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Extended Spectrum β-lactamase Producing *Klebsiella a*n Emerging Threat to Human Health, Epidemiology and Detection Methods: A Review

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A B S T R A C T

Klebsiella species are opportunistic pathogens responsible for a wide range of hospital and community-acquired infections, including pneumonia, urinary tract infections, septicemia, and liver abscesses. The global emergence of multidrug-resistant (MDR) *Klebsiella*, particularly those producing extended-spectrum β-lactamases (ESBLs), poses a significant public health challenge. ESBLs hydrolyze β-lactam antibiotics, rendering extended-spectrum cephalosporins and monobactams ineffective. The widespread use and misuse of antibiotics in human medicine and agriculture have accelerated the development and dissemination of resistance, facilitated by horizontal gene transfer through plasmids, transposons, and integrons. Among ESBLs, TEM, SHV, and CTX-M types are most prevalent, with CTX-M-15 emerging as a dominant variant, especially in Asia, including India. *Klebsiella* species are the primary reservoirs of ESBL genes and are frequently implicated in nosocomial outbreaks. Accurate detection and molecular characterization of these strains are essential for epidemiological surveillance and outbreak control. Molecular typing techniques such as SDS-PAGE and RAPD are valuable tools for studying clonal relationships and resistance gene dissemination. Despite the high prevalence of ESBL-producing *Klebsiella* in India, limited data exist for regions of Maharashtra. This study was undertaken to determine the phenotypic prevalence and molecular epidemiology of ESBL-producing *Klebsiella* species in these regions, establishing a baseline for resistance surveillance and control strategies.

Introduction

Klebsiella species are opportunistic pathogens that cause hospital and community acquired infections such as pneumonia, urinary tract infection, septicemia, soft tissue infections, liver abscess, burn wound infections and meningitis. In fact nosocomial infections associated with *Klebsiella* species have shown an increase in most part of

the world (Fung *et al.,* 2002). Multidrug-resistant strains possessing extended-spectrum β-lactamases (ESBLs) has become an increasing problem worldwide. The overuse and in some cases, misuse of antibiotics in humans and in animal husbandry has been cited as a responsible factor in the development of drug resistance in all bacterial species. The advancing age, hospitals cross infection, the food chain trade and human migrations have contributed to increase the risk for community-acquired ESBL (Brisse *et al.,* 2006).

Beta-lactam antibiotics are commonly used to treat bacterial infections. This group of antibiotics includes penicillins, cephalosporins, carbapenems and monobactams. With the high and improper use of antibiotics, particularly the third generation or extended spectrum cephalosporins, leads to the development of bacterial resistance mediated by β-Lactamases which subsequently led to the development of ESBL producing bacteria. Hence these new β-Lactamases were coined extended spectrum β-Lactamases. ESBLs are enzymes that mediate resistance to extended spectrum e.g., third generation cephalosporins as well as monobactams such as aztreonam (CLSI, 2010). These enzymes catalyze the hydrolysis of the β-lactam ring of antibiotic, thereby destroying the antimicrobial activity. ESBLs have been reported worldwide in many different genera of *Enterobactericeae* and *Pseudomonas aeroginosa* (Friedman *et al.,* 2005).

However ESBLs are more prevalent in *Klebsiella* species than any other enterobacterial species and outbreaks of infection caused by ESBL producing *Klebsiella* species have been widely reported (Duman *et al.,* 2005). ESBL producing organisms are often resistant to several other classes of antibiotics, as the plasmids with the gene encoding ESBLs often carry other resistance determinants. Initially ESBL producing organisms were isolated from nosocomial infections but these organisms are now also being isolated from community. The colonization rate for *Klebsiella*is low in healthy individuals in the general population. But it is increased in hospitalized patients especially with long care facilities, health care manipulations like use of catheters (Yusha'u *et al.,* 2010).

TEM-1 was the first plasmid mediated β-Lactamase reported in Gram-negative bacteria and described in the early 1960s. Afterwards it was detected from *Klebsiella* in Europe 1980, in Germany 1983, and in France 1985 (Perez *et al.,* 2007). At the same time another plasmid mediated β-Lactamase SHV-1 was found in *K. pneumoniae* and *E.coli* (Paterson and Bonomo, 2005). Genetic control of β-Lactamase production resides either on plasmids or on the chromosome are often in association with integrons, while expression is either constitutive or inducible (Chiang and Liaw, 2005). The development of extended spectrum cephalosporins in the early 1980s was regarded as a major addition to our therapeutic problem in the fight against β-Lactamase mediated bacterial resistance. The emergence of *K. pneumoniae* resistant to ceftazidime and other cephalosporins (CTX-M) seriously compromised the efficacy of these life saving antibiotics (Perez *et al.,* 2007).

ESBLs are inhibited *in-vitro* by β-Lactamase inhibitors such as clavulanic acid and tazobactam. Some ESBLs are derived from earlier, broad-spectrum β-Lactamases (e.g., TEM, SHV and OXA enzyme families) and differ from the parent enzyme by a few point mutations, which confer an extended spectrum of activity. More recently another family of ESBLs, the CTX-M types, has emerged and these ESBLs are becoming increasingly common (Hawkey, 2008).

