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Review Article

AmpC β-Lactamases in Enterobacteriaceae - A Mini Review

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Abstract: Beta-lactamases are enzymes that confer resistance to β-lactam antibiotics. Both Gram-positive and Gram-negative bacteria produces these enzymes. There are about 3000 enzymes that initially emerged from bacteria found in the environment to protect themselves from the natural β-lactam. After the 1980s, many transmissible enzymes were detected periodically, resistant to cephalosporins, monobactam, and carbapenems. These enzymes were classified based on function and molecular structure. Among them AmpC β-lactamases were found to be resistant to β-lactams and β-lactamases inhibitors. They are class C cephalosporinases that confer resistance to the first, second, third generation cephalosporins and cephamycin, and also resistance to beta-lactamases inhibitors such as sulbactam, tazobactam, and clavulanic acid. Family Enterobacteriaceae comprises many organisms that cause community and nosocomial infections, such as *Escherichia coli, Klebsiella pneumoniae, Citrobacter spp, Enterobacter aerogenes, and Salmonella species*. Beta-lactamases are produced by Enterobacteriaceae, where AmpC beta-lactamases are found to be one of the mechanisms. Different types of AmpC beta-lactamases: mutation/attenuation in the chromosome, induced plasmid-mediated AmpC beta-lactamases. Some Enterobacteriaceae, like *Enterobacter*, carry it on their chromosome, and some other Enterobacteriaceae has plasmid-mediated AmpC beta-lactamases. This type of resistance has led to increased mortality and morbidity. It is challenging to detect these AmpC beta-lactamases in diagnostic settings. Still, the detection of AmpC β-lactamases is cumbersome, and no approved methods are found in CLSI guidelines. But the prevalence of AmpC beta-lactamases has increased drastically in Asia. The review aims to give an overview of AmpC β-lactamases. The objective of this review is to review the evolution, types, detection methods, recent world epidemiology, treatment options, and current updates of the AmpC beta-lactamases.

Keywords: AmpC β-lactamases, Gram-negative bacteria, plasmids, β-lactamases, Enterobacteriaceae, Antibiotic resistance

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1. INTRODUCTION

AmpC β-lactamases have been known since 1940s and belong to Ambler class C cephalosporinases, resistant to penicillin, extended-spectrum cephalosporins, and β-lactamases inhibitors. They are three types of AmpC producers[1]. They are chromosomally encoded AmpC genes (inducible) in organisms such as Enterobacter *cloacae*, Serratia *marcescens (S. marcescens)*, *Citrobacter freundii (C. freundii), Pseudomonas aeruginosa (*2) chromosomal due to a mutation in promoter and attenuator regions in organisms like *Escherichia coli (E. coli), Acinetobacter baumannii* (3) plasmid-mediated AmpC β-lactamases resistance in organisms such as *Klebsiella pneumoniae (K. pneumoniae)*, *Salmonella* species.[2]

2. ORIGIN

The history of these β-lactamases is way back 2 million years ago, even before the introduction of antibiotics in medicinal use, reflecting the advancement of resistance components to natural β-lactams created by organisms for survival (Hall B G., 2004). The first enzyme was reported in *E. coli*, the capability of degrading penicillin in 1940.[2] It was classified as molecular class C by Ambler molecular classification of β-lactamases. The active site of the AmpC was serine residue in the protein; However, the serine is an active site for ESBL. Also, the protein sequence is different, leading to differences in the degradation of β-lactams.[3] They belong to group 1 in the functional classification of β- lactamases.[4] The chromosomal genes were found in some enteric microbe which got transferred to other enteric microbes through plasmids leading to the formation of plasmid-mediated β-lactamases (Table 1 & Table 2). The prevalence of AmpC is less prominent than ESBL.

Table 1: Genetic origin of different plasmid-mediated AmpC β-lactamases.

Type	Origin
CMY-2 clusters)	*Citrobacter spp*
Other CMY (CMY-1,9,8,11,10,19), MOX	*Aeromonas spp.*
MOX-4	*Vibrio fluvialis*
DHA 1 &2	*Morganella morganii*
ACC	*Hafnia alveli*
FOX	*Aeromonas cavaie*
MIR-1	*Enterobacter cloacae*
MIR-2, MIR-3	*Aeromonas spp.*
ACT	*E. absuria*

Table 1 illustrates the names of different plasmid-mediated AmpC beta-lactamases and the genetic origin of these genes from the chromosomal-induced organisms.

Table 2: Origin of AmpC beta-lactamases

AmpC β -lactamases	Year	Origin	Detected in first Isolate
CMY-1	1989	South Korea	*K. pneumoniae*
MIR-1	1990	United states	*K. pneumoniae*
MOX-1	1993	Japan	*K. pneumoniae*
LAT-1	1993	Greece	*K. pneumoniae*
FOX-1	1994	Argentina	*K. pneumoniae*
CMY-2	1994	France	*S. senftenberg*
MOX-2	1995	France	*K. pneumoniae*
CMY-1	1996	Greece	*K. pneumoniae*
DHA-1	1997	Saudi Arabia	*S. enteriditis*
ACT-1	1997	United states	*K. pneumoniae*
ACC-1	1999	Germany	*K. pneumoniae*
FOX-4	2000	Spain	*E. coli*
CFE-1	2004	Japan	*E. coli*

Table 2 illustrates the origin of the different AmpC beta-lactamases from different countries, the origin it was identified, and from which nation and organism it was isolated. There are about six families of plasmid-mediated AmpC beta-lactamases.

