of ESBL positive *E.coli* isolates(%)** | **Percentage of ESBL positive *E.coli* Isolates (%)** |
|---|---|---|
| Surgical | 6 | 10.52 |
| Medical | 34 | 59.64 |
| Intensive Care Unit | 17 | 29.82 |
| **Total** | **57** | **100** |

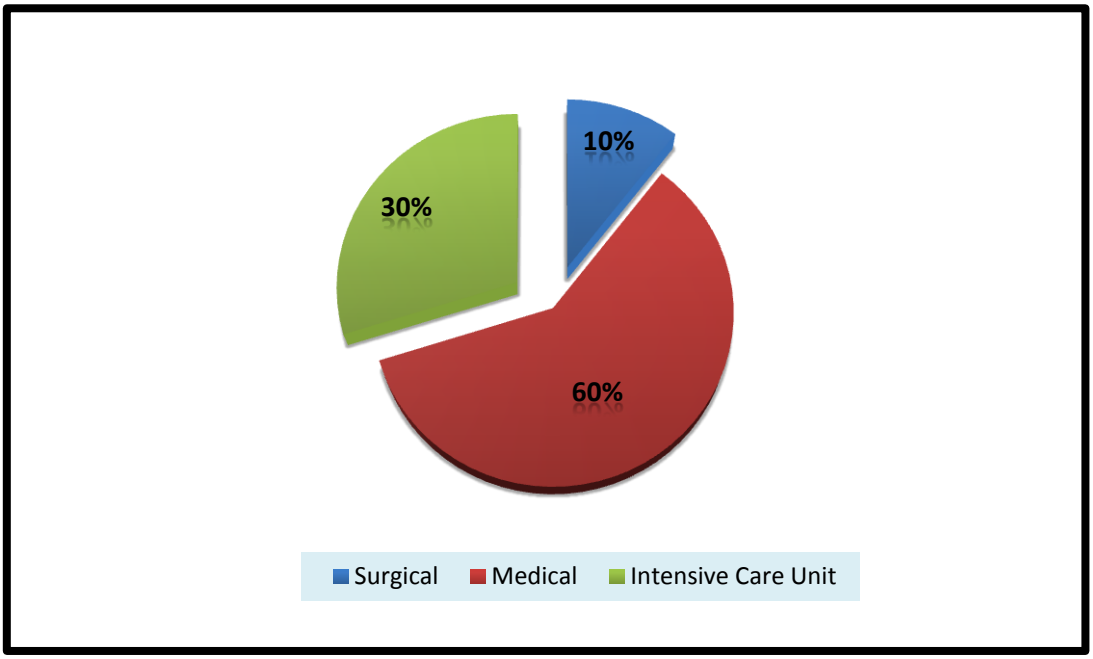


**Chart 8:** Ward wise distribution of ESBL positive isolates

## 5.9 Gender wise distribution of ESBL positive isolates

In the test group 33 (57.80%) out of 57 ESBL positive *E.coli* were isolated from male patients and 24 (42.10%) were from female patients. While in the control group 3 (60.00%) out of 5 ESBL positive isolates were from males and 2 (40.00%) were healthy females.

**Table 14:** Gender wise distribution of ESBL positive isolates

| **Gender** | **Test group (%)** | **Control group (%)** |
|---|---|---|
| Male | 33 (57.80) | 3 (60.00) |
| Female | 24 (42.10) | 2 (40.00) |
| **TOTAL** | **57 (100.0 )** | **5 (100)** |

