

Review article

BETA-LACTAMASES ENZYMES : MECHANISM AND CLASSIFICATION

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ABSTRACT : Increased resistance of bacteria towards different classes of antimicrobial agents become a critical concern and challenge now days to treat bacterial infections around the world. There are many mechanisms of bacterial resistance towards antimicrobial agents; one of the most important mechanisms is producing hydrolyzing enzymes such as β -lactamases, which inhibit a wide range of Beta-lactams that represented a drug of choice for many serious bacterial infections. Hence, this article reviews the β -lactam structures, classifications and the mechanisms that make bacteria resist to β -lactams. In addition to β -lactamases classifications groups and sub groups, history of these classification and their action on β -lactams, with highlighting the role of Extended spectrum β -lactamases, especially CTM-X group and Metallo- β -lactamases.

Key words : Beta-lactams, beta-lactamases, CTX-M, metallo- β -lactamases, extended spectrum Beta lactamases

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INTRODUCTION

Beta-lactam antimicrobial agents contain a beta-lactam ring that inhibits cell wall synthesis of bacteria by binding to penicillin binding proteins (PBPs), which are membrane proteins. β -lactams comprise a very large family which distinguished by the structure of the ring attached to the β -lactams ring and the side chain to this rings (Fernandes *et al*, 2013). The mechanism of resistance of bacteria toward β -lactam antimicrobial demonstrate by alteration in target site, alteration in access to the target site or production of β -lactamases. β -lactamases are bacterial enzymes that are able to hydrolyze beta-lactam antimicrobial drugs, which are the commonest cause of bacterial resistance to β -lactam antimicrobial agents. β -lactamases have been classified by their hydrolytic spectrum, susceptibility to inhibitors and whether they are encoded by chromosome or plasmids. In general, they are classified either on biochemical and functional characteristics of enzymes or on the molecular structure of enzyme. β -lactamases divide into four classes: A, B, C and D. Each including types that are usually plasmid-mediated or chromosomal. These distinctions are blurred because it is increasingly appreciated that most 'plasmid-mediated' types are in fact genetic escapes from the chromosomes of other

species (Livermore *et al*, 2006 and Drawz *et al*, 2010). Widespread use of antimicrobials have led to selection of bacteria producing extended spectrum β -lactamases (ESBL) and Metallo-Beta lactamases (MBL) mainly. ESBLs are characterized by their ability to hydrolyze penicillins, early cephalosporins, oxyimino-thiazolyl cephalosporins (including third and fourth generation cephalosporins) and monobactams, but not cephamycins or carbapenems. They are inhibited by suicide-inhibitors such as clavulanic acid, tazobactam and sulbactam (Rupp *et al*, 2003). MBLs producing bacteria hydrolyze all carbapenems including meropenem, imipenem, doripenem and ertapenem has a broad spectrum of activity against serious infection of some bacteria, while they are resistance to hydrolysis by most β -lactamases, including ESBL. MBLs are able to efficiently hydrolyze all β -lactams with the exception of monobactams and aztreonam. These enzymes require divalent cations, usually zinc ion, as metal cofactors for enzyme activity and are inhibited by metal chelators such as ethylenediamine tetra acetic acid (EDTA). MBL belongs to a group β -lactamase, which requires divalent cations of zinc as cofactors for enzyme activity. These have potent hydrolysing activity not only against carbapenem but also against other β -lactam antibiotics; approximately

nine different types of acquired MBL have been defined. Based on molecular studies, carbapenem-hydrolyzing enzymes are classified into four groups A, B, C and D. The MBLs belong to group B of Ambler classification (Walsh *et al*, 2005; Palzkill *et al*, 2013 and Mojica *et al*, 2016).

This article aims to summarize the important role of beta-lactamase enzymes that increase bacterial resistance towards antimicrobial agents, especially the role of ESBLs and MBLs; which become a serious concern in treatment of bacterial infections.

Beta lactamases

The β -lactamase enzymes have been created via microorganisms, and they have the ability for breaking β -lactam ring in the β -lactam antimicrobial agents, making them inactive and therefore leading to β -lactam resistance. Up to now, there are over five-hundred β -lactamases were indicated and created via different bacteria. Also, the β -lactamases were specified as the major common mechanism of resistance contributing to general resistance between bacteria, particularly Gram negative bacteria, that has been mediated through expressed chromosomally or plasmid-mediated. With regard to Gram negative organisms, which are chromosomally located, inducible expression related to beta-lactamases was frequent, yet, the spread of beta-lactamases was often associated to plasmid mediated ESBL, particularly the CTX-M family (Rahman *et al*, 2018). In addition, the β -lactamases were the major cause regarding bacterial resistance to the β -lactam antimicrobial agents. Their prevalence eliminated the benzylpenicillin utility against the *Staphylococci* and has massively damaged that related to ampicillin against the enterobacteria and *Haemophilus* and *Neisseria* spp. Furthermore, new enzymes, along with new production modes that are related to old enzymes, are presently threatening the value regarding extended-spectrum cephalosporins against the enterobacteria. Furthermore, the β -lactamases of Gram positive species were extracellular, yet, based on growth conditions, a few enzymes might be adhering to the cytoplasmic membrane. Then again, beta-lactamases regarding Gram negative species were mainly periplasmic, even though a few extracellular releases might happen, mediated through leakage instead of secretion (Livermore, 1995).

β -lactamases action

Some β -lactamases are using ions of zinc for disrupting the β -lactam ring, yet more numbers are operating through serine ester mechanism. PBPs are reacting with the β -lactams for providing serine esters,

yet, dissimilar to other esters created via β -lactamases, they don't readily hydrolyze; such distinction is blurred in some conditions: a few β -lactamase inhibitors create stable serine esters with the β -lactamases; while, some PBPs rapidly deacylated and their weak β -lactam-hydrolyzing activity might be protecting the bacterial cell in the case when the drug entry was adequately restricted through impermeability (Livermore, 1995).

β -lactamases classification

Till lately, β -lactamases were categorized via their susceptibility to inhibitors, hydrolytic spectrum, and if they were encoded through the chromosome or through plasmids. Massive weight was put on if cephaloridine has been hydrolyzed more quickly compared to benzylpenicillin, or the other way around and on if the enzyme was inactivated via cloxacillin, aztreonam, clavulanate, or *p*-chloromercuribenzoate. Typically, the β -lactamases were majorly categorized base on 2 schemes (Shaikh *et al*, 2015; Rahman *et al*, 2018 and Livermore, 1995):

- 1) The molecular classification or Ambler molecular classification is on the basis of enzymes' protein homology, into 4 classes A, B, C and D enzymes; such enzymes are using serine for the β -lactam hydrolysis as well as class B metalloenzymes, which require divalent zinc ions (metal ion) for substrate hydrolysis.
- 2) Bush-Jacoby-Medeiros functional classification, that group various β -lactamases based on their functional properties related to enzymes, for instance, the substrate as well as inhibitor profiles. It is an approach correlating the β -lactamases with phenotypes in the clinical isolates.

According to Rahman *et al* (2018), the most recent updated functional classification comprises 3 groups (Table 1).

- Group-1 (class C), cephalosporinases
- Group-2 (classes A & D), inhibitor resistant, broad spectrum, serine carbapenemases, and extended spectrum β -lactamases,
- Group-3 (class B), metallo β -lactamases

Classification history

In the year 1989, Bush suggested the main reorganization, with more updating in the year 1995. Also, the revised scheme of Bush classified beta-lactamases through their substrate preference among oxacillin, penicillin, cephaloridine, carbenicillin, expanded spectrum cephalosporins, also imipenem as well as via their susceptibility to the inhibition through clavulanate.