3. HYDROLYTIC PROPERTIES OF AmpC BETA LACTAMASES

AmpC beta-lactamases are class C cephalosporinases found on many Gram-negative organisms' chromosomes. They are resistant to penicillin, 1st, 2nd, and 3rd generation cephalosporins, aztreonam, and cephamycins but resistant to beta-lactamase inhibitors such as tazobactam, clavulanic acid, sulbactam and generally sensitive to carbapenems and 4th generation cephalosporins. Apart from chromosomal AmpCs, many plasmids-mediated AmpCs have emerged and are grouped into six families.

4. TYPES OF AmpC β-LACTAMASES

1. Chromosomal
2. Plasmid-mediated

5. CHROMOSOMAL AMPC β-LACTAMASES

AmpC is a protein encoded by the chromosomes of a few

microorganisms. Genes encoding AmpC β-lactamases are placed on the chromosomal DNA of important Gram-negative organisms, especially in enteric bacteria. The organism which carries the chromosome of AmpC is *S. marcescens, Enterobacter spp., C. freundii, and Morganella morganii*. The level of expression of chromosomal AmpC genes differs in different species. This type of expression is common in clinical settings. The AmpC genes are overexpressed by different mechanisms: constitutive and inducible. Organisms like *E. coli* do not have an inducible AmpC gene; any mutations in their attenuator or promoter region will lead to overexpression of AmpC. Genes for the production of AmpC are present in the chromosome of many species[5], but it is produced at very low levels, which is not detectable. Mutation in AmpC promoter regions leads to overexpression of the AmpC enzyme, which results in treatment failure. *E. coli* can obtain the genes for AmpC enzyme production from other species. But some organisms *(e.g., E. cloacae, C. freundii, S. marcescens, or P. aeruginosa*) have inducible AmpC genes on the chromosome, which leads to over-expression and are called derepressed mutants. The mechanism of AmpC is closely related to the recycling process of cell walls. During the cell wall synthesis, in Gram-negative, e.g., *E. coli* degrades 40-50% of the peptidoglycan. The degraded products (1, 6,-anhydromuropeptides) are recycled. These released 1, 6,-anhydromuropeptides are transported from the periplasm into the cytoplasm through a transmembrane permease (AmpG). Consequently, the anhydromuropeptides are hydrolyzed by AmpD, which is cytoplasmic amidase for further recycling, which produces UDP-pentapeptides and transported to periplasm for recycling; only few 1,6 anhydropeptides are available for AmpR to transcribe the bla_{AmpC} to produce AmpC β- lactamases.

1. Induced

In the presence of β-lactams, anhydromuropeptides increase in the periplasm and are transported to the cytoplasm. AmpD recycles anhydromuropeptides present in the cytoplasm. The excess anhydromuropeptides bind to the transcriptional regulator of AmpC (AmpR), which produces the AmpC β-lactamases.

2. Derepressed

1. In the AmpR mutants, the cell wall degradation products behave like AmpR activating ligands, causing AmpC expression.
2. In AmpD mutant strains, unhydrolyzed anhydromuropeptides accumulate in the cell's cytoplasm, leading to the AmpR activation and resulting in the semi-constitutive or constitutive expression of AmpC. AmpD-dependent constitutive over-expression of AmpC occurs at a frequency of 10^{-6} in defective strains. The regulation of AmpC production is depicted in figure1.