The true incidences of ESBL'sis difficult to determine because of the difficulty in detecting ESBL production and due to inconsistencies in testing & reporting (Yusha'u *et al.,* 2010). The prevalence of ESBLs in Europe is higher than in the USA but lower than in Asia and South America. In Asia pacific region plasmid borne ESBLs were reported 62% and 75% in *E.coli* and *Klebsiella* species, respectively (Bell *et al.,* 2007). However, In India, in 2006 CTX-M-15 was found 73% in *E.coli* and 72% in *Klebsiella* species (Sharma *et al.,* 2010).

Klebsiella species are genetically heterogeneous and strains within species and subspecies can be discriminated by number of methods including biotyping, antibiogram, phage typing, bacteriocins typing and molecular typing methods (Brisse *et al.,* 2006). From the epidemiological point of view, it is often necessary to determine the clonality of the strains.

This is particularly important in endemic and epidemic nosocomial outbreaks of *Klebsiella* infection to improve the management of such out breaks (Poduschun and Ullmann, 1998). Molecular typing methods, as applied to the genus *Klebsiella*, have increasingly become an integral part of both clinical and research microbiology

laboratories. Genotyping methods are divided into two methods; protein based methods and nucleic acid based methods. SDS-PAGE (Sodium dodecyl sulphate polyacrylamide gel electrophoresis) method has been used to subtype *Klebsiella* (Costas *et al.,* 1990). They compared SDS-PAGE with capsular serotyping and concluded that SDS-PAGE protein profiles could be used as an effective method. RAPD (Randomly amplified polymorphic DNA) technique is powerful tool for genetic studies.

This technique utilizes low-stringency PCR amplification with single primers of arbitrary sequence to generate strain-specific arrays of anonymous DNA fragments. RAPD is very helpful for rapid local investigation of the sources and routes of local spread and applicable to study the *Klebsiella* epidemiology (Tribuddharat *et al.,* 2008).

Proper use of antibiotics is very important for various reasons. It reduces unnecessary expenses, reduces development of resistance to useful and life saving antibiotics, and minimizes many side effects. Proper use of antibiotics is ensured by formulating an antibiotic policy. ESBL screening as a routine test has not yet been practiced. ESBL occurs at an alarming rate among *Klebsiella* isolates among the hospitalized patients which can result in an outbreak in the community that may be difficult to treat, Hence it becomes more important to study the general characteristics, taxonomy, virulent factor and detection methods of ESBL,s very crucial which provided a base line survey for drug resistance pattern and the molecular epidemiology and similarity of ESBL producing strains.

The genus *Klebsiella*

General characteristics: *Klebsiella* is a genus of Gram-negative bacteria characterized by its lack of motility and spores. It is oxidase-negative and capable of fermenting lactose. A defining trait of *Klebsiella* is its thick, polysaccharide capsule, which gives its colonies a shiny, mucoid appearance when grown on agar. The bacterial cells are rod-shaped, typically measuring between 0.3–1 µm in width and 0.6–6 µm in length, and may be observed as single cells, in pairs, or arranged in short chains. These organisms are facultative anaerobes, forming large, dome-shaped, and sticky colonies. When cultured on MacConkey agar, the colonies appear red with a spreading pigment, indicating glucose fermentation accompanied by acid production. *Klebsiella* species are commonly found in various environments, including as part of the normal intestinal microbiota in humans and animals, and as free-living organisms in soil, water, and plant material (Brooks, 2007).

Taxonomy: *Klebsiella* is well known as a causative agent of community-acquired pneumonia. The genus was named in honor of Edwin Klebs, a German microbiologist of the 19th century. The organism was first described by Friedländer in 1882, following its isolation from a fatal case of pneumonia. Later, Trevisan formally named the genus *Klebsiella*, placing it within the family *Enterobacteriaceae*. Initially, the genus was divided into three main species based on biochemical characteristics and clinical significance: *Klebsiella pneumoniae*, *Klebsiella ozaenae*, and *Klebsiella rhinoscleromatis*, each linked to specific diseases. However, DNA-DNA hybridization studies later demonstrated that *K. ozaenae* and *K. rhinoscleromatis* are better classified as subspecies of *K. pneumoniae* (Hansen *et al.,* 2004). *Klebsiella oxytoca* was originally isolated from spoiled milk and later identified as a separate species distinct from *K. pneumoniae* (Jain *et al.,* 2007). Likewise, *Klebsiella planticola* and *Klebsiella trevisanii* were reclassified as a single species—*K. planticola*—based on DNA sequence homology. Notably, both have recently been isolated from human clinical samples (Brisse *et al.,* 2006). Due to their close genetic relationships with *Klebsiella* species, *Enterobacter aerogenes* and *Calymmatobacteriumgranulomatis* were reclassified as *Klebsiella mobilis* and *Klebsiella granulomatis*, respectively (Bennett *et al.,* 2001). Additionally, the species *K. terrigena*, *K. planticola*, and *K. ornithinolytica* were recently transferred to a newly established genus, *Raoultella* (Drancourt *et al.,* 2001).

Pathogenesis and clinical importance: The *K. pneumoniae* are medically most important species of the genus *Klebsiella* followed by *K oxytoca* and *K. rhinoscleromatis* have also been demonstrated in human clinical specimens. Extended-spectrum β-lactamases producing Gram-negative bacteria have been associated with increased mortality, length of hospitalization and hospital costs (Schwaber *et al.,* 2006). In humans, *Klebsiella* species may colonize the skin, pharynx, or gastrointestinal tract. They may also colonize sterile wounds, urine and may be regarded as normal flora in many parts of the colon, intestinal and biliary tract (Brisse *et al.,* 2006). *Klebsiella* species are rarely found on human skin as do not find good growth condition and regarded as transient members of the flora.