Table 1 : Different schemes of classification schemes and representatives related to extended spectrum beta-lactamase enzymes.

Ambler (molecular) class	Bush & Jacoby group (2009)	Substrate/target	Inhibition profile		Member examples
			Clavulanic acid	Tazobactam	
A	2a	Penicillins	Yes	No	PC-1
	2b	Penicillins, a few first generation cephalosporin	Yes	No	SHV-1, TEM-1, TEM-2
	2be	Monobactam, extended spectrum cephalosporin	Yes	No	SHV-2, TEM-3, PET-1, CTX-M-15, VEB-1
	2br	Penicillins	No	No	SHV-10, TEM-30,
	2ber	Monobactam, extended spectrum cephalosporin	No	No	TEM-50
	2c	Carbenicillin	Yes	No	CARB-3, PSE-1,
	2ce	Cefepime, Carbenicillin	Yes	No	RTG-4
	2e	Extended spectrum beta lactams	Yes	No	CepA
	2f		Changing	No	1M1-1, KPC-2, SME-1
B	3a	Carbapenems	No	Yes	VIM-1, IMP-1, IND-1, CcrA, NDM-1
	3b	Carbapenems	No	Yes	S -1, CphA,
C	1	Cephalosporines	No	No	P99, AmpC, CMY-2, ACT-1, MIR-1, FOX-1,
	1e	Cephalosporines	No	Yes	CMY-37, GC1,
D	2d	Colxacillin	Changeable	No	OXA-10, OXA-1,
	2de	Extended spectrum cephalosporin	Changeable	No	OXA-15, OXA-11,
	2df	Carbapenems	Variable	No	OXA-48, OXA-23

(Livermore, 1995 and Ghafourian *et al*, 2015). Early classifications related to β -lactamases are on the basis of many phenotypic (physiological) parameters like susceptibility to inhibitors, hydrolytic spectrum, hydrolysis rate and if they have been plasmid mediated or chromosomal. Susceptibility to cloxacillin, aztreonam, clavulanic acid, or pchloromercuribenzoate is considered.

1. Sawai T : Sawai *et al* (1968) attempted the first beta-lactamases' classifications. They indicated cephalosporinases and penicillinases via utilizing antisera as another separation way.

2. Sykes and Richmond : Initially suggested in the year 1970 via Richmond and Jack, it has been expanded subsequently in the year 1973 through Sykes and Richmond. β -lactamases have been categorized into 5 groups on the basis of substrate profile. Subsequently, it has been extended in the year 1976 via Matthew and Sykes, in which the plasmid mediated β -lactamases are differentiated through isoelectric focusing.

3. In 1981, Inoue and Mitsuhashi suggested another scheme that involved “cefuroxime hydrolyzing

β -lactamases” in addition to cephalosporinase and penicillinase.

4. Jacoby, Bush, and Medeiros phenotypic classification : In the year 1989, Karen Bush suggested another classification on the basis of enzymes' action on substrates (oxacillin, penicillin, cephaloridine, carbenicillin, imipenem and expanded-spectrum cephalosporins,) along with the susceptibility to inhibition through clavulanic acid. In addition, the classification is updated more in the year 1995 via George Jacoby, Karen Bush and Antone Medeiros and after that revised again in the year 2009. It includes four 4 groups and many sub-groups in each one of the groups (Ghafourian *et al*, 2015).

Group-1

Group-I (Ambler Class C) β -lactamases (also referred to as AmpC enzymes), such group showed high-resistance to β -lactamase inhibitors such as clavulanate and majorly observed in chromosomes. Also, they were inhibited through aztreonam and cloxacillin, yet not via clavulanic acid. Enzymes in group-1 were observed in *P. aeruginosa* and *Enterobacteriaceae* family. Researches

showed a shift of enzymes from chromosome to plasmid in a few strains, including *Klebsiella* spp and *E. coli*. Group-I producer β -lactamases were highly-resistance to β -lactam/ β -lactamase inhibitor combinations, cephamycins, penicillins, also 1st, 2nd and 3rd generation cephalosporins, while they have high sensitivity to carbapenems and cefepime.

Group-2

Group-2 (Ambler Class A) enzymes were harbored via a plasmid; also, they might be simply transmitted into various bacterial cells, resulting in rapid resistance to these enzymes. The inhibitors of β -lactamase, like sulbactam, clavulanic acid and tazobactam are inhibiting the original group-2 enzymes. Such group enzymes were SHV and TEM. TEM1 has been identified initially in the year 1965 in the *Enterobacteriaceae* family. Then, it was spread to bacteria like *Neisseria*, *Haemophilus* and *Vibrio* spp. SHV1 has been discovered in the year 1979 and was majorly recognized in *E. coli* and *Klebsiella* spp. Group-2 enzymes might be hydrolyzing the ampicillin, and 1st, 2nd and 3rd generations cephalosporins and monobactams (extended spectrum β -lactamases or ESBL) and they were highly-resistance to β -lactamase. Group-2 includes cephalosporinases, penicillinases, serine carbapenemases and broad-spectrum β -lactamases; such enzymes were typically inhibited through clavulanic acid. Traditional beta-lactamases, KPC-type, OXA-type and ESBLs belong here.

Group 3

They are found often in the *Bacteroides fragilis*, *P. aeruginosa* and *Stenotrophomonas maltophilia*, which include the metallo- β -lactamases (MBL) having requirements for an ion of Zn at the active site. Unlike serine β -lactamases, MBLs are of a poor hydrolytic ability for the mono-bactams and aren't inhibited by the tazobactam or the clavulanic acid. They're inhibited by the chelators of the metal ion like the EDTA, 1,10-phenanthroline, or dipicolinic acid. Those metalloenzymes were sub-divided according to either one of the structures (sub-classes B-1, B-2 and B-3) or function (sub-groups 3a & 3b).

Sub-group 3a includes the main plasmid-encoded families of the MBL, like VIM and IMP enzymes. Those enzymes are part of the molecular sub-class B-1, requiring 2 atoms of Zn for their hydrolytic activity. It includes as well, the enzymes of the sub-class B-3 MBLs like the CAU1, L1, GOB1, and FEZ1.

Sub-group 3b: which includes a smaller MBL group, preferentially hydrolyzing the carbapenems, unlike the cephalosporins and penicillins. They include only 1 atom

of Zn in the active sites. They are corresponding to the molecular sub-group B-2. E.g., CphA, Sfa1.

Group 4

The β -lactamases of the Group 4 contain the unusual penicillinases that have not been inhibited by the clavulanic acid, 4 of those enzymes show high hydrolysis rates with the carbenicillin, cloxacillin or both. A number of them show irregular behaviors, according to the involvement of the metal ion. Whether or not those enzymes represent a different β -lactamase molecular class is unknown.

5. Ambler's Molecular classification : The β -lactamases classification was at first based upon either the functional properties of enzymes or their fundamental structure. The molecular classification was suggested in 1980 by R.P. Ambler, who had taken under consideration the β -lactamases amino acid sequence. Those β -lactamases are divided to 4 classes, every one of which includes the types which are often chromosomal or plasmid-mediated (Table 2). None-the-less, those distinctions have been blurred due to the fact that it is appreciated increasingly that the majority of the 'plasmid-mediated' types are actually genetic escapes from other species' chromosomes (Livermore, 1998).

