"PHENOTYPIC DETECTION OF EXTENDED SPECTRUM β-LACTAMASE, METALLO β- LACTAMASE, AmpC β-LACTAMASE AMONG ESCHERICHIA COLI AT TERTIARY CARE HOSPITAL"

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LIST OF ABBREVIATIONS USE

1) E. coli -Escherichia coli
2) ESBL- Extended spectrum beta- lactamases
3) AmpC-class C β-lactamases
4) MBL- Metallo beta- lactamases
5) TEM- a β-lactamase enzyme named after a Greek patient emoneira
6) SHV-Sulf-hydryl variable
7) CTX-M-Cefotaxim-munich
8) OXA-Oxacillinases
9) Viz-Vedelicet
10) GIM- German imipenemase
11) IMP- Imipenemases
12) SIM- Seoul imipenemase
13) VIM - Verona integron-encoded metallo beta-lactamases
14) NDM- New Delhi-metallo beta-lactamases
15) UPEC- Uropathogenic E coli
16) DECP- Diarrheagenic E coli
17) MAEC-Meninitgis causing E coli
18) ETEC- Enterotoxigenic E. coli
19) EIEC- enteroinvasive E. coli
20) EHEC- enterohemorrhagic E. coli
21) EPEC- enteropathogenic E.coli
22) EAggEC- enteroaggregative E. coli
23) DAEC- diffusely adherent E. coli
24) LT- Heat-labile toxin

25) ST- heat-stable toxin

26) ETEC- by enterotoxigenic E. coli

27) STEC- Shiga toxin-producing E. Coli

28) HUS-Hemolytic uremic syndrome

29) CDT- Cytolethal Distending Toxin

30) T3SS- Type III Secretion System

31) ICU-Intensive care unit

32) CRE- carbapenem-resistant Enterobacteriaceae

33) TLA-Total laboratory automation

34) MIC-Minimum inhibitory concentration

35) CLSI- Clinical Laboratory Standard Institute

36) MRSA-Methicillin-resistant staphylococcus aureus

37) µg-Microgram

38) ml-mililitre

39) MHA-Muller hington agar

40) EDTA- ethylenediaminetetraacetic acid

41) E-test- Epsilometer Test

42) Bla- β-Lactamase gene

43) U/Kg- unit/kilogram

44) DDS-double disc synergy

45) SFO-Subfornical organ

46) BES-Bioelectrochemical s

ABSTRACT

Background: Production of β-lactamase enzymes by Gram-negative bacteria (E coli) is the most common mechanism to acquire drug resistance to β-lactam antibiotics. Limitations in detecting extended spectrum β-lactamases (ESBL) and Amp-C β-lactamases Metallo β-lactamases have contributed to the uncontrolled spread of bacterial resistance and are of significant clinical concern.

Objective : To detect ESBL, AmpC AND MBL among E coli isolates

Materials and Methods: A total of 104 isolates (E coli) were selected for detection of ESBL, AmpC and MBL producers These isolates were phenotypically screened and confirmed by confirmatory test by using the Kirby Bauer disk diffusion method.

Result: Among 104 isolates (80.8%) ESBL (cefotaxime) producers, (12.5%) Ampc (cefoxitin) producers, and (76.0%) were MBL producers , colistin showed (100%) sensitive followed by MEM(75%), IPM(74%), AN(65.4%),ETP(62.5%), AMC (60.5%), SFP (53.8%), TZP(48.1%), TIC (48.1%), FOS (47.1%), CAZ (47.1%), TGC (46.2%), FT(36.4%), CRO (31.7%), NOR (30.8%), FOX (27.9%), OFL (24%), CIP (22.1%), NA (20.2%), GM (19.2%), FEP (16.3%), CFM (15.4%), AM (13.5%), CF (7.7%), CXM (1%).towards E coli

Conclusion : The present study highlights the necessity to identify the MDR β-lactamases stains for effective therapy in severe as well as mild bacterial infections, thereby enabling to reduce the risk of MDR in Tertiary care hospital and community settings. Further, similar studies in specific geographical regions may be encouraged to have a brief idea of organism-based antibiotic susceptibility patterns and β-lactamase production for effective management and treatment

regimes Hence Early detection of β- lactamases among E coli avoid treatment failure and spread of MDR

Keywords: E coli, ESBL, AmpC, MBL

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INTRODUCTION

1. INTRODUCTION

Escherichia coli

Escherichia coli (E. coli) is a member of the Enterobacteriaceae family, a facultative pathogen residing as the most common facultative anaerobic organism in the intestine of humans. E. coli is part of their normal gut flora, usually present as commensals. While E. coli is naturally present in the human gut, particular strains transmitted through food can result in severe gastrointestinal tract infections.(1)

Pathogenic E. coli infections can spread throughout the body or affect the surfaces of the mucous membranes. The infectious nature of these organisms is caused by different virulence characteristics, including their capacity to create poisons such as enterotoxins and their ability to attach, multiply, and colonize the small intestine. The primary clinical symptoms of an E. coli infection include urinary tract infections, enteric or diarrheal illnesses, sepsis or meningitis, pyelonephritis, cystitis, and asymptomatic bacteriuria. It is one of the most frequently isolated organisms from blood. Neonatal meningitis and wound infections, including necrotizing fasciitis, are serious, although more uncommon, infections caused by E. coli. It is one of the leading causes of nosocomial Infections like urinary tract infections, Endocarditis, Central Nervous System Infections, Gastrointestinal Infections, Pneumonia, Neonatal Infections, Soft Tissue Infections etc., and community-acquired infections in humans.(2)

The lactam family of antibiotics has been used more frequently to treat E. coli infections, and antibiotic-resistant strains have started to appear. The emergence of producers of ESBL, MBL, and AmpC poses a severe clinical crisis.(3)

ESBL:

Extended-spectrum-lactamase enzymes are plasmid-borne enzymes with hydrolytic activity against monobactams and expanded-spectrum cephalosporins, such as ceftazidime and cefotaxime, but not against cephamycins and carbapenems. However, lactamase inhibitors prevent ESBL activity (e.g., clavulanic acid). These enzymes are the result of TEM-1, TEM-2, and SHV-1 mutations. The Bush-Jacoby-Medeiros classification system places ESBLs in group 2be, which indicates that they are descended from group 2b-lactamases (such as TEM-1, TEM-2 and SHV-1); This shows that lactamases have an extended spectrum. The extended-spectrum lactam antibiotics can hydrolyze extended-spectrum lactam antibiotics can be hydrolyzed by ESBLs because of a variety of mutations that they have. TEM, SHV- type ESBLs can still hydrolyzed penicillins, but they are not as effective as the parent enzymes were. The increased activity against extended-spectrum cephalosporins caused by the active site expansion may also make ESBLs more susceptible to -lactamase inhibitors. ESBL-producing organisms are of great clinical importance as they have been associated with various types of infections viz: Bacteremia, intra-abdominal infection, urinary tract infections particularly in the (community setting), and respiratory tract infections. They are readily inactive diseases. ESBL-producing organisms are known to acquire co-resistance to other antibiotics, such as tetracyclines, co-trimoxazole, trimethoprim, aminoglycosides, and quinolones, further limiting therapeutic options.

AmpC:

The resistance that AmpC β-lactamases provide to cephalosporin in the oxyiminogroup (cefotaxime, ceftazidime, ceftriaxone), 7-α-methoxy-cephalosporin (cefoxitin or cefotetan), and monobactam makes them clinically relevant. Most importantly, they are not inhibited by β-lactamase inhibitors. Since these enzymes are usually linked to several antibiotic resistances, few treatment alternatives are available. These enzymes may also provide carbapenem resistance in a strain with reduced outer membrane permeability. Many species of the Enterobacteriaceae

family, such as Enterobacter, Shigella, Providencia, Citrobacter freundii, Morganella morganii, Serratia marsescens, and Escherichia coli, have genes for AmpC β-lactamases on their chromosomes.The origin of plasmid-mediated AmpC β-lactamases can be traced back to the chromosomal transfer of the inducible AmpC β-lactamase genes. Following the transfer, isolates of Salmonella species, Escherichia coli, Klebsiella pneumoniae, Citrobacter freundii, Enterobacter aerogenes, and Proteus mirabilis were shown to have plasmid-mediated AmpC β-lactamases. (5)

When treating severe infections brought on by multiresistant Enterobacteriaceae—especially those that produce Amp C beta-lactamase and extended-spectrum beta-lactamase (ESBL)—carbapenems are the most effective therapeutic option. Because there is currently a limited selection of antibiotics available to treat infections caused by these bacteria, the rise of carbapenem-resistant bacteria has raised serious concerns.(6)

The primary cause of carbapenem resistance in gram-negative bacteria is the synthesis of carbapenemases, which hydrolyze carbapenem. Another significant factor contributing to carbapenem-resistant Enterobacteriaceae is the overexpression of the enzyme AmpC beta-lactamase in a porin-losing bacterium.(7)

MBL:

Over the past few decades, a steady number of acquired carbapenemases have been reported, which fall into three of the four recognized kinds of beta-lactamases: either Ambler molecular groups A and D (serine carbapenemases) or Ambler molecular class B (metallo beta-lactamases).(8)

Class B enzymes include carbapenemases, which have therapeutic significance. The metallo beta-lactamases (MBL) include the Imipenemases (IMP) family of carbapenemases, the Seoul

imipenemase (SIM), the German imipenemase (GIM), the Verona integron–encoded metallo beta-lactamases (VIM), and the New Delhi-metallo beta-lactamases (NDM) enzymes. These enzymes are classified as class B enzymes. There have been reports of metallo beta-lactamase enzymes belonging to the IMP or VIM class worldwide. (9)

MBL confers resistance to all beta-lactam antibiotics except monobactams. These zinc-dependent beta-lactam hydrolyzing enzymes are characterized by resistance to beta-lactamase inhibitors like clavulanic acid, sulbactam, and tazobactam. They are distinct from other beta-lactamases in that they do not compete with penicillin-binding proteins for their mode of action. MBL enzymes, whose genes can be chromosome- or plasmid-borne, are often situated in integrons and pose a serious risk of substantial dissemination among the gram-negative fraternity. (10) High morbidity and mortality are associated with invasive infections caused by MBL-producing gram-negative isolates. The emergence of metallo beta-lactamase (MBL)-producing gram-negative bacilli poses a therapeutic challenge and is a severe concern for infection control in a hospital environment. In our healthcare setting, carbapenem-resistant Enterobacteriaceae strains were increasingly being isolated, and hence, this study was taken to determine the prevalence of MBL among Enterobacteriaceae.(11)

So, this study is to evaluate the presence of the Extended Spectrum beta-lactamase, AmpC beta-lactamase, and Metallo beta-lactamase-producing strains among clinical isolates of Escherichia Coli.

2. AIMS & OBJECTIVES OF THE STUDY

AIMS: To evaluate the presence of the Extended Spectrum β- Lactamase, AmpC β- Lactamase, and Metallo β- Lactamase producing strains among clinical isolates of Escherichia coli.

OBJECTIVES:

1) Isolation and Identification of E coli from all clinical samples of patients in tertiary care hospitals.
2) To study the multi-drug resistance pattern of the identified E coli isolates.
3) To detect ESBLs among E coli isolates.
4) To detect AmpC beta- lactamase among E coli isolates.
5) To detect MBLs among E coli isolates.

2. Escherichia coli :

The versatile microbe Escherichia coli (E. coli) belongs to the genus Escherichia and family Enterobacteriaceae. Theodore Escherichia, a German pediatrician and bacteriologist, initially identified the bacteria in 1885 and named it Bacterium coli commune. It was later given the name Escherichia coli.(12) E. coli is the most common facultative anaerobe In human intestines. It is also a vital component of the intestinal flora that keeps the physiology of a healthy host alive. E. coli is a type of bacteria that is part of the typical gut flora found in the lower intestines of humans. When found in the large intestine, it helps with food absorption, waste processing, and vitamin K creation. It is a regular resident of the human digestive system, which is frequently kept innocuously small.(13) Most E. coli strains are not considered pathogens; however, when gastrointestinal barriers are breached, even “non-pathogenic” strains of E. coli can cause illness. These strains can also act as opportunistic pathogens in immunocompromised hosts. (14)

E. coli infections can spread throughout the body or affect the mucosal surfaces.(16) However, dangerous strains of E. coli exist, and among the isolated bacterial pathogens, the most common pathogenic E. coli illnesses can spread throughout the body or affect the mucosal surface. E. coli is highly well-adapted to induce a variety of human diseases.(15) Additionally, it is one of the main reasons why people with underlying illnesses get dangerous infections. A human bacterial pathogen such as E. coli expresses several virulence factors that aid in the initial colonization process, avoid the host’s immune system, adapt the bacterial metabolism and catabolism to the new environment, and extract vital nutrients like iron needed for pathogenesis. Pathogenic Most E. coli is entirely safe and frequently seen in human lower intestines. Because they transfer from the skin, an average pair of underwear has 370,000 E. coli bacteria. The bacteria were considered solely commensals, or naturally occurring, benign organisms of the large intestine

until the 1940s, when they were linked to a baby diarrhoea outbreak. (17) According to estimates, E. coli comprises about 1% of the microbial biomass in faeces. Since E. coli is usually present in animals, it is unsurprising that these microorganisms can enter the food chain and end up in consumer goods.(18)

PATHOGENESIS

In the intestines and other body areas where there is some abnormality or weakness of defense, the intestinal commensal strains of Escherichia coli frequently cause opportunistic infections. Infection with intrinsically pathogenic strains of Escherichia coli leads to three common clinical syndromes: (i) urinary tract infection, (ii) enteric/diarrheal illness, and (iii) sepsis/meningitis.(21)

Uropathogenic E coli (UPEC)

One of the frequent Enterobacteriaceae family members that causes urinary tract infections is E. coli. Uropathogenic E. coli is the causative agent of 70% to 95% of community-acquired and 50% of nosocomial urinary tract infections, according to studies (UPEC). E. Coli is the cause of 80% to 90% of UTIs in children. The majority of E. coli isolates (31% of all isolates) in the research study conducted in our country came from urine from extraintestinal sources, and the infection was more common in women (41/72). The urinary tract is where microorganisms from the perineal area or excrement rise to reach the bladder. Due to the shorter urethra in females, bladder infections are fourteen times more likely than in males. The typical clinical manifestations of pyelonephritis, cystitis, and asymptomatic bacteriuria.(22)

Diarrheagenic E coli (DECPs)

The most well-known feature of E. coli as a pathogen is its capacity to cause intestinal illnesses. Globally, E. Coli is a leading cause of gastroenteritis. E. Coli can seriously infect people and various animals, including dogs, horses, and sheep. Six groups of E. coli cause diarrheal disorders; these strains are known to be essential sources of gastrointestinal diseases, ranging from mild diarrhea to situations resembling dysentery. Some strains are known to be human

pathogens. Every class has unique pathophysiological characteristics and belongs to a certain serological subgroup. Enterotoxigenic (ETEC), enteroinvasive (EIEC), enterohemorrhagic (EHEC), enteropathogenic (EPEC), enteroaggregative (EAggEC), and diffusely adherent (DAEC) E. coli are among the several diarrheagenic serotypes of the bacteria that have been identified. Several of the primary enterotoxigenic E. coli that cause diarrhoea. (23)

Sepsis / Meningitis causing E coli (M A E C)

Additionally, E. coli is a significant cause of nosocomial bacteremia; studies have shown that its incidence rates range from 10% to 20%. It is also the most crucial cause of gram-negative meningitis in neonates and one of the top five causes of nosocomial bloodstream infections. One of the most frequent causes of infections is E. coli, frequently seen in bedsores, cholecystitis, peritonitis, appendix abscesses, and septic wounds. It can cause meningitis in newborns and infect the lower respiratory tract. It can also result in bacteriemia, endotoxic shock, and surgical or other weakened patients receiving antibiotics to which they are resistant.(24)

Treatment of E coli Infection:

Depending on the severity of the infection, antibiotics may be administered parenterally or orally to treat E. coli infections. Antimicrobial susceptibility testing in laboratories is crucial since there is a wide range in the antibacterial activities of sulphonamides, ampicillin, cephalosporin, fluoroquinolones, and aminoglycosides on enteric bacteria. The most popular treatment for E. coli infections is β-lactam antibiotics.(25)

Antibiotics, which are β-lactams, are among the most commonly prescribed drugs globally. Over the last 20 years, resistance to these agents has emerged, leading to a severe clinical crisis. One primary mechanism of β-lactamase resistance to β-lactam antibiotics, especially in gram-negative harmful bacteria, is present.(26)

The last two decades have seen a concerning trend in the resistance of the E. coli and K. pneumoniae species to extended-spectrum cephalosporins, such as cefotaxime, ceftazidime, and

ceftriaxone. Among ESBL manufacturers, resistance to co-trimoxazole, tri-methoprene, and fluoroquinolones is commonly seen. Very few medications are available for treating a patient with an isolate that produces ESBLs. Even though ESBL-producing E. coli will seem responsive to penicillins, cephalosporins, or aztreonam treatment in vitro, these antibiotics may need to be revised in the clinic.(27)

Virulence factors of E coli:

The bacterial species Escherichia coli, or E. coli, is diverse. At the same time, some of its members are harmless and found naturally in the human gut, and others can cause illnesses ranging from minor infections to severe gastroenteritis. Pathogenic E. coli strains' virulence factors are essential to their capacity to spread disease. Below is a summary of some critical virulence factors:

Adhesins: E. coli can attach to host cells due to surface proteins called adhesins, which make proliferation and invasion easier. Adhesins come in two varieties: non-fimbrial adhesins and fimbriae, or pili. P and type 1 fimbria are crucial for attachment to specific receptors on host tissues.

TOXINS:

Enterotoxins: These poisons affect the digestive system and might result in symptoms like vomiting and diarrhoea. Heat-labile toxin (LT) and heat-stable toxin (ST), which are produced by enterotoxigenic E. coli (ETEC), are factors in diarrheal illness.

Shiga Toxins: These toxins, which are produced by E. Coli O157:H7 and other Shiga toxin-producing E. Coli (STEC), destroy the lining of blood vessels and induce haemolytic uremic syndrome (HUS) and bloody diarrhoea.

Cytolethal Distending Toxin (CDT): Certain pathogenic Escherichia coli strains produce CDT, which causes cell cycle arrest and cell enlargement, increasing inflammation and tissue damage.

Capsules: Certain pathogenic strains of Escherichia coli generate capsules that aid in their ability to avoid the host immune response by blocking phagocytosis. These polysaccharide-containing capsules have been linked to an increased risk of infection.

Iron Acquisition Systems: Usually retained by host proteins, iron is generally limited in the host environment and is detrimental to bacterial growth. Iron-regulated outer membrane proteins and siderophores, tiny molecules that chelate iron, are two of the many mechanisms pathogenic E. coli have developed to obtain iron from their hosts.

Type III Secretion System (T3SS): Some pathogenic strains of Escherichia coli use a device called T3SS, which resembles a needle, to inject bacterial effector proteins straight into host cells. The bacteria can penetrate host cells, modify the immune response, and increase their chances of survival and replication by manipulating host cell signaling mechanisms through these effector proteins.

Biofilm Formation: The ability of E. coli to produce biofilms on both biotic and abiotic surfaces increases their resistance to antimicrobial agents and host immune defenses. Bacterial populations organized and covered in an extracellular polymeric matrix are called biofilms

Flagella: E. coli may travel towards favorable conditions and away from hazardous ones because flagella facilitates movement. Motility can help spread across the host and assist in expanding its tissues.