Community-acquired pneumonia is a very severe fatal illness with a rapid onset of high fever, haemoptysis, bulging interlobar fissure and cavitary abscesses. *K. pneumonia* caused primary liver abscess (PLA) is an important emerging infection. This syndrome is characterized by high mortality and in some cases causes PLA with sepsis and bacteremia and complicated with meningitis or endophthalmitis (Fang *et al.,* 2005). Schelenz *et al.,* (2007) reported few cases of acute septic arthritisdue to extended-spectrum β-Lactamase (ESBL) producing *K. pneumoniae.* Moreover, *K. oxytoca* considered as an opportunistic infection in laboratory rodents is among the top 4 pathogens that cause infection among patients in neonatal intensive care units (Bleich *et al.,* 2008). Ozena, a chronic atrophic rhinitis caused by *K. pneumoniae* subsp. *Ozaenae* characterized by necrosis of the mucosa and mucopurulent nasal discharge (Strampfer *et al.,* 1987). *K. pneumoniae* subsp. *Ozaenae* may cause invasive infections, especially in immunosuppressed hosts such as bacteraemia with or without meningitis, otitis, mastoiditis, urinary tract infections, wound infections, corneal ulcers, pneumonia, or brain abscess (Brisse *et al.,* 2006).

Pathogenicity factors of *Klebsiella:* The pathogenic mechanism of *Klebsiella* infection involves a number of bacterial factors that contribute to the pathogenesis of these bacteria. The pathogenecity of *Klebsiella* involves the group of five factors.

A. Capsular Antigens: Capsule polysaccharide (CPS) is recognized as one of the most important virulence factors of *Klebsiella*spp. The capsular repeating subunits are consisting of four to six sugars and very often uronicacids (Brisse *et al.,* 2006). The K2 capsular polysaccharide has been reported to contain glucose, mannose, and N-acetyl-glucuronic acid (Arakawa *et al.,* 1991). The capsular material forms thick bundles of fibrillose structures covering the bacterial surface in massive layers to protect the bacterium from phagocytosis by macrophage, and prevents killing of the bacteria by bactericidal serum factors (Lin *et al.,* 2004).

Capsular serotypes K1 and K2 are considered to be the predominant virulent strains of *K. pneumoniae*. Capsular type K1 is most frequently associated with acute pneumonia, but K2, K3, K4, K5, and K6 can also be involved (Brisse *et al.,* 2006). K1 has been further investigated as the most common serotype isolated from patients with *K. pneumoniae* liver abscess and endophthalmitis (Chuang *et al.,* 2006).

B.Pili (Fimbriae): Pili are nonflagellar, filamentous projections on the bacterial surface. These structures are up to 10 mm long and have a diameter of 1 to 11 nm; they consist of polymeric globular protein subunits with a molecular mass of 15 to 26 kDa (Podschun and Ullmann, 1998). Adhesion to mucosal and epithelial cell surfaces is often the first step in the development of colonization and infection. The adhesive properties in the *Klebsiella* are generally mediated by different types of pili. More than 80% of clinical isolates of *K. pneumoniae*, but few *K. oxytoca* strains, express type-1 fimbriae. Strains of *K.pneumoniae* commonly express three types of pili known as types 1 (common pili), 3and 6.

Type 1 fimbriae are well studied and found in the most of enterobacterial species. A type 1fimbriae are an important virulence factor in *K. pneumoniae* causing urinary and respiratory tract infection (Podschun and Ullmann, 1998). Type 3 fimbriae are characterized by their ability to agglutinate erythrocytes treated with tannic acid. Furthermore, type 3 fimbriae are also believed to contribute to the formation of extended extracellular structures known as biofilms. Biofilms serve as structural anchors, barriers to contact with host defences and as impediments to antibiotics (Bortz *et al.,* 2008).

C. Lipopolysaccharide: The lipopolysaccharide (LPS) molecule is composed of three distinct sections; lipidA, a core polysaccharide and a side chain O-antigen (O-Ag) polysaccharide. Nine O-antigen types are distinguished in *K. pneumoniae*, O1 being the most frequent. The most important role of the O-antigen is to protect *K. pneumoniae* from complement mediated killing (Brisse *et al.,* 2006). The lipid A anchors the LPS molecule into the outer membrane and is also anendotoxin, stimulating the immune system through agonism of Toll-like receptor 4(TLR4) which is present on macrophages, dendritic cells and other cell types inducing NF-kB mediated production of cytokines (Alexander and Rietschel, 2001).

D.Siderophores: Iron is essential for bacterial growth. Bacteria secure their supply of iron in the host by secreting high-affinity, low-molecular-weight iron chelators, called siderophores. Siderophores are capable of solubilizing and importing the required iron bound to host proteins. Two different groups of siderophores are mostly produced in the genus *Klebsiella*, phenolates (enterobactin) (also known as enterochelin) and hydroxamates (aerobactin). All strains were found to produce enterochelin, only a few could produce

aerobactin. Nassif in 1986 showed that aerobactin is an essential factor of pathogenecity. The *Klebsiella*spp. produced third siderophore called "yersiniabactin," which is encoded by the *Yersinia* high-pathogenecity island, butits prevalence, role in the *Klebsiella* and pathogenesis are unknown (Brisse *et al.,*2006).