At first, only 2 classes (*i.e.* A & B) have been described for accounting for the serine and metallo-beta lactamases, respectively. Class C cephalosporinases have been explained in 1981 by Grundstrom and Jaurin. In the late 1980s, class D oxacillin-hydrolyzing enzymes have been included in classification. Nowadays, β -lactamases are classified to 4 classes, which are A, B, C and D. The molecular phylogeny of enzymes has been obtained from sequences of the amino-acid. According to the similarity of the sequence, sub-divisions in the class were made through the illustration of a dendrogram (Livermore, 1998).

1) Class A beta-lactamase

This class includes the plasmid-mediated and chromosomal penicillinases, carbapenemases and cephalosporinases, which have serine in the active sites. It comprises the early and conventional beta-lactamase types in addition to the beta-lactamases of the extended-spectrum. The following enzymes are in this group:

- Penicillinases (PC-1), hydrolyzing the benzyl-penicillin with higher efficiency compared to the cephalosporins. Those have been inhibited with clavulanic acid.
- The extended-spectrum beta-lactamases (SHV2, TEM3, CTX-M15), hydrolyzing the cephalosporins at a 10% higher rate compared to the penicillinases.

Table 2 : β -lactamase classes.

Molecular class and functional mechanism	Types that are normally chromosomal and ubiquitous in species or group	Types that are normally plasmid-, transposon- or integron-mediated
Class A Serine β -lactamases	SHV-1, LEN-1 and K1 in <i>Klebsiella</i> spp.; chromosomal cefuroximes of <i>Proteus vulgaris</i> ; chromosomal β -lactamases of <i>Bacteroides</i> spp.	Staphylococcal penicillinase; TEM, SHV, VEB, PER and CTX-M penicillinases and ESBLs; KPC, IMI/NMC and SME carbapenemases
Class B Metallo- β -lactamases	L1 enzyme of <i>Stenotrophomonas maltophilia</i> ; chromosomal enzymes of some <i>Chryseobacterium</i> spp. and <i>Aeromonas</i> spp.; CcrA enzyme found in 1–3% of <i>Bacteroides fragilis</i> isolates.	IMP, VIM and SPM types
Class C Serine β -lactamases	Chromosomal AmpC enzymes of <i>Escherichia coli</i> , <i>Shigella</i> spp., <i>Enterobacter</i> spp., <i>Citrobacter freundii</i> , <i>Morganella morganii</i> , <i>Providencia</i> spp. and <i>Serratia</i> spp.	CMY-1, LAT-1, BIL, MOX, ACC, FOX and DHA types ^a
Class D Serine β -lactamases	Chromosomal (along with other β -lactamases) in <i>Acinetobacter</i> spp. (OXA-51-like); <i>P. aeruginosa</i> (OXA-50) and some <i>Aeromonas</i> spp. (e.g. OXA-12).	Most OXA types, excluding those detailed here as chromosomal

Those have the ability to hydrolyze the monobactam and the oxyimino-cephalosporins and have a susceptibility to the inhibition by the clavulanic acid.

- Penicillinases and the early cephalosporinases (TEM1, TEM2, SHV1), hydrolyzing the penicillinases and the early cephalosporinases in an equal way. Those have been inhibited with clavulanic acid.
- The extended-spectrum cephalosporinases (TEM50), hydrolyzing the extended spectrum cephalosporins and monobactams, but have resistance to the tazobactam, clavulanic acid, and sulbactam.
- Penicillinases (TEM30, SHV10) that have resistance to the inhibition with the tazobactam, sulbactam and clavulanic acid.
- Carbenicillinases (PSE1, CARB3), preferentially hydrolyzing the carbenicillin and stay susceptible to the clavulanic acid.
- The extended spectrum cephalosporinases (Cep-A) that have the ability to hydrolyze the cephalosporins but not the aztreonam. Those have been inhibited by the clavulanic acid.
- Carbapenemases (KPC2, IMI1, SME1), hydrolyzing the oxyimino-cephalosporins, carbapenemases and cephamycins. They vary in their susceptibility to the clavulanic acid. Those enzymes can usually be observed in the *Serratia marcescens*, *K. pneumoniae* and *Enterobacter cloacae*. The genes for those enzymes are encoded chromosomally in the *S. marcescens* and *E. cloacae*, while its plasmid encoded in the *K. pneumoniae*.
- Carbenicillinases (RTG4), also hydrolyzing the cefpirome and cefepime, however, is inhibited with the clavulanic acid.

2) Class B beta-lactamase

Those are carbapenemases that have Zn^{+2} ions at the active site, which have also been referred to as the Metallo beta-lactamase. They have the ability for

hydrolyzing the cephalosporins, carbapenems, penicillins and have the resistance to the inhibitions by common inhibitors of the beta-lactamase. None-the-less, their hydrolytic activities don't extend to the aztreonam. *Bla* genes reside on plasmid, integrons, and chromosomes. In the *Chryseobacterium* spp., *Bacillus* spp. and *Stenotrophomonas maltophilia* MBL has been encoded in a chromosomal manner. In the *K. pneumoniae*, *A. baumannii* and *P. aeruginosa*, MBL, the encoding of the MBL has been carried out by the mobile genetic elements.

3) Class C beta-lactamases

Those can be defined as cephalosporinases with the serine in the active sites. Which are often encoded by genes that are located on the chromosome of the bacteria; however, the enzymes that have been encoded by the plasmid have turned quite common. It has been encoded chromosomally in the *Citrobacter* spp and *Enterobacter* spp; however, in the *Salmonella* spp, *Proteus* spp and *Klebsiella* spp, it's usually mediated by the plasmid. The isolates that produce those enzymes have resistance against the oxyimino-cephalosporins, aminopenicillins, beta-lactamase inhibitors, and cephamycins. The over-expression results in conferring the resistance against the cephalosporins of broad-spectrum, which includes ceftazidime, ceftriaxone, cefotaxime and has been considered as an issue, particularly in the infections as a result of the *E. cloacae* and *E. aerogenes*. The enzymes of the AmpCs result in the poor hydrolyzing of the cefepime and have been inhibited by the oxacillin, aztreonam, and cloxacillin. The chromosomal AmpC in the Gram-negative types of bacteria are usually represented at a low (*i.e.*, "repressed") level, however, may be "de-repressed" with the mutations in the *ampD* and *ampR* genes or through inductions with specific beta-lactam types, especially the ceftioxitin.

4) Class D beta-lactamase

Those enzyme types have been classified at first as

“oxacillinases” due to the fact that they are capable of hydrolyzing the oxacillin at a minimal rate of 50% that of the benzylpenicillin. This is why those enzymes have been referred to as the OXA-type beta lactamase. First reported in 1991 from Turkey, those enzymes have conferred resistance to the cephalosporins and penicillins. The OXA-type ESBLs (OXA11, OXA15) converse the resistance to the oxyimino-cephalosporins. The OXA-type carbapenemase types (OXA23, OXA48) are capable of hydrolyzing the carbapenems. The majority of the OXA enzymes have resistance against the inhibition by sulbactam, tazobactam, and clavulanate. None-the-less, there have been a few exceptions- OXA2 and OXA32 are inhibited with the tazobactam, however, not clavulanate and sulbactam, and OXA53 is inhibited with the clavulanate. Nearly 240 OXA-type beta-lactamases were explained yet. The molecular classification of Ambler seems commonly accepted compared to Bush’s phenotypic classification as a result of its phylogenetic relations amongst the enzymes as well as its simplicity.