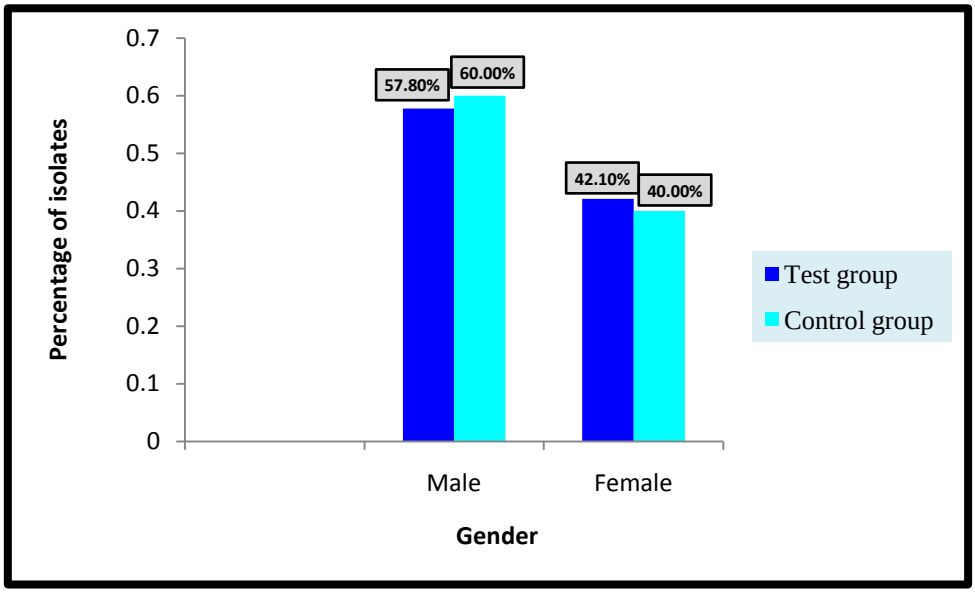


**Chart 9:** Gender wise distribution of ESBL positive isolates

## 5.10 Antibiogram of ESBL positive isolates

It was found that ESBL positive isolates have shown minimum susceptibility to the third generation cephalosporins. No ESBL positive isolate was sensitive to Cefoperazone. The ESBL positive isolates were resistant to most of the group of antibiotics such as Cefoxitin (80.70%), Ofloxacin (77.19%), Cefepime (68.42%), Cotrimoxazole (61.40%), and Doxycycline (59.64%).

**Table 15:** Antimicrobial susceptibility pattern of ESBL positive isolates

| **Antimicrobial agent** | **Number of sensitive ESBL positive *E.coli* isolates (N=62)** | **Percentage of sensitive ESBL positive *E.coli* isolates (%)** | **Number of resistant *E.coli* isolates (N=62)** | **Percentage of resistant ESBL positive *E.coli* isolates (%)** |
|---|---|---|---|---|
| Cefoperazone | 3 | 4.84. | 59 | 95.16 |
| Ceftazidime | 4 | 6.45 | 58 | 93.54 |
| Ceftriaxone | 3 | 4.84 | 59 | 95.16 |
| Cefpodoxime | 0 | 0.00 | 62 | 100.0 |
| Ceftazidime + Clavulanic acid | 58 | 93.54 | 4 | 6.45 |

| | | | | |
|---|---|---|---|---|
| Piperacilin +Tazobactum | 60 | 96.77 | 2 | 3.33 |
| Cefepime | 43 | 69.35 | 19 | 30.64 |
| Cefoxitin | 36 | 58.06 | 26 | 41.93 |
| Ofloxacin | 12 | 19.35 | 50 | 80.64 |
| Gentamycin | 16 | 25.81 | 46 | 74.19 |
| Cotrimoxazole | 07 | 11.29 | 55 | 88.70 |
| Doxycycline | 13 | 20.97 | 49 | 79.03 |