Invasins: Proteins known as invasive sins help pathogenic E. coli invade host cells. They interact with receptors on host cells, setting off signaling cascades that encourage internalization of the bacterium.(28)

By facilitating colonization, host defense evasion, tissue injury, and disease progression, these virulence factors together add to the pathogenicity of E. coli strains. Creating plans to treat and prevent E. coli infections requires understanding these variables

Antimicrobial Agents:

The terms “antimicrobial agent” and “antimicrobial” are mainly used to refer to chemically generated antibacterial medications and antibiotics, which are compounds produced by microorganisms that either kill or inhibit other microorganisms. When applicable, they also include antiviral and antifungal agents. The invention of Salvarsan, a medication made from arsenic, by Paul Ehrlich at the turn of the 20th century marked the beginning of the history of antimicrobial agents. Although penicillin’s effects were discovered by Alexander Fleming in 1928, it wasn’t until the 1940s that the drug could be synthesized with any degree of effectiveness. Until the late 1960s, the development of new antibiotics happened relatively quickly. These included trimethoprim, tetracyclines, βlactams, aminoglycosides, sulphonamides, macrolides, glycopeptides, and quinolones. No new class of antimicrobial medication was introduced between 1968 and 2000. (29)

Despite the large number of drugs, the targets for the antimicrobial substances are surprisingly few. The main targets or basic mechanisms for antimicrobial action on the bacterial cell include interference with cell wall synthesis (e.g., β-lactams), inhibition of protein synthesis (e.g., macrolides), interference with nucleic acid synthesis (e.g., quinolones), inhibition of a metabolic pathway (e.g., trimethoprim-sulfamethoxazole), and disruption of bacterial membrane structure (e.g., polymyxins).

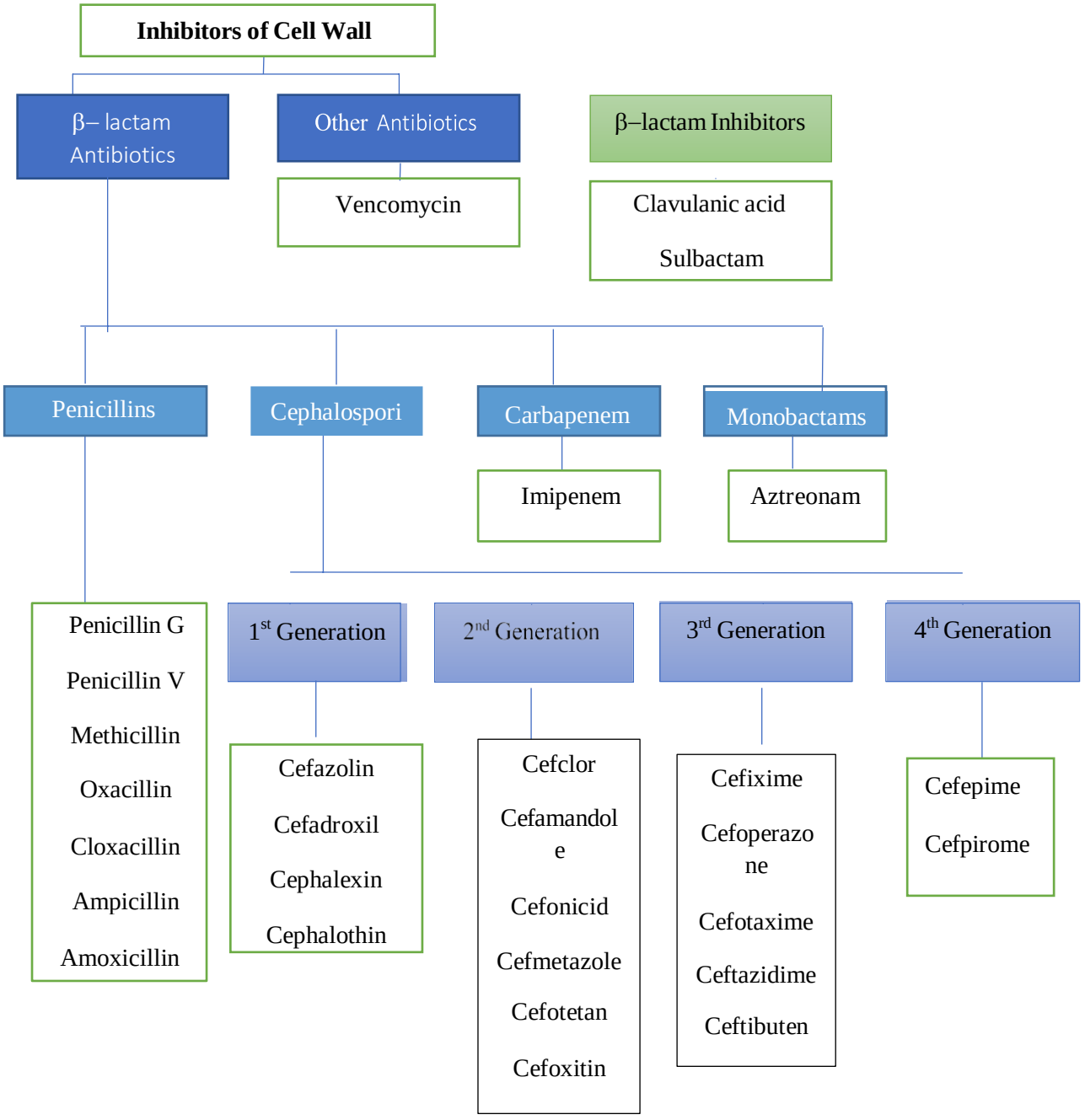

Fig no : 1**Summary of Antimicrobial Agents Affecting Cell Wall Synthesis(30)**

cephalosporins

Belonging to the β-lactam antibiotic class, cephalosporins are broad-spectrum antibiotics. The first cultures of Cephalosporium acremonium were used to isolate cephalosporin compounds. To combat the penicillin resistance that was being seen, synthetic cephalosporins were developed in the early 1960s. Bactericidal in nature, cephalosporins work the same way as other β-lactam antibiotics.

Based on the agent's introduction and activity range, cephalosporins may be categorized into four groups: first, second, third, and fourth generation. Fourth generation β-lactams have good actual broad-spectrum activity against Gram-negatives and Gram-positives. Second-generation cephalosporins have increased Gram-negative and somewhat less Gram-positive activity; third-generation antimicrobials have improved Gram-negative and variable Gram-positive activity; and first-generation agents generally have good Gram-positive activity and relatively modest coverage for Gram-negative organisms. Occasionally, second-generation cephamycins are categorized with cephalosporins. (31)

Beta- lactamases (β-lactamases

Nowadays, bacterial resistance to β-lactam antibiotics has become a widespread issue and is growing at a substantial rate. β-lactam antibiotic-induced antibiotic resistance can arise from many methods. The most prevalent and significant way by which bacteria can develop resistance to β-lactams is by expressing β-lactamases, such as plasmid-mediated AmpC enzymes, extended-spectrum β-lactamases (ESBLs), and carbapenemases that hydrolyze β-lactamases. (32)

The above Figure illustrates the division of the β-lactamase family according to their molecular properties and functioning. Before the standard sequencing of genes, distinct βlactamases' biochemical properties were identified, enabling the family to be divided into four categories

(Bush et al., 1995; Wright, 2005). Members of group 3 are metallo-β-lactamases, whereas those in groups 1, 2, and 4 are serine-β-lactamases. Four main categories, known as the Ambler classes A–D, have also been identified by classification based on molecular features, such as amino acid homology. These groups exhibit a good correlation with the functional scheme but lack information regarding the enzymatic activity. Ambler class B β-lactamases are all metallo-enzymes that need zinc as a cofactor for their catalytic activities, whereas Ambler classes A, C, and D comprise the βlactamases with serine at their active site.

Enzymes are classified into four categories: narrow, moderate, comprehensive, and ESBLs. According to a widely accepted definition, broad spectrum β-lactamases can provide resistance against cephalosporins and penicillins and are not inhibited by tazobactam or clavulanic acid. β-lactamase inhibitors prevent the ESBLs, which impart resistance to penicillins, first-, second-, and third-generation aztreonam, but not carbapenems.(33)

ESBLs:

Origin:

For many years, it was known that a small number of Escherichia coli and Klebsiella pneumoniae strains produced beta-lactamases of the TEM and SHV types, which are non-ESBL. While ceftriaxone, cefotaxime, and ceftazidime were specifically formulated to withstand degradation by these enzymes, they do not hydrolyze ampicillin.

However, during the 1980s, non-ESBL β-lactamases underwent a mutation involving amino acid replacement, which resulted in ESBLs with altered specificity for breaking down oxyiminocephalosporins. This, in turn, increased resistance.

E. coli and K. pneumoniae were exposed to the newly developed ESBLs, TEM, and SHV. They possessed co-resistance to cotrimoxazole, aminoglycosides, and fluoroquinolones. In 1990,

reports of K. pneumoniae and ESBL E. coli were reported in several US hospitals. Then, in K. pneumoniae, SHV ESBL took its place. 1989 saw the publication of CTX-M, a novel ESBL.(34)

Definition:

Compared to its simple parent βlactamases, Extended-Spectrum β-lactamases (ESBLs) can hydrolyze a broader range of β-lactam antibiotics, which is why they are called that. Antibiotics hydrolyze when their β-lactam ring's amide bond is attacked by ESBLs, which have serine at their active site. Plasmid-mediated β-lactamases are what they are. They can render β-lactam antibiotics with an oxyimino group inactive, including oxyimino-monobactam (aztreonam) and oxyimino-cephalosporins (such as ceftazidime, ceftriaxone, and cefotaxime). They do not affect cephamycins or carbapenems. β-lactamase inhibitors often inhibit them, including carbapenems like tazobactam, sulbactam, and clavulanate. Generally, β-lactamase inhibitors like clavulanate, sulbactam, and tazobactam. (35)

In the Bush-Jacoby-Medeiros functional categorization, ESBLs are found in subgroups 2be (Amber's Class A) and 2d (Amber's Class D) (Bush et al. 1995). The "e" in 2be indicates that the β-lactamases have an extended spectrum. The 2be designation means these enzymes are developed from group 2b β-lactamases (TEM-1, TEM-2, and SHV-1). Cephamycins and carbapenems exhibit stability for ESBLs. β-lactamase inhibitors, such as clavulanic acid, suppress them. Frankfurt, Germany, reported the first ESBL incident in 1983. Resistance to additional antibiotic families, including aminoglycosides, trimethoprim/sulfamethoxazole, and fluoroquinolones, is frequently observed in conjunction with ESBL development.(36)

The risk factors for developing drug resistance resulting from ESBL-producing organisms are similar to those associated with other prevalent drug resistance development pathways, including prior antibiotic exposure, recent surgery, instrumentation, ICU admission, extended hospital stays, and nursing home admission. The selection of genes (bla) encoding these enzymes, which

has resulted in the formation of the ESBLs, is thought to be primarily caused by the widespread use of aztreonam and third-generation cephalosporins. The new enzymes' substrate affinity and profile were significantly altered by minimal alterations in the original genes' sequences compared to the parental enzymes. The bacteria that produce ESBL are usually linked to multidrug resistance because of the plasmids that drive it.(37)

TEM - β-lactamases:

Derivatives of TEM-1 and TEM-2 βlactamases make **ESBL TYPES:**

ESBLs are classified into two categories. Variants of TEM-1 or SHV-1 that differ by a few changes in amino acids make up the first of these categories (type I). Enzymes unrelated to TEM-1 or SHV-1 are in the second group (type II). The most common family of type II ESBLs is the plasmid-encoded CTX-M family, which is expanding. up the TEM family of ESBLs. First discovered from an E. coli strain in 1965, TEM-1 is one of the most common β-lactamases in the Enterobacteriaceae family. Ampicillin resistance is present in isolates harbouring TEM-1 βlactamases. The quantity and diversity of extended-spectrum TEM derivatives have increased dramatically. The first TEM variation to exhibit enhanced action against extended-spectrum cephalosporins was TEM-3, initially described in 1987. There are currently more than 100 different TEM-type ESBL variants with distinct amino acid sequences. There are currently around 165 different bla TEM gene variations. Specific TEM β-lactamase mutants, such as gene variations resistant to inhibitors such as clavulanic acid, are being found. TEM-type ESBLs, (38)

SHV – β-lactamases:

More than all other types of ESBLs, the SHV type is most frequently discovered in clinical isolates. Klebsiella species appear to be the source of SHV β-lactamases. Sulphydryl variable, or SHV, was initially identified in 1972 for SHV-1 and in the early 1980s for SHV-2 in Germany.

In contrast to TEM-type β-lactamases, SHV-1 has a comparatively small number of derivatives. Glycine at position 238 is replaced with serine in the bulk of SHV variants that exhibit an ESBL phenotype. There are already over 120 documented ESBL variants of SHV worldwide, all based on distinct amino acid substitutions. Currently, a variety of Enterobacteriaceae and non-fermenters have been found to harbour SHV-type ESBLs. Both nonfermentors and Enterobacteriaceae. (39)

CTX – M β-lactamases:

The most prevalent non-TEM, non-SHV ESBL is known to be the CTX-M family of extended-spectrum β-lactamases, which was recently characterized. Members of the CTX-M family can differ by up to 20% in their amino acid sequence, making it far more variable than the SHV and TEM families.

The hydrolytic solid activity of these β-lactamases against cefotaxime is reflected in the name CTX. These enzymes preferentially hydrolyze cefotaxime over ceftazidime and hydrolyze cephalothin more effectively than benzylpenicillin. Variability has been observed in the MICs of azitreonam. B -lactamases of the CTX-M type highly efficiently hydrolyze Cefipime. The β-lactamase inhibitor tazobactam inhibits them more effectively than clavulanate and sulbactam. They are instances of β-lactamase genes ordinarily found on Kluyvera species' chromosomes that are acquired by plasmids instead of mutations.(40)

OXA - β-lactamases:

The emerging class of ESBLs known as OXA-type β-lactamases, or oxacillin-hydrolysing, is noteworthy. They are members of functional group 2d and molecular class D. Calvulanate inhibits them poorly. The OXA-type ESBLs were first found in isolates of Pseudomonas

aeruginosa from a single Ankara, Turkey, hospital. Many recent OXA-type ESBLs have been identified, mostly in isolates of Pseudomonas aeruginosa from France and Turkey. (41)

Other ESBLS:

Numerous other kinds of β-lactamases have also been reported, either integron-associated or mediated by plasmids. None of the known β lactamases have these simple point mutations. Their geographic diversity is what makes them unique. Additionally, PER, VEB, BES, SFO, and TLA are uncommon ESBLs identified in Enterobacteriaceae.(42)

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ESBLs Detection:

Laboratory testing methods that can reliably detect the presence of these enzymes in clinical isolates are desperately needed, as the proportion of bacterial isolates that produce ESBLs is rising. Regretfully, for various reasons, identifying organisms that produce ESBL is difficult and complex. Factors such as the enzymes' heterogeneity, variable activity against possible

substrates, co-existence with other β-lactamases, and variables that alter their expression all contribute to the difficulty of accurately detecting ESBL synthesis in clinical isolates. Two things are concerning about this**.**

1) **The therapeutic implications:** Clinical results are significantly impacted by infections causing ESBL. They are linked to increased costs, more extended hospital stays, and high rates of morbidity and mortality. Patients with ESBL-producing infections typically have a higher chance of not responding to treatment. When cephalosporins are used for severe infections caused by ESBL-generating organisms, the failure rate is significant and can surpass 90%. This is especially true when the MICs of the utilized cephalosporins are higher (e.g., 4 or 8 µg/ml) yet still fall within the susceptible range. According to the Clinical Laboratory Standard Institute (CLSI), MICs < 8µg/ml demonstrate cephalosporin susceptibility. The national breakpoints determine how differently cephalosporin resistance is reported. The discovery of ESBLs resulted from certain ESBL-producing microbes appearing vulnerable to cephalosporins. (44)

2) **The aspects of infection control and epidemiology:** This is a crucial argument supporting continued attempts to identify ESBLs. Due to ignorance and subpar laboratory detection and reporting, the severity of the issue is underappreciated despite many findings indicating that ESBL incidence is rising globally.

Prevalence monitoring is crucial to assess the scope of the issue and facilitate the implementation of suitable infection control measures. These actions can stop epidemics as well as manage endemic conditions.(45)

Diagnostic Problems of ESBLs Detection:

Detection of ESBL is not straightforward for many reasons:

1) In contrast to methicillin-resistant Staphylococcus aureus (MRSA), it lacks a straightforward marker for its existence. Since ceftazidime is increasingly being

substituted by cefepime in hospitals, ceftazidime resistance is no longer a reliable indicator of the existence of ESBLs and is no longer evaluated.

2) Not every producer of ESBL exhibits universal resistance to all extended-spectrum β-lactams. Their substrate specificity varies, and they might not show signs of resistance to their substrate phenotypically.
3) Multiple ESBLs or other distinct enzymes that can modify the antibiotic resistance phenotype, such as Metallo β-lactamases and AmpC β-lactamases, may be present in an ESBL producer.
4) The inoculum effects significance for determining MICs (46)

ESBLs Detection methods:

There are two main categories of ESBL detection methods: molecular detection methods (genotypic method) and clinical microbiological methods (phenotypic method). The choice of antibiotics to test is crucial since ESBLs have varying degrees of activity against different cephalosporins, making them difficult to detect.