5. The phylogenetic tree of Ogawara Hiroshi

According to amino acid sequences of the 47 β -lactamases and computer-aided analyses, a phylogenetic tree was suggested in 1993 by O. Hiroshi. Based on the tree, the beta-lactamases have been categorized into 6 groups. Group1 beta-lactamases include plasmid-mediated enzymes from the gramnegative bacteria and the chromosomal beta-lactamase from the *Rhodopseudomonas capsulate* and *K. pneumoniae*. Enzymes from Group2 included the chromosome-mediated beta-lactamases from the *Yersinia enterocolitica*, *Streptomyces*, *K. oxytoca* and *Citrobacter diversus*. The enzymes from Group3 included the chromosome mediated β -lactamases from other types of the gram negative bacteria. Group4 beta-lactamase has been composed of the metallo-beta-lactamases. Group5 has been composed of the OXA-type beta-lactamases. Group 6 has been composed of the chromosome-encoded β -lactamases from the gram-positive bacteria (Kong *et al*, 2009).

Genetic distribution of β -lactamases

A) Chromosomal β -lactamses

There are 2 chromosomal-encoded β -lactamase types: Constitutive and Inducible. The constitutive exists at a predictable level in every circumstance, while the latter has been repressed in the absence of the antibiotics, and it is induced with the existence of the β -lactam agents (Kong *et al*, 2009). The analyses of the chromosomal-mediated beta-lactamases showed that in the species of bacteria possessing a certain transcription regulatory

system, β -lactamase synthesis can be induced, while in the strains that do not have this system, the syntheses are often constitutive, where the class A enzymes are constitutive (Livermore, 1995).

The gram negative organisms have the chromosomally determined β -lactamases and the produced β -lactamase types are usually species-specific. Inducible β -lactamase expression of the Gram negative bacteria has suggested the fact that this bacterial resistance mode involves no one-step single-protein approach. In fact, the genetic researches that have been carried out on the *Citrobacter freundii*, *E. coli* and *E. cloacae* have shown that a minimum of 5 genes that are have been found in 3 loci participate directly in the resistance of the β -lactam. Those are *ampR*, *ampC*, *ampE*, *ampG* and *ampD* (Kong *et al*, 2009). The *Enterobacter aerogenes*, *Enterobacter cloacae*, *Morganella morganii*, *Citrobacter freundii*, *Providencia* spp., *Serratia* spp., *Pseudomonas aeruginosa* as well as other fluorescent pseudomonads, having inducible class C enzymes, which are; *Proteus vulgaris*, *Citrobacter diversus* and *Burkholderia* (i.e., *Pseudomonas cepacia*) having inducible class A enzymes; *Stenotrophomonas* (*Xanthomonas*) *maltophilia* having an inducible class B carbapenem-hydrolyzing β -lactamase as well as unsequenced Bush group 2-e cephalosporinases and the *Bacillus cereus* having about 3 inducible β -lactamase types (Livermore, 1995).

B) Plasmid-Mediated β -lactamases

Plasmids are double strands, extrachromosomal DNAs maintained as independent molecules by most of the bacterial genera. Genes located on various plasmids can express characteristics such as antibiotics resistance, enterotoxin production, heavy metal resistant (Ramawat *et al*, 2000). Throughout the last 2 - 5 decades, the plasmid-mediated β -lactamases became widespread in *Enterobacteria*, *Staphylococci*, *Neisseria gonorrhoeae* and *Haemophilus influenza*. More than 75 enzymes appear now on plasmid-mediated β -lactamase lists, even though several of them are sequence variations of a small number of the prevalent types. Generally, the plasmid-mediated β -lactamases differ from chromosomal types; however, there is a number of overlaps. Particularly, *bla*_{SHV} is one of the common plasmid types (Livermore, 1995). More than 30 plasmid-mediated β -lactamases (usually the penicillinases) were discovered in the Gram negative bacterial types and categorized based on some features like the substrate specificity, molecular weight, and isoelectric point. Common are OXA, TEM, PSE, and SHV types.

However, there were only 2 plasmid-mediated cephalosporinase reports from the *Proteus mirabilis*. The *AmpC* enzymes that have been encoded with the genes which escaped from *Enterobacter* and *Citrobacter* spp. Chromosomes. There is a possibility that every plasmid mediated β -lactamase has been of chromosomal origins, even though source organisms for numerous of the types are still not known. The plasmid-mediated enzyme distribution is a reflection of the elements' transmissibility to which their genes were spread (Livermore, 1995).

C) Transposons

The DNA segment may be moving around and inserted at a number of the sites in the phage of DNA of the bacteria (Ramawat *et al*, 2000). Numerous enzyme genes are on the transposons, which results in the facilitation of the dissemination amongst a variety of organisms and plasmids. For instance, the TEM β -lactamases have been first found in 1965 to be encoded by the entero-bacterial plasmids; however, by 1969, they had spread to the *P. aeruginosa*, by 1973 to the *Vibrio cholera* and by 1974 to *Haemophilus* and *Neisseria* species. The "secondary β -lactamase" term will be utilized in the present research for describing enzymes like those, encoded with the chromosomal inserts and supplementary to normal chromosomal organism β -lactamases (Livermore, 1995).

β -Lactamases of extended-spectrum

Classically, the ESBLs have been defined as the enzymes that are created with specific bacterial species which have the ability for hydrolyzing the extended spectrum beta lactam medications; cephalosporins and penicillins, however, not cephamycins or carbapenems by the hydrolytic activity and they have been inhibited by the inhibitors of the β -lactamase, which it is the clavulanic acid (Rahman *et al*, 2018; Nakamura *et al*, 2012 and Wiegand *et al*, 2007). Within β -lactamases, ESBLs were worthy of the attention of clinicians and the scientific community over the past 30 years, since they had been first described in 1983. Generally, the medications like ceftazidime, ceftriaxone and cefotaxime are medications of choice to treat the serious infections that have been caused by the members of family *Enterobacteriaceae*. Fundamentally, ESBLs are found in the *K. pneumoniae*, *E. coli* and *P. mirabilis*; however, they may happen in different *Enterobacteriaceae* family members and in some of the non-enteric organisms. They are usually encoded in plasmids (that may be transferred easily between the isolates), transposon and/or integrin (Cantón *et al*, 2012; Amaral *et al*, 2009 and Chanawong *et al*, 2007). They are plasmid born and identified as a result of

the fact that they are capable of hydrolyzing the early cephalosporins, penicillins, oxyimino-thiazolyl cephalosporins (which include the 3rd and 4th cephalosporin generations) and monobactams, however, not the cephamycins (like the cefoxitin), or carbapenems (which include ertapenem, imipenem, doripenem, or meropenem). They're inhibited with the suicide-inhibitors like tazobactam, sulbactam and clavulanic acid. The *AmpC* β -lactamases, unlike the ESBL, hydrolyze broad and extended cephalosporins' spectrum; however, they aren't inhibited with the clavulanic acid or any other inhibitors of the beta-lactamase (Turner, 2005 and Rupp *et al*, 2003). ESBL genes coding is usually located on the mobilizable or self-transmissible broad- host range plasmid types; the majority of the ESBL evolve with the genetic mutation from the native β -lactamases, in particular, the TEM1, TEM2 and SHV1, besides other genes of resistance, conferring the resistance to the antimicrobials like the sulphonamides and amino-glycosides (Rahman *et al*, 2018; Ghafourian *et al*, 2015, Nakamura *et al*, 2012 and Wiegand *et al*, 2007). Antimicrobial agent resistant problems like the ESBLs may considerably differ according to the patient populations, geographic areas, time periods, in addition to approached to collect and test the strains (Ho *et al*, 2005). Due to the fact that the enzyme has been active against a wide variety of the beta-lactam antibiotic types, this and the subsequent enzymes that have an equivalent capability have been designated ESBLs. Up until now, there have been more than 130 TEM-typed and more than 50 SHV-types beta-lactamases, and it seems that the new ones are detected weekly. Other ESBL types not belonging to SHV or TEM families began to be reported as well, and those have been referred to as the "CTX-M" (which highlights their higher activity towards the cefotaxime compared to the ceftazidime). Up until now, more than 40 of those enzymes were reported (Turner, 2005 and Livermore *et al*, 2006).