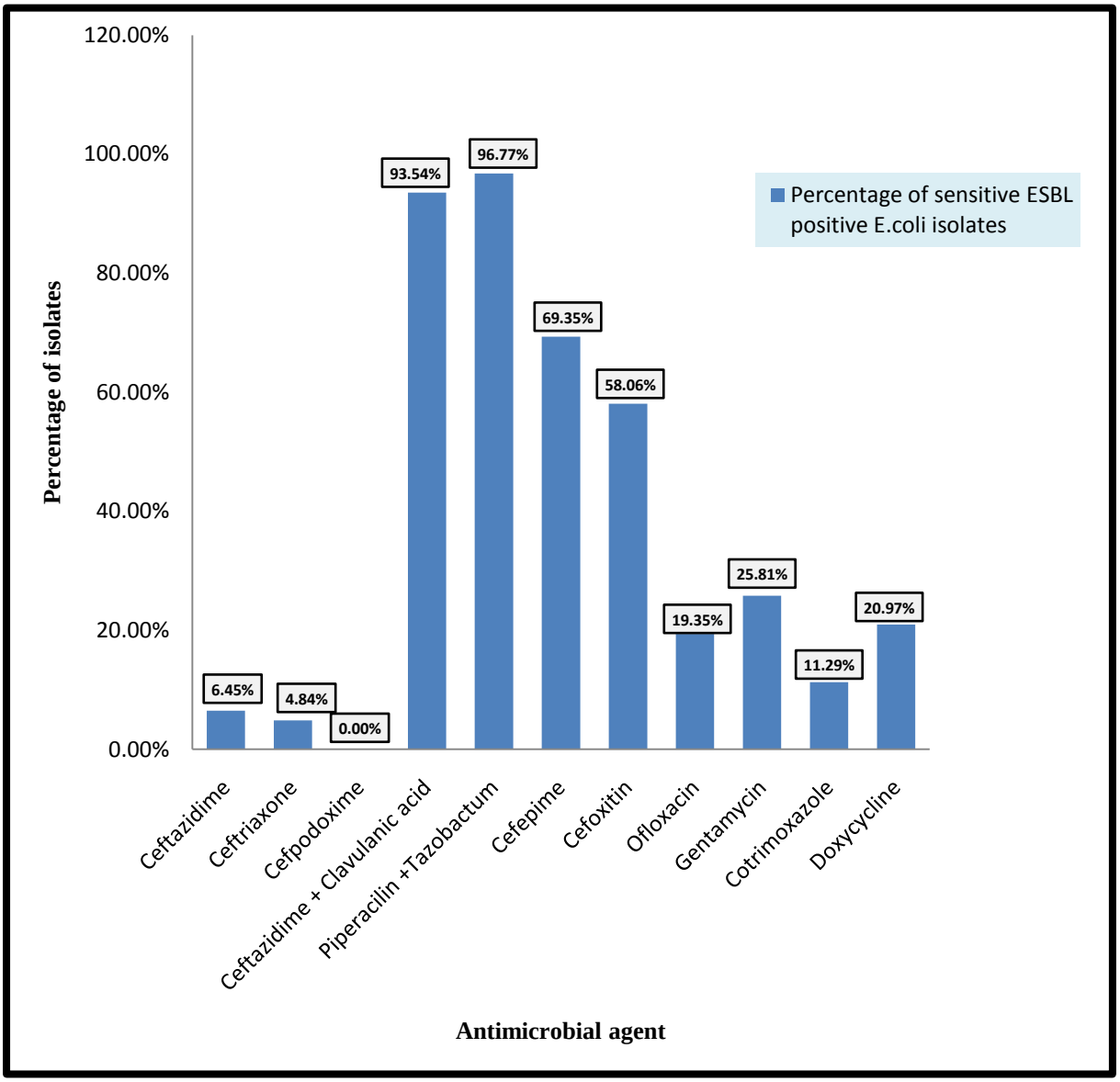


**Chart 10:** Antibiogram of ESBL positive test group isolates (N=62)

## 5.11 Antibiogram of ESBL negative isolates

Out of fifty eight ESBL negative *E.coli* isolates the combination drugs showed maximum sensitivity (100%) to ESBL negative *E.coli* isolates. The third generation cephalosporins also showed sensitivity to more than 90% of the isolates. The ESBL negative isolates did not show a significant resistant to other class of antimicrobials such as Ofloxacin (17.24%), Gentamicin (25.86%), Cotrimoxazole (29.31%).

**Table 16:** Antimicrobial susceptibility pattern of ESBL negative *E.coli* isolates

| **Antimicrobial agent** | **Number of sensitive ESBL negative *E.coli* isolates (N=58)** | **Percentage of sensitive ESBL negative *E.coli* isolates (%)** | **Number of resistant ESBL negative *E.coli* isolates (N=58)** | **Percentage of resistant ESBL negative *E.coli* isolates (%)** |
|---|---|---|---|---|
| Cefoperazone | 55 | 94.83 | 3 | 5.17 |
| Ceftazidime | 56 | 96.55 | 2 | 3.44 |
| Ceftriaxone | 55 | 94.83 | 3 | 5.17 |
| Cefpodoxime | 57 | 98.27 | 1 | 1.72 |
| Ceftazidime + Clavulanic acid | 58 | 100.0 | 0 | 0.00 |
| Piperacilin +Tazobactum | 58 | 100.0 | 0 | 0.00 |
| Cefepime | 57 | 98.27 | 1 | 1.72 |
| Cefoxitin | 53 | 91.38 | 5 | 8.62 |
| Ofloxacin | 48 | 82.76 | 10 | 17.24 |
| Gentamycin | 43 | 74.13 | 15 | 25.86 |
| Cotrimoxazole | 41 | 70.69 | 17 | 29.31 |
| Doxycycline | 51 | 87.93 | 7 | 12.06 |