Phenotypic Methods:

While several techniques have been put forward to identify ESBLs in clinical isolates, it is crucial to remember that none of the methods that depend on the β-lactamase's phenotypic expression will identify every isolate that produces ESBLs. They are predicated on the fact that ESBLs impart resistance to oxyimino-β-lactams (such as ceftriaxone, cefotaxime, ceftazidime, and aztreonam) and that this resistance can be blocked by a β-lactamase inhibitor (typically clavulanate). Numerous assessments have been suggested. Primary screening and confirmatory tests are the categories into which phenotypic tests are separated.(47)

a) **Screening test :**

Disk diffusion methods:

CLSI suggested disc diffusion techniques to detect ESBL development by Proteus mirabilis, Klebsiella species, and E. coli. Uses include ceftriaxone, aztreonam, cefpodoxime, cefotaxime, and ceftazidime. Ceftazidime, cefuroxime, cefotaxime, ceftriaxone, cefpodoxime, and aztreonam are antibiotic isolates with decreased susceptibility that show promise as ESBL producers. The diagnosis should be confirmed by phenotypic confirmatory testing. When utilizing these methods as a screening test for the generation of ESBLs, the revised disc diffusion and MIC interpretive criteria given by CLSI should be used. With cefpodoxime, ceftazidime, aztreonam, or ceftriaxone, these criteria can be used to test for the generation of ESBL by Klebsiella species and E. coli. The likelihood of ESBL formation is indicated by the MIC of one of the antimicrobial drugs specified being more than two µg/ml.(48)

***Screening by dilution tests**:*

CLSI has proposed dilution methods to screen for the generation of ESBL by E. coli and Klebsiella spp. To screen for ESBL, CLSI advises using ESBL breakpoints for the indicator medications ceftriaxone, cefotaxime, ceftazidime, cefpodoxime, and aztreonam. Screening criteria proposed by CLSI are MIC ≥ 8µg/ml for cefpodoxime and MIC ≥ 8µg/ml for ceftazidime, cefotaxime, ceftriaxone, and aztreonam. A phenotypic confirmatory test is advised if the first screen yields positive results. (49)

a) **Phenotypic Confirmatory tests**:

Combination Disk Method:

Combination discs for cephalosporin and clavulanate are utilized. The CLSI suggests using cefotaxime (30µg) or ceftazidime (30µg) discs, both with and without clavulanate (10µg), to establish ESBL presence in Klebsiella and E. coli phenotypically—the conduct recommends conducting a test on Mueller-Hinton agar with confluent growth. Phenotypic proof of ESBL generation is defined as a difference of ≥ 5mm between the zone diameters of either cephalosporin discs or their respective cephalosporin/clavulanate discs. Since ceftazidime alone

has failed to detect organisms that produce CTX-M, it is recommended that both antibiotic discs be used. (50)

Broth microdilution Method:

Broth microdilution assays employing ceftazidime (0.25 to 128 μg/ml), ceftazidime plus clavulanic acid (0.25 to 128 μg/ml), cefotaxime (0.25 to 64 μg/ml), and cefotaxime plus clavulanic acid (0.25 to 64 μg/ml) can also be used for phenotypic confirmatory testing. Two antibiotics should be used together. Using accepted practices, the test is conducted. If the MIC of any cephalosporin in the presence of clavulanic acid decreases by ≥ 3-2 fold when serially diluted, it is deemed phenotypically confirmed. It is essential to adhere to CLSI quality control guidelines for both screening and confirmatory testing. (51)

C) Commercial methods:

E- test for ESBL:

The E-test ESBL strip is a two-sided strip that looks for a decrease in the minimum inhibitory concentration (MIC) of cephalosporins when clavulanate is added to one side of a dual oxyimino-β lactam gradient. Cefotaxime and ceftazidime strips are readily available, which enhances the detection of ESBL types that hydrolyze cefotaxime preferentially. This technique is helpful for ESBL production screening as well as phenotypic confirmation. As a phenotypic confirmatory test for ESBL, the stated sensitivity is 87 to 100%, while the specificity is 95 to 100%. The test's shortcomings include its expensive nature, inability to detect minute zone abnormalities, and inconclusive results. (52)

Automated antimicrobial susceptibility test systems:

ESBL testing is also carried out by automated antimicrobial susceptibility test systems (Vitek, MicroScan, and BD Phoenix). Cefotaxime and ceftazidime, both alone and together, are used in the Vitek ESBL test. A positive test result is shown by a specified decrease in the cefotaxime or ceftazidime wells containing clavulanate compared to the growth in the well-

containing cephalosporin alone. The method's sensitivity and specificity are higher than 90%. Results that are falsely negative have been noted. MicroScan panels with combinations of β-lactamase inhibitors + ceftazidime or cefotaxime have shown excellent reliability. The Phoenix ESBL test detects the synthesis of ESBLs by measuring the growth response to cefpodoxime, ceftazidime, ceftriaxone, and cefotaxime with or without clavulanate. Usually, the findings are ready in six hours. These systems cost too much to use regularly. (53)

d) **Other methods:**

Double disk diffusion test:

In the 1980s, the first detection test disclosed was called double disc synergy (DDS) or Jarlier double disc approximation. Disc diffusion testing, or DDS, involves placing 30 μg antibiotic discs containing ceftazidime, ceftriaxone, cefotaxime, and aztreonam on a plate 30 mm (from center to center) apart from the disc containing amoxicillin/clavulanate (20μg/10μg). Synergy is understood to be the presence of an ESBL when the antibiotic's inhibitory zone extends clearly toward the clavulanate-containing disc. Based on a review of DDS, cefpodoxime has been recommended as the expanded spectrum cephalosporin of choice, with sensitivities and specificities ranging from 79% to 97% and 94% to 100%, respectively. There have been cases of isolates carrying TEM-12, SHV-2, and SHV-3 producing false-negative results. In solitary.(54)

Cephalosporin/clavulanate Combination disks on Iso-Sensitest agar:

A disc diffusion approach using ceftazidime/clavulanate and cefotaxime/clavulanate combination discs with semi-confluent growth on iso-sensitize agar has been suggested by the British Society for Antimicrobial Chemotherapy for phenotypic confirmation of ESBL presence. Each combination's zone diameter is compared to cephalosporin alone, and a ratio of cephalosporin/calvulanate zone size to cephalosporine zone size is computed. ESBL is present

when the ratio is 1.5 or above. The assay does not detect the production of ESBL by bacteria generating SHV-6. (55)

***Agar supplemented with clavulanate**:*

Mueller-Hinton agar plates supplemented with 4μg/ml clavulanate and clavulanate-free Mueller-Hinton agar plates are inoculated with antibiotic discs containing 30μg of ceftazidime, 30μg of cefotaxime, 30μg of ceftriaxone, and 30μg of aztreonam. A β-lactam zone width difference of ≥10 mm between the two mediums is favorable for developing ESBLs. For ceftazidime, the specificity is 100%, and the sensitivity is 93–96%. According to several researchers, sensitivity has decreased. The requirement to prepare clavulanate-containing medium fresh is a significant drawback of the test. After 72 hours, clavulanic acid's efficacy starts to diminish. (56)

Disk replacement method:

A Mueller-Hinton plate containing the test organism is infected with three amoxicillin/clavulanate discs. These antibiotic discs are removed and replaced with discs containing ceftazidime, cefotaxime, and aztreonam at the exact location after an hour at room temperature. At least 30 mm away from these sites, control discs containing these three antibiotics are concurrently positioned. The test is considered positive when the zone increases by > 5 mm between the amoxicillin/clavulanate replacement discs and the control discs. This method's main flaw is that the second stage, which happens one hour after the first plate inoculation, makes it unsuitable for busy clinical microbiology laboratories.(57)

***Three-dimensional test** :*

Without depending on the proof of β-lactamase inhibitor-induced β-lactamase inactivation of the β-lactamases, it provides phenotypic evidence of ESBL-induced inactivation of aztreonam or extended-spectrum cephalosporins. The test relies on the test organism's culture's capacity to reshape the inhibitory zone surrounding an oxyimino-β-lactam disc. Although it was found that

this test was sensitive, it is practically undesirable because it requires more labor and is more difficult to use than other approaches. (58)

Emerging Epidemiology of Antibiotic Resistance:

Approximately half of the antimicrobial drugs used in people today are β-lactam antibiotics, and their use has led to the selection of β-lactam-resistant bacteria. The majority of resistance is a result of β-lactamases, which are enzymes that break down β-lactams. The fact that Enterobacteriaceae that produce ESBLs frequently co-express resistance to classes of antibiotic drugs other than those hydrolyzed by the ESBLs is a significant factor that restricts the range of effective medicines against these organisms. This has been demonstrated in tetracyclines, aminoglycosides, trimethoprim/sulfamethoxazole, and fluoroquinolones.

Even though some cephalosporins may seem active in vitro, the corresponding clinical results could be better. The preferred agent is thought to be carbapenems.

Monobactams, carbapenems, and β-lactamase inhibitor/β-lactam combos have become more common as ESBL-positive bacterial infections become more common. However, resistance to these medications has also been observed in recent years. Meropenem and imipenem are the carbapenems that can be used in India. Little information is available on the prevalence of carbapenem resistance in clinical isolates from our nation, especially in E. coli.
According to the information, E. coli may be developing a substantial new component of antibiotic resistance that may limit available treatments and have a negative clinical effect. Selecting the best course of treatment may be facilitated by organizing and translating complex microbiological data into therapeutically relevant categories. The problem of bacterial drug resistance spreading and advancing may have a temporary solution if previously used antibiotics that have seen little clinical use in recent decades are re-evaluated for their potential antimicrobial activity and clinical effectiveness against today's resistant microorganisms.

Enterobacteriaceae members are resistant to several widely used medications, including E. coli. Because more strains of E. coli are becoming resistant to antibiotics, treating urinary tract infections is becoming more challenging. A 22% resistance to trimethoprim-sulfamethoxazole was found in a prospective study of antibiotic resistance of E. coli from women with urinary tract infections. E. coli isolated from a urine sample demonstrated a substantial rise in the percentage of ampicillin resistance in another in vitro study conducted in Spain. Multidrug resistance up to 41.8% has been seen in E. coli isolated from urine and faecal flora in Iran. Tetracycline, trimethoprim-sulfamethoxazole, and ampicillin were resistant in 90%, 77%, and 62% of E. coli isolates found in Mexico. Studies conducted in Europe have also revealed.

Most tertiary care hospitals in India experienced severe resistance to infections caused by Klebsiella and E. coli. Other Enterobacteriaceae that are multiresistant also become established. Now, nosocomial and residential care facility isolates of K. pneumonia and E. coli with variable prevalence constitute the main ESBLs observed globally. There may also be differences amongst institutions in the same community. In the United States, Canada, the United Kingdom, France, Italy, Japan, Poland, Romania, Russia, Turkey, Greece, Korea, China, Pakistan, and other countries, ESBL-producing E. Coli has been isolated. Within 904 nosocomial isolates of E. coli and K. pneumoniae obtained from 28 Russian hospitals, 78 (15%) of the E. coli were positive for the ESBLS phenotype (Edelstein, 2003). In another study conducted in two Greek hospitals, the percentage of E. coli that produced ESBL was reported to be 20%. Other prevalence studies were conducted in China, the Philippines, Korea, and Pakistan, and 18%,

The development of ESBL-producing E. coli and other Enterobacteriaceae in ICU and other hospital wards has been linked to the heavy dosage and kind of antibiotics administered, particularly second and third-generation cephalosporins.

Like other nosocomial germs, this spreads between patients through healthcare workers' hands and equipment. Patients who have received incorrect antibiotic treatment may have preventable treatment failure and increased costs due to an infection with ESBL-producing bacteria. Enterobacteriaceae that produce ESBLs are typically isolated from nosocomial specimens. However, research on patients treated outdoors in the USA, Canada, and Europe has revealed that ESBL-producing E. coli and other Enterobacteriaceae also appear in these patients.(59)

Treatment of ESBL:

In the past, carbapenem was the medication of choice for treating infections brought on by the ESBLE and AmpC enzymes. Compared to fluoroquinolones and cephalosporins, the treatment failure rate for ESBL was lower.

With a few exceptions, such as the protease tribe and Serratia, polymyxin and colistin are effective against Enterobacteriaceae. It has been applied to strains with carbapenem resistance.

It has been discovered that combinations of colistin are more effective than monotherapy. When used alone as opposed to in combination, it was linked to more fatal occurrences. Combining tigecycline with carbapenems improved the outcome by lowering the death rates.

It was expected to mix colistin with fosfomycin, tigecycline, and carbapenems. The nephrotoxicity of colistin is the only issue.

ESBL-producing Enterobacteriaceae are treated with tigecycline. Treatment for ESBL infections involves tigecycline, which appears effective against CRE isolates. Yet, large dosages are required to treat pneumonia and BSI.

It has been discovered that aminoglycosides, either alone or in combination, are efficient against ESBL and carbapenem-resistant Enterobacteriaceae (CRE) isolates. All of the medications are shown to be sensitive, except for organisms that produce 16S rRNA methyltransferases. It has

been discovered to be more successful than tigecycline and polymyxin in treating UTIs. Its toxicity makes it ineffective when used alone or in conjunction with beta-lactamase to treat sepsis.

AmpC and ESBL producers can be treated with amikacin. A novel medication called plazomicin exhibits strong anti-ESBL and anti-AMPC properties.

Enterobacteriaceae that produced ESBLs were successfully treated with fosfomycin. Fosfomycin has been used in multicentric research to treat XDR strains.

In addition to being used with other medications to treat VAP and BSI, it has also been used to treat simple UTIs. For some patients with MDR strains, fosfomycin can be utilized, even though it is not the first line of treatment for severe infections.

Temocillin works as well as imipenem to treat CTX-M isolates because it is active against enteric bacteria that secrete ESBL and AmpC beta-lactamases.

Cephamycins, including cefotetan, cefoxitin, and moxalactam can be used instead of carbapenems to treat UTIs and only ESBL producers.

The beta lactam-beta lactamase inhibitors (BLBLIS) that are fatal to ESBL producers include augmentin, ticarcillin-sulbactam, piperacillin-tazobactam, cefoperazone-sulbactam, ampicillin-sulbactam, and ticarcillin-sulbactam. Depending on the continent, their resistance degree varies.

ESBL-producing K. pneumoniae and E. coli isolates have both been treated with ceftolozane-tazobactam. Treatment for ESBLs with ceftazidime and avibactam is successful.

UTIs can be treated using ESBL producers that are vulnerable to fluoroquinolones. ESBLcUTI is treated with cotrimoxazole 60)

AmpC beta lactamases:

Origin:

The development of resistance components to natural beta-lactams produced by bacteria dates back more than two million years, predating antibiotics in medicine.

Creatures for habitation enzyme that degrades penicillin were initially identified in E. coli in 1940. Ambler's molecular classification of beta-lactamases placed it in molecular class C. Beta-lactam degradation varies depending on the protein sequence. However, the active site of AmpC was a serine residue in the protein. Interestingly, the same serine is also the active site for ESBL. Within the functional classification of beta-lactamases, they belong to group 1 (Bush and Jacoby, 2010). A particular intestinal bacterium included the chromosomal genes. (61)

Penicillins:

In 1928, Alexander Fleming isolated the first penicillins from Penicillium notatum. In the beginning of 1930, Penicillium notatum's antibacterial qualities were once again identified. Ernst Boris Chain initially proved the antibacterial efficacy of penicillin in 1940 when he used it to treat experimentally afflicted pigs. Since then, penicillin has been administered to human patients, and the infection has been successfully treated. Every member of the penicillin class has a beta-lactam ring structure, which is necessary for their antibacterial activity. They are all derivatives of 6-aminopenicillanic acid.

Penicillins bind to bacterial enzymes known as penicillin-binding proteins (PBPs) and activate cell wall autolysis by preventing the peptidoglycan crosslink in the bacterial cell wall from emerging.

Penicillins are classified according to the additional chemical substitutes added to their side chains. These substitutions primarily cause variations in the penicillins' bioavailability and spectrum of activity by extending their activity towards Gram-negative bacteria in comparison to penicillin G. (62) Based on the spectrum of activity, four subclasses of penicillins were

identified: broad-spectrum or antipseudomonal penicillins, very-narrow spectrum (also known as penicillinase-resistant penicillins), extended-spectrum or aminopenicillins, and narrow-spectrum or natural penicillins.(63)

Classification of penicillin:

Narrow spectrum	beta-lactamase susceptible	benzylpenicillin, benzathine benzylpenicillin, procaine benzylpenicillin, phenoxymethylpenicillin
	beta-lactamase resistant	cloxacillin (dicloxacillin, flucloxacillin), oxacillin, meticillin, nafcillin
Broad spectrum	Aminopenicillins	amoxicillin, ampicillin, epicillin
	Carboxypenicillins	carbenicillin, ticarcillin, temocillin
	Ureidopenicillins	azlocillin, piperacillin, mezlocillin
	Other	mecillinam, sulbenicillin

Fig no :2 Classification of penicillin

Cephalosporin group of drugs:

Beta-lactam antibiotics such as cephalosporins prevent bacteria's cell walls from forming.Dr. Abraham discovered cephalosporin C in 1948 while studying a fungus called Cephalosporium acremonium. Newton and Abraham refined and isolated the compound at Oxford University in 1955. The beta-lactamase produced by Gram-negative bacteria was stable against cephalosporin C. Numerous cephalosporins have been created and released onto the market. These antibiotics are bactericidal because they destroy germs when taken as directed. They share similarities with penicillins, both pharmacologically and structurally. In place of the five-membered thiazolidine rings seen in penicillins, cephalosporins have a beta-lactam ring structure that is infused with a six-membered sulfur-containing dihydrothiazine ring. A system of classification between the generations of cephalosporins(64)

Classification of Cephalosporin:

There are several generations of cephalosporins. The overall trend is that cephalosporins of lower generations exhibit more excellent Gram-positive activity, whereas those of higher generations exhibit more excellent Gram-positive activity. The exception is the medication cefepime, which is equal to third-generation cephalosporins in Gram-negative activity and first-generation cephalosporins in Gram-positive activity. Cephalosporins can be categorized into a spectrum, generation, chemical structure, resistance to beta-lactamases, and clinical pharmacology. Generation-based classification, however, is the most well-known kind. Based on their microbiological spectrum, cephalosporin medications are classified into distinct generations: the first, second, third, fourth, and fifth generations of people.

First Generation cephalosporins:

First-generation cephalosporins primarily target Gram-positive bacteria like streptococci and staphylococci, with a narrow range of activity. They don't have much of a gram-negative spectrum, either. While enterococci are not as susceptible to first-generation cephalosporins, they are often more effective against aerobic Gram-positive cocci, such as methicillin-sensitive Staphylococcus aureus. Cephalothin, cephapirin, cephradine, and cefazolin are the first-generation cephalosporins currently accessible for parenteral usage. Cefazolin is superior to the others in two key ways: its serum concentration is significantly higher, and it has a much longer half-life. All of the medications in this class have the same bacterial spectrum. Upper respiratory tract infections are treated with these medications.

Second Generation cephalosporins:

Second-generation cephalosporins are generally more effective against some Gram-negative bacteria and anaerobes and less effective against Gram-positive cocci (such as streptococci and staphylococci). Cefamandole, cefoxitin, cefuroxime, cefonicid, cefotetan, and ceforanide are a few of the second-generation cephalosporins available for parenteral usage. When treating abdominal infections, cefuroxime, cefaclor, and cephamycins (cefoxitin) are excellent options because they are more active against Gram-negative organisms but less effective against staphylococci. With a long half-life, cefonicid, ceforanide, and cefotetan can be used once or twice daily.

Similar actions are shown in cefoxitin, cefotetan, and cefonicid.

Regarding Haemophilus influenza and some Enterobacteriaceae, cefamandole has increased action. Cefoxitin, or cefotetan, frequently causes Bacteroides fragilis to become sensitive.

Third Generation cephalosporins:

others. The preferred medications for infections caused by Bacteria that are not Gram-negative Due to their broad spectrum of action, gram-positive and gram-negative bacteria can be effectively combatted using third-generation cephalosporin medications. Yet, gram-negative bacteria are the target of their maximum activity. This class of beta-lactam antibiotics improved beta-lactamase's stability and gram-negative cell wall penetration. Compared to earlier generations, they possess more advantageous pharmacologic qualities. Pseudomonas aeruginosa, which includes Enterobacter species and Serratia spp., and Enterobacteriaceae, which includes producers of beta-lactamases, are more susceptible to the actions of third-generation cephalosporins. Regarding B. fragilis, most, except ceftizoxime and moxalactam, are inactive. As of right now, third-generation cephalosporins contain the following medications: cefotaxime, moxalactam, cefoperazone, ceftazidime, cefsulodin, ceftizoxime, ceftriaxone, and cefmenoxime, among

Fourth Generation cephalosporins:

The broadest spectrum of activity is seen by fourth-generation cephalosporins, which exhibit comparable activity to first-generation cephalosporins against Gram-positive pathogens. Additionally, compared to third-generation cephalosporins, they are more resistant to beta-lactamases. Both cefepime and cefpirome have substantial levels of activity against various resistant bacteria that have historically been challenging to eradicate. The fourth age group When used in conjunction with aminoglycosides, cephalosporins (such as cefepime and cefpirome) are effective against pseudomonas.

Fifth Generation cephalosporins:

These antibiotics have a broad spectrum. Decisive action against aerobic Gram-negative bacteria and high activity against aerobic Gram-positive bacteria are the characteristics of fifth-generation

medications. Fifth-generation cephalosporins include ceftobiprole and ceftaroline. Ceftobiprole possesses potent antipseudomonal properties and seems less prone to the emergence of resistance. Acute bacterial skin and skin structure infections, including methicillin-resistant S. aureus, have been treated with ceftaroline.(65)

Detection of AmpC beta-lactamases::

CLSI has not established strict guidelines for evaluating AmpC synthesis in gram-negative organisms. Nonetheless, their detection is crucial for controlling the illness and supplying epidemiological data. Here, we list a few phenotypic.