The ESBLs functional definition below has been provided in 2009 by Giske *et al* (2009) "ESBL has been characterized functionally as the β -lactamases (which belong to Bush's functional group 2be and the Ambler molecular class A), which is capable of hydrolyzing and causing the resistance to a variety of newer β - lactam antibiotic types, which includes extended-spectrum (or 3rd generation) cephalosporins (i.e., cefotaxime, ceftazidime and ceftriaxone) as well as the monobactams (i.e., the aztreonam) at a rate which is $\geq 10\%$ of that for the benzyl-penicillin, however, not cephamycins (i.e., cefotetan and cefoxitin) and carbapenems (i.e., meropenem, ertapenem and imipenem) and for being

inhibited by the inhibitors of the β -lactamase, like the clavulanic acid, while the ESBLs do not have the ability of efficiently hydrolyzing the cephamycins (*i.e.*, cefotetan or cefoxitin) or the carbapenems (meropenem or imipenem)." (Pitout *et al*, 2008 and Turner, 2005).

1) ESBL_A (class A ESBLs)

Inclusive of the SHV-, TEM-, GES-, CTX-M- and VEB-ESBL. Which has been sub-divided into high and low prevalence ESBL_A. Those enzymes are usually transferable horizontally and may be inhibited or inactivated with the clavulanic acid.

2) ESBL_M

Named for the miscellaneous ESBL that was sub-divided to ESBL_{M-C} (class C; plasmid-mediated Amp-C) and ESBL_{M-D} (class D; OXA-ESBL). The acquired Amp-C are the most commonly found ESBL in that class.

3) ESBL_{CARBA}

(which has been named for the ESBL having hydrolytic activity towards the carbapenems) that was sub-divided to:

- ESBL_{CARBA-A} (class A carbapenemase)
- ESBL_{CARBA-B} (class B carbapenemase)
- ESBL_{CARBA-D} (class D carbapenemase)

Lee *et al* (2010) have suggested an overall ESBL definition. Ambler Class A ESBL has been referred to as the aESBL, the class C enzymes with the extended spectrum as the c-ESBLs and the class D extended-spectrum type of the enzymes of the OXA as the d-ESBLs. Concerning that definition a-ESBLs include the SHV-ESBLs, TEM-ESBLs, GES-ESBLs, CTX-M-ESBLs and VEB-ESBLs; the d-ESBLs include OXA-ESBLs and c-ESBLs include the AmpC-ESBLs. Presently, the ESBLs are of high importance in the medical area. As a result of their ability to provide the bacterial strains with the resistance to the cephalosporins, workhorse hospital antimicrobial factor that has been applied for several illness types as antibiotics of the first line. Any identification delay and failure in the treatment of severe infection cases that result from the ESBL producer bacteria results in an increase in the mortality and morbidity. Due to the fact that the ESBL producers bacteria usually exhibit multi-drug resistances, like the resistance to the fluoroquinolones and amino glycosides, there are limited therapeutic options that are related to those strain types. Obviously, there is an increasing ESBLs producer prevalence, which causes higher degrees of mortality, morbidity and healthcare-related expenses (Ghafourian *et al*, 2015).

Genotypic detection

Nowadays, molecular techniques have a significant role in lab screening, monitoring and tracking the spread of numerous organisms that produce the CTX-M enzymes from hospitals and the community. The molecular method that is often utilized is the *bla* TEM and *bla* SHV gene PCR, succeeded by the sequencing, which is important for the discrimination between non-ESBL parent enzyme types (such as TEM-1, TEM-2 or SHV-1) and various types of the TEM or SHV ESBL (such as TEM-3, SHV-2 and others). Determining ESBLs in clinical isolates which are associated with the SHV and TEM enzymes is a complex procedure due to the point mutations as a result of the changes in the amino acid in SHV and TEM sequences, causing the increase in the enzymes activity spectrum, like the TEM-1, TEM-2 and SHV-1 (Shaikh *et al*, 2015 and Bradford, 2001). In contrast to the SHV and TEM types of the ESBLs; the PCR amplification of the CTX-M-specific products with no sequencing, in the diagnosis of the clinical isolates as one of the ESBL producing isolates, often gives adequate evidence on the fact that the *bla*-CTX-M gene is responsible for such phenotype (Shaikh *et al*, 2015).

ESBLs types

The majority of the ESBL types are SHV or TEM enzyme derivatives. Presently, there are 25 SHV-type β -lactamases as well as 90 TEM-type β -lactamases; with those two enzyme groups, a few point mutations at chosen loci in gene give rise to extended-spectrum phenotypes (for the amino acid sequences of SHV, OXA, and TEM inhibitor-resistant or extended-spectrum β -lactamase). The differences between the β -lactamase enzyme types is substitution of the amino acids producing various enzyme phenotypes. Substitutions happen more often amongst the SHV, OXA and TEM enzymes in the specified positions of the amino acids. Combining the altered amino acids results in producing various β -lactamase enzyme phenotypes with various abilities for hydrolyzing the cephalosporin of the 3rd generation and results in increasing the resistance level to the inhibitors of the β -lactamase (Ghafourian *et al*, 2015 and Bradford, 2001). According to Rahman *et al* (2018), it has been considered that 2 fundamental strategies of the evolution were deployed by a variety of the Gram-negative bacteria like the *K. pneumoniae* and *E. coli*:

- 1) variety of mutants with the extended specificity of the substrate from already prevalent SHV and TEM beta-lactam types
- 2) uptake and capture of the new wide variety of the β -lactamase genes from naturally existing meta-

genome, coding for the enzymes that have been endowed naturally with the activity of the ESBL.

1) TEM

The encoded of the TEM is usually performed by the Gram-negative bacteria. Nearly 90% of resistance to the ampicillin in the Gram-negative bacteria result from the TEM encoded genes as well as its responsibility for penicillin and ampicillin resistance which has been observed in the *N. gonorrhoeae* and *H. influenzae* in increasing numbers. TEM-type ESBL types are usually the plasmid mediated obtained from the mutations in conventional TEMs (*i.e.* TEM1 and TEM2) genes with multiple or single substitution of the amino acid around active site (Rahman *et al*, 2018 and Livermore, 1995).

- TEM1 has the ability of hydrolyzing the penicillins as well as the early cephalosporins like the cephaloridine and cephalothin.
- TEM2, the first TEM1 derivative had one substitution of the amino acid from original beta-lactamase. Which has resulted in shifting isoelectric point from a pI of 5.40 to 5.60, however, it caused no change to the profile of the substrate.
- TEM3, which has been reported first in 1989, has been the 1st TEM-type -lactamase which has shown the phenotype of the ESBL (Livermore, 1995).