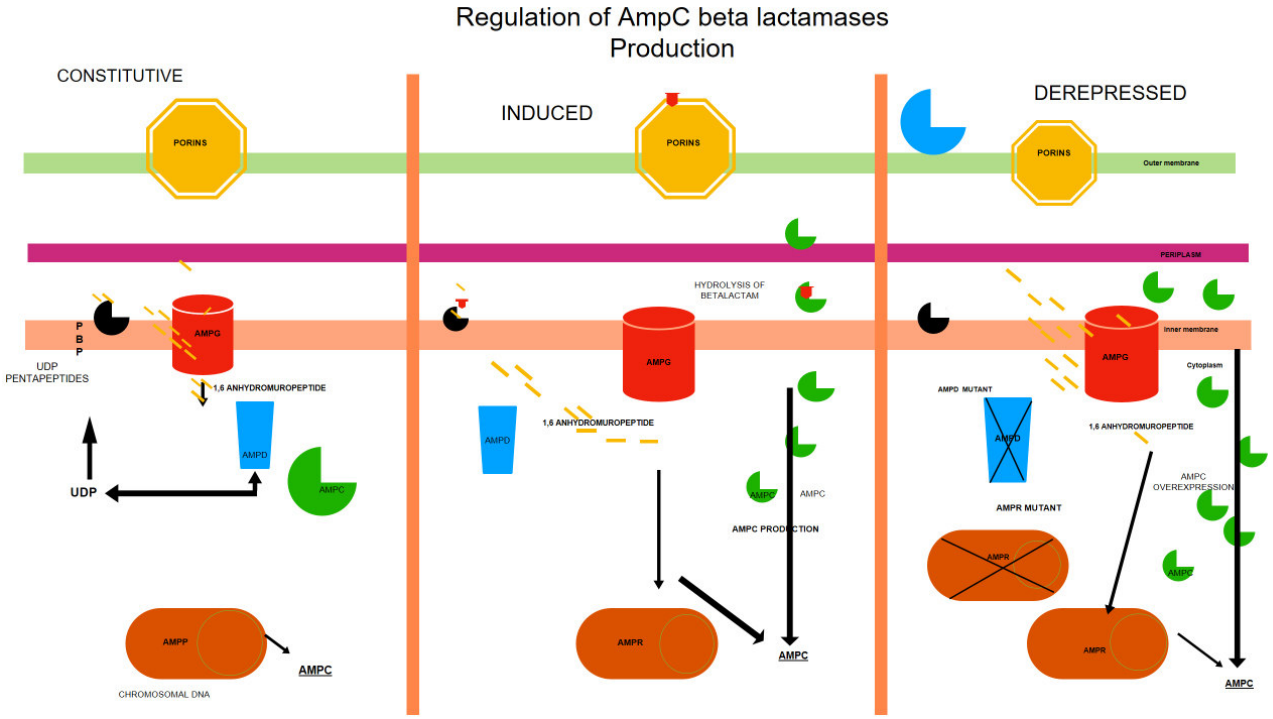

Fig1: Regulation of AmpC β -lactamases

6. THREE DIMENSIONAL STRUCTURE OF AMPC β-LACTAMASES [2]

AmpC enzymes have remarkably comparable three-dimensional structures. On one side of the molecule is a α-helical domain, and on the other, α/β domain. With the reactive serine residue at the amino terminus of the central α-helix, the active site is in the middle of the enzyme at the left edge of the five-stranded β-sheet. The R1 site, which accommodates the R1 side chain of the β-lactam nucleus, and the R2 site, which accommodates the R2 side chain, can be further split into the active site. The Ω-loop surrounds the R1 site, and the H-10 and H-11 helices of the R2 loop surround the R2 site. Though AmpC enzymes structure is close to that of class A group of enzymes, class C enzymes have a larger open binding site, which reflects their improved capacity to accommodate the bulkier side chains of cephalosporins and improved resistance pattern. Along with Ser64, other important catalytic residues for AmpC enzymes include Lys67, Tyr150, Asn152, Lys315, and Ala318; changes at these locations significantly reduce enzymatic activity. Most of these crucial residues are located near the active site of the folded

protein, where Tyr150 serves as a momentary catalytic base and Lys67 hydrogen bonds to Ser64.

7. PLASMID MEDIATED AmpC β LACTAMASES (PMABL)

It was identified first in 1979 from *Proteus mirabilis*[6] *and* later recovered in a *K. pneumoniae* from a wound sample of a patient and named CMY-1[7]. CMY-1 was able to degrade cephamycin and was not inhibited by sulbactam, tazobactam, clavulanate, and MIR-1[8], identified also had a similar profile. This gene has a 90% identified with the *Enterobacter cloacae* chromosomal gene. In 1989, another enzyme was isolated from a Pakistan patient in the UK and named BIL-1[9]. Many genes were identified in later years was isolated from *E. coli* and was subsequently shown to transfer resistance to three different genera. Many plasmid-mediated genes were identified and named. CMY-2 type was the generally reported PMABL on earth.[1] They were found both in hospital and community strains. The plasmid-mediated AmpCs β-lactamases degrade penicillin, cephalosporins, and monobactam except for cefepime and carbapenams. The PMABL is formed from chromosomal genes that are transferred from different organisms. These enzymes were reported in Enterobacteria. The six different families are commonly reported: ACC, FOX, MOX, DHA, CIT, and EBC, which are based on the genetic origin[9-11]; several varieties are found in these families., e.g., 7 varieties in FOX, 43 varieties in CMY, 4 varieties in ACC, MIR, and LAT, MOX, and ACT. The resistance profile of plasmid-mediated enzymes was similar to chromosomal AmpC β-lactamases. The most prevalent PMABL was CMY-2e. ACC, CMY, and DHA are prevalent in hospital-based infections.

The genetic element involved in the AmpC genes mobilization is ISEcp1which is involved in the mobilization of the chromosomal gene to the plasmid location for CMY, ACC-1&4 genes, ISCR1 associated with bla_{CMY-1}, bla_{DHA-1}, bla_{MOX-1} gene and IS 26 associated with bla_{CMY-3}. Due to genetic modification, AmpC β-lactamases with extended activity are named extended-spectrum AmpC (ESACs), which show increased activity against oxyimino cephalosporins. Generally, modifications occur at the β loop or R2-loop, which expands the R2 binding site, or accessibility to the substrate in the R1 side chain is increased.

8. EPIDEMIOLOGICAL FEATURES[12]

Plasmid-mediated LAT-2 (CMY-2) enzymes were discovered in clinical isolates of *Enterobacter aerogenes* in Greece, and *K. pneumoniae* and *E. coli* in France[13], and plasmid-mediated ACC-1 was discovered in clinical isolates of *P. mirabilis* and E. coli urine sample in France. 13 Ceftriaxone resistant *Salmonella* arose in the US due to plasmid-mediated CMY-2 - beta-lactamases. In eight separate states between 1996 and 1998, in symptomatic individuals[14]. There were several *Salmonella* serotypes found in pigs and cattle[15]. A 12-year-old child from Nebraska was infected with a ceftriaxone-resistant *S. enterica* serotype Typhimurium spread from calves[16] The widespread dissemination of bacteria that produce plasmid-determined cephalosporinases is a startling trait. They have been discovered in North America, Saudi Arabia, Algeria, Tunisia, India, Japan, Pakistan, South Korea, France, Germany, Greece, Italy, Sweden, the United Kingdom, Argentina, Guatemala, and the United States. Similar to the importation of ESBL-producing strains, AmpC genes were imported between Asian countries and European countries. Most organisms that produce plasmid-determined AmpC enzymes have been obtained from hospitalized patients (Critical care patients, surgery patients, cancer patients, transplantation patients) except a few *Salmonella* strains and sporadic *K. pneumoniae* isolates. Mainly the organism was obtained from blood, wounds, sputum, or stool. Cefoxitin, moxalactam, cefmetazole, cefotetan, and imipenem were among the β-lactam antibiotics that most patients were treated. Many bacteria with AmpC enzymes also produce ESBL, like TEM-1, TEM-3 and SHV-5, and CTX-M. *K. pneumoniae* produces sporadic AmpC outbreaks, e.g., MIR-1 (CMY-2)-like enzyme, ACC-1, and ACT-1.