Phenotypic tests:

- **Screening for cefoxitin**: (CLSI guidelines). Mueller Hinton agar (MHA) is swabbed with the test organism, a cefoxitin disc is placed on top of it, and the mixture is incubated at 370 °C for 16–18 hours. Diminished The cefoxitin test susceptibility is employed as a screening tool. However, carbapenamases in K. pneumoniae and E. coli with abnormalities in their outer membrane porins can also generate it.
- **AmpC disc test:**

  A saline or EDTA-impregnated disc is positioned next to a cefoxitin disc in the strain's lawn culture, and a few colonies are spread across the impregnated or plain disc. The generation of AmpC is indicated if distortion happens in the zone of inhibition.(66)

- **Three-dimensional test**:

On MHA agar, E. coli ATCC 25922 is cultivated in a broth and swabbed like grass. Cefoxitin is placed on the plate, and next to it, an agar surface is slit circularly by 3 mm, and a well is

pipetted with the test strain suspension. For the synthesis of the AmpC enzyme, distortion of the inhibitory zone is recommended. It is possible to make adjustments such as adding the test organism pellet, which is centrifuged, frozen, and thawed five times, and creating a radial slit.(67)

- **Modified Hodge test:**

E. Coli ATCC 25922 is swabbed as a lawn on MHA plates. Test strains are inoculated from the plate's periphery to the disc's edge, with cefoxitin (30 μg) retained in the plate's middle. The cloverleaf model produced by oblique growth is favorable for AmpC generation. It is thought that isolates with no distortion in the cefoxitin zone are negative for AmpC synthesis. (68)

- **AmpC Induction Test:**

In this test, the bacteria are exposed to a substance that induces AmpC expression, like cefoxitin or cloxacillin, and their resistance to cephalosporins is then monitored. AmpC beta-lactamases may be present if the bacteria show signs of enhanced resistance following exposure to the inducer.(69)

- **AmpC E-test:**

This quantitative technique uses E-test strips with a gradient of cephalosporin concentration. The antibiotic's minimum inhibitory concentration (MIC) is ascertained by placing the strip on an agar plate infected with the test organism. When compared to isolates of the wild type, a higher MIC could indicate the generation of AmpC. (70)

Treatment:

Since most of the time, the bacteria that produce AmpC beta-lactamases are resistant to most antibiotics, choosing the right antibiotic for treatment might be challenging.

AmpC-producing bacterial infections cannot be treated with penicillins, cephalosporins, beta-lactam-beta inhibitors, or other antibiotics due to the danger of mutation induction. According to studies, poor clinical outcomes have been linked to cefotaxime, ceftazidime, and piperacillin-tazobactam.

When Enterobacteriaceae that generate ampicillin cause bacteremia, piperacillin-tazobactam is utilized; however, one must first determine the Pitts bacteremia score.

In general, cefepime exhibits susceptibility to conventional techniques because of its limited ability to promote the creation of ampicillin. However, cefepime MICs dramatically increase, as the high inoculum test indicates. Therefore, cefepime must be used with caution.

It has been discovered that temocillin, a ticarcillin derivative, works well to treat Enterobacteriaceae.

It has shown efficiency against AmpC beta-lactamases mediated by chromosomes or plasmids.

While it isn't available in the US, aminopenicillin has been shown in vitro to be efficacious against E. coli.

Carbapenems like imipenem, meropenem, and ertapenem can be utilized to treat ampC infections. However, bacteria with porin defects and carbapenemases have been found to produce AmpC beta-lactamases. It has been shown that there is decreased susceptibility to imipenem with a MIC range of 8 to 128 µg/ml.

hyperproduce AmpC, such as Enterobacter, E. coli, Klebsiella, and Citrobacter.(71)

Metallo β- Lactamases (MBL):

Origin:

In 1966, Metallo β-lactamase was initially recognized as β-lactamase in Bacillus cereus after evidence that metal chelators such as EDTA might block the activity of cephalosporins. The first MβL determinants were identified on a chromosome and produced by rare curiosities, including Legionella germanic, B. cereus, and Flavobacterium odoratum, which had little clinical significance.

In 1991, P. aeruginosa from Japan was the source of the first report of transferable MβL. Later, MβLs were associated with other clinically significant taxa, including Serratia, Bacteroides, and Pseudomonas. Commercially available β lactamase inhibitors are ineffective against class B β-lactamases. However, metal ion chelators like EDTA can inhibit them. Because these enzymes can hydrolyze almost all kinds of β-lactams, including carbapenems, they are exciting and concerning. There have also been reports of several clinical Burkholderia cepacia isolates that produce inducible metalloenzymes (PCM-I) and exhibit preferential hydrolysis of carbapenems and imipenems. A small subset of B. fragilis isolates has been demonstrated to produce the resistance-enhancing chromosomal metalloenzyme CfiA/CcrA. (72)

Definition:

The most effective drugs to treat serious infections caused by multiresistant Enterobacteriaceae are carbapenems, especially those that produce the enzymes Amp C and extended-spectrum beta-lactamase (ESBL). Because there are currently few antibiotics available to treat infections caused by these bacteria, the rise of carbapenem-resistant bacteria has raised serious concerns. The synthesis of carbapenemases, which hydrolyze carbapenem, is the primary cause of resistance to carbapenem in gram-negative bacteria. Hyperproduction of the AmpC beta-lactamase enzyme in

an organism with porin loss is another significant factor contributing to carbapenem-resistant Enterobacteriaceae(73)

Over the past few years, a steady number of acquired carbapenemases have been reported, which fall into three of the four recognized types of beta-lactamases: either Ambler molecular classes A and D (serine carbapenemases) or Ambler molecular class B (metallo beta-lactamases). Class B enzymes are the carbapenemases that have therapeutic significance. The metallo beta-lactamases (MBL) include:

- The Imipenemases (IMP) family of carbapenemases.
- The Seoul imipenemase (SIM).
- The German imipenemase (GIM).
- The Verona integron-encoded metallo beta-lactamases (VIM).
- The New Delhi-metallo beta-lactamases (NDM) enzymes.

These enzymes are classified as class B enzymes. There have been reports of metallo beta-lactamase enzymes belonging to the IMP or VIM class worldwide. The discovery of New Delhi metallo beta-lactamase 1 (NDM-1) in Klebsiella pneumoniae recovered from a Swedish patient earlier this year has drawn interest worldwide.

Except for monobactams, MBL confers resistance to all beta-lactam antibiotics. Resistance to beta-lactamase inhibitors such as clavulanic acid, sulbactam, and trobactam characterizes these zinc-dependent beta-lactam hydrolyzing enzymes. Their mode of action is not in competition with penicillin-binding proteins, which sets them apart from other beta-lactamases.MBL enzymes pose a significant danger of significant spread across the gram-negative community since their genes, which can be plasmid- or chromosome-borne, are frequently located in integrons. High rates of morbidity and mortality are linked to invasive infections brought on by gram-negative isolates that produce MBL.(74)

Epidomology of Metallo β- lactamase:

In the last ten years, acquired MβLs have been found in clinical isolates from Asia, Europe, North America, and South America, revealing the global scope of the issue as well as an unexpected diversity of enzymes. The IMP-type and VIM-type enzymes, known as multiple variations, are currently the most common and widely distributed acquired MβLs. Additional forms of obtained MβLs, such as SPM-1, GIM-1, and SIM-1, have also been recognised. Peleg and associates (2005) initially documented the appearance and swift spread of an acquired MβL determinant in an Australian hospital context, a continental resistance BlaIMP-4, an allelic variant of blaIMP-1 gene previously found in clinical isolates of Acinetobacter, was the MβL gene implicated in the outbreak.(75)

Metallo beta-lactamase detection:

Combined disc diffusion test:

Mueller Hinton agar plates were infected with isolates meeting the 0.5 McFarland turbidity standard, and the combined disc diffusion test identified MBL production. Two 10 mcg imipenem discs were placed on the Mueller Hinton agar plate, and 10 mcg of 0.5 M EDTA solution was added to one of them to achieve the required concentration (750 mcg of disodium EDTA dihydrate per disc). Following 16–18 hours of air incubation at 35˚C, the imipenem and imipenem-EDTA disc inhibition zones were compared. If the diameter of the inhibitory zone increased by more than 7 mm while using imipenem plus EDTA disc compared to imipenem disc alone, the isolate was deemed positive for MBL generation.(76)

Modified Hodge test (MHT):

Indicator organism On Mueller Hinton agar, Escherichia coli ATCC 25922 was grown in a lawn culture at a 0.5 McFarland turbidity standard (1:10 dilution). Once the plate had dried, an imipenem disc (10 mcg) was positioned in the center. After that, the test strains were heavily inoculated in a straight line from the disc's edge to the plate's edge, and they were incubaed for an entire night at 35˚C. After incubation, the test isolate was considered to have produced

carbapenemase if there was an indentation along the streak line like a clover leaf. On the other hand, the lack of indentation was seen negatively for the synthesis of carbapenemase. (77)

Treatment :

Cefiderocol:

All families of carbapenemases (KPC, OXA, NDM, VIM, and IMP) are resistant to cefiderocol, a new siderophore cephalosporin with exceptional broad-spectrum action and stability. Porin channels are unnecessary because they enter the bacterial cell through the iron transporters. Many beta-lactamases, including MBLs, can hydrolyze it without breaking it down. Based on a 4 mg/L breakpoint, more than 72 percent of NDM producers, 91.7% of VIM producers, and 87% of IMP producers can infect Enterobacterales that produce MBL. Its MIC50, however, is 1 to 4 mg/L for NDM producers—that is, extremely near the breakpoint. Additionally, it is essential to understand that various testing methods may cause future Enterobacteria MIC values to vary.

Tetracyclines:

The majority of blaNDM Enterobacterales infections can be successfully treated with tetracyclines such as tigecycline; nevertheless, durable plasmids resistance has been reported. In vitro, tigecycline is still effective against the majority of other Enterobacterales that produce MBL. According to one animal model, tigecycline at 50 or 100 mg dose by itself is insufficient to treat pneumonia caused by Enterobacterales that produce NDM. It caused bacterial regrowth when used in monotherapy at such humanized dosages.

Aminoglycosides:

Aminoglycosides quickly kill bacteria. MBL strains frequently exhibit resistance because of enzymes that alter aminoglycosides. MBL-producing Enterobacterales were rarely susceptible to amikacin, while one-third of the bacteria in a recent Greek study were susceptible to gentamicin.

Plazomycin is active in over 80% of VIM and almost half of NDM-producing Enterobacteria and can elude enzymes. The medication was approved by the FDA in 2018 after it was successfully evaluated for CRE infections, primarily KPC. Regretfully, it has yet to be made widely available. Aminoglycosides cannot be used as empirical therapy due to their high resistance rate. It might be used in conjunction with other antibiotic treatments based on known infections.

Polymixins:

With an MBL-producing Enterobacteria MIC90 of 1 mg/L, polymixins are effective against these bacteria in over 90% of cases. Remember that it has a natural inactivity against Serratia spp., Morganella, Proteus, and Providencia types. Before 2015, it was among the essential medications for treating MBL infections. High dosages (i.e., 75 to 150,000 U/Kg/d with a maximum dose of 12 MUI per day) of intravenous colistin should be administered in the intensive care unit. The limits of colistin's therapeutic range are pretty small, and elevated levels of the drug can cause harm to the kidneys and brain. Several studies conducted in the last several years have recommended against using colistin when an alternate treatment is available for gram-negative infections that are difficult to treat. The risk of acute renal injury was shown to be higher while following a regimen based on colistin.(78)

4. MATERIALS AND METHOD

Study Site: This study was carried out in the Department of Microbiology of SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE, VIJAYAPURA

Study Design- This is Cross- Sectional study (Hospital Based)

Study Period- 1 year

Sample Size:

With anticipated Proportion of E coli 68% (ref) Tewari R, Mitra SD, Ganaie F, Venugopal N, Das S, Shome R, et al. Prevalence of extended-spectrum β-lactamase, AmpC β-lactamase and metallo β- lactamase mediated resistance in Escherichia coli from diagnostic and tertiary health care centers in south Bangalore, India. Int J Res Med Sci 2018;6:1308-13., the study would require a sample size of **104 samples** with E. Coli isolates 95**%** confidence level and 10% absolute precision**.**

(Referred: Statulator software ttp://statulator.com/SampleSize/ss1P.html)

Formula used

$$n = z^2 \frac{p*q}{d^2}$$

Where Z= Z statistic at α level of significance

d_2= Absolute error

P= Proportion rate

q= 100-p

Inclusion Criteria: Clinical samples showing a growth of E. coli will be included in the study.

Exclusion Criteria: Clinical samples which will not show growth of E. coli will be excluded from the study.

Collection and processing of clinical samples

The present study was conducted by collecting clinical samples received for the bacteriological laboratories of SHRI B.M . PATIL MEDICAL COLLEGE, HOSPITAL, AND RESEARCH CENTRE, VIJAYAPURA During the tenure of this study we processed a number of different clinical samples namely Viz. urine, stool, CSF, body fluids/aspirates, pus, blood, and swabs from various anatomical sites.

The samples were collected by following the standard procedure described below.

Samples such as urine and stool were collected in a sterile wide-mouth container with a tightly fitted screw cap lid. The samples such as CSF, body fluids/aspirates, pus, and blood were collected by needle aspiration technique. In some of the cases, the samples were collected by swabbing from various anatomical sites using a sterile cotton swab procured from Hi media. All the samples were collected as per the guidelines mentioned by Isenberg (1998) and WHO Manuals (1980)

Isolation and Characterization of E .coli:

Isolation and cultivation of bacteria:

- A loopful of sample was streaked aseptically on a MacConkey agar plate for selective isolation & differentiation of pathogens and then the media were incubated overnight at 37°C in an incubator.

- On a Selective and Differential media MacConkey agar plate were streaked and incubated at 37°C for 18-20 hours. The typical colonies showing only rose pink color lactose fermenter colonies were selected for our study Gram staining, test were performed.

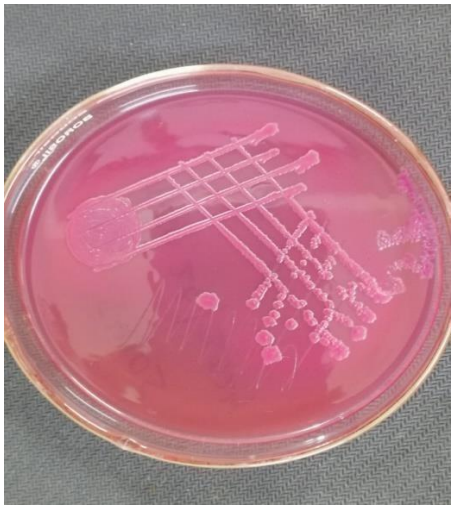


Fig no :3 LF colonies of E coli

Characterization of isolates:

- The typical colonies were further characterized by their biochemical characteristics using Gram staining and multi-test biochemical media as follows,

A series of biochemical tests especially selective for *E. coli* i.e Indole (I) test, Citrate utilization test (C) Ureases test (U) Triple Sugar fermentation test (TSI), etc were performed for confirmatory identification

GRAM STAINING:

1. The smear was made on a clean heat-free glass slide in a drop of normal saline.
2. The smear was dried in air and fixed by flaming
3. The slide was placed, with a smear upwards over a staining rack.
4. The smear was covered with a gentian violet stain which was left to act for one minute
5. The slide was held at a steep slope with the help of forceps. Gram's iodine solution was poured from one end and the gentian violet stain was washed off.
6. . The smear was covered with fresh iodine solution and the slide was left

horizontally on the rack for one minute.

7. . The smear was decolorized with acetone for 2-3 seconds and washed with water.
8. The smear was covered with 0.5% safranine and left to act for 30 seconds.
9. The slide was washed thoroughly with water, dried, and examined under oil Immersion

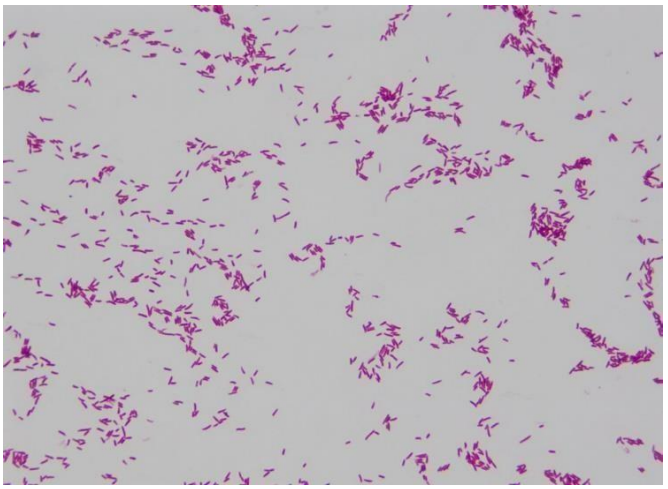


Fig no:4 Pink rod-shaped bacilli randomly arranged

Catalase test

Principle:- A drop of hydrogen peroxide (3% H_2O_2) is added to (or when the colony is mixed with a drop of H_2O_2 placed on a glass slide) any catalase-producing bacteria, bubbles appear due to the breakdown of H_2O_2 by catalase to produce oxygen

Interpretation:

Catalase positive- Bubbles are produced

Catalase negative- No bubbles

Fig no: 5 Catalase test _positive for E coli

Oxidase test

Principle – This test depends on the presence in bacteria, of an enzyme cytochrome oxidase which catalyzes the oxidation of reduced cytochrome by molecular oxygen.

Procedure - Used 1% tetra-methyl paraphenylene diamine dihydrochloride freshly prepared solution on a piece of filter paper. Then rubbed a few colonies of the test organism on it the smeared area turned purple within 10 seconds. A positive control should be included

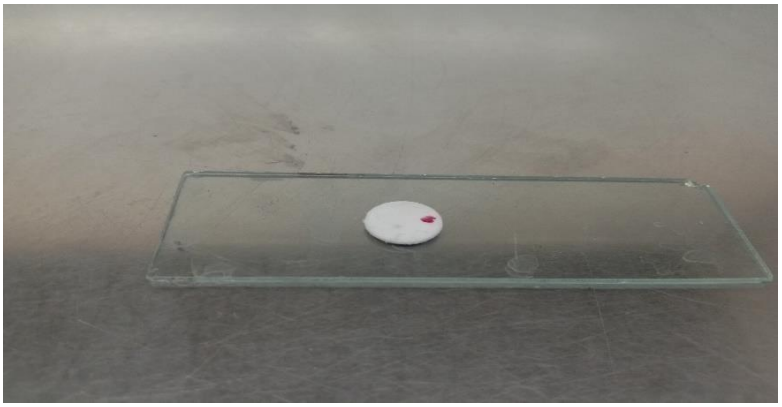


Fig no:6 Oxidase test is negative for E coli

Interpretation

Positive- Development of deep purple color within 10 seconds

Negative- No color change

BIOCHEMICAL TESTS:

Indole test

Principle: - Certain bacteria that possess the enzyme tryptophanase degrade the amino acid tryptophane to indole, pyruvic acid, and ammonia. When Indole reacts with the aldehyde group of (4-dimethyl amino Benzaldehyde, iso amyl alcohol, hydrochloric acid) known as Kovacs reagents result in the formation of a red color ring complex.