Escherichia coli, which has been isolated from one patient called Temoneira (which is where the term TEM has come from) in Greece, harboring resistance that has been encoded by the *TEM1* has been the first report ever in 1965. TEM1 has the ability to hydrolyze the penicillins and first cephalosporin generation however, it does not have the ability for attacking oxyimino cephalosporin. TEM2 has been obtained from original enzymes of the TEM1 due to single or multiple mutations of the amino acid sequences. They all have the same hydrolytic profile, however, every one of them has a distinct iso-electric point, which is why it has not been viewed as ESBL. TEM3 has been discovered initially in France in 1984 in the *K. pneumoniae*. It has been the first beta-lactamase TEM-type which has shown the phenotype of the ESBL. At first, it has been referred to as the CTX1, due to its activities against the cefotaxime and it has been discovered to be harboring a new plasmid mediated beta-lactamase coined. the enzyme, however, it is presently referred to as the TEM3, which differs from the TEM2 with the double replacements of the amino acid. Shortly after, the TEM5 and TEM4 have been discovered 3 & 4 amino acid differ from the TEM1 of

the parent. TEM12 has been the 1-st TEM-type ESBL that has been found in the *Klebsiella oxytoca*, which has been isolated in 1982, in Liverpool, U.K. (Rahman *et al*. 2018; Ghafourian *et al*, 2015 and Shaikh *et al*, 2015). Since that first report, more than 90 extra derivatives of the TEM were described (for the sequences of the amino acid for SHV, OXA and TEM extended-spectrum and inhibitor-resistant beta-lactamase types), some of those beta-lactamases can be described as enzymes that are inhibitor resistant, however, most new derivatives are the ESBLs (Rupp *et al*, 2003 and Bradford, 2001). The list is growing as well for the TEM as novel types are reported from nearly all over the world with the number increasing to 223 (14th of Jan. 2018) (Rahman *et al*, 2018). The most common TEM type ESBLs are discovered in the *K. pneumoniae* and *E. coli*. None-the-less, the TEM-type ESBLs were given in different *Enterobacteriaceae*, like the (*Morganella morganii*, *Enterobacter cloacae*, *Proteus mirabilis*, *Enterobacter aerogenes*, *Salmonella* spp and *Proteus rettgeri*) as well as the rest of the Gram-negative bacteria and in the *Non-Enterobacteriaceae* as well, they're in the *P. aeruginosa* (Ghafourian *et al*, 2015 and Livermore, 1995).

2) SHV

The SHV types beta-lactamase can be seen most often in the *Klebsiella* species; SHV1 beta-lactamase is found most often in the *K. pneumoniae*, in addition to that they can found in many different *P.aeruginosa*, *Acinetobacter* spp and *Enterobacteriaceae*. SHV1 confers the resistance to a wide range of the penicillin types like the tigecycline, piperacillin and the ampicillin, however, not to oxyimino replaced cephalosporin types. It has the responsibility for about 20% of plasmid-mediated resistance to the ampicillin in the *K. pneumoniae* spp. In numerous, *K. pneumoniae* strains, *bla*_{SHV1} or an associated gene has been integrated to the chromosome of the bacteria, however, usually contained by the plasmid. None-the-less, several species were shown to be carrying the SHV1 gene in the chromosome. Even though it was theorized that the encoding of the gene SHV1 can be existing as a part of the transposable element, it wasn't proven yet (Rahman *et al*, 2018; Ghafourian *et al*, 2015; Shaikh *et al*, 2015 and Livermore, 1995). Unlike CTX-M and TEM, the SHV can be in a few types. Up until now, 193 different variants, which have been based upon the composition of the amino acid sequence, were available and reported in database. In addition to that, the variations which were seen in the *bla*_{SHV-1} for giving the rise to SHV types, which take place in smaller number of the positions in structural gene. Most SHV types that possess a phenotype of the ESBL have

been identified by substituting a serine for the glycine at the location of 238. An on-line database of The Lactamase Engineering Database (<http://www.laced.uni-stuttgart.de/>) has yet 132 assigned types of the SHV with the functional and structural properties. In addition to that, the amino acid substitution and its position has been restricted to a narrow and limited area, in contrast to the wide CTX-M area (Rahman *et al*, 2018 and Rupp *et al*, 2004).

The majority of the strains have the SHV on the plasmid by replacing the serine into glycine at the 238 position. Moreover, some of the strains change the lysine into the glutamate at the 240 position, several types that are associated with the SHV5 also have a lysine substitution for the glutamate at the 240 position. The interesting fact is that Gly-238Ser as well as the Glu-240Lys substitutions of the amino acid mirror the ones that have been observed in the TEM-type ESBL. Serine residue at the 238 position is of a high importance to hydrolyze the ceftazidime as well as the residue of the lysine is of a high importance to hydrolyze the cefotaxime (Ghafourian *et al*, 2015).

3) OXA

The enzymes of the OXA-type are an additional growing ESBL family. They have been termed that way due to their oxacillin-hydrolyzing capabilities. Those beta-lactamase types have been identified with the rates of the hydrolysis for the oxacillin and cloxacillin > 50% compared to that for the benzyl penicillin. Those beta-lactamase types are different from SHV and TEM enzymes in the fact that they are a part of the functional group 2d and the molecular class D, which exhibit the oxacillin-hydrolyzing abilities. The OXA type beta-lactamases is massively increasing according to the variations of the amino acid sequence, and up until now, 498 types were reported and provided in the data-base (Rahman *et al*, 2018; Shaikh *et al*, 2015 and Bradford, 2001). OXA-type enzymes were mainly reported in the *P. aeruginosa* they happen mainly in the *P. aeruginosa*, however, they were found in a wide range of other types of the Gram-negative bacteria. Actually, the most widespread OXA-type beta-lactamase have been discovered first in the isolates of the *P. aeruginosa* from one hospital in Ankara, Turkey. In France, an innovative OXA10 derivative (numbered OXA28) has been discovered in an isolate of the *Pseudomonas aeruginosa*. An innovative ESBL (OXA18) as well as an extended-spectrum derivative of narrow spectrum OXA13 beta-lactamase (which has been numbered as OXA19) were found as well in the *pseudomonas aeruginosa* isolates in France (Shaikh *et al*, 2015).

The majority of those enzymes have the resistance to the cephalothin, ampicillin and ceftazidime; with the high hydrolytic activities against the cloxacillin and oxacillin, and the fact that they have been inhibited poorly by the clavulanic acid and can't be degrading newer cephalosporin types so that they aren't considered as ESBL types (Rahman *et al*, 2018 and Bradford, 2001). Not every OXA enzyme is ESBL; actually, some of those (group 2-d) are plain oxacillinases, some of them have the phenotype of the ESBL (group 2-de) and some of them are carbapenemase (i.e. group 2-df). Of 244 known OXA-type beta-lactamases, a minimum of 16 have been known to be having a phenotype of the ESBL (Rupp *et al*, 2003; Drawz *et al*, 2010 and Sridhar, 2012). OXA beta-lactamase family has been created at first as phenotypic instead of a genotypic group for a small number of the beta-lactamases that had a certain profile of the hydrolysis. Which is why, there has been a 20% sequence homology amongst some members of that family. Nonetheless, the latest additions to that family have shown a homology level to one or several existing OXA beta-lactamase family members (Bradford, 2001).