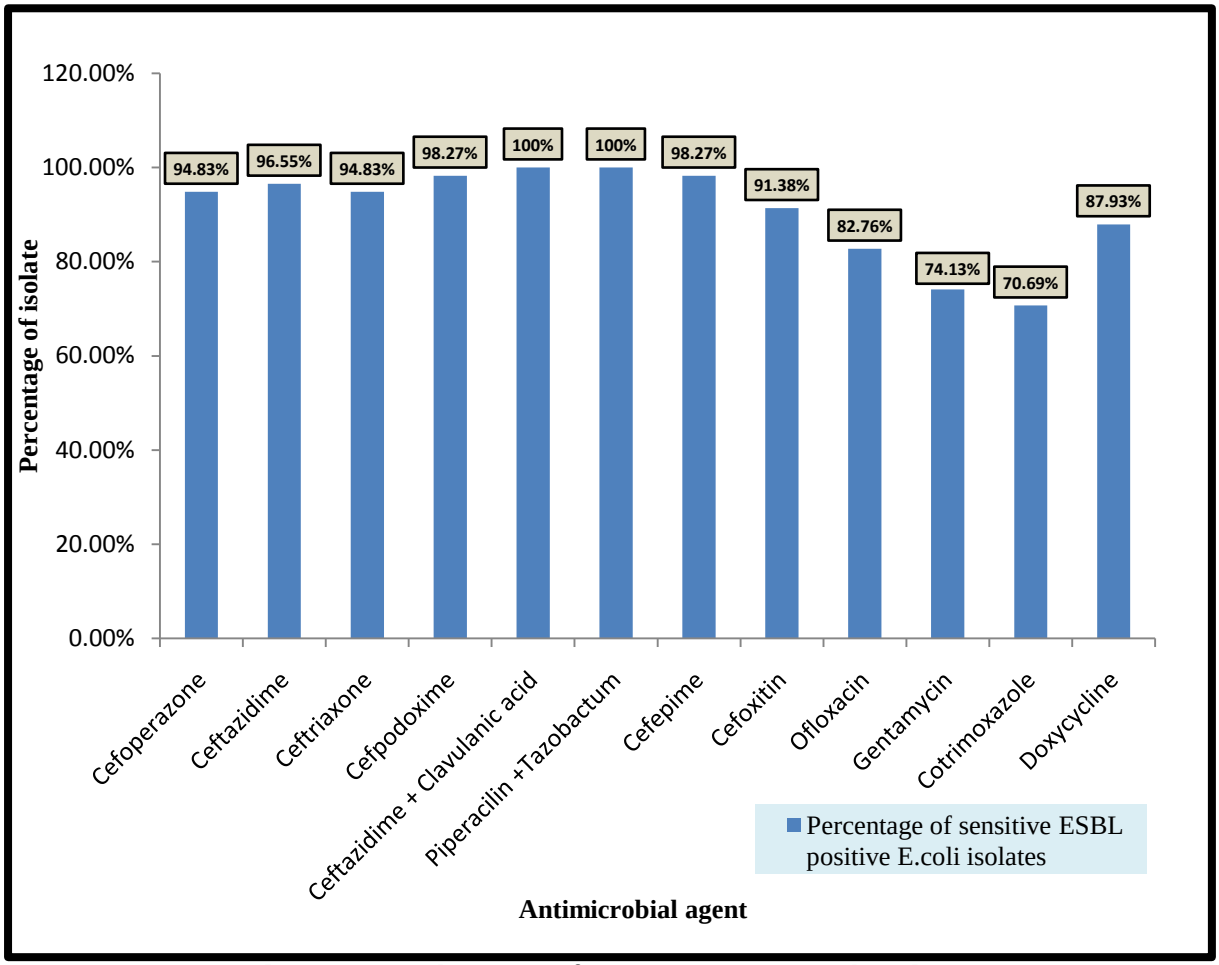


**Chart 11:** Antibiogram of ESBL negative *E.coli* isolates

## 5.12 Comparison of antibiogram of ESBL positive isolates and ESBL negative isolates

A comparative analysis of the antimicrobial susceptibility of ESBL positive *E.coli* isolates and ESBL negative *E.coli* isolates showed that ESBL negative isolates were sensitive to most of the antimicrobial agent used whereas the ESBL positive isolates were resistant to third generation cephalosporins and other class of antimicrobial agents such as Ofloxacin, Gentamicin and Cotrimoxazole.