E. Other Factors: There is many other factors play a minor role in pathogenecity of *Klebsiella*spp. such as cytotoxins, enterotoxins and haemolysin. The little information is known about heamolysis produced by *Klebsiella*as it is non hemolytic for human RBC and haemolytic in rabbit blood agar (Brisse *et al.,* 2006).

Typing of *Klebsiella* isolates: *Klebsiella* species are genetically heterogeneous within species and subspecies. The importance of typing methods is to obtain information about endemic and epidemic nosocomial outbreaks of *Klebsiella* infections and to determine the clonality of the strains. There are two methods have been used in *Klebsiella* typing.

Phenotypic Methods

a. Biotyping: Biotyping is based on biochemical reactions and environmental tolerance. It is suitable method of typing for smaller not optimally equipped laboratories. It is useful in assessing outbreaks of *Klebsiella* but it is considered to be of little use in epidemiological studies. Biotyping can be carried out by using API 20E system with supplementary tests. *Klebsiella* strains are often currently identified by using automated instruments based on classical biochemical tests, such as the Vitek and API systems (Podschun and Ullmann, 1998).

b. Serotyping: Serotyping is a reaction of specific antiserum to surface-exposed antigen determinant. Serotyping is currently the most widely used technique for typing *Klebsiella* species. The immune determinant of *Klebsiella* is the capsule. *Klebsiella*e usually have well developed polysaccharide capsules, which give their colonies their characteristic mucoid appearance. *Klebsiella*e also possesses 12 different O-antigen types, but they are difficult to classify because they are covered by the heat stable capsules (Hansen *et al.,* 2002). The techniques used in Serotyping are, countercurrent imunoelectrophoresis and immnofluroescence. The Quellung test depends on the adsorption of capsular antibodies onto the CPS, which results in a change in the refractive index. But disadvantages of serotyping are (i) the occurrence of serological cross-reactions among the 77 capsule types, (ii) the weak reaction due to a weak antigen which affects interpretation, (iii) time consuming methodology, (iv) lack of commercially available anti-capsule antisera, and (v) occurrence of non-typable isolates (Brisse *et al.,* 2006). PCR amplification of the capsular antigen gene cluster (cps) was followed by digestion with the restriction enzyme *HincII* (cps PCR-RFLP analysis).

c. Phage Typing: Bacteriophage typing is based on the susceptibility of bacterial strains to a panel of bacteriophages. Phage typing of *Klebsiella* was first developed in the 1964 by Milch and Deak (1964). Phage typing has never become widespread because it shows poor typing rate of 19 to 67%; the lack of standardization and inoculum concentration; the limited availability of bacteriophages; the stability of bacteriophages must be evaluated and maintained over time. For these reasons, bacteriophage typing is useful mainly as secondary method in combination with serologic testing but not used as an alternative to capsule typing (Brisse *et al.,* 2006).

d. Bacteriocin Typing: Bacteriocins are bactericidal substances, usually proteins, produced by bacteria to inhibit the growth of other bacteria through inhibition of protein and nucleic acid synthesis and uncouple electron transport from active transport of thiomethyl-β-D-galactoside and potassium. Bacteriocins technique has become the method of choice for typing of organisms belonging to *Klebsiella* genus because only 34% of all *Klebsiella* isolates produce bacteriocins.

e. Antibiogram: Changes in antibiogram may reflect spontaneous point mutation. Thus, isolates that are epidemiologically related and otherwise genetically indistinguishable may show different antimicrobial susceptibilities (Riley, 2004).

Multilocus Enzyme Electrophoresis (MLEE): Multilocus enzyme electrophoresis based on metabolic enzymes that are highly conserved and unlikely to change quickly in a given species. The main application of MLEE in epidemiology is in the global surveillance of pathogens because the technique is based on gene whose variation accumulates slowly over time.

Molecular Typing Methods: Molecular typing methods, as applied to the genus *Klebsiella*, have increasingly become an integral part of both clinical and research

microbiology laboratories. Microbial genotyping techniques consider valuable tools to distinguish bacterial strains or clones. Genotyping methods are divided into two methods; protein-based methods and nucleic acid-based methods.

a. Protein based method

SDS-PAGE: SDS-PAGE method has been used to subtype *Klebsiella*by (Costas *et al.,* 1990). Who did compare it with capsular serotyping and concluded that SDS-PAGE protein profiles could be used as an effective method.

b. Nucleic acid based methods

i) PCR amplification and sequencing: *K.oxytoca* can be differentiated from other *Klebsiella* species by a specific PCR targeting the *pehX*gene involved in pectin degradation (Kovtunovych *et al.,* 2003).

ii) Pulsed-field Gel Electrophoresis (PFGE): PFGE may be used for genotyping or genetic fingerprinting. It is commonly considered a gold standard in epidemiological studies of pathogenic organisms. PFGE analysis is recommended to address fine-scale epidemiological studies and can detect chromosomal rearrangements by mobile elements with rapid evolutionary rates (Vimont *et al.,* 2008).