9. SUSCEPTIBILITY PATTERNS

Strains with plasmid-mediated AmpC enzymes were consistently resistant to aminopenicillins (ampicillin or amoxicillin), carboxypenicillins (carbenicillin or ticarcillin), and ureidopenicillins (piperacillin) and, among the penicillins, these strains were susceptible only to amdinocillin or temocillin. The enzymes provided resistance to cephalosporins in the oxyimino group (ceftazidime, cefotaxime, ceftriaxone, ceftizoxime, cefuroxime) and the 7-α-methoxy group (cefoxitin, cefotetan, cefmetazole, moxalactam). MICs were usually higher for ceftazidime than for cefotaxime and cefoxitin. They are susceptible to cefepime, cefepirome, and carbapenems. Changes in antibiotic accessibility to the enzyme can significantly alter the susceptibility profile. Imipenem MICs can reach 64 g/ml, and meropenem MICs can reach 16 g/ml in *K. pneumoniae* strains containing plasmids defining AmpC enzymes when outer membrane porin channels are lost. Cefepime and cefpirome MICs in these isolates become inoculum dependent, and at inocula of 10^7/ml, MICs can approach 256 g/ml[17]. In current days, even cefepime resistance has been developed. Though AmpC enzymes are after resistant the sulbactam/tazobactam, it is susceptible to piperacillin-tazobactam.

10. ENZYMATIC PROPERTIES

The pI range for plasmid-mediated AmpC-type β-lactamases is 6.4 to 9.4. After isoelectric focusing, AmpC enzymes in clinical isolates with multiple β-lactamases can be detected by differential suppression of nitrocefin reactivity with 5 mg of cefoxitin/ml[18] or by bioassay-based detection of cefoxitin hydrolysis. While a small number of plasmids, such as DHA-1 and DHA-2, include AmpR and AmpC genes and are inducible, the majority of plasmid-mediated AmpC genes, such as MIR-1, are expressed constitutively even in the presence of a complete induction system. The Plasmid-mediated AmpC β-lactamases have an apparent molecular weight that ranges from 38 to 42 kDa and contain 378, 381, 29, 41, 382, or 386 amino acid residues. The relative Vmax values for cephalothin and cephaloridine were higher than those for ampicillin and penicillin, and there was greater activity with penicillin than with ampicillin as well as low hydrolysis rates for oxyimino- or methoxy-compounds. The Km values for cefoxitin, cefotetan, cefotaxime, moxalactam, or aztreonam, on the other hand, were often lower than those for penicillin or ampicillin and significantly lower than the Km values for cephaloridine, cephalothin, or cefepime. Like group 1 cephalosporinases, plasmid-mediated AmpC enzymes were inhibited by low concentrations of aztreonam, cefoxitin, or cloxacillin and only by high concentrations of clavulanate. Sulbactam and, particularly, tazobactam were more effective inhibitors. The amino acid sequence of the enzymes revealed an active-site serine in the motif Ser-X-X-Lys (where X is any amino acid)

at residues 64 to 67 of the mature protein. A Lys-Ser/Thr-Gly motif has been found at residues 315 to 317 and plays an essential role in forming the tertiary structure of the active site. A tyrosine residue at position 150 forms part of the class C-typical motif Tyr-X-Asn and is also important (but not essential) for the catalysis of β-lactam hydrolysis.

11. GENETIC FEATURES

AmpC genes are located on different plasmid sizes ranging from 7 to 180 kb. Many of these plasmids are self-transmissible, and only a few are transferred by transformation. Plasmids encoding AmpC enzymes often carry multiple other resistances, including resistance to aminoglycosides, chloramphenicol, sulfonamide, tetracycline, and trimethoprim. FOX-type enzyme containing plasmids carried a gene resistant to fluoroquinolone. AmpC enzymes producing isolates may also produce other beta-lactamases, e.g., TEM and, SHV, CTX. The gene for AmpC enzyme on the chromosome is transposed by a transposon, e.g., ACT-1, MIR. The blc and sugE genes located downstream from AmpC on the *C. freundii* chromosome are close to the *C. freundii*-type blaCMY-5 gene according to the mapping of the gene in plasmid pTKH11[19]. A putative insertion element that may have played a role in gene capture has replaced the AmpR gene that was located upstream of AmpC on the chromosome. Other plasmids that encode *C. freundii*-type AmpC enzymes have a similar organizational structure as far as they have been sequenced. It suggests that they originated directly from the *C. freundii* chromosome and then underwent an accumulation of mutations in the AmpC gene to produce the current array of CMY and LAT-type enzymes, supported by phylogenetic analysis. Numerous resistance genes, such as those for the Ambler class A, B, and D β-lactamases, are found in gene cassettes with a downstream 59-base region that serves as a particular recombination site for inclusion into integrons[20]. According to an analysis of published sequences, AmpC genes identified on plasmids are not connected to 59-base elements. A site-specific integrase, two copies of qacE-1sull, an aminoglycoside resistance gene (aadA2) with its downstream 59-base region, and a putative recombinase (ORF341) are all present in an integron that contains the DHA-1 structural and regulatory genes on plasmid pSAL-1. This integron has characteristics with the In6 and In7 plasmids pSa and pDGO100 that lack the bla gene regarding a genomic organization.