4) PER-type ESBLs

PER-type ESBLs are sharing only about 25 to 27% of the homology with the known SHV-type and TEM-type ESBLs. PER1 beta-lactamase performs an efficient hydrolysis of the cephalosporins and penicillins and has the susceptibility to the inhibition of the clavulanic acid. PER1 has been initially found in France, in 1991 in an isolate of the *Pseudomonas aeruginosa*, which have been obtained from patient who has been transported from Turkey and after that in the isolates of the *Acinetobacter* and *S. enterica* serovar *Typhimurium* too. In Turkey, up to 46% of the nosocomial *Acinetobacter* spp. isolates and 11% of the *P. aeruginosa* have been discovered to be producing the PER1. PER2, sharing 86% homology with the PER1 was found in the *E. coli*, *S. enterica* serovar *Typhimurium*, *Proteus mirabilis*, *K. pneumoniae* and *V. cholerae*. PER1 was detected as well in Turkey, in the *Acinetobacter* spp and *Salmonella* spp. It was reported as well in Algeria, in *P. stuartii* and *P. vulgaris*. PER2, having a 87% homology with the PER1, was reported in Argentina. PER3 has been found in an *Aeromonas punctata* isolate in France. PER4 has been found in the *P. vulgaris*, PER5 in the *A. baumannii*, PER6 in an environmental *Aeromonas* species and PER7 in the *Acinetobacter baumannii*. A total of 7 PER-types were described. The PER-type enzymes have given a high resistance level to the ceftazidime, however, a low resistance level to the rest of the penicillins and cephalosporins (Shaikh *et al*, 2015 and Sridhar, 2012).

5) GES type

The GES1 has been described first in an isolate of the *K. pneumoniae* from neonatal patient who has just been transported to France from French Guiana). GES1 has hydrolytic activities against the penicillins as well as the cephalosporins of extended-spectrum, however, not towards the carbapenems or cephamycins, and it has been inhibited by the inhibitors of the beta-lactamase. Those enzymatic characteristics are resembling the ones of other class A ESB�; which is why, the GES1 has been characterized as an ESB� member (Shaikh *et al*, 2015).

6) VEB-type ESB�s

VEB1 (*i.e.* the Vietnamese extended spectrum β -lactamase) has been described initially in a strain of *E. coli* that has been isolated in one of the French hospitals in 1996, from a four-month old Vietnamese boy's pus. It has been reported then, in several of the gram negative bacteria like the *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis*, *A. baumannii*, *C. freundii*, *E. cloacae*, *P. stuartii*, as well as others. VEB1 ESB� is common in the South-east Asia, however, it was reported as well from Belgium, France, England, Iran, Bulgaria, Algeria, Kuwait, Korea, India and China. It is known for quite a little homology of the amino acid to other known types of the ESB�; the maximum being a 38% homology with the PER1 and PER2. In the majority of the cases, the *bla*_{VEB-1} is located on a cassette of the gene, presenting on an integrin of the class 1. Being present on the self-transmissible large plasmid types, the *bla*_{VEB-1} has been spread amongst Enterobacteriaceae. *A. baumannii*, resulting in the production of the VEB1 ESB� was implicated in the outbreaks of the hospital in a number of the countries, which include Korea, China and France. Presently, 7 VEB-type ESB�s were characterized. (Sridhar, 2012).

7) CTX-M β -lactamases

A new plasmid-mediated ESB� family, constituting the ESB�'s fast growing family CTX-M CTX-Ms have been named because they have extended activity towards the cefotaxime, in comparison with the cefazidime, up until now, 172 types of the CTX-M were reported. They are specially capable of the hydrolysis of the cephalothin in a more efficient way compared to the cefotaxime and benzyl-penicillin better than the ceftazidime. However, some of the CTX-M-type β -lactamases have shown a resistance to that medication. In addition to that, the resistance to the Aztreonam has been characterized as variable. The CTX-M-type β -lactamases have the ability of hydrolyzing the cefipime (Rahman *et al*, 2018; Ghafourian *et al*, 2015; Pitout *et al*, 2008; Livermore *et*

al, 2006 and Bonnet, 2004). They were mostly discovered in the *E. coli*, *Klebsiella pneumonia* and *Salmonella enterica* serovar *Typhimurium* strains (Bradford, 2001 and Bonnet, 2004), they have been characterized lately in *Acinetobacter baumannii*, *Vibrio Cholerae*, or *aeromonas hydrophila*, however, were described as well in other Enterobacteriaceae species (Shaikh *et al*, 2015 and Amaral *et al*, 2009).

The majority of early ESB�s have been ceftazidimases for the most part, without or with little hydrolytic activity towards the cefotaxime. ESB� have been different in the variability of the substrates, with some of the enzymes hydrolyzing one or several types of the cephalosporin over others. The first enzyme that had the property of the cefotaximase and susceptibility to the ceftazidime, has been reported in Japan in 1986 from an isolate of the *E. coli* that has been taken from a dog which has been utilized for studying the pharmacokinetics of the cephalosporin. It has been designated as FEC1. On the successive sequencing of the amino acid, it has been characterized similar to the CTX-M3 beta-lactamase. The initial cefotaximase property clinical isolate has been reported from the *E. coli* that has been grown from the ear discharge of a four-month baby in 1989 in Munich, Germany. It has been referred to as the CTX-M1 (M for Munich and CTX for cefotaximase). In that same year, a different cefotaximase has been designated as MEN1 (after the name of the patient) has been noticed in the isolate of the *E. coli* that has been obtained from an Italian patient in France. It has been found after that to be similar to the CTX-M1. In the year of 1989, the cefotaximases have been noticed in *S. typhimurium* that has been obtained from septicemia, enteritis and meningitis patients in Argentina. The amino acid sequencing has shown that it only had a 84% homology with that of the CTX-M1 and has been designated as CTX-M2 (Sridhar, 2012).

The chromosomal β -lactamases genes from some of the species of *Kluyvera* (widespread in environment), were proposed as ancestors of every one of the CTX-M clusters and *bla*_{CTX-M} gene mobilization to a different genera of the bacteria took place by the re-combinatorial events that have been mediated with the ISCR-1, ISEcp1 or the phage associated elements (Amaral *et al*, 2009 and Bonnet, 2004). Insertion of ISEcp1 element frequently found in the upstream region of the *bla*_{CTX-M} genes have a significant impact on mobilizing those genes (Chanawong *et al*, 2007). The plasmid encoded *bla*_{CTX-M} enzymes are a representation of a significant class A beta-lactamase sub-group that hydrolyze a wide range of the antibiotics of the β -lactam, which cause a wider

beta-lactamase (ESBL) phenotype range that has been increasingly discovered in the species of the enterobacteria (Monstein *et al*, 2009). The fast and wide CTX-M enzymes' spread all over the world, can be attributed mostly to frequent *bla*_{CTX-M} genes' association to the genetic mobile elements, like the epidemic plasmids, insertion sequences and/or transposons that are located upstream *bla*_{CTX-M} responsible as well for expressing the gene (Amaral *et al*, 2009). The enzymes of the CTX-M are different from the majority of the SHV and TEM ESBLs with a considerably higher hydrolytic activities against the cefotaxime compared to it against the ceftazidime and a different profile of susceptibility to the inhibitors. In addition to that, the spectrum of the activity amongst the enzymes of the CTX-M has been unusually heterogeneous. Substrate spectrum modulation of the enzymes of the CTXM-type with the point mutations is possible, like what occurs with SHV- and TEM- type enzymes. Particularly, several CTXM mutants with an increase in the activity of the ceftazidimase were explained. Those were possibly chosen by massive utilization of the ceftazidime in the clinical practices. Mutations in those types happen in 2 structural elements, delimiting beta-lactam-binding sites (Rossolini *et al*, 2008).