iii) Plasmid profile analysis: Plasmid based typing methods rely on differences in molecular weight and the number of different plasmids a bacterium carries. This is one of the first molecular typing methods for epidemiological studies of nosocomial infections. This method has been widely used to differentiate *K.pnuemoniae* isolates in epidemiological studies. It is of limited value because genetically unrelated isolates may harbor the same plasmids and related isolates may lose unstable plasmids (Gori *et al.,* 1996).

iv) Randomly amplified polymorphic DNA (RAPD): The first use of RAPD was in 1996. This technique utilizes low-stringency PCR amplification with single primers of arbitrary sequence to generate strain-specific arrays of anonymous DNA fragments. RAPD technique may be used to determine taxonomic identity, assess kinship relationships, analyze mixed genome samples, and create specific probes.

v) Restriction fragment length polymorphism (RFLP): Identification of the isolates were confirmed by *gyrA*PCR-RFLP using restriction enzymes *Hinc*II, *Taq*I and *Hae*III of the 441-bp fragment of the *gyrA*gene, and the940-bp fragment of the RNA polymerase β subunit gene (*rpoB)* allowing for the identification of *K. pneumoniae* species and *K. oxytoca* genetic groups (Brisse *et al.,* 2004).

vi) Multilocus sequence typing (MLST): MLST is a nucleotide sequence-based method used for characterizing the genetic relationships among bacterial isolates. It provides computerized data that allow multi-user international databases available. MLST is more appropriate for strain phylogeny and large-scale epidemiology (Vimont *et al.,* 2008).

vii) Repetitive sequence-based PCR (rep-PCR): It uses primers targeting noncoding repetitive elements interspersed throughout the bacterial genome. Standardized, commercially available kits containing primers and PCR master mix reagents are marketed as DiversiLab systems which automated the detection and analysis by using micro fluidics for rapid detection in a single day, and digitized the corresponding information in a software package. The standardized digitized gel images are stored for comparison between runs and between laboratories (Pitout *et al.,* 2009).

Antimicrobial Agents

Historical perspective: In 1928, Sir Alexander Fleming observed antibacterial substance producing *Penicillium* mould. He named the filterable active agent penicillin. Penicillin-G was first β lactam antibiotics in clinical use, which leads to the development of modern antibiotics (Goldsworthy and McFarlane, 2002). Cephalosporins are β lactam antibiotics originally isolated from the mould *Cephalosporium.* They differ from penicillins in having dihydrothiazine ring fused with β lactam ring. This gives more opportunities for biochemical modifications, in order to expand the spectrum of activity and improves the pharmacokinetic properties of the drug (Knothe *et al.,* 1983).

β-Lactam Antibiotics: The β-lactam antibiotics can be divided into six different groups, the penicillins, cephalosporins, carbapenems, cephamycins, monobactams, and β-Lactamase inhibitors (Smet *et al.,* 2008) as shown in Table 1.2. β-lactam antibiotics contain a β-lactam ring which is a heteroatomic ring structure consisting of three carbon atoms and onenitrogen atom. The β-lactam ring of natural or semisynthetic penicillins is fused with a thiazolidine ring. In cephalosporins, the β-lactam ring is merged with adihydrothiazine ring as shown in Fig 1.1. In the carbapenems, the β-lactam ring is combined with ahydroxyethyl side chain, lacking an oxygen or sulphur atom in the bicyclic nucleus. In contrast to the antibiotics, clavulanic acid, a β-Lactamase

inhibitor is composed ofa β-lactam ring fused with an oxazolidine ring (Amyes, 2010).

Mechanism of action of β-lactam antibiotics

β-Lactam antibiotics act on bacteria by inhibiting the bacterial enzymes, transpeptidases and carboxypeptidases, located in the cytoplasmic membrane which catalyses synthesis of the cross-linked peptidoglycan.

These enzymes are commonly called penicillin-binding-proteins (PBPs) (Spratt, 1994). β – Lactam antibiotics only kill growing bacteria. They bind to PBPs, these are enzymes involved in cell wall synthesis. In Gram-negative bacteria this is in the periplasm.

The β-lactam antibiotics are analogues of the terminal amino acid (D-alanyl-D-alanine) residues on the precursor NAM/NAG-peptide subunits of the peptidoglycan layer. In the presence of the β-lactam antibiotics, the transpeptidases and carboxypeptidases react with acyl-D-alanyl-D-alanine to form alethal serine-ester-linked acyl (penicilloyl, cephalosporoyl) enzyme complex. This disrupts the synthesis of the cell wall and makes the growing bacteria highly susceptible to cell lysis and death (Wilke *et al.,* 2005).

Mechanisms of resistance to β-lactam antibiotics: Resistance to antimicrobials is natural biological phenomenon. Resistance genes encode various mechanisms which allow microorganisms to resist the inhibitory effects of specific antimicrobials. Among bacteria there are four major ways to avoid the bactericidal effect of β-lactam antibiotics.

PBPs modifications

Alterations of PBPs have been described in both Gram-negative and Gram-positive organisms, assuming a more important role in Gram-positive bacteria. There are several PBP-mediated mechanisms of β-lactam resistance, including:

- ✓ Point mutations altering an amino acid.
- ✓ The acquisition of foreign PBP resistant to β-lactam antibiotics
- ✓ Recombination between susceptible PBPs and those of less susceptible species.
- ✓ Overexpression of a PBP.