12. MOLECULAR ASPECTS

Regardless of their genetic origin, AmpC β-lactamases have identical hydrolytic characteristics. These enzymes often have low Vmax and high Km values for the third-generation cephalosporins. The plasmid-encoded AmpC β-lactamases MIR-1 and MOX-1, as well as the kinetic values of the *Serratia marcescens.* AmpC β-lactamase reported for ceftazidime are significant outliers.4–7 These data are commonly used due to the lack of consistency among laboratories in AmpC enzymatic investigations. The exact functions these enzymatic activities play in the overall resistance pattern of organisms will thus not be clear, and comparisons of data obtained on certain enzymatic activities between laboratories would not be possible. Even though various AmpC β-lactamases have slightly varying hydrolytic characteristics, until the AmpC β-lactamase is produced at high levels, organisms harboring these enzymes are not resistant to third-generation cephalosporins.1 It is well known that chromosomal AmpC gene expression is induced by β-lactam antibiotics like cefoxitin and imipenem but is only marginally (if at all) induced by third- or fourth-generation cephalosporins. These organisms include *Citrobacter freundii, Enterobacter cloacae, Morganella morganii, Hafnia alvei, and Serratia marcescens.*[8–10] The DNA-binding protein AmpR is required for induction, reversible after the inducing substance has been withdrawn. *E. cloacae* and *C. freundii*, two model organisms, have been used to examine the mechanisms of AmpC gene expression.[3, 11–14] A current study documenting AmpC expression in *S. marcescens.* The contributions of gene copy number and promoter strength on total AmpC gene expression were discussed in a recent study. The relative copy number of many AmpC genes represented by plasmids has been determined using a novel technique. Therefore, investigations did not support the widely held hypothesis that high-copy plasmids mediate the high-level expression of AmpC genes carried by plasmids. After adjusting for copy number, analysis of gene expression revealed that AmpC expression in the absence of AmpR produced much greater.

13. CLINICAL IMPLICATIONS OF PLASMID-ENCODED AmpC-MEDIATED RESISTANCE

The clinical microbiologists' most pressing issue is finding Gram-negative microbes with plasmid-encoded AmpC-mediated resistance. Although there are no established recommendations for detecting this resistance mechanism, clinical laboratories need to address this problem just as much as they do for detecting ESBLs. Cefoxitin-resistant AmpC producers should be distinguished from cefoxitin-resistant non-AmpC producers. Differentiating between these two types of organisms might influence the available treatment choices, with carbapenems being used for cefoxitin-resistant AmpC producers and extended-spectrum cephalosporins being used for cefoxitin-resistant non-AmpC, non-ESBL producers. The distinction between these sorts of organisms would affect the use of cephalosporins and carbapenems, which would also impact the selection pressure driving ESBL, AmpC, or plasmid-encoded class resistance to carbapenems. The emergence of AmpC β-lactamases encoded by inducible plasmids adds another warning sign for difficult detection. It is widely recognized that AmpD mutations, which encode an inducible chromosomal AmpC, are related to the derepressed phenotype of organisms. The fact that most Gram-negative organisms encode AmpD is not widely recognized. Because there is no discernible phenotype in the absence of an inducible chromosomal AmpC, spontaneous AmpD mutations that should occur in clinical isolates of *E. coli, K. pneumoniae, and Salmonella* spp. have not been identified. When plasmid-encoded inducible AmpC genes are produced in the presence of AmpD mutations, it is expected that clinical isolates of *E. coli* and *K. pneumoniae* would exhibit considerable increases in ESBL MICs. In addition to isolates from people, plasmid-encoded AmpC β-lactamases have also been discovered in isolates from companion animals like dogs and livestock like swine and cattle. The importance of precisely identifying this resistance mechanism is increased by these additional sources of isolates that produce AmpC. To stop the spread of plasmid-encoded AmpC-mediated resistance within the hospital, hospital-based clinical labs should screen isolates from community-based patients before hospitalization, according to a source of AmpC-mediated resistance in the community. Studies to monitor plasmid-encoded AmpC β-lactamase genes obtained from the population are necessary. Using phenotypic susceptibility testing, it is challenging to differentiate between

species that produce ESBLs and those that produce plasmid-encoded AmpC β-lactamases. Cefoxitin resistance may be a sign of AmpC-mediated resistance but may also be a sign of decreased membrane permeability. To assist in distinguishing between cefoxitin-resistant non-AmpC producers and cefoxitin-resistant AmpC producers, certain phenotypic assays are available, like the three-dimensional test and the AmpC disc test. Furthermore, β-lactamase inhibitors can be used to locate potential producers of AmpC. None of these assays are standardized; therefore, screening a lot of isolates can take some time. To identify cefoxitin-resistant non-AmpC producers from cefoxitin-resistant AmpC producers, a recently developed multiplex PCR for the identification of plasmid-encoded AmpC genes has proven beneficial as a quick screening technique. In order to identify cefoxitin-resistant non-AmpC producers from cefoxitin-resistant AmpC producers, a multiplex PCR for the identification of AmpC genes carried on plasmids has recently been developed. The data produced by the multiplex PCR approach may detect the AmpC gene and identify the family of AmpC genes in the resistant organism, separating potential inducible AmpC producers from non-inducible AmpC producers. This PCR-based technique can also discriminate between *E. coli* isolates containing an 'imported' AmpC gene and isolates that produce excessive amounts of chromosomal AmpC. The capacity to identify the kind of AmpC or ESBL may help with hospital infection control and the doctor's ability to administer the best antibiotic, reducing the selection pressure that leads to antibiotic resistance. To identify cefoxitin-resistant non-AmpC producers from cefoxitin-resistant AmpC producers, a multiplex PCR for identifying AmpC genes carried on plasmids has recently been developed. The data produced by the multiplex PCR approach may detect the AmpC gene and identify the family of AmpC genes in the resistant organism, separating potential inducible AmpC producers from non-inducible AmpC producers. This PCR-based technique can also discriminate between *E. coli* isolates containing an 'imported' AmpC gene and isolates that produce excessive amounts of chromosomal AmpC. The capacity to identify the kind of AmpC or ESBL may help with hospital infection control and the doctor's ability to administer the best antibiotic, reducing the selection pressure that leads to antibiotic resistance. A priority becomes adequate surveillance when one considers the potential to discriminate between organisms generating ESBLs, plasmid-encoded AmpC β-lactamases, or synthesis of both enzymes in a single organism; molecular testing must be used in the clinical laboratory. To regulate the Gram-negative β-lactamase resistance mechanisms we currently confront and, for the first time, to prevent the formation of a new type of β-lactamase, the ESACs, surveillance is essential. There has been a lot of advancement in our understanding of AmpC β-lactamases during the past 25 years. However, reality shows that we have not succeeded in stopping the proliferation of this resistance mechanism because of our ignorance. The clinical consequences of patients infected with organisms generating plasmid-encoded AmpC β-lactamases, as well as AmpC production and detection of resistance mechanisms in the clinical context for both outpatients and inpatients, require more study.