**Table 17:** Comparative analysis of antibiogram of ESBL positive and ESBL negative *E.coli* isolates

| **Antimicrobial agent** | **Percentage of sensitive ESBL positive *E.coli* isolates (%)** | **Percentage of sensitive ESBL negative *E.coli* isolates (%)** |
|---|---|---|
| Cefoperazone | 4.84 | 94.83 |
| Ceftazidime | 6.45 | 96.55 |
| Ceftriaxone | 4.84 | 94.83 |
| Cefpodoxime | 0 | 98.27 |
| Ceftazidime + Clavulanic acid | 93.54 | 100 |
| Piperacilin +Tazobactum | 96.77 | 100 |
| Cefepime | 69.35 | 98.27 |
| Cefoxitin | 58.06 | 91.38 |
| Ofloxacin | 19.35 | 82.76 |
| Gentamycin | 25.81 | 74.13 |
| Cotrimoxazole | 11.29 | 70.69 |
| Doxycycline | 20.97 | 87.93 |

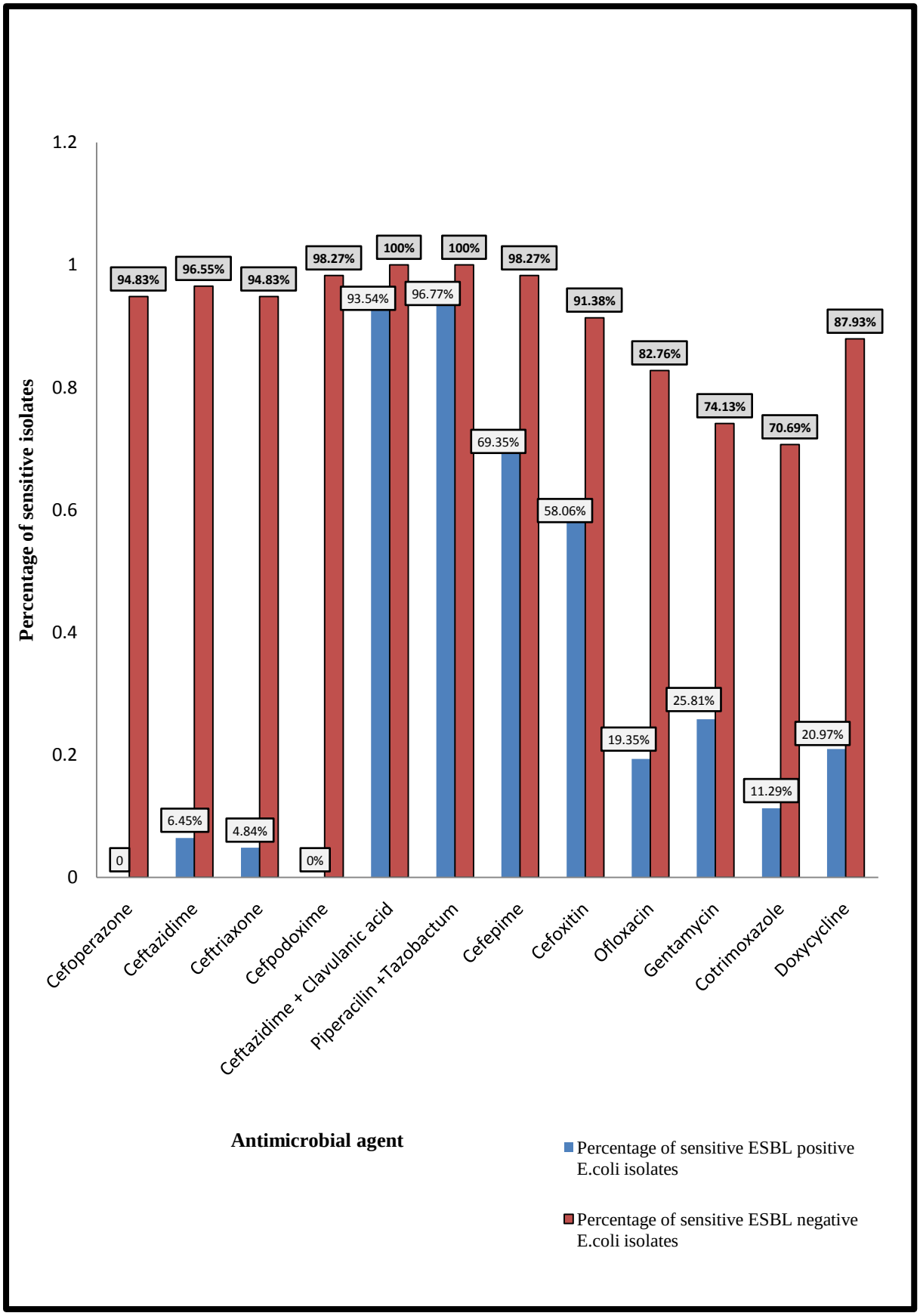


**Chart 12:** Comparison of antibiogram of ESBL positive and ESBL negative *E.coli*

# CHAPTER 6: DISCUSSION

The continued evolution of antimicrobial resistance in the hospital threatens to seriously compromise the ability of health care workers to treat serious infections. Gram negative bacteria, in particular *Enterobacteriaceae*, have acquired or selected many genes of resistance in the past two decades and are often resistant to third-generation cephalosporins, since they carry extended-spectrum beta-lactamases. ESBL producing organisms are presently one of the major causes of hospital-acquired infections. The gut plays a prominent role in the development of antibiotic resistance, allowing the hidden selection and multiplication of resistant micro-organisms in the hospitals, long-term health care facilities, and community (27). The emergence of resistant micro-organisms in the gut may be related to the ingestion of highly pathogenic microorganisms or to antibiotic-induced alterations in the gut microbiome (64).

*E.coli* is a predominant bacterial normal flora of the gastrointestinal tract and various studies from India have quoted high prevalence of ESBLs among *E.coli* (18, 65). The prevalence of ESBL-producing Gram negative isolates in India is alarmingly high ranging from 19–69 % (66-68). In various studies conducted across India the prevalence rate of ESBL-producing *E.coli* was found to be 41–62 % (69-71).