Outer membrane (OM) permeability-based resistance: The OM of Gram -ve bacteria is a barrier to both hydrophilic and hydrophobic compounds. In Gram +ve bacteria the β-lactam can easily reach the cytoplasmic membrane, whereas in Gram-negative the crossing of the outer membrane is essentially done through protein channels. The porins are divided into two classes: In *E.coli,* OmpC and OmpF are non-specific porins, while *K.pneumoniae* strains usually express OmpK35 and OmpK36 are usually no porin at all (Hernandez-Alles *et al.,* 1999).

Efflux pump: is the third mechanism involved in resistance to β-lactam antibiotics. These proteins transport the antibiotic from within the cell to the external environment. A characteristic of efflux pumps is the variety of molecules they may transport, due to poor substrate specificity (Poole, 2004).

Enzyme production: The fourth and most important mechanism of resistance to β-lactam antibiotics is the production of β-Lactamase enzymes. The β-lactamases confer significant antibiotic resistance to their bacterial hosts by hydrolysis of the amide bond of the β-lactamring. Classes A, C, and D include enzymes that hydrolyze their substrates by forming an acyl (penicilloic or cephalosporoic) enzyme through an active site serine, whereas class B β-lactamases are metalloenzymes that utilize at least one active-site zinc ion. These enzymes are especially important in Gram-negative bacteria as they constitute the major defense mechanism against β-lactam-based drugs (Wilke *et al.,* 2005).

Emergence of resistance to β-lactam antibiotics began even before the development of penicillin. The first identification of β-Lactamase enzyme came from *E.coli* before the use of penicillin in medical practice (Abraham and Chain, 1940). Many genera of Gram-negative bacteria naturally possess chromosomal-mediated β-lactamase. These enzymes and penicillin-binding proteins (PBPs) are thought to have evolved from a common ancestor, which probably assist the bacteria in competition with other naturally producing β-lactams bacteria (Ghuysen, 1991). The first plasmid mediated β-Lactamase in Gram-negative bacteria, TEM-1, was described in 1965 (the designation “TEM” came from the patient’s name, Temoniera) (Datta and Kontomichalou, 1965). At the same time, another plasmid-mediated β-lactamase, known as “SHV-1” (sulfhydryl variable) was found in *K. pneumoniae* and *E.coli*. The genes encoding β-lactamases can be located on the bacterial

chromosome, on plasmids, on transposons, or on integrons. These mobile genetic elements enable β-lactamases dissemination to other members of the *Enterobacteriaceae* family, *Haemofelus influenzae, Niesseria gonorrhoeae, and Pseudomonas aeruginosa*, andrise the incidence of multi-drug resistant bacteria with complex resistance patterns (Paterson and Bonomo, 2005). The mutations in TEM and SHV β-lactamases gave them expanded spectrum of activity against oxyimino-β-lactams (oxyimino-cephalosporins) and then these enzymes were called extended-spectrum β-lactamases (ESBLs) (Gniadkowski, 2008).

Extended spectrum β-lactamases: Extended spectrum β-lactamases (ESBLs) are "β-lactamases capable of conferring bacterial resistance to the penicillins, first-, second- and third-generation cephalosporins, and aztreonam (but not the cephamycins or carbapenems) by hydrolysis of these antibiotics, and which are inhibited by β-Lactamase inhibitors such as clavulanic acid" (Paterson and Bonomo, 2005).

The first ESBL was identified by Knothe *et al.,*(1983) in a nosocomial *K.pneumoniae* strain isolated in Germany in 1983; since then over 500 variants of the clavulanic acid-inhibited form (TEM, SHV, CTX-M, OXA) have been described worldwide. They are most prevalent in *Klebsiella*spp. and their epidemiology reflects a mixture of mutations, plasmid transfer and or clonal spread ESBLs are typically plasmid encoded but also present on chromosome, often in association with integrons (Livermore and Woodford, 2006). The most common ESBL phenotypes arise from point mutations in the *bla*TEM, *bla*SHV, or *bla*CTX genes resulting in alterations of the primary amino acid sequence of the enzyme (Paterson, 2006). These mutations usually occurred at position 104 (TEM), 146(SHV), 156 (SHV), 164 (TEM), 167 (CTX-M), 169 (SHV), 179 (SHV and TEM), 205 (TEM), 237 (TEM), 238 (SHV and TEM) and 240 (TEM, SHV and CTX-M) (Gniadkowski, 2008). Many of the organisms that harbour ESBLs are also resistant to other classes of antibiotics, such as aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol, and sulfonamides (Bonnet, 2004).

Classification and nomenclature of β-Lactamases: One of the most used classification schemes is Ambler's (Ambler *et al.,* 1991) based upon amino acid sequences. He classified the β-lactamases into four molecular classes, A, B, C and D. Moreover, Bush *et al.,* (2010) extended his classification scheme of 1989 attempted tocorrelate the functional characteristics with the molecular structure recognizes four major β-Lactamase classes, one of which (Group 2) is split into eight subgroups. Finally, Bush and Jacoby (2010) updated his classification scheme of 1995 by adding new functional subgroups to the scheme as a result of identification of new major β-lactamase families' variants. β-Lactamases are categorized based on similarity in amino acid sequence (Ambler classes A through D) or on substrate and inhibitor profile classification schemes are widely accepted as shown in above Table 2.