Procedure: The test was done by inoculating peptone water broth with the test organism and incubated at 37°C for 18-24 hours. Then 0.5 ml of Kovacs reagents was added down the inner wall of the tube and gentle shaking

Interpretation:

Indole Positive: A red coloured ring near.

Indole Negative: Yellow coloured ring

Citrate utilization test

Principle – It detects the ability of a few bacteria to utilize citrate as the sole source of carbon for their growth, with the production of alkaline metabolic products. A citrate test is performed on a citrate-containing medium, such as Simmons or koser medium.

Procedure - A well-isolated colony was picked up and inoculated as a single streak on the slant surface of the simmon citrate agar tube. The tube was incubated at 37°C for 18 to 24 hours.

Interpretation:

A positive result was interpreted as blue colour and/or streak of growth.

Urease production test

Principle: This test detects the ability of an organism to produce enzyme urease which splits urea into ammonia. Ammonia makes the medium alkaline and thus phenol red indicator changes to pink/red.

Procedure: The test was done by heavily inoculating the test organism into a slant contain Christensen's urea agar and incubated at 37°C for 18- 24 hours.[365]

Interpretation: -

Urease Positive: Pink color.

Urease negative: No color change (Colorless)

Triple Sugar iron agar test (TSI)-

Principle – It is used to detect the ability of an organism to attack specific carbohydrates included in a growth medium, with or without the production of gas, along with the determination of possible hydrogen sulphide production.

Procedure- TSI is a composite medium that contains three carbohydrates sucrose, glucose, lactose, and also ferric acid for testing H_2S production. The concentration of sucrose and lactose is 10 times that of glucose in the medium. Phenol red is used as an indicator. The test organism is inoculated with a pure bacterial culture by a straight wire and pierced deep in the butt (stab culture) and then inoculated on the entire slope of the medium. The tube is then incubated at 37° C for 18-24 hrs

Slant/butt	Color	Utilization
Alkaline/acid(K/A)	Red/Yellow	Only Glucose fermented
Acid/acid(A/A)	Yellow/Yellow	Glucose fermented. Lactose and/ or sucrose also fermented
Alkaline/alkaline(K/K)	Red/Red	No fermentation of glucose, lactose or sucrose

Fig no : 7 INTERPRETATION OF BIOCHEMICAL TESTS (E coli)

- **Indole test (I) - Positive**
- **Urease production test (U) - Negative**
- **Citrate utilization test (C) - Negative**
- **Triple Sugar iron agar test (TSI) - A/A (acid/acid)**

Antimicrobial susceptibility testing –

Bacterial identification will be performed by the Vitek 2 compact system (bioMerieux, France) with the GN cards, according to the manufacturer's instructions. Susceptibility of the isolates to antimicrobial agents will be tested with AST-N235 cards for urine isolates, other than urine, AST-N405 for fermenter isolates, and gram-negative identification cards (GNID) in Vitek 2 compact system (bioMerieux, France). Additionally, antibiotic susceptibilities will be determined by the Kirby- Bauer disk diffusion method and the results will be interpreted according to the guidelines of the Clinical Laboratory Standard Institute. The antibiotic discs used will be ceftazidime ceftriaxone, ciprofloxacin, levofloxacin, gentamicin, imipenem meropenem, piperacillin-tazobactam, amoxicillin/ clavulanic acid, aztreonam, ceftriaxone,

ciprofloxacin , levofloxacin gentamicin, imipenem, meropenem , piperacillin-tazobactam, cefoxitin , cefuroxime, amoxicillin/ clavulanic acid, aztreonam, colistin Ceftazidime,cephoperazon sulbactam, ciprofloxacin, imipenem meropenem, piperacillin-tazobactam cefoxitin ampicillin-sulbactam, tigecycline, and colistin will be used

- **inoculum preparation**

- Colonies of similar morphology (5-7 in number) were selected and taken up from agar plates.
- The inoculums were incubated at 37 ºC for 2-6 hrs, Turbidity of the broth medium was adjusted by using 0.5 McFarland standards

- **Inoculation of test plates**

- A sterile cotton swab was used dipped into the inoculum and rotated several times.
- The swab was then pressed to remove an excess volume of inoculum from the swab inside the test tube wall above the fluid level
- Muller- Hinton agar plate plates were used and lawn inoculums were streaked over the surface of an agar plate. The streaking was done three times to get a good distribution of inoculums at an angle of 60 º.

- **Application of disks to an inoculated agar plate**

- The predetermined drug discs were placed on the surface of an inoculated agar plate. The discs were pressed firmly to attain full contact with the agar
- The plates were then incubated at 37 ºC within 15 minutes of placing the discs.

- **Reading of results:**

- The plates were examined for a zone of inhibition after 16-18 hrs of incubation. The zone measurement was done by using a caliper or ruler that was placed on the backside of the Petri plate

➢ **Interpretation of results**:

- The plates were examined and zone interpretation was done as sensitive, intermediate and resistant according to given standard zones (CLSI guidelines)

1) Phenotypic detection of ESBL

a) Screening Tests :

All the E.coli isolates will be screened for ESBLs by disc diffusion method. all the isolates will be tested for susceptibility to antibiotic discs containing ceftazidime cefotaxime (30 g) as part of the presumptive test to identify possible ESBL producers. According to the CLSI recommendations, the following interpretation of the results Is made up of zones of inhibition of 27 mm for cefotaxime indicating ESBL generation. Confirmatory tests will be performed on the isolates that are less vulnerable or resistant.

b) Confirmatory Tests :

The ESBL-producing E. Coli isolates will be confirmed by the Clinical and Laboratory Standard Institute (CLSI) phenotypic confirmatory test of the combined disc assay method. On a Muller Hinton agar plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards, one disc of each of the antibiotics , cefotaxime (30g), and one in combination with clavulanic acid (10g) will be placed at a distance of 20 mm and incubating overnight at 37°C. The zone diameter for each antimicrobial drug tested with clavulanic acid increased by about 5mm compared to its zone when tested alone for the ESBL-producing bacteria. The quality control strain utilized is E. coli ATCC 25922

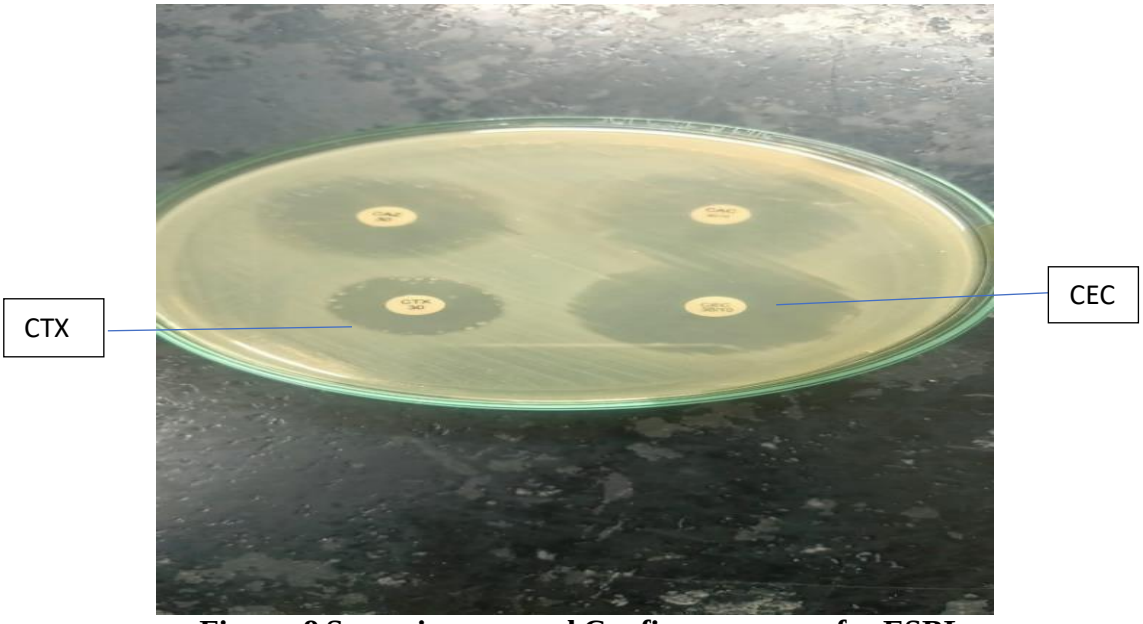


Fig no :8 Screening test and Confirmatory test for ESBL

Cefotaxime(CTX) =Cefotaxime/Clavulanic acid (CEC)

2) Phenotypic detection of AmpC

a) Screening Tests :

The isolates will be screened for presumptive AmpC production by testing their susceptibility to cefoxitin (30µg) antibiotic discs using the Kirby Bauer disk diffusion method. By employing the Kirby Bauer disc diffusion method to assess the isolate's resistance to cefoxitin (30 g) antibiotic discs, the isolates were screened for possible AmpC synthesis. All isolates with an inhibitory zone diameter of less than 14mm for cefoxitin were classified as AmpC positive and underwent a confirmatory test.

b) Confirmatory Tests

AmpC producers were confirmed by a phenotypic confirmatory test of the combined disc assay method.16 One disc of cefoxitin (30µg) alone and one in combination with cloxacillin (200µg)

were placed at a distance of 20mm on a Muller Hinton agar plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards and incubated overnight at 37°C. The E. coli strains demonstrating a zone diameter around the cefoxitin+cloxacillindisc≥5mmthan the zone diameter around the cefoxitin disc alone were considered as AmpC producers. E. coli ATCC 25922 were used as a quality control strain

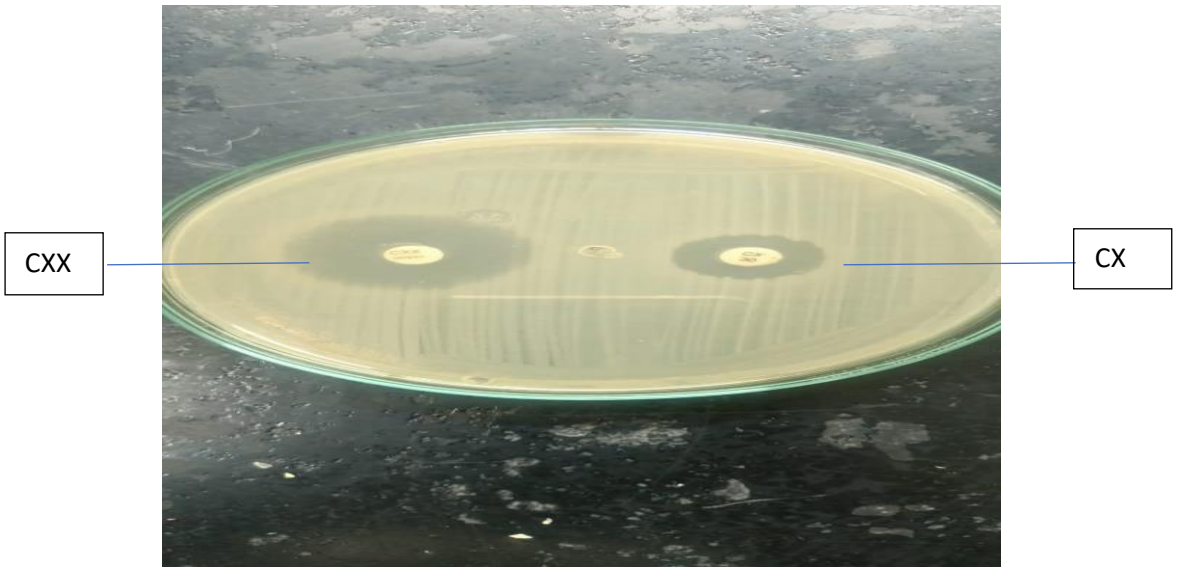


Fig no :9 Screening test and Confirmatory test for AmpC

Cefoxitin (CX) = Cefoxitin/Cloxacillin (CXX)

3) Phenotypic detection of MBL

a) Screening Tests :

All the *E. coli* isolates will be screened for MBL production by testing their susceptibility to imipenem (10µg) and meropenem (10µg) antibiotic discs using the Kirby Bauer disk diffusion method. All isolates with a screen-positive result for MBL and an inhibitory zone diameter of less than 19 mm underwent a confirmatory test

a) Confirmatory Test

All screen-positive E. coli isolates were confirmed of Metallo- β -lactamase production as described by.17 One disc of imipenem (10µg) alone and one in combination with EDTA

(750µg/mL) were placed at a distance of 20mm on a Muller Hinton agar plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards and incubated overnight at 37°C. The E. coli strain demonstrating a zone diameter ≥7mm around the imipenem/EDTA disc compared to that of the imipenem disc alone was considered to be positive for the for the presence of MBLs. E. coli ATCC 25922 is used as a quality control strain.

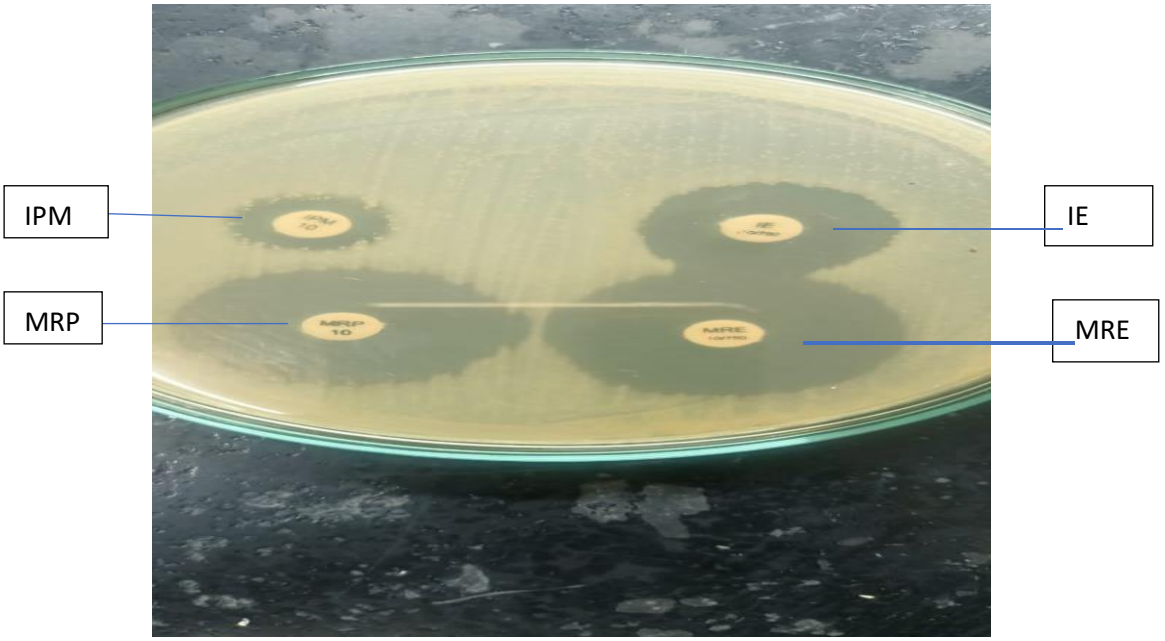


Fig no: 10 Screening test and Confirmatory test for MBL

Imipenem (IPM) = Imipenem/EDTA (IE)

Meropenem (MRP) = Meropenem/EDTA (MRE)

RESULTS

Table no : 2 Distribution of Gender

Gender	No.of patients	Percentage
FEMALE	45	43.3
MALE	59	56.7
Total	104	100.0

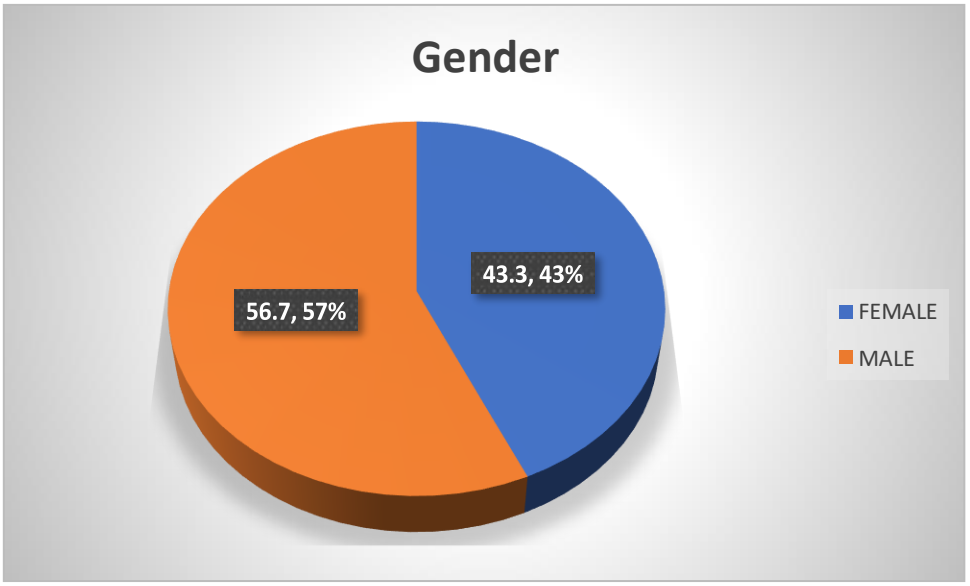


Fig no:11 Distribution of isolates according to gender

According to this report, The males were found to be more predominant 59(56.7) than female patients 45(43.3) affected by various infection of e coli

Table no: 3 Distribution of Age

< 10	7	6.7
10 - 19	10	9.6
20 - 29	12	11.5
30 - 39	9	8.7
40 - 49	7	6.7
50 - 59	21	20.2
60 - 69	25	24.0
70+	13	12.5
Total	104	100.0

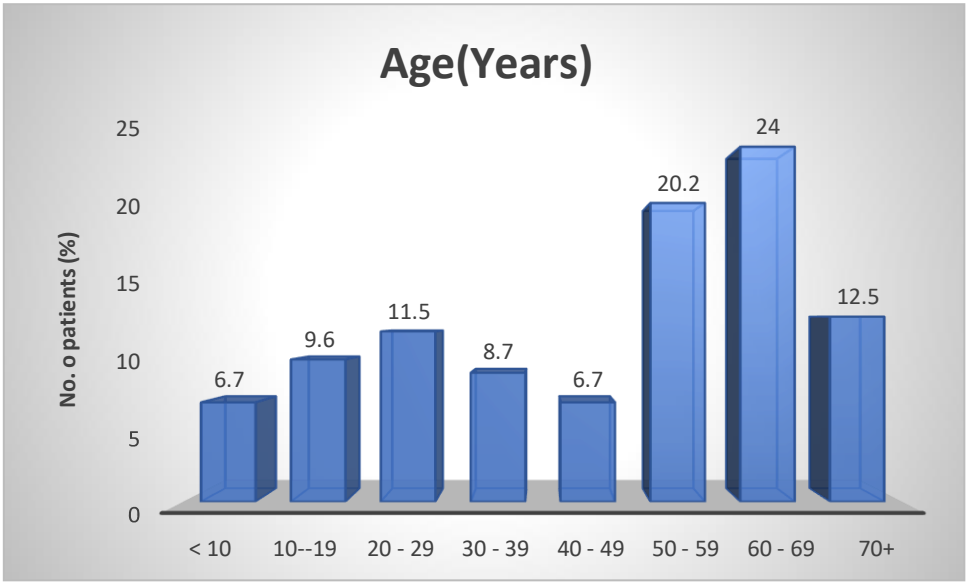


Fig no: 12 Age distribution among patients with E coli infection

Among the study population highest number of patients 104(100.0%) were infected by E coli between the age group 60-69 (24.0%) and followed by 50-59(20.2%) patients between 20-29 (11.5%), patients between 10-19 (9.6%), patients between 30-30 (8.7%) , patients between 40-49 (6.7%), patients between <10 (6.7%)