14. HEALTH THREAT OF ESBL/AmpC β-LACTAMASE

The pAmpC-producing organisms have large plasmids which carry genes that cause resistance to first-line antibiotics such as quinolones, cotrimoxazole, and aminoglycosides. Infections due to pAmpC-producing Enterobacteriaceae have led to few therapeutic options, which increase morbidity and mortality in the patients [21].

15. ANIMALS TRANSMISSION

The primary reservoir of the ESBL/AmpC-producing organism is still controversial. Especially plasmid-mediated AmpC beta-lactamases have increased drastically over the past two decades. The transmission of these organisms in the community, hospitals, and homes suggests the intestinal colonization of this organism serving as a reservoir[22]. These resistant organisms are isolated from animal food[23], farm animal[24], vegetables[25], petting zoo[26], and in surface water, wastewater, and sea water[27]

16. RISK FACTORS OF ESBL- OR AmpC-PRODUCING BACTERIAL STRAINS

1. Prior Antibiotic usage[28], especially oxyiminocephalosporins.
2. Prolonged hospital stay[29]
3. Indwelling medical devices (urinary catheters)
4. Colonization with ESBL/pAmpC β-lactamases producing organism.

17. CONTROLLING OPTIONS

Rational use of the antibiotics
Limiting the use of hospitalization stay
Prevention of usage of antibiotics in food and animal farms.

18. PLASMID-MEDIATED BETA-LACTAMASES

Due to the selective pressure, there is a wider range of resistance determinants, and antibiotic resistance has increased. The increased use of amoxy clavulanate and tazobactam, sulbactam has exerted additional selective pressure and has various mechanisms to counteract that, and one such response is AmpC beta-lactamases. Though AmpC gene production is beneficial but in a location in chromosomes has limited options. Many plasmid-mediated AmpC β-lactamases have increased since the 1900s, and in the 2000s have increased drastically. *Klebsiella* spp., tends to acquire plasmids, and hence the prominence has increased. The exact nature of the chromosomal AmpC into plasmids is unknown, but transposons and plasmids spread the genes within the species and among the genus through trans conjugation. All resistance determinants carrying plasmids are integrated with integrons and transposons and form the gene cassette. The majority of plasmids are self-transferrable. Few are non-self-transmissible; such plasmids are transferred by conjugation (conjugative transposons) or resistance plasmids. The major site of transfer is the intestinal tract. Many rectal screening multidrug resistance screening test has proven such transfer in *E.coli, Proteus mirabilis*, and *Klebsiella* spp. Occurrence of plasmid transfer of plasmids between *K. pneumoniae* and *Salmonella* in the intestine. The transfer of plasmid-mediated AmpC β-lactamases has been found in organisms that originally did not possess the genes. Hence the acquisition of these genes among *E. coli, Klebsiella, and Salmonella* spp., restricts the therapeutic alternatives for treating infections in these organisms. The AmpC beta-lactamases may implicate major health implications. The plasmid-borne enzymes have 6 families. 4 descend from enterobacterial AmpC beta-lactamases, while the fifth is *Aeromonas.* As a result, it is

possible to predict that AmpC β-lactamases carried by plasmids in Enterobacteriaceae will eventually present issues with antibacterial treatment. It may be predicted that the prevalence of AmpC genes carried by plasmids will increase significantly if an AmpC gene is successfully acquired by a transposon similar to the TEM-1 and TEM-2 β-lactamases, which are the most common plasmid-encoded β-lactamases in gram-negative bacteria.