Active site: The β-lactamases are divided into two classes; serine and metallo β-lactamases that do not share sequence or structural homology. In classes A, C and D an active siteserine and a molecular mass of approximately 29,000 Da, is responsible for the β-lactam hydrolysis (Bradford, 2001). The three classes of serine β-lactamases, A, C and D share similarity on the protein structure level, which proves that they are derived from common ancestor (Hall and Barlow, 2004).

Types of ESBLs

a. TEM β-lactamases: TEM-1, was first reported in 1965 from an *E.coli* isolate. Plasmid mediatedTEM-1 is the most prevalent β-lactam inactivating enzyme found in entericbacilli especially in *E.coli* and *K. pneumoniae*. They are also found with increasing frequency in other Gram-negative species (Bradford, 2001).TEM-1 is able to hydrolyze ampicillin more than carbenicillin, oxacillin orcephalothin and is inhibited by clavulanic acid (Leflon-Guibout, 2000). Based upon different combinations of changes, currently 195TEM-type enzymes have been reported. TEM and SHV are transferred by both plasmid and chromosome. TEM-2 the first derivative of TEM-1, had a single amino acid substitution at position 39 (Gln39→Lys) from the original TEM-1 β-Lactamase (Jacoby and Carreras, 1991). TEM-3, originally reported in 1987 was the first TEM-type β-Lactamase that displayed the ESBLs phenotype. TEM12 reported in 1982 from *Klebsiella oxytoca* as it carries the gene on plasmid which is responsible for ceftazidime resistance. TEM enzymes confer a phenotypic resistance to β-Lactamase inhibitors conferring an inhibitor resistant TEM (IRT) phenotype. Currently, more than 28 *bla*TEM gene variants are resistant to inhibitors like clavulanic acid. Amino acid substitutions in TEM at positions Met69, Ser130, Arg244, Arg275 and Asn276 are usually associated with the resistance to β-Lactamase inhibitors (Drawz and Bonomo, 2010).

b. SHV β-lactamases: SHV stands for Sulfhydril variable.SHV-1 shares 68 percent of its amino acids with TEM-1 and has a similar overall Structure. SHV-1 was first described in 1972 and called Pit-2 from the author's name Pitton (Pitton, 1972). SHV-1 confers resistance to ampicillin, amoxicillin, carbenilcillin and ticarcillin (Livermore, 1995). The SHV-1 betalactamaseis most commonly found in *K. pneumoniae* and is responsible for up to20% of the plasmid-mediated ampicillin resistance in this species. ESBLs in this family also have amino acid changes around the active site, most commonly at positions 238 and 240 (Farkosh, 2007). A chromosomal copy of *bla*SHV-1 or *bla*SHV-11 or close relatives, encoding non-extended-spectrum enzymes, considered as native to the great majority of *K.pneumoniae* strains (Lee *et al.,* 2006). SHV enzymes were also detected in other *Enterobacteriaceae* as a plasmid-mediated β-lactamases (Sabate *et al.,* 2002). Plasmid-mediated *bla*SHV genes are possibly mobilized from genome to plasmid mediated by IS*26*facilitating the mobilization of chromosomal sequences containing resistance genes (Miriagou *et al.,* 2005). The first of these enzymes capable of hydrolyzing the newer β-lactams SHV-2 was found in a single strain of *K. ozaenae* isolated in Germany (Knothe *et al.,* 1983). Sequencing of the SHV-2 gene showed that only one amino-acid substitution of Gly238→Ser was differed from SHV-1.More than 60 SHV varieties are known. They are the predominant ESBL type in Europe and United States and are found worldwide.SHV-5 and SHV-12 are among SHV the most common β lactamase responsible for outbreaks of nosocomial infections in several countries (Kliebe *et al.,* 1985).

c.CTX-M β lactamases: In the second half of the 1980s, non-TEM and non-SHV plasmid class A ESBLs enzymes have been reported, among them the CTX-M type β-lactamases were first detected in Japan in 1986 from cefotaxmine resistant *E.coli* (the enzyme was initially named TOHO-1 and was later changed to CTX-M). During the 1990s, general dissemination and occasional nosocomial outbreak, mostly of CTX-M-2-producing *Enterobacteriaceae*, were reported from South America (Peirano and Pitout, 2010). However, since 2000 *E.coli* producing CTX-M β-lactamases have emerged worldwide as an important cause of community-onset urinary tract infections (UTIs)and this has been called the CTX-M pandemic. These enzymes were named for their greater activity against cefotaxime than otheroxyimino-β-lactam substrates (e.g., ceftazidime, ceftriaxone, or cefepime) (Pitout *et al.,* 2008). These enzymes are not very closely related to TEM or SHV β-lactamases inthat they show only approximately 40% identity with these two commonly isolated β-lactamases. The change at position 102 mainly enhances resistance to Ceftazidime, while the change at position 236 predominantly augments resistance to Cefotaxime, with a slight effect for Ceftazidime (Rahman *et al.,* 2004). More than 96CTX-M enzymes are currently known, they are currently divided into 5 clusters on the basis of amino acid sequence: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9 andCTX-M-25 (Bonnet, 2004). CTX-M-15 belongs to the CTX-M-1 cluster and is derived from CTX-M-3 by one amino acid substitution at position 240 (Asp→Gly). CTX-M enzymes are susceptible to β-Lactamase inhibitors, although a low-level of resistance to the combination of clavulanic acid with amoxicillin and ticarcillin could be observed. CTX-M-14 was able to hydrolyze sulbactam, clavulanate and tazobactam retain their ability to inactivate this enzyme (Ishii *et al.,* 2007).