Table no: 4 Distribution of location

Patient Location	No.of Isolates	Percentage
SURGERY	44	40.3
UROLOGY	17	16.3
MEDICINE	16	15.4
OBGY	8	7.7
EMERGENCY MEDICINE	6	5.9
PAEDIATRIC	4	4.0
ICU	3	2.9
ORTHOPAEDICS	2	2.0
DERMATOLOGY	1	1.0
ENT	1	1.0
OPHTHALMOLOGY	1	1.0
RESPIRATORY MEDICINE	1	1.0
Total	104	100.0

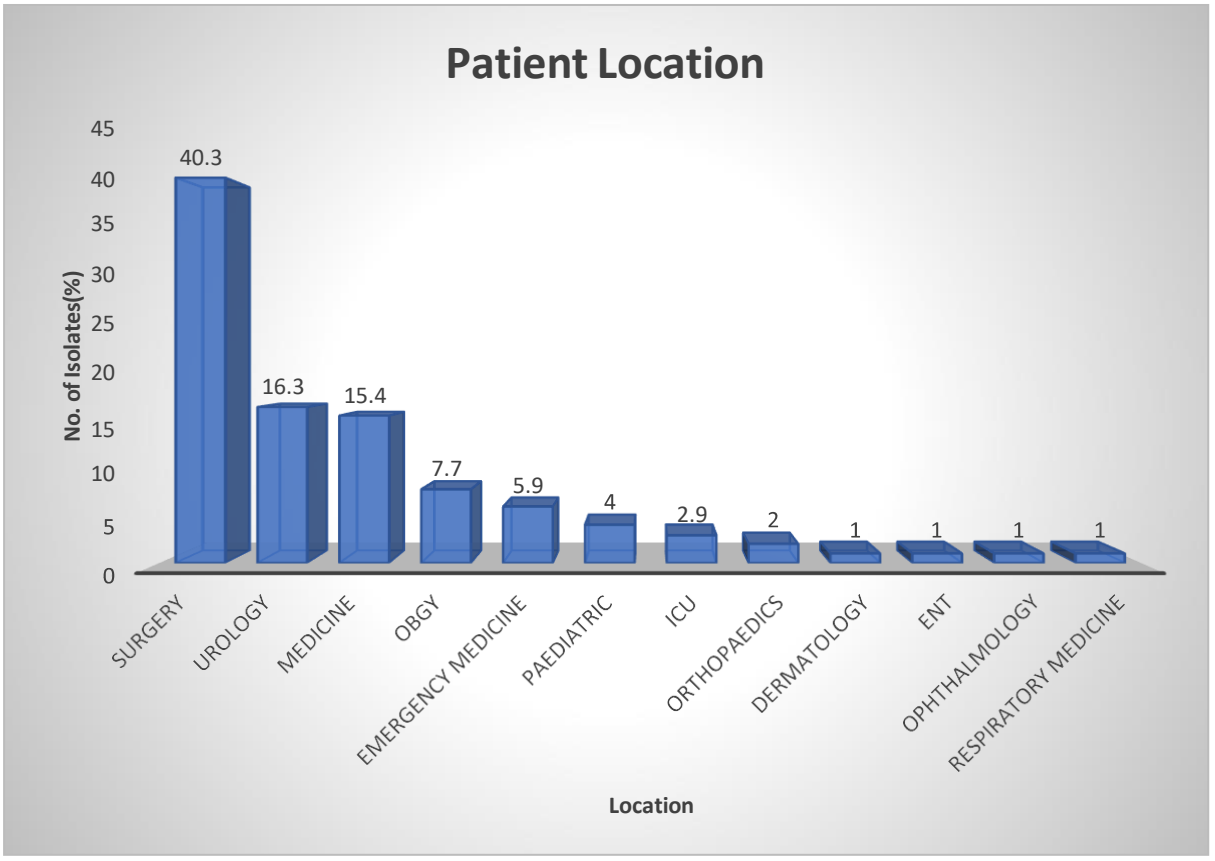


Fig no: 13 Distribution of location

Out of 130 clinical samples 104 isolates of Escherichia coli were found.the data showed that majority of Escherichia coli was found from Surgery followed by Urology, Medicine, OBGY, Emergency medicine, Paediatric, ICU, Orthopaedics,Dermatology, ENT, Opthomology, Respiratory medicine.

Table no :5 Distribution of specimens

Specimen Type	No.of Isolates	Percentage
URINE	49	47.1
PUS	44	42.3
SPUTUM	3	2.9
SWAB	2	1.9
WOUND	2	2.0
BLOOD	1	1.0
ET TUBE	1	1.0
PERIAPPENDIALAR COLECTION	1	1.0
PERITONEAL FLUID	1	1.0
Total	104	100.0

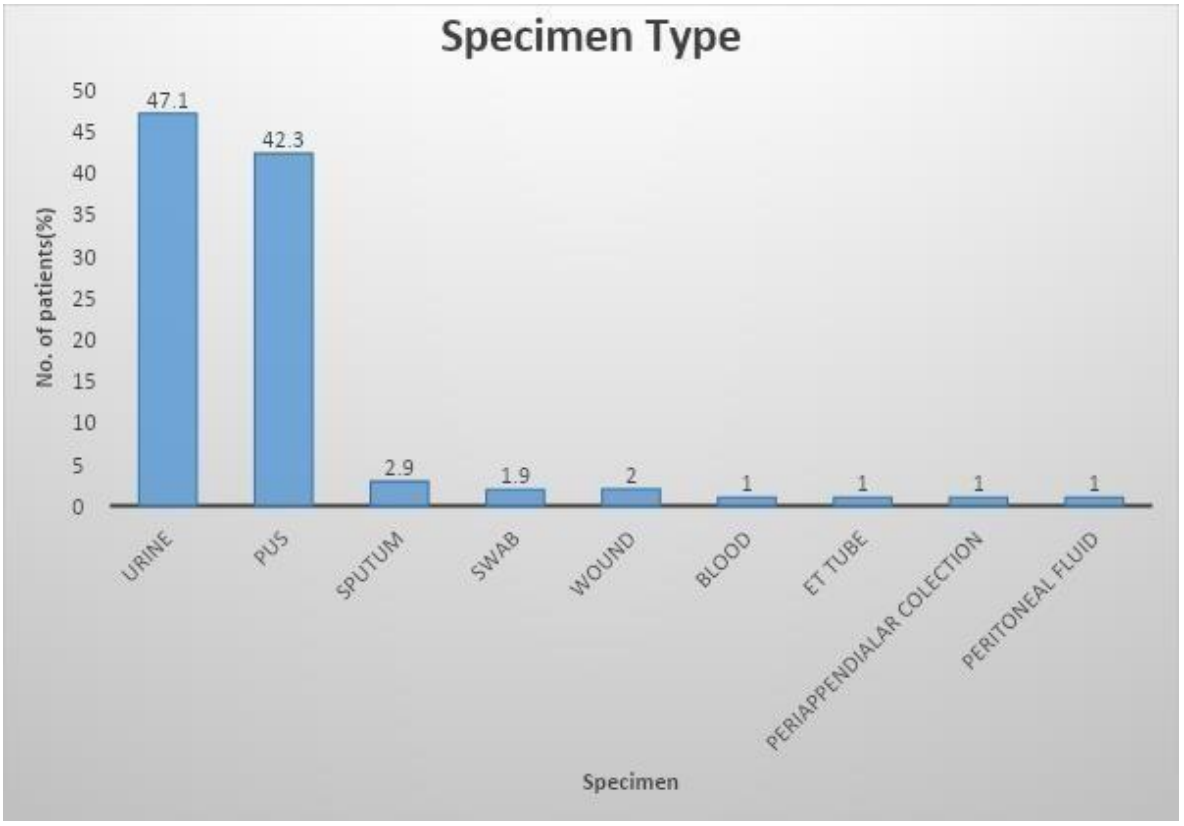


Fig no:14 Distribution of specimens

Out of 130 clinical samples 104 isolates of Escherichia coli were found.the data showed that majority of Escherichia coli was found from urine followed by pus sputum,swab,wound,Blood,Endotrachial tube,Periappendicular collection,Peritonial fluid

Table no :6 Distribution of ABST sensitive pattern

Antibiotics	Sensitive	
	No. of Isolates	%
AM-INTERPRETATION	14	13.5
AMC-INTERPRETATION	63	60.5
AN-INTERPRETATION	68	65.4
CAZ-INTERPRETATION	49	47.1
CF-INTERPRETATION	8	7.7
CFM-INTERPRETATION	16	15.4
CIP-INTERPRETATION	23	22.1
CRO-INTERPRETATION	33	31.7
CS-INTERPRETATION	104	100.0
CXM-INTERPRETATION	1	1.0
ETP-INTERPRETATION	65	62.5
FEP-INTERPRETATION	17	16.3
FOS-INTERPRETATION	49	47.1
FOX-INTERPRETATION	29	27.9
FT-INTERPRETATION	41	39.4
GM-INTERPRETATION	20	19.2
IPM-INTERPRETATION	77	74.0
MEM-INTERPRETATION	78	75.0
NA-INTERPRETATION	21	20.2
NOR-INTERPRETATION	32	30.8
OFL-INTERPRETATION	25	24.0
SFP-INTERPRETATION	56	53.8

TGC-INTERPRETATION	48	46.2
TIC-INTERPRETATION	50	48.1
TZP-INTERPRETATION	50	48.1

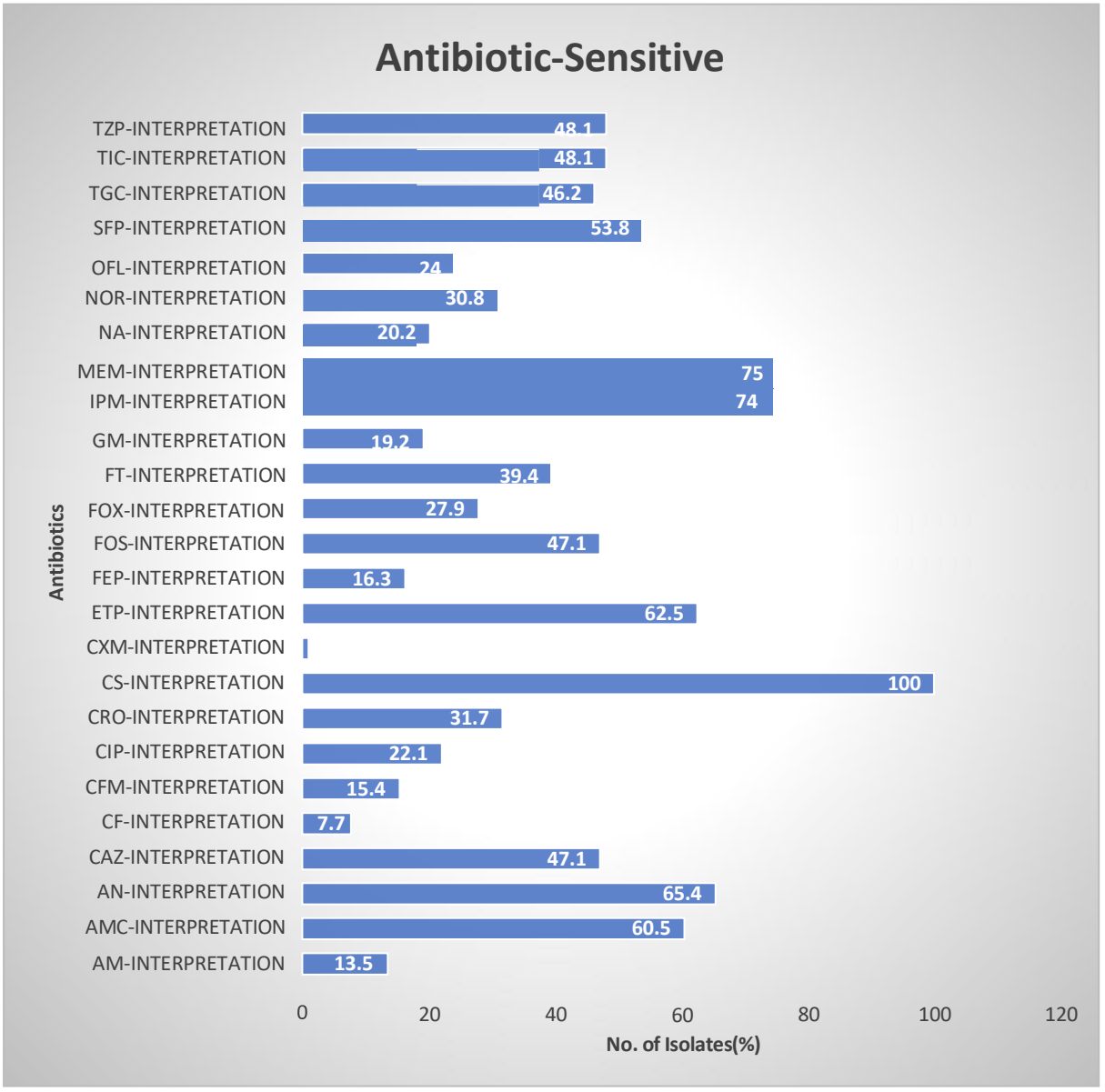


Fig no :15 Distribution of ABST sensitive pattern

Out of 104 isolates colistin showed 100% sensitive and followed by other 23 antibiotics

Table no:7 : PHENOTYOIC DETECTION OF ESBL BY SCREENING AND CONFIRMATORY TEST

INTERPRETATION	Screening test		Confirmatory test	
	CEFOTAXIME - INTERPRETATION		CEFOTAXIME/CLAVULANIC ACID INTERPRETATION	
	No.of Isolates	Percentage	No.of Isolates	Percentage
NEGATIVE	12	11.5	56	53.8
POSITIVE	92	88.5	48	46.2
Total	104	100.0	104	100.0

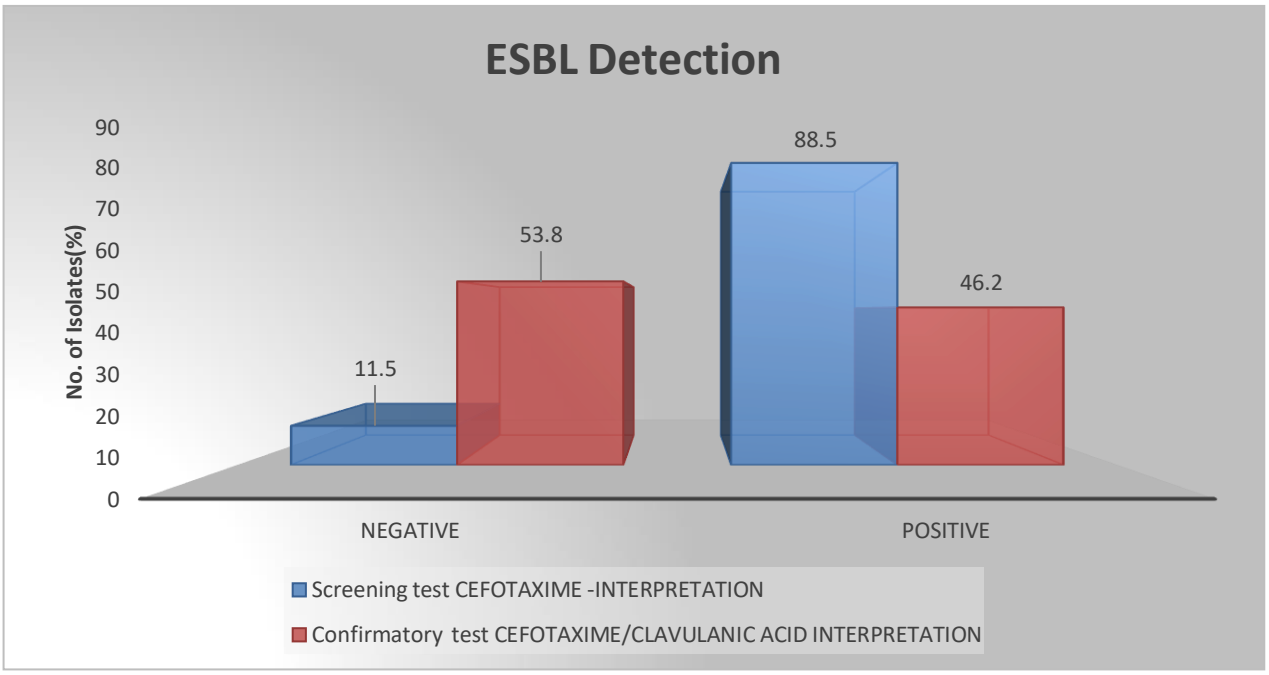


Fig no: 16 PHENOTYOIC DETECTION OF ESBL BY SCREENING AND CONFIRMATORY TEST

Phenotypic detection of ESBL:

The increase of inhibition zone 22mm around the cefotaxime(30µg) alone was interpreted as ESBL positive according to the CLSI guidelines, In this test 88.5% are positive and 11.5% are negative for screening test, similarly the increase of inhibition zone >5mm around cefotaxime(30µg)+clavulanic acid(10µg)alone was interpreted as ESBL positive according to the CLSI guidelines,In this test 80.8% are positive and 19.2% are negative for confirmatory test.