19. CURRENT CHALLENGES[30]

AmpC β-lactamases are a class C enzyme typically produced by bla genes on the bacterial chromosome, though plasmid-borne AmpC enzymes are now more common. Penicillins, β-lactamase inhibitors like clavulanate and tazobactam, and the majority of cephalosporins, including cefoxitin, cefotetan, ceftriaxone, and cefotaxime, are often ineffective against organisms expressing the AmpC β-lactamase. AmpC enzymes weakly hydrolyze a broad-spectrum cephalosporin called cefepime, and carbapenems easily inactivate them. Now a day's, cefepime resistance also have been found. Avibactam is particularly effective at inactivating AmpC cephalosporinases and other bacteria, *K. pneumoniae, Klebsiella oxytoca, Proteus mirabilis, and Salmonella* spp., lack these enzymes. Inducers and substrates for AmpC β-lactamase include benzylpenicillin, ampicillin, amoxicillin, and cephalosporins such as cefazolin and cephalothin. Strong inducers include cefoxitin and imipenem. Ceftriaxone, Ceftazidime, Cefepime, Cefuroxime, Piperacillin, and Aztreonam are weak inducers and substrates, but they can be hydrolyzed with sufficient enzyme expression. As a result, AmpC hyperproduction significantly raises the MICs of oxyimino-β-lactams that are weakly inducing. As a result of the high degree of AmpC expression that is already induced by strong inducers, however, the MICs of these drugs do not alter significantly with regulatory changes. A few β-lactamase inhibitors are also inducers, including clavulanate, which strangely can seem to increase AmpC-mediated resistance in an inducible organism despite having no inhibitory effect on AmpC β-lactamase activity. In clinically significant Gram-negative bacteria, AmpC enzyme production is often suppressed (or "repressed") but can be "derepressed" by induction with specific b-lactams, especially cefoxitin, and imipenem. A good inducer of AmpC β-lactamases is sulbactam, but not tazobactam. Although they have been the focus of extensive research, the genetic foundations of this regulation are not the subject of this review. *Citrobacter, Salmonella,* and *Shigella* are examples of Enterobacteriaceae members that produce clinically significant amounts of AmpC enzymes that are resistant to inhibition by clavulanate and sulbactam.

20. EVOLUTION

In the past 80 years, we have discovered that the ongoing evolution of substrate specificity meets every new β -lactam introduced. The "long view" forecasts that these enzymes will continue to change and take on new shapes. Maybe one day, we'll figure out why this happens and how to stop it. Future research in this field must be flexible and open to novel ideas. We still don't fully understand all the correlates of activity and resistance or the mechanism underlying the emergence of novel structural variants. The current problems will require the use of new technology.

Factors determining β-lactamases expression

1. Effective expression of the efflux pump reduces antibiotic accumulation and enhances the resistance enzyme activity.
2. Reduced expression of outer membrane porin activity reduces the intake of antibiotics, and these ESBL isolates have developed resistance to cefepime and imipenem in AmpC isolates.
3. Apart from this, resistance patterns also differ depending on the bacterial host and environment. Some ESBL has lost intrinsic resistance, which can be corrected by gene dosage or increased activity of promoter region.

Treatment

Control of Outbreak

1. Restriction of usage of third-generation cephalosporins
2. Following the infection control practices strictly
3. Isolation procedures with minimization of antibiotic usage.
4. Using piperacillin-tazobactam, Cefepime-amikacin, and imipenem as empirical therapy

21. ENTEROBACTERIACEAE Producing AMPC - LACTAMASES [31]

Amp beta-lactamases were presented and produced by chromosomally encoded organisms like S*erratia marcescens, Hafnia alveli, Citrobacter freundii, Morganella morganii,* and *Providencia stuartii* produced chromosomal mediated AmpC beta-lactamases. *E. coli* produces a low amount of AmpC beta-lactamases in *Klebsiella* spp. And *salmonell*a produces AmpC beta-lactamases due to acquiring plasmid-mediated AmpC beta-lactamases. Cephamycin-resistant *E. coli* due to plasmid-derived AmpC beta-lactamases has also been found. The earliest reports of plasmid-mediated AmpC β-lactamase-producing organisms date back to the 1980s. These enzymes (e.g., CMY, ACT, FOX, ACT, and DHA types) are derivatives of the chromosomally encoded AmpC cephalosporinases of bacteria such as *Enterobacter spp. C. freundii, M. morganii, Aeromonas spp*., and *Hafnia alvei* are not inhibited by the "classical" β-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam. But several inhibitors, such as cloxacillin and boronic acid, have the power to block chromosomal and plasmid-mediated AmpC β-lactamases. Limited treatment alternatives are usually available since the genes are frequently contained on huge plasmids that also carry other antibiotic resistance genes. There were known to be spread in hospitals, which later transferred to the community. *Salmonella enterica* serotype Newport which produces CMY-2 has been linked to community-associated acquisition and infections by enterobacteria with plasmid-mediated AmpC β-lactamases in Canada and USA. Consuming undercooked meat and handling pet treats have been linked to *the Salmonella enterica* serotype. Newport infections that produce CMY-2 have been identified. Population-based research from the Calgary Health Region indicated that women had a fivefold greater risk of infection and that 61% of the 369 patients with community-associated infections caused by isolates resistant to cephamycin had AmpC-producing *E. coli*. Sequencing revealed that these enzymes were CMY-2 after PCR revealed that 34% of the samples were positive for black genes. The study concluded that AmpC-producing *E. coli* is an emerging pathogen in the population that frequently causes urinary tract infections in older women in this broad Canadian region. It was followed by articles from Washington and Nebraska, respectively, that demonstrated the presence of Enterobacteriaceae in outpatient clinics in the USA that

generate the CMY, ACC, and DHA kinds of AmpC β-lactamases.

22. DETECTION OF AMPC β-LACTAMASES

Though are no certain guidelines laid by CLSI for testing AmpC production in Gram-negative organisms. A Cefoxitin screening test was used to screen the isolates, followed by phenotypic and molecular methods. There were various methods devised by different scientists: several Phenotypic tests such as AmpC disk test, AmpC disk test with EDTA, Modified three-dimensional test, cefoxitin-agar, beta-lactam inhibitor assay like cloxacillin, boronic acid and its derivatives and E-test strips combination. AmpC disk test and modified three-dimensional test were more sensitive in detecting the AmpC beta-lactamases in Enterobacteriaceae. It was found to be high in sensitivity and specificity to inhibitor-based tests. The gold standard method for detecting plasmid-mediated beta-lactamases. There are few methods to detect the production of AmpC β-lactamases that could provide epidemiology.