Detection of organisms harbouring ESBLs provides clinicians with helpful information. Treatment of infections caused by ESBL-producing organisms with extended-spectrum cephalosporins or aztreonam may result in treatment failure even when the causative organisms appear to be susceptible to these antimicrobial agents by routine susceptibility testing (5, 12).In addition, patients colonized or infected with ESBL-producing organisms should be placed under contact precautions to avoid hospital transmission (72).

The present study was undertaken to know the prevalence of faecal carriage of ESBL producing *E. coli* in patients admitted in hospital and in healthy individuals. Our study included 120 *E.coli* isolates from faecal samples of 100 hospitalized patients (test group) and 20 randomly selected healthy individuals (control group). Of the 100 patients studied in test group maximum 28 were in age group 0-10 years followed by 22 in the age group 11-20 years. In the age group 51–60 years, there were 15 patients; 11 in 21 – 30 years and 9 in 31 - 40 years group. There were only 7 patients in age group 41- 50 years. In the control group out of 20 individuals, maximum 5 (25%) were in age group 0 – 10 years and only 2 each in age groups 31-40 years and 51 – 60 years (Table 6) (Chart 1).

As per the gender distribution in the present study, In the test group 58 out of 100 patients were male and 42 were females while in the control group, 12 individuals out of twenty (60%) were males and 8 out of 20 (40%) were females (Table 7) (Chart 2).

Ward distribution of the samples showed that maximum numbers of the samples that are 52 out of 100 were received from patients admitted in various medical wards of the hospital. These included medicine, paediatrics and psychiatry wards. Of the 100 samples 30 were of ICU patients and 18 samples were from surgical departments which included Surgery, Obstetrics and Gynaecology and Orthopaedics departments (Table 8) (Chart 3).

Review of literature shows considerable variation in the prevalence of ESBL producing *E.coli* worldwide. The prevalence of ESBL among various clinical isolates varies greatly in different geographical areas and is rapidly changing over time. In the West, ESBL production in *Enterobacteriaceae* varies from 5% to 52% and in other Asian countries from 10% to 46.5% (5, 74). Other studies from India have reported a high prevalence of ESBL production ranging from 41.0 to 63.6% in *E. coli* and 40 to 83.3% in *K. pneumoniae* (74-76).