CTX-M beta-lactamases can be considered typical ESBL types belonging to the Bush's group 2-be as well as the Ambler's class A (Rossolini *et al*, 2008). Those enzyme types aren't quite tightly associated with the SHV or TEM beta-lactamase types in the fact that they only exhibit about 40% of the identity with those 2 often isolated beta-lactamases. (Bradford, 2001).

There has been a minimum of 172 types of the CTX-M that were described yet. With the use of the relatedness of the amino-acid sequence. They have been exclusively discovered in functional group2 and considered to be originating from the chromosomal ESBL genes that have been discovered in the *Kluyvera* spp, which is one of the opportunistic pathogens of the *Enterobacteriaceae* discovered in environment. A phylogenetic tree was constructed according to the sequence of the amino acid for the determination of relatedness amongst CTX-M β -lactamase members. The CTX-M was classified to 6 groups, which are (CTX-M1, CTX-M2, CTX-M8, CTX-M9, CTX-M25 and KLUC, which have been named after the 1st member of the group which has been described. Cluster naming has been based upon the first member of the group which has been described (Rahman *et al*, 2018 and Shaikh *et al*, 2015)

Some of the members are:

- CTX-M1 cluster: CTX-M-1, -3, -10, -12, -15, -22, -23
- CTX-M8 cluster: CTX-M-8, -40, -63
- CTX-M2 cluster: CTX-M-2, -4, -5, -6, -7, -20, -76, -77
- CTX-M25 cluster: CTX-M-25, -26, -39, -41, -91
- CTX-M9 cluster: CTX-M-9, -14, -15, -16, -17, -18, -19

Members in a group have more than 94% relatedness amino acid and less than or equal to 90% of the relatedness over groups. The beta-lactamase CTX-M gene is encoding a 291 amino acid enzyme. One change in the amino acid in the *bla* CTX-M would constitute a new type of the CTX-M (Rahman *et al*, 2018; Sridhar, 2012; Pitout *et al*, 2008 and Bonnet, 2004).

8) Metallo- β -lactamases (MBLs)

The MBLs have been classified initially in 1980 from the serine beta-lactamases in the classification which has been suggested by Ambler. In the year of 1989, Bush had additionally classified the MBL to a separate group (*i.e.* group 3) based on the functional characteristics, according to: profiles of the substrate (particularly the imipenem hydrolysis), their sensitivity to the EDTA, as well as their lack in the inhibition by the serine inhibitors of the beta-lactamase. Such classification is still the advised reference system for the beta-lactamases in general and has been upgraded in 1995 and modified additionally in the year of 1997 for the purpose of accommodating the growing group three enzymes that are being continuously classified (Walsh *et al*, 2005).

The β -lactamases have been categorized to 4 classes A, B, C and D, according to the sequence of the amino acid. Enzymes from Class A, Class C and Class D utilize an active-site serine nucleophile as well as a transient covalent reaction which is intermediate for the catalysis of beta-lactam antimicrobial agent hydrolysis. However, class B includes the MBLs have a wide range of the substrates and are capable of catalyzing the hydrolysis of virtually every beta-lactam antimicrobial agent except the mono-bactams. The MBLs are inactivated by the chelators of the metal like the EDTA, they aren't inhibited by the mechanism-based inhibitors like the: sulbactam, tazobactam or asclavulanate that are efficient against the serine-based. In addition to that, they aren't inhibited effectively with the NXL104, inhibiting the enzymes of classes A & C (Mojica *et al*, 2016 and Palzkill *et al*, 2013).

(MBLs) perform the catalysis of the hydrolysis of the beta-lactam antimicrobial agents by the use of a non-covalent approach, where 1 or 2 bound zinc ion equivalents result in the promotion of nucleophilic

hydroxide formation, which is a substitute for Ser residue which is utilized by the classes A, C, & D. In MBLs, Zn-containing active site has been positioned in a shallow groove, which has been formed at central beta sheets' interface. The crystal structures of the B-1 beta-lactamases show a di-nuclear or mono nuclear cluster of the metal ions where Zn-1 is often coordinated with the tetra-hedral geometry, and Zn-2 with the trigonal bipyramidal geometry in the absence of the substrate or product ligands. In the mono-zinc B-1 MBL structures, Zn-1 site is occupied by sole Zn ion. In the di-zinc structures, the B1 MBLs with different affinities for every one of the sites, Some of the crystal structures of B-1 beta-lactamase NDM1 has shown as well a 3rd ion of the zinc (Zn₃) that is bound at the active site's edge, positioned about 8Å from di-nuclear Zn cluster. Nonetheless, Zn3's functional relevance was not specified and it can be an artefact or crystallization (Mojica *et al*, 2016).

As a family, MBLs show a wide variety of the sequences (usually less than 25% identities of the amino acid), each one of them exhibits a similar fold of the protein and have the same mechanistic characteristics as some of the enzymes. The X-ray structures of multiple MBLs show that the MBLs that have structural similarity are part of a larger super-family of the metallo-proteins with different biological functions beyond the hydrolysis of the beta-lactams, which have been designated as a superfamily of metallo-hydrolase/oxidoreductase in structural Protein (SCOP) data-base classifications. And they have a characteristic $\alpha\beta/\beta\alpha$ sandwich fold with active site that is located at interface between the domains where an internal mixed beta-sheet sandwich has been surrounded by the solvent-exposed α -helices. Such scaffold can support about 6 residues at active site, coordinating either 1 or 2 Zn ions, which have been considered as central to catalytic mechanisms. MBL $\alpha\beta/\beta\alpha$ fold-type is encompassing a larger metalloproteins' super-family with a wide range of the biological functions beyond the hydrolysis of the beta-lactam (Mojica *et al*, 2016 and Palzkill *et al*, 2013).

The Class B *bla* (MBLs) have been classified to 3 sub-classes that have been characterized based on the differences in Zn shell of coordination and based upon the homology of the primary amino acid sequence. There is rather a low sequence identity (*i.e.*, less than 20%) between sub-classes that may be producing unreliable alignments of the sequence over the sub-classes according to Mojica *et al* (2016):

- 1) Sub-class B-1: which is binding 1 ion of the zinc (Zn₁) with 3 His residues (which are H-116, H-118 and H-196) and a 2nd ion of the zinc (Zn₂) with 3 distinctive residues, which notably include a Cys (D-120, C-221 and H-263). That sub-class contains the most clinically-relevant members; like IMPs (imipenemase), VIMs (*i.e.*, Verona integrin-encoded MBLs), and the NDMs (*i.e.*, the New Delhi MBLs).
- 2) Sub-class B-2: have a Zn₁ binding site with 1 altered residue (N-116, H-118 and H-196); however, they maintain a similar site of the Zn₂ (D-120, C-221, and H-263). This sub-class contains the smallest number of members that are produced by different *Aeromonas*' species.
- 3) Sub-class B-3: which has a varied binding site of Zn₁ (H/Q-116, H-118, H-196) as well as a distinctive Zn₂ binding site, lacking a Cys residue (D-120, H-121, H-263).

CONCLUSION

This article summarizes the concern of Beta-lactamases as one of most critical factors that increasing the resistance of bacteria towards antimicrobial agents that deals with the treatment of wide spectrum of infectious diseases. The ability of beta-lactamases's genetic material to be mobilized and transferred in addition to changing the chemical structure by mutations leading to generate a huge groups and subgroups of these enzymes, which increase the difficulty of facing up the problem of bacterial resistance towards the drugs which will fail to treat bacterial infections leading to increase the mortality rate.

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