23. PHENOTYPIC TESTS

1. Cefoxitin screening test: [32] (CLSI guidelines).

The test organism swabbed on Mueller Hinton agar (MHA), cefoxitin disc is placed on it and incubated at 37 ° C for 16-18 hours. Reduced Susceptibility to the cefoxitin test is used as a screening test. But it can also be produced by carbapenamases, in *E. coli* and *K. pneumoniae* having outer membrane porins defects.

2. AmpC disk test:[33]

A saline or EDTA-impregnated disc is placed adjacent to the cefoxitin disc in the lawn culture of the strain; few colonies are smeared over the impregnated or plain disc. If distortion occurs in the zone of inhibition, it indicates AmpC production.

3. Three dimensional test:[34,35]

E. coli ATCC 25922 is grown in a broth and swabbed as a lawn on MHA agar. Cefoxitin is kept on the plate, and near it, a circular slit of 3 mm is made on the agar, and suspension of test strains is pipetted into a well. Distortion of the zone of inhibition is indicated for the production of AmpC enzyme. Modifications include a radial slit and adding the test organism pellet, which is centrifuged, frozen, and thawed five times.

4. Modified Hodge test[36]

In MHA plates, *E. coli* ATCC 25922 is swabbed as a lawn. In the center of the plate, Cefoxitin (30µg) is kept, and test strains are inoculated from the periphery of the plate to the edge of the disc. The oblique growth-producing cloverleaf model is positive for AmpC production. Isolates that had no distortion around the cefoxitin zone are considered to be negative for AmpC production.

24. INHIBITOR BASED METHOD

1. Phenyl boronic acid test: [37]

Boronic acid derivatives are added to the beta-lactam disk (cefoxitin), placed near cefoxitin in an MHA plate inoculated with the suspected organism, and incubated for 18hrs at 37 ℃. Enhanced zone of inhibition around the boronic acid/cefoxitin compared to the beta-lactam indicates the AmpC production. Philip E. Coudron (2005)[38] has reported that phenyl boronic acid is useful for detecting plasmid-mediated resistance. The sensitivity (60-70%) and specificity (45-98%) are being evaluated.

2.Cloxacillin test:[39]

Cloxacillin is placed between cefotaxime and ceftazidime, used to detect AmpC production.

25. GENOTYPIC TEST

- ***Multiplex PCR***[11]

Plasmid-mediated beta-lactamases are detected based on the current gold standard multiplex PCR, which targets the six families of AmpC using six primer sets.

- ***Multiplex asymmetric PCR-based array*** [40]

It detects both plasmid-mediated AmpC beta-lactamases and mutants. Ongoing research is trying to automate AmpC beta-lactamases detection by modification of this array method.

26. TREATMENT

Organisms producing AmpC β-lactamases are generally resistant to various antimicrobial agents. Hence the selection of the antibiotic for treatment is tough. β-lactam and β-lactam inhibitors must not be used to treat AmpC-producing organism infections because they carry the risk of induction and mutants. Studies have shown poor clinical outcomes for cefotaxime, ceftazidime, and piperacillin-tazobactam. Piperacillin-tazobactam can be used in bacteremia produced by AmpC-producing Enterobacteriaceae, but Pitts's bacteremia score has to be assessed. Generally, cefepime is susceptible when conventional methods are used because it is a poor inducer of AmpC production. But the high inoculum test shows a drastic increase in cefepime MICs[41, 42]. Hence the usage of cefepime must be done carefully. Temocillin is active against both chromosomal/plasmid-mediated AmpC β-lactamases[43, 44]. Carbapenems like imipenem, meropenam, and ertapenam can treat AmpC infections.[45] But carbapenamases and porin defect strains producing AmpC β- lactamases have emerged. Fluoroquinolone therapy has been used for non-life-threatening infections such as UTIs. The drugs like fosfomycin, tigecycline, colistin, polymyxin, aminoglycosides, and double carbapenem are used to treat AmpC-producing Enterobacteriaceae infections.[46] Tigecycline can be used in isolates like *Enterobacter* spp*, E.coli, Klebsiella* Spp, and *Citrobacter,* which hyperproduce AmpC[47].

27. CONCLUSION

AmpC β-lactamases are important cephalosporinases encoded on the chromosome of Enterobacteriaceae, which mediate resistance to 1st and 2nd-generation cephalosporins, penicillin, and β- lactam inhibitors. Overexpression in these enzymes in *Enterobacter* species leads to cefotaxime, ceftriaxone, and ceftazidime resistance. Plasmid-mediated AmpC β- lactamases are found in *E. coli, Klebsiella* spp*, and P. mirabilis*, which confers resistance to many antibiotics. AmpC β-lactamase detection in pathogens is important for effective antibiotic therapy. Several methods for screening and confirmation are evaluated and evolving. Still, no CLSI guidelines are there for detecting AmpC

production; this underestimates the prevalence rate of AmpC β-lactamases. So developing a diagnostic algorithm for detection is the need of the hour.

28. AUTHORS CONTRIBUTION STATEMENT

All authors have made a substantial, direct, and intellectual contribution to the work and approved it for publication. Bindu.D contributed to the data extraction, analysis, and article preparation. Chitralekha saikumar contributed by being a part of writing the article.

29. CONFLICT OF INTEREST

Conflict of interest declared none.

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