In the present study overall prevalence rate of ESBL producing *E.coli* isolated from the faecal samples was found to be 51.67% (62/ 120). In the hospitalized patients ESBL positive *E.coli* faecal carriage prevalence rate was 57% (57/100) while in the healthy individuals it was 25% (05/20) (Table 11) (Chart 6). Our findings of overall high prevalence of ESBL are in coherence with observations of other studies from India (74-76).

High prevalence rate of 57% in hospitalized and 25% in healthy individuals as compared to studies from other parts of the world as well as India explain the geographical variation in the prevalence of the isolate. Low prevalence rates of 6%, 6.3%, 5.8% and 6.4% were reported in independent studies from France, Germany, Switzerland and Japan respectively (17,77-79).High prevalence rates of faecal carriage of ESBL producing *E.coli* were reported by different authors from different geographical locations; 50% from China (17), 63.3% Egypt (80) and 65.7% from Thailand (78).It also varies as per the antibiotic prescribing practices of the area. Easy availability of antibiotics over the counter might be the contributing factor in high incidence of ESBL positive commensal *E. coli* in our area of Northern India.

Age related prevalence of ESBL producing *E.coli* indicated maximum incidence of 36.80% in the age group 0-10 years followed by 24.56% in 11–20 years age group. Lowest prevalence was observed in age group 41–50 years (Table 12) (Chart 7). Observations of many studies showed that age does not affect the prevalence rate of faecal ESBL producing *E.coli*(25, 26).Though few studies have indicated more prevalence in adults as compared to children (81, 48) and on the contrary another study quoted higher rate in children[82]. In our study children were more prone to harbour ESBL positive commensal *E coli.*

Most of the strains isolated from samples from the department of Medical were positive for ESBL producing *E.coli* (59.64%) followed by a carriage of 29.82% of ESBL producing *E.coli* isolates from Intensive care unit. The isolates obtained from the Department of surgery shows 10.52% of ESBL producing *E.coli* isolates (Table 13) (Chart8).

It was also observed in the present study that more number of male patients, 33/57 (57.80%) were carrying ESBL producing *E.coli* in their gut as compared to the females, 24/57 (42.10%) (Table 9) (Chart9). Most of the studies done on risk factors for ESBL producing *E.coli* colonization have documented no significant correlation between the gender and the prevalence of ESBL producing *E.coli* (83).

Comparative analysis of susceptibility pattern of ESBL positive isolates and ESBL negative isolates showed significant difference in the susceptibility towards various antimicrobial groups. ESBL negative strains were more sensitive to most of the antibiotics while resistance to various groups of antibiotics was more pronounced in ESBL producing *E.coli* (Table12) (Chart12).

ESBL production coexists with resistance to several other antibiotics. ESBLs are encoded by plasmids, which also carry resistant genes for other antibiotics. Co-resistance to quinolones and aminoglycosides is common (84). In our study co resistance was found with Co-Trimoxazole (61.40%), Gentamicin (33.33%) and Fluroquinolones (45.62%) (Table10) (Chart10). Varsha et al. reported 91.17%, 100% and 94.91% resistance respectively to Gentamicin, Cotrimaxozole and Ciprofloxacin in ESBL producers (85).

Various studies have shown the coresistance with these groups of antibiotics as mentioned below:

**Table 18:** Coresistance in ESBL positive isolates

| **S.No.** | **Various studies** | **Coresistance with other antimicrobial agent** | | |
|---|---|---|---|---|
| | | **Cotrimaxozole** | **Gentamicin** | **Fluroquinolones** |
| 1 | Miranda et al. (86) | 70.50 % | 33.00% | 50.90% |
| 2. | Reuland et al. (45) | 70.00% | 29.00% | NA |
| 3. | Andriatahina*et al.*(54) | 93.30% | 73.30% | 86.70% |
| 4. | Valverde et al. (87) | 50.00% | NA | 53.50% |
| 5. | Isendahl et al. (88) | 94.00% | 43.40% | 81.90% |
| 6. | Blom et al. (48) | 77.00% | 24.00% | NA |
| 7. | Valenza et al (79) | 62.10% | 28.90% | 51.60% |
| 8. | Fernández-Reyes et al. (82) | 41.20% | NA | 64.70% |
| 9. | **Present study** | 61.40% | 33.33% | 45.62% |

High resistance to non β-lactam antibiotics by ESBL producing strains poses a threat of treatment failure by these drugs and also minimizes the therapeutic choice to carbapenems and hence the emerging resistance to carbapenems is a phenomenon of great concern to combat infections by multidrug resistant bacteria (2). Although beta lactam/ beta lactamase inhibitors combinations have been suggested as treatment option for ESBL producers, these drugs must be given in high doses (89).