d. OXA β-lactamases: The OXA β-lactamases differ from the TEM and SHV enzymes and they belong to class D (2d) according to Ambler classification (Ambler *et al.,* 1991). The OXA group mainly occurs in *Acinetobacter* and *Pseudomonas* species. The OXA β-lactamases attack the oxyimino-cephalosporins and have a high hydrolytic activity against oxacillin, methicillin and cloxacillin more than benzylpenicillin, inhibitedless efficiently by clavulanate and their activity is inhibited by NaCl (Poirel and Nordmann, 2008). Most OXA-type β-lactamases are not regarded as ESBLs, because they do not hydrolyse the extended-spectrum cephalosporins. Currently, over than 180 different variants of OXA enzymes have been identified on the protein level. Whereas most of the genes encoding class D oxacillinases have commonly been found on plasmids incorporated as gene cassettes in integrons, several chromosomal encoded oxacillinases have been reported (Heritier *et al.,* 2005).

Table.1 Groups and examples of β lactam antimicrobial agents

β-lactam group	Examples of antimicrobial agents
Penicillin	Penicillin G
	Penicillinase resistant penicillins: methicillin, nafcillin, oxacillin, cloxacillin
	Aminopenicillins: ampicillin, amoxicillin
	Carboxypenicillins: carbinicillin, ticarcillin
	Ureidopenicillins: azalocillin, mezalocillin, piperacillin
Cephalosporin	First generation: cefazolin, cephalothin, cephalexin
	Second generation: cefuroxime, cefaclor, cefamandole, cephamycins
	Third generation : cefotaxime, ceftriaxome, cefpodixime, ceftizoxime, cefoperazone, ceftazidime
	Fourth generation : cefepime, cefpirome
Carbapenem	Imipenem, meropenem, ertapenem
Monobactam	Aztreonam

Table.2 Classification of β-Lactamase scheme

Ambler class	Bush-Jacoby group	Distinctive Substrates	Inhibited by		Representive enzymes
C	1	Cephalosporins	CA/TZB	EDTA	AmpC,P99,ACT-1, CMY-2,FOX-1,MIR-1
			-	-	
C	1e	Cephalosporins	-	-	GC-1,CMY-37
A	2a	Pencillins	+	-	PC1
A	2b	Pencillins,early cephalosporins	+	-	TEM-1,TEM-2,SHV-1
A	2be	Extended-spectrum cephalosporins,monobactams	+	-	TEM-3,SHV-2,CTX-Ms,PER,VEB
A	2br	Pencillins	-	-	TEM-30,SHV-10
A	2ber	Extended-spectrum cephalosporins,monobactams	-	-	TEM-50
A	2c	Carbencillin	+	-	PSE-1,CARB-3
A	2ce	Carbencillin,cefepime	+	-	RTG-4
D	2d	Coxacillin	V	-	OXA-1, OXA-10
D	2de	Extended-spectrumCephalosporins	V	-	OXA-11, OXA-15
D	2df	Carbapenems	V	-	OXA-23, OXA-48
A	2e	Extended-spectrumCephalosporins	+	-	CEPA
A	2f	Carbapenems	V	-	KPC-2,IMI-1,SME-1
B	3a (B1)	Carbapenems	-	+	IMP-1,VIM-1,IND-1,CcrA
	(B2)				L1,CAU-,GOB-1,FEZ1
B	3b(B3)	Carbapenems	-	+	CphA,Sfh-1
Unknown	4	-			
(V)- Variable, (+) -Yes, (-) - NO, CA -Clavulanic acid, TZB- Tazobactam					

Others: A variety of other β-lactamases which are plasmid-mediated or integrons associated class- A enzymes have been recently discovered. ESBLs, such as PER, VEB, GES, and IBC have been described but are uncommon and have been found mainly in *P. aeruginosa, E.coli* and *K.pneumoniae* and at a limited number of geographic sites. PER-1 in isolates in Turkey, France, and Italy; VEB-1 and VEB-2 in strains from

Southeast Asia; and GES-1, GES-2, andIBC-2 in isolates from South Africa, France, and Greece (Brisse *et al.,* 2006).

Extended-spectrum β-lactamase (ESBL)-producing bacteria are increasingly prevalent in hospitals due to antibiotic pressure or transfer of colonized patients. These organisms spread through clonal expansion or horizontal gene transfer via plasmids, often carrying ESBL genes on mobile elements like transposons and integrons. Initially confined to hospital settings, ESBL-producing strains are now common in community infections.

The selective pressure of β-lactam use has driven the evolution of ESBLs, mainly from TEM and SHV families, particularly in *E. coli* and *K. pneumoniae*. Among ESBL types, CTX-M enzymes have become globally dominant due to their association with mobile genetic elements.

ESBL prevalence varies by region—higher in Europe than the U.S., and highest in Asia and South America. *K. pneumoniae* is the primary ESBL producer in Eastern Europe, with rising cases in Poland, Turkey, and Romania. CTX-M-15 is notably spreading across Europe. In Asia, especially India, ESBL rates in *Klebsiella* species can reach 60%, underscoring the rapidly evolving and unpredictable nature of ESBL epidemiology.

Author Contributions

M. G. Thosar: Investigation, formal analysis, writing—original draft. P. V. Gadakh: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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