Table no:8 PHENOTYOIC DETECTION OF AmpC BY SCREENING AND CONFIRMATORY TEST

INTERPRETATION	Screening test		Confirmatory test	
	CEFOXITIN - INTERPRETATION		CEFOXITIN+CLOXACILLIN INTERPRETATION	
	No.of Isolates	Percentage	No.of Isolates	Percentage
NEGATIVE	36	34.6	91	87.5
POSITIVE	68	65.4	13	12.5
Total	104	100.0	104	100.0

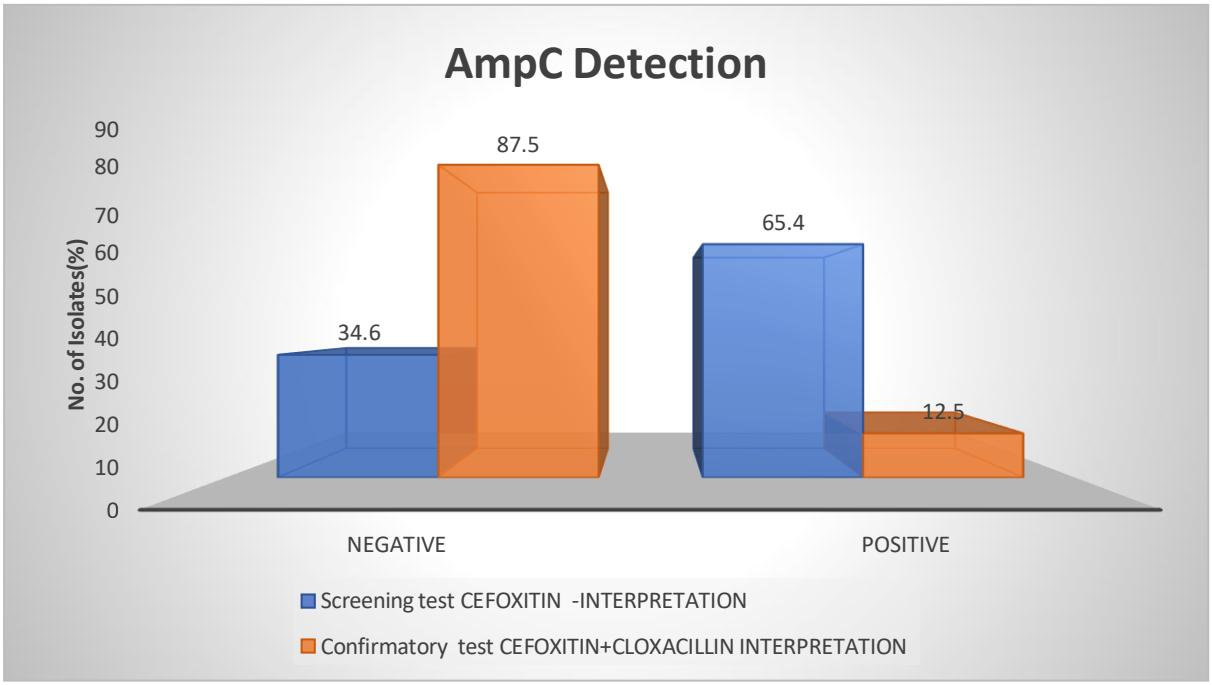


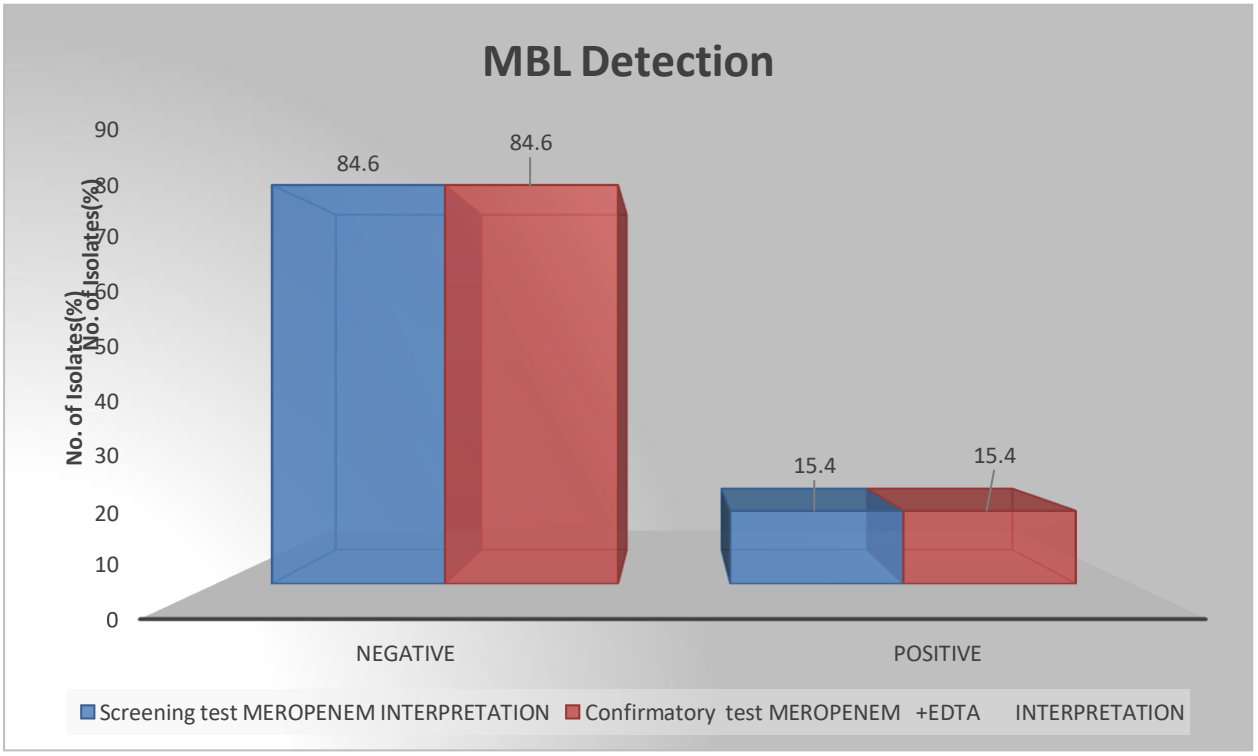
Fig no : 17 PHENOTYOIC DETECTION OF AmpC BY SCREENING AND CONFIRMATORY TEST

Phenotypic detection of AmpC:

The increase of inhibition zone <12mm around the cefoxitin(30µg) alone was interpreted as AmpC positive according to the CLSI guidelines, In this test 65.4% are positive and 34.6% are negative for screening test, similarly the increase of inhibition zone >=5mm around cefoxitin(30µg)+cloxacilin(200µg)alone was interpreted as AmpC positive according to the CLSI guidelines, In this test 12.5% are positive and 87.5% are negative for confirmatory test.

Table no:9 **PHENOTYOIC DETECTION OF MBL BY SCREENING AND CONFIRMATORY TEST**

	MEROPENEM INTERPRETATION		MEROPENEM +EDTA INTERPRETATION	
	No.of Isolates	Percentage	No.of Isolates	Percentage
NEGATIVE	88	84.6	88	84.6
POSITIVE	16	15.4	16	15.4
Total	104	100.0	104	100.0



ig no: 18 **PHENOTYOIC DETECTION OF MBL BY SCREENING AND CONFIRMATORY TEST**

The increase of inhibition zone <19mm around the Meropenem(10µg) alone was interpreted as MBL positive according to the CLSI guidelines, In this test 15.4% are positive and 84.6% are

negative for screening test, similarly the increase of inhibition zone >=7mm around

Meropenem(10µg)+EDTA (750µg)alone was interpreted as MBL positive according to the CLSI guidelines, In this test 15.4% are positive and 84.6 % are negative for confirmatory test.

The increase of inhibition zone <19mm around the Imipenem(10µg) alone was interpreted as MBL positive according to the CLSI guidelines, In this test 57.7% are positive and 42.3% are negative for screening test, similarly the increase of inhibition zone >=7mm around

Imipenem(10µg)+EDTA (750µg)alone was interpreted as MBL positive according to the CLSI guidelines, In this test 76.0% are positive and 24.0 % are negative for confirmatory test.

DISCUSSION

The discovery and development of antibiotics was undoubtedly one of the greatest advances of modern medicine. unfortunately the emergence of antibiotic resistance bacteria is threatening the effectiveness of many antimicrobial agents. And it has increases the hospital stay of the patients which in turn causes economic burden.

In the present study, an attempt was made to know the rate of ESBL, AmpC and MBL producing E coli, isolated from urine, pus, sputum, endotracheal tube, Blood and other body fluid samples and to know antibiogram at Shri B.M. Patil medical college, hospital and research centre vijayapur.

Patients with various E coli admitted or attending out patient department between march 2022 to Dec 2023 were included in the study`. various samples were collected from 104 patients with suspected E coli infections. 104 isolates(100%) yielded growth of E coli. The isolates were obtained from 59(56.7) male patients and 45(43.3) female patients.

We observed isolation of E coli from various infection more common in the age group 60-69(24.0%) followed by 50-59 (20.2%) patients between 40-49(6.7%) patients between <10(6.7%)

In the present study, rate of isolation of E coli was higher in surgery ward (40.3%), as compared to other wards and followed by urology(16.3%), medicine(15.4%), OBGY(7.7%), Emergency medicine(5.9%), Paediatric(4.0%), ICU(2.9%), Orthopaedics(2.0%),Dermatology(1.0%), ENT(1.0%0, Opthalmology(1.0%), Respiratory medicine(1.0%). The majority of the isolates are from out patient department.

In a study by M Kibret E. coli was isolated from 446 (14.2%) samples. The highest isolation rate was obtained from urine samples 203 (45.5%). High resistance rates to erythromycin (89.4%), amoxicillin (86.0%) and tetracycline (72.6%) were documented. However, significantly high degree of sensitivity rates to nitrofurantoin (96.4%), norflaxocin (90.6%), gentamicin (79.6%)

and ciprofloxacin were recorded ($p<0.001$). Multiple antimicrobial resistances of 74.6% and increased resistance rates to all antimicrobials except ciprofloxacin were also recorded.

In a present study colistin showed 100% sensitive and followed by above mentioned antibiotics as shown in the Table no:6.

In a past study by Kaiser Ahmed et al , ESBL producing E. coli. Various isolates of E. coli were obtained from patients admitted or attending Out Patient Department (OPD) over a period of 2 years from 1st August, 2005 to 31st July, 2007. In this study, 221 E. coli were subjected to screening by using cefotaxime, ceftazidime and ceftriaxone 30 g discs. Among them, 211 were screen positive for potential ESBL productions which were further subjected to confirmatory tests by phenotypic methods: Double Disc Synergy Test (DDST), Phenotypic Confirmatory Disc Diffusion Test (PCDDT) and E-test. 55.9% (118/211) of E. coli isolates were positive for ESBL production from different clinical specimens, maximum number being from urine (72.9%).The maximum number of ESBL producing isolates were from inpatients (71.2%) followed by outpatient (28.8%). Resistance pattern of ESBL positive isolates showed resistance to 3rd and 4th generation cephalosporins (97.5 to 99.2%), quinolones (93.1 to 100%) and aminoglycosides (65.2%) in that order respectively.

In a study by Mohammed Jaafaru subjected 47 E. coli isolates to the disc diffusion method to determine their ESBL production. Among them, 19 (40.4 %) E. coli isolates were found to be positive as ESBL producers To confirm the 19 ESBL-producing E. coli isolates; we used the double disc synergy technique with antibiotic discs containing cefotaxime (30 μg), amoxicillin+clavulanic acid (20 μg+10 μg) and ceftazidime (30 μg). However, out of the 19 ESBL-producing E. coli isolates identified earlier, only 16 (34.0 %) were confirmed to be positive based on the improved zone of inhibition of more than 5.0 mm observed against the antibiotic discs used.

In a present study among 104 isolates of E coli , ESBL (cefotaxime)production was determined by phenotypic method by screening and screening test was confirmed by confirmatory test using Kirby Bayer's test (standard disk diffusion test), (46.2%) are confirmed as ESBL producers, zone of inhibition should be >5mm ,According to CLSI guidelines.

In a study by Dhanashree P Inamdar ,Out of 140 isolates tested, 80(57%) were positive (resistant) for screening test by cefoxitin. Out of them 61(76.2%) were Escherichia coli, 16 (20.1%) isolates were Klebsiella pneumoniae and 3 (3.75%) were Enterobacter sp. Phenotypic confirmatory methods by Cefoxitin Cloxacillin Double disc Synergy (CC-DDS) test showed zone difference of >4mm in 38(47.5%) isolates , by Phenylboronic acid method (PBA) > 5mm zone difference was observed in 34(42.5%) isolates, similar to study done by Handa, et al. where they had 59% isolates tested positive by screening. Escherichia coli was the commonest isolate in our study followed by Klebsiella pneumoniae as most of our clinical isolates were from urine sample followed by pus. This study is similar to study done by Polfuss et al. in 2011 where Escherichia coli was the commonest isolate. Our study is in contrast to study done by Soha et al. in 2015 where Klebsiella pneumoniae were common isolates.Phenotypic confirmatory tests showed maximum detection by Cefoxitin Cloxacillin Double disc Synergy (CC-DDS) test in 38(47.5%) isolates.

In a present study among 104 isolates of E coli only (12.5%) were AmpC(cefoxitin+ Cloxacillin)producers, zone of inhibition should be >5mm.According to the CLSI guidelines.

In a study by Fahimeh et al. multi-drug resistance (MDR) and ESBL production were observed in more than 54.9, 36.2 and 11.7% of commensal *E. coli* isolates, respectively. Out of six isolates resistant to imipenem and meropenem, four isolates were phenotypically detected as MBL producers. Two and one *E. coli* strains carried the $bla_{\text{NDM-1}}$ and $bla_{\text{VIM-}}$

In study by Kulkarni et al ,Out of 393 isolates, 130 (33.07%) isolates were resistant to imipenem on screening of which 71 (18.06%) were Klebsiella pneumoniae and 59 (15.01%) were E. coli. About 43.66% Klebsiella pneumoniae isolates and 40.67% E. coli isolates were MBL-positive by the combined disc test. Using the E-test, MBL production was found to be 46.47% and 45.76% in Klebsiella pneumoniae and E. coli, respectively.[2] genes, respectively and were able to transmit imipenem resistance through conjugation.

In a present study ,Among 104 isolates of E coli (15.4%) were MBL (Meropenem) producers

According to the CLSI guidelines, zone of inhibition shoulde be >5mm.

CONCLUSION

The members of Enterobacteriaceae in this geographical region showed high multidrug resistance. A high prevalence of β-lactamases and their co-production were also found among the Enterobacteriaceae family, mainly in and E. coli isolates. The present study highlights the necessity to identify the MDR β-lactamases stains for effective therapy in severe as well as mild bacterial infections, thereby enabling to reduce the risk of MDR in Tertiary care hospital . In our present study the prevalence of ESBL was 46.2%, AmpC 12.5% and MBL 15.4% among E coli isolates Further, similarstudies in specific geographical regions may be encouraged to have a brief idea of organism-basedantibiotic susceptibility patterns and β-lactamase production for effective management and treatment regimes.

Hence Early detection of β- lactamases among E coli avoid treatment failure and spread of MDR.

BIBLIOGRAPHY

1) Martinson JN, Walk ST. Escherichia coli residency in the gut of healthy human adults. EcoSal plus. 2020 Dec 31;9(1):10-128.
2) Kaper JB, Nataro JP, Mobley HL. Pathogenic escherichia coli. Nature reviews microbiology. 2004 Feb;2(2):123-40.
3) Shaikh S, Fatima J, Shakil S, Rizvi SM, Kamal MA. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi journal of biological sciences. 2015 Jan 1;22(1):90-101.
4) Castanheira M, Simner PJ, Bradford PA. Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection. JAC-antimicrobial resistance. 2021 Sep 1;3(3):dlab092.
5) Philippon A, Arlet G, Jacoby GA. Plasmid-determined AmpC-type β-lactamases. Antimicrobial agents and chemotherapy. 2002 Jan;46(1):1-1.
6) Rodríguez-Baño J, Gutiérrez-Gutiérrez B, Machuca I, Pascual A. Treatment of infections caused by extended-spectrum-beta-lactamase-, AmpC-, and carbapenemase-producing Enterobacteriaceae. Clinical microbiology reviews. 2018 Apr;31(2):10-128.
7) Codjoe FS, Donkor ES. Carbapenem resistance: a review. Medical Sciences. 2017 Dec 21;6(1):1.
8) Queenan AM, Bush K. Carbapenemases: the versatile β-lactamases. Clinical microbiology reviews. 2007 Jul;20(3):440-58.
9) Sahuquillo-Arce JM, Hernández-Cabezas A, Yarad-Auad F, Ibáñez-Martínez E, Falomir-Salcedo P, Ruiz-Gaitán A. Carbapenemases: a worldwide threat to antimicrobial therapy. World Journal of Pharmacology. 2015 Mar 9;4(1):75-95.

10) Tan X, Kim HS, Baugh K, Huang Y, Kadiyala N, Wences M, Singh N, Wenzler E, Bulman ZP. Therapeutic options for metallo-β-lactamase-producing enterobacterales. Infection and drug resistance. 2021 Jan 18:125-42.

11) Boyd SE, Livermore DM, Hooper DC, Hope WW. Metallo-β-lactamases: structure, function, epidemiology, treatment options, and the development pipeline. Antimicrobial agents and chemotherapy. 2020 Sep 21;64(10):10-128.

12) Friedmann HC. Escherich and Escherichia. EcoSal Plus 6. doi: 10.1128/ecosalplus. ESP-0025-2013; 2014.

13) M Eltabey S, H Ibrahim A, M Zaky M, M Saleh M. Antimicrobial Susceptibility and Resistance Profile of Escherichia coli Isolates from Patients at Suez Canal University Specialized Hospital. Alfarama Journal of Basic & Applied Sciences. 2024 Jan 1;5(1):63-75.

14) Kaper JB, Nataro JP, Mobley HL. Pathogenic escherichia coli. Nature reviews microbiology. 2004 Feb;2(2):123-40.

15) Vila J, Sáez-López E, Johnson JR, Römling U, Dobrindt U, Cantón R, Giske CG, Naas T, Carattoli A, Martínez-Medina M, Bosch J. Escherichia coli: an old friend with new tidings. FEMS microbiology reviews. 2016 Jul 1;40(4):437-63.

16) Pakbin B, Brück WM, Rossen JW. Virulence factors of enteric pathogenic Escherichia coli: A review. International journal of molecular sciences. 2021 Sep 14;22(18):9922.

17) M Eltabey S, H Ibrahim A, M Zaky M, M Saleh M. Antimicrobial Susceptibility and Resistance Profile of Escherichia coli Isolates from Patients at Suez Canal University Specialized Hospital. Alfarama Journal of Basic & Applied Sciences. 2024 Jan 1;5(1):63-75.

18) Bergwerff AA, Debast SB. Modernization of control of pathogenic micro-organisms in the food-chain requires a durable role for immunoaffinity-based detection methodology—a review. Foods. 2021 Apr 11;10(4):832.

19) Compaore MK, Kpoda SD, Bazie RB, Ouedraogo M, Valian M, Gampene ML, Yakoro A, Nikiema F, Belemlougri A, Meda NS, Meda NI. Microbiological quality assessment of five common foods sold at different points of sale in Burkina-Faso. Plos one. 2022 Apr 14;17(4):e0258435.

20) Szabó S, Feier B, Capatina D, Tertis M, Cristea C, Popa A. An overview of healthcare associated infections and their detection methods caused by pathogen bacteria in Romania and Europe. Journal of clinical medicine. 2022 Jun 4;11(11):3204.

21) Pakbin B, Brück WM, Rossen JW. Virulence factors of enteric pathogenic Escherichia coli: A review. International journal of molecular sciences. 2021 Sep 14;22(18):9922.

22) Terlizzi ME, Gribaudo G, Maffei ME. UroPathogenic Escherichia coli (UPEC) infections: virulence factors, bladder responses, antibiotic, and non-antibiotic antimicrobial strategies. Frontiers in microbiology. 2017 Aug 15;8:280574.

23) Gomes TA, Elias WP, Scaletsky IC, Guth BE, Rodrigues JF, Piazza RM, Ferreira L, Martinez MB. Diarrheagenic escherichia coli. brazilian journal of microbiology. 2016;47:3-0.

24) Shao Q, Chen D, Chen S, Ru X, Ye Q. Escherichia coli Infection Sepsis: An Analysis of Specifically Expressed Genes and Clinical Indicators. Diagnostics. 2023 Nov 27;13(23):3542.

25) Kibret M, Abera B. Antimicrobial susceptibility patterns of E. coli from clinical sources in northeast Ethiopia. African health sciences. 2011;11:40-5.

26) Pandey N, Cascella M. Beta Lactam Antibiotics.[Updated 2022 Feb 5]. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. 2022.

27) Shaikh S, Fatima J, Shakil S, Rizvi SM, Kamal MA. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi journal of biological sciences. 2015 Jan 1;22(1):90-101.

28) Pakbin B, Brück WM, Rossen JW. Virulence factors of enteric pathogenic Escherichia coli: A review. International journal of molecular sciences. 2021 Sep 14;22(18):9922.

29) Kaiser KG, Delattre V, Frost VJ, Buck GW, Phu JV, Fernandez TG, Pavel IE. Nanosilver: An old antibacterial agent with great promise in the fight against antibiotic resistance. Antibiotics. 2023 Jul 31;12(8):1264.