Amongst the ESBL producers susceptibility to third generation cephalosporins revealed Ceftazidime and Cefoperazone (97.82 percent) to be more sensitive in screening ESBL production versus Ceftriaxone (94.73 percent) and Cefotaxime (94.73 percent) (Table 15) (Chart 10). Confirmation of ESBL production was done using combined disc method. All the screening test positive strains for ESBL were positive with the combined disc method also. It further stressed on the documented fact that usage of more than one antibiotic of 3$^{rd}$ generation cephalosporin increases the sensitivity of screening method (13).

It is apparent from our findings that faecal carriage of ESBL producing *E.coli* is high in our hospitalized patients. As normal microflora of the gut, these organisms could be a threat to the patients who have been colonised, and to the patients who are in the vicinity. This could also pose a challenge to hospital infection control practices. These results should be taken in consideration when treating these groups of patients if they develop severe sepsis or septic shock.

# CHAPTER 7: CONCLUSIONS

Colonization with multi drug resistant isolates, including ESBL producing isolates, is one of the significant risk factor for infection. Therefore importance of detection of carriers of antimicrobial resistant bacteria in hospitalized patients as well as in community is of utmost value. Our data suggests alarmingly high prevalence of faecal carriage of ESBL producing *E.coli* in indoor patients. Antibiotic selection pressure in hospital may be the contributing factor for the presence of large number of carriers harbouring resistant bacteria. By minimizing selective pressure through more judicious use of antibiotics, we may well be able to maintain antimicrobial susceptibility patterns at a level we can tackle with.

It was observed that healthy individuals also carried the commensal ESBL producing *E.coli* in their gut in a high percentage. The occurrence of these ESBL positive *E.coli* strains as colonizers in the community indicates a reservoir outside the hospitals that should be taken seriously regarding implementation of screening and hygiene precautions for prevention of infections with these drug resistant bacteria.

Therefore, it is prudent to suggest that as a routine, hospitalized patients especially admitted to critical case areas such as ICUs, may be screened for colonisation with ESBL producing organisms. Strict adherence to patient hygiene and infection control practices may be enforced to curtail hospital acquired infections. Rational use of antibiotics would substantially decrease pressure on the gut microflora and thereby limit acquisition of resistant genes among these microorganisms. There is a need to have stringent local and national research and surveillance efforts to monitor resistance pattern of commensal *E.coli.*

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# CHAPTER 9: APPENDICES

## 9.1 APPENDIX 1: LIST OF ABBREVIATIONS

| | |
|---|---|
| $^0$C: | degree Celsius |
| %: | Percentage |
| ATCC: | American type culture collection |
| CAI: | Community acquired infections |
| CLSI: | Clinical and laboratory standard institute |
| CTX-M: | Cefotaximase Munich |
| *E.coli* | *Escherichia coli* |
| ESBL: | Extended spectrum beta lactamase |
| GIT: | Gastrointestinal tract |
| GNB: | Gram negative bacteria |
| HAI: | Hospital acquired infections |
| $H_2O_2$: | Hydrogen peroxide |
| KPC: | *Klebsiella pneumonia* carbapenamase |
| MBL: | Metallo beta lactamases |
| mcg: | micrograms |
| MDR-TB: | Multi drug resistant *Mycobacterium tuberculosis* |
| ml: | millilitres |
| mm: | millimetres |
| N: | Number |
| OXA: | Oxacillinase-type beta-lactamase |

| | |
|---|---|
| psi: | per square inch |
| SHV: | Sulfhydril variable, a type of beta-lactamase |
| spp. | Species |
| TEM: | Temoneira, a type of beta-lactamase named after the first patient |
| UK: | United Kingdom |
| US: | United States |
| UTI: | Urinary tract infections |
| VRE: | Vancomycin resistant *Enterococcus* |