30) Sarkar P, Yarlagadda V, Ghosh C, Haldar J. A review on cell wall synthesis inhibitors with an emphasis on glycopeptide antibiotics. Medchemcomm. 2017;8(3):516-33.

31) Omole AE, Awosika AO, Patel P. Cefuroxime. InStatPearls [Internet] 2024 Jan 11. StatPearls Publishing.

32) Mancuso G, Midiri A, Gerace E, Biondo C. Bacterial antibiotic resistance: The most critical pathogens. Pathogens. 2021 Oct 12;10(10):1310.

33) Bush K, Jacoby GA. Updated functional classification of β-lactamases. Antimicrobial agents and chemotherapy. 2010 Mar;54(3):969-76.

34) Shaikh S, Fatima J, Shakil S, Rizvi SM, Kamal MA. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi journal of biological sciences. 2015 Jan 1;22(1):90-101.

35) Shaikh S, Fatima J, Shakil S, Rizvi SM, Kamal MA. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi journal of biological sciences. 2015 Jan 1;22(1):90-101.

36) Bush K, Jacoby GA. Updated functional classification of β-lactamases. Antimicrobial agents and chemotherapy. 2010 Mar;54(3):969-76.

37) Chen Q, Li D, Beiersmann C, Neuhann F, Moazen B, Lu G, Müller O. Risk factors for antibiotic resistance development in healthcare settings in China: a systematic review. Epidemiology & Infection. 2021 Jan;149:e141.

38) Bradford PA. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clinical microbiology reviews. 2001 Oct 1;14(4):933-51.

39) Liakopoulos A, Mevius D, Ceccarelli D. A review of SHV extended-spectrum β-lactamases: neglected yet ubiquitous. Frontiers in microbiology. 2016 Sep 5;7:219996.

40) Cantón R, González-Alba JM, Galán JC. CTX-M enzymes: origin and diffusion. Frontiers in microbiology. 2012 Apr 2;3:20597.

41) Evans BA, Amyes SG. OXA β-lactamases. Clinical microbiology reviews. 2014 Apr;27(2):241-63.

42) Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. Clinical microbiology reviews. 2005 Oct;18(4):657-86.

43) Sawa T, Kooguchi K, Moriyama K. Molecular diversity of extended-spectrum β-lactamases and carbapenemases, and antimicrobial resistance. Journal of intensive care. 2020 Jan 28;8(1):13.

44) Pana ZD, Zaoutis T. Treatment of extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBLs) infections: what have we learned until now?. F1000Research. 2018;7.

45) Castanheira M, Simner PJ, Bradford PA. Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection. JAC-antimicrobial resistance. 2021 Sep 1;3(3):dlab092.

46) Husna A, Rahman MM, Badruzzaman AT, Sikder MH, Islam MR, Rahman MT, Alam J, Ashour HM. Extended-Spectrum β-Lactamases (ESBL): Challenges and Opportunities. Biomedicines. 2023 Oct 30;11(11):2937.

47) Bradford PA. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clinical microbiology reviews. 2001 Oct 1;14(4):933-51.

48) Kothari A, Kumar S, Omar BJ, Kiran K. Detection of extended-spectrum beta-lactamase (ESBL) production by disc diffusion method among Pseudomonas species from various clinical samples. Journal of family medicine and primary care. 2020 Feb 1;9(2):683-93.

49) Reddy P, Malczynski M, Obias A, Reiner S, Jin N, Huang J, Noskin GA, Zembower T. Screening for extended-spectrum β-lactamase-producing Enterobacteriaceae among high-risk patients and rates of subsequent bacteremia. Clinical infectious diseases. 2007 Oct 1;45(7):846-52.

50) Iqbal R, Ikram N, Shoaib M, Muhammad JA, Raja TM, Abid AN, Aanam A, Bushra I, Faiza N. Phenotypic cofirmatory disc diffusion test (PCDDT), double disc synergy test (DDST), E-test OS diagnostic tool for detection of extended spectrum beta lactamase (ESBL) producing Uropathogens. J Appl Biotechnol Bioeng. 2017;3(3):344-9.

51) Reddy P, Malczynski M, Obias A, Reiner S, Jin N, Huang J, Noskin GA, Zembower T. Screening for extended-spectrum β-lactamase-producing Enterobacteriaceae among high-risk patients and rates of subsequent bacteremia. Clinical infectious diseases. 2007 Oct 1;45(7):846-52.

52) Linscott AJ, Brown WJ. Evaluation of four commercially available extended-spectrum beta-lactamase phenotypic confirmation tests. Journal of clinical microbiology. 2005 Mar;43(3):1081-5.

53) Poulou A, Grivakou E, Vrioni G, Koumaki V, Pittaras T, Pournaras S, Tsakris A. Modified CLSI extended-spectrum β-lactamase (ESBL) confirmatory test for phenotypic detection of ESBLs among Enterobacteriaceae producing various β-lactamases. Journal of clinical microbiology. 2014 May;52(5):1483-9.

54) Cormican MG, Marshall SA, Jones RN. Detection of extended-spectrum beta-lactamase (ESBL)-producing strains by the Etest ESBL screen. Journal of Clinical Microbiology. 1996 Aug;34(8):1880-4.

55) Rawat D, Nair D. Extended-spectrum β-lactamases in Gram Negative Bacteria. Journal of global infectious diseases. 2010 Sep 1;2(3):263-74.

56) Chon JW, Kim HS, Kim DH, Kim H, Choi IS, Oh DH, Seo KH. Modification of Karmali agar by supplementation with potassium clavulanate for the isolation of Campylobacter from chicken carcass rinses. Journal of food protection. 2014 Jul 1;77(7):1207-11.

57) Kader AA, Kumar A, Krishna A, Zaman MN. An accelerated method for the detection of extended-spectrum β-lactamases in urinary isolates of Escherichia coli and Klebsiella pneumoniae. Saudi Journal of Kidney Diseases and Transplantation. 2006 Oct 1;17(4):535-9.

58) Kaur J, Chopra S, Mahajan G. Modified double disc synergy test to detect ESBL production in urinary isolates of Escherichia coli and Klebsiella pneumoniae. Journal of clinical and diagnostic research: JCDR. 2013 Feb;7(2):229.

59) Cortés-Cortés G, Arenas-Hernández MM, Ballesteros-Monrreal MG, Rocha-Gracia RD, Barrios-Villa E. Epidemiology of antimicrobial resistance and virulence factors of emerging and re-emerging bacteria. Frontiers in Cellular and Infection Microbiology. 2024 Mar 6;14:1387087.

60) Pana ZD, Zaoutis T. Treatment of extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBLs) infections: what have we learned until now?. F1000Research. 2018;7.

61) Jacoby GA. AmpC β-lactamases. Clinical microbiology reviews. 2009 Jan;22(1):161-82.

62) Sanders CC, Bradford PA, Ehrhardt AF, Bush K, Young KD, Henderson TA, Sanders Jr WE. Penicillin-binding proteins and induction of AmpC beta-lactamase. Antimicrobial agents and chemotherapy. 1997 Sep;41(9):2013-5.

63) Alcock BP, Huynh W, Chalil R, Smith KW, Raphenya AR, Wlodarski MA, Edalatmand A, Petkau A, Syed SA, Tsang KK, Baker SJ. CARD 2023: expanded curation, support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance Database. Nucleic acids research. 2023 Jan 6;51(D1):D690-9.

64) Esposito C, Kamper M, Trentacoste J, Galvin S, Pfister H, Wang J. Advances in the cystic fibrosis drug development pipeline. Life. 2023 Aug 30;13(9):1835.

65) Jacoby GA. AmpC β-lactamases. Clinical microbiology reviews. 2009 Jan;22(1):161-82.

66) Black JA, Moland ES, Thomson KS. AmpC disk test for detection of plasmid-mediated AmpC β-lactamases in Enterobacteriaceae lacking chromosomal AmpC β-lactamases. Journal of clinical microbiology. 2005 Jul;43(7):3110-3.

67) Coudron PE, Moland ES, Thomson KS. Occurrence and detection of AmpC beta-lactamases among Escherichia coli, Klebsiella pneumoniae, and Proteus mirabilis isolates at a veterans medical center. Journal of clinical microbiology. 2000 May 1;38(5):1791-6.

68) Amjad A, Mirza IA, Abbasi SA, Farwa U, Malik N, Zia FJ. Modified Hodge test: A simple and effective test for detection of carbapenemase production. Iranian journal of microbiology. 2011 Dec;3(4):189.

69) Gupta G, Tak V, Mathur P. Detection of AmpC β lactamases in gram-negative bacteria. Journal of laboratory physicians. 2014 Jan;6(01):001-6.

70) Peter-Getzlaff S, Polsfuss S, Poledica M, Hombach M, Giger J, Böttger EC, Zbinden R, Bloemberg GV. Detection of AmpC beta-lactamase in Escherichia coli: comparison of three phenotypic confirmation assays and genetic analysis. Journal of clinical microbiology. 2011 Aug;49(8):2924-32.

71) Jacoby GA. AmpC β-lactamases. Clinical microbiology reviews. 2009 Jan;22(1):161-82.

72) Meini MR, Llarrull LI, Vila AJ. Evolution of metallo-β-lactamases: trends revealed by natural diversity and in vitro evolution. Antibiotics. 2014 Sep;3(3):285-316.

73) Meletis G. Carbapenem resistance: overview of the problem and future perspectives. Therapeutic advances in infectious disease. 2016 Feb;3(1):15-21.

74) Boyd SE, Livermore DM, Hooper DC, Hope WW. Metallo-β-lactamases: structure, function, epidemiology, treatment options, and the development pipeline. Antimicrobial agents and chemotherapy. 2020 Sep 21;64(10):10-128.

75) Boyd SE, Livermore DM, Hooper DC, Hope WW. Metallo-β-lactamases: structure, function, epidemiology, treatment options, and the development pipeline. Antimicrobial agents and chemotherapy. 2020 Sep 21;64(10):10-128.

76) Picao RC, Andrade SS, Nicoletti AG, Campana EH, Moraes GC, Mendes RE, Gales AC. Metallo-β-lactamase detection: comparative evaluation of double-disk synergy versus combined disk tests for IMP-, GIM-, SIM-, SPM-, or VIM-producing isolates. Journal of clinical microbiology. 2008 Jun;46(6):2028-37.

77) Amjad A, Mirza IA, Abbasi SA, Farwa U, Malik N, Zia FJ. Modified Hodge test: A simple and effective test for detection of carbapenemase production. Iranian journal of microbiology. 2011 Dec;3(4):189.

78) Timsit JF, Wicky PH, de Montmollin E. Treatment of severe infections due to metallo-betalactamases enterobacterales in critically ill patients. Antibiotics. 2022 Jan 24;11(2):144.

79) Kibret M, Abera B. Antimicrobial susceptibility patterns of E. coli from clinical sources in northeast Ethiopia. African health sciences. 2011;11:40-5.

80) Ahmed K, Thokar MA, Toboli AS, Fomda BA, Bashir G, Maroof P. Extended spectrum-β-lactamase mediated resistance in Escherichia coli in a tertiary care hospital in Kashmir, India. Afr J Microbiol Res. 2010 Dec 18;4(24):2720-8.

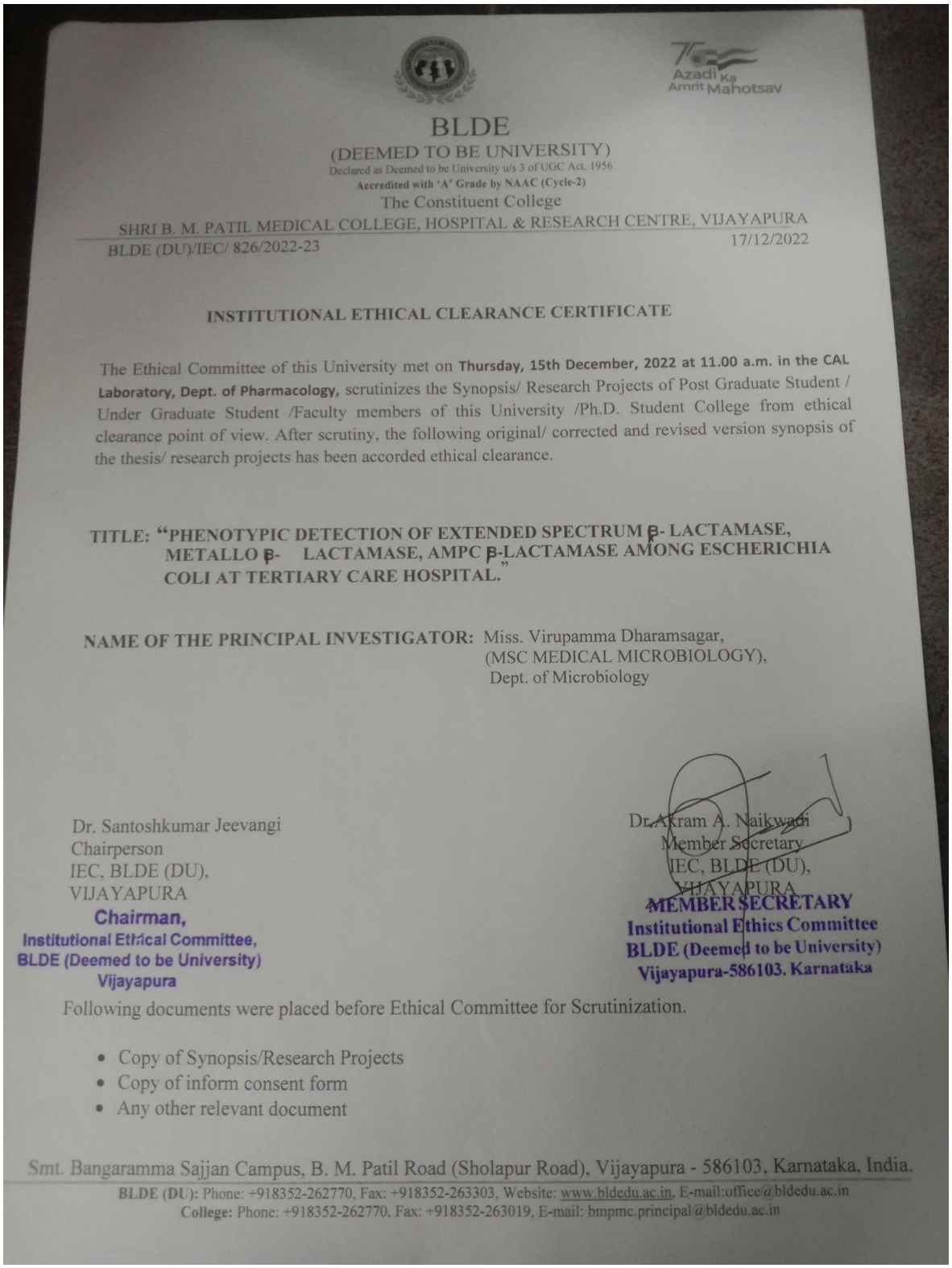
81) Ja'afaru M, Gaure A, Ewansiha J, Adeyemo O. Prevalence of Extended Spectrum β-Lactamases-Producing Escherichia coli Isolated from Clinical Samples and Their Antibiotic Resistance Pattern. Journal of Medical Microbiology and Infectious Diseases. 2023 Mar 10;11(1):34-40.

82) Inamdar DP, Anuradha B. Seroprevalence of transfusion transmissible infections among blood donors at tertiary care hospital in southern Telangana-A 3 year cross sectional study. Indian J Microbiol Res. 2018 Oct;5(4):446-50.

83) Zeighami H, Haghi F, Hajiahmadi F, Kashefiyeh M, Memariani M. Multi-drug-resistant enterotoxigenic and enterohemorrhagic Escherichia coli isolated from children with diarrhea. Journal of Chemotherapy. 2015 Jun 1;27(3):152-5.

84) Kulkarni SS, Mulay MV. Phenotypic detection of metallo-beta-lactamase production in clinical isolates of Escherichia coli and Klebsiella pneumoniae in a tertiary care hospital. MGM Journal of Medical Sciences. 2022 Apr 1;9(2):149-53.

ANNEXURE – I INSTITUTIONAL ETHICAL CERTIFICATE



BLDE
(DEEMED TO BE UNIVERSITY)
Declared as Deemed to be University u/s 3 of UGC Act, 1956
Accredited with 'A' Grade by NAAC (Cycle-2)
The Constituent College
SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

BLDE (DU)/IEC/ 826/2022-23 17/12/2022

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this University met on **Thursday, 15th December, 2022 at 11.00 a.m. in the CAL Laboratory, Dept. of Pharmacology,** scrutinizes the Synopsis/ Research Projects of Post Graduate Student / Under Graduate Student /Faculty members of this University /Ph.D. Student College from ethical clearance point of view. After scrutiny, the following original/ corrected and revised version synopsis of the thesis/ research projects has been accorded ethical clearance.

TITLE: "PHENOTYPIC DETECTION OF EXTENDED SPECTRUM β- LACTAMASE, METALLO β- LACTAMASE, AMPC β-LACTAMASE AMONG ESCHERICHIA COLI AT TERTIARY CARE HOSPITAL."

NAME OF THE PRINCIPAL INVESTIGATOR: Miss. Virupamma Dharamsagar,
(MSC MEDICAL MICROBIOLOGY),
Dept. of Microbiology

Dr. Santoshkumar Jeevangi
Chairperson
IEC, BLDE (DU),
VIJAYAPURA
Chairman,
Institutional Ethical Committee,
BLDE (Deemed to be University)
Vijayapura

Dr. Akram A. Naikwadi
Member Secretary
IEC, BLDE (DU),
VIJAYAPURA
MEMBER SECRETARY
Institutional Ethics Committee
BLDE (Deemed to be University)
Vijayapura-586103. Karnataka

Following documents were placed before Ethical Committee for Scrutinization.

- Copy of Synopsis/Research Projects
- Copy of inform consent form
- Any other relevant document

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ANNEXURE – II

SCHEME OF CASE TAKING:

Name:

Age:

Sex:

Occupation:

Residence:

Contact no:

OPD/IP NO:

Lab No:

Other clinical history;

1. Clinical history:

2. Previous Treatment history :- History of any previous visit or admitted. Earlier medication history or any current medications.

If the patient is involved in any surgery.

If the patient has a history of a long stay in the hospital.

3. Laboratory Diagnosis : From all Clinical isolates we obtain E. Coli and observe ABST by standard Antibiotic disks used as follows.

ANNEXURE – III

INFORMED CONSENT FORM

GUIDE: DR. Smitha Bagali MD

PG STUDENT: Virupamma Dharmasagar [MSC MEDICAL MICROBIOLOGY]

Purpose Of Research:

I have been informed that this study is bacteriological based and for studying the antibiogram of the study organism. This study was carried out in a tertiary care center in BLDE Hospital Vijayapura. I have been given free choice for participation in this study. The study will help in giving appropriate treatment to the patient, and this will enhance better patient management.

PROCEDURE: I understand that I will undergo a detailed history, after which necessary investigations will be done.

Risk And Discomforts:

I understand that I may experience some discomfort during the sampling procedure. The procedures of this study are not expected to exaggerate those feelings which are associated with the usual course of study.

Benefits:

I understand that my participation in the study as one of the study subjects will help the researcherto identify antibiotic resistance and the prevalence of serotype. The study will have more indirect benefits to me than the potential benefits of the study for choosing appropriate antibiotic management.

I have explained to Mr./Mrs._____the purpose of the research, the procedures required and the procedures required possible risk factors to the best of my ability.

_____

Miss. Virupamma Dharmasagar DATE:

(investigator)