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Review of Beta lactams

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β-Lactam antibiotics structure

β-lactam antibiotics are a class of antibiotics that are used in clinical practice in both oral and parenteral forms. β-lactam antibiotics have become the most widely used therapeutic class of antimicrobials due to their broad antibacterial spectrum and excellent safety profile. [1]. They have a beta ring [2]. β-lactam antibiotics inhibit bacterial cell wall biosynthesis and are the most commonly used antibiotics for treating a wide range of bacterial infections. [3]. These target proteins catalyze the formation of the peptidoglycan that makes up the bacteria's cell wall. PBP mutations have been linked to resistance to beta-lactam antibiotics [4]. They have radically changed the landscape of the fight against bacterial infectious diseases since their introduction in the 1930s. [5]. β-lactam antibiotics are classified into several classes, including penicillins (penams), cephalosporins (cephems), carbapenems, monobactams, and beta-lactamase inhibitors. The 3-carbon and 1-nitrogen ring (Beta-lactam Ring) of these medications, Beta lactam antibiotics, is extremely reactive from a biochemical standpoint [6]. But for individual structures,

1. penicillins: 6-aminopenicillanics acid, also known as 6-APA, is a basic bicyclic compound with a basic bicyclic structure. The β-lactam and thiazolidine rings are formed by condensation of L-cysteine and D-valine, forming an enclosed dipeptide. [7]. Penicillins are classified as either natural or semi-synthetic. Penicillins derived from penicillin culture solutions are known as natural penicillins. Penicillium is grown in culture and only the nucleus is synthesized to make semi-synthetic penicillins.This type of penicillin has a wider spectrum of action, is effective against gram-negative bacteria, and is not penicillinase - resistant. [8].

2. Cephalosporins are 7-amino-cephalosporanic acid derivatives with a penicillin-like structure. They contain a beta-lactam ring. They are penicillinase-resistant and relatively

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stable in dilute acid.The first-generation cephalosporins include cephalothin, cefazolin, and cephalexin, and are all active against most gram-positive cocci.They are more effective against gram-positive bacteria, than gram-negative bacteria, and their antimicrobial activity against gram-negative bacteria increases in the second generation.The third generation has the greatest antibacterial activity against gram-negative bacteria and the least activity against gram-positive bacteria.Fourth-generation cephalosporins are zwitterions that can penetrate the outer membrane of gram-negative bacteria and act similarly to first-generation cephalosporins against gram-positive bacteria. [9].

3.Carbapenems: have a five-membered ring similar to penicillin, but the sulfur at C-1 is replaced by a carbon atom, and a double bond between C-2 and C-3 is introduced [10]. They have the broadest antimicrobial spectrum of any beta-lactam and are mostly used to treat infections caused by aerobic gram-negative bacteria [11]. A carbapenem ring linked to a beta-lactam ring distinguishes them, providing protection against most beta-lactamases. [6].

4. Monobactams: The beta-lactam ring exists independently and is not fused to another ring.[6].Monobactams are monocyclic beta-lactams with a moiety of 2-oxoazetidine-1-sulfonic acid. Aztreonam is the only monobactam currently approved by the FDA. [12].

5-Beta-lactamase inhibitors: are sulfone derivatives of clavulanic acid and penicillanic acid, both of which are beta-lactam compounds in and of themselves. [13]. (Figure 2)

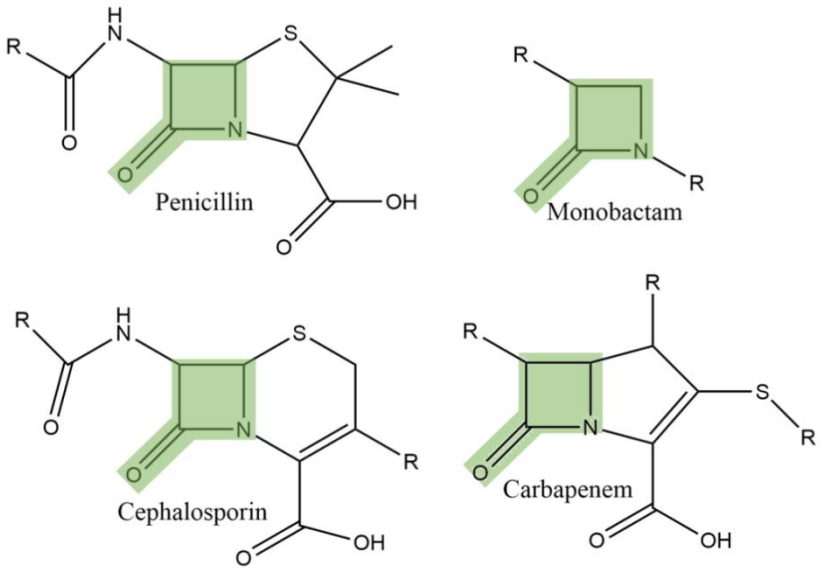

Fig1: Chemical structures of Beta lactams

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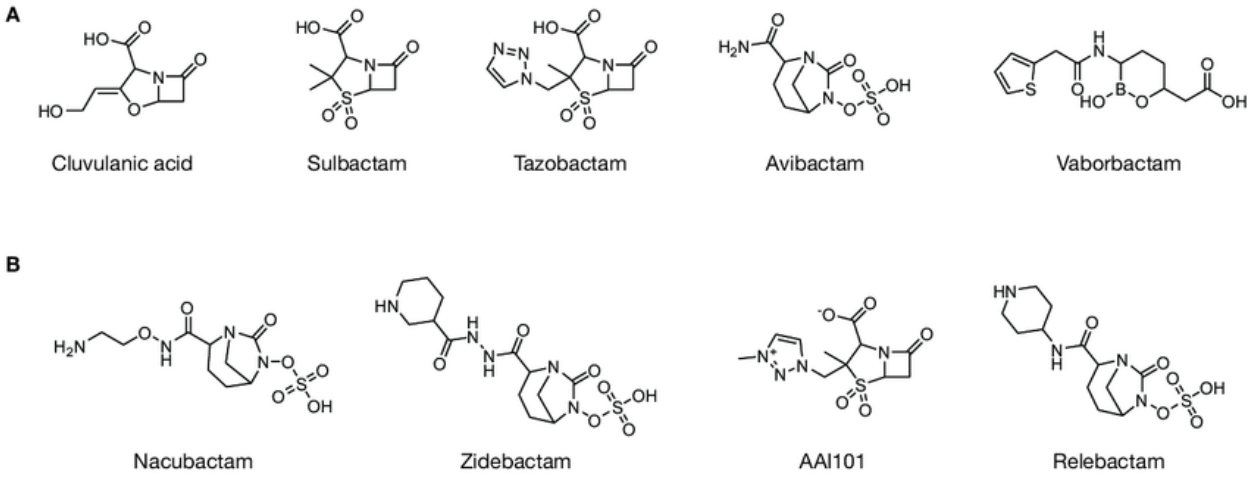

Fig2: Chemical structures of prominentBeta-lactamase inhibitors

2.1 Mechanism of action of β-Lactams

Beta-lactams exert antibacterial activity by inhibiting bacterial cell walls, peptidoglycan synthesis, and the precise function of penicillin-binding proteins, also known as transpeptidases. [14]. The peptidoglycan molecule is a structural component of the bacterial cell and periplasm. In addition to stiffness, it protects bacterial cells from excessive internal osmotic pressure and provides them with a defined shape. PBP catalyzes the cross-linking of amino acids from neighboring chains, creating a mesh between the inner and outer membranes in the periplasmic space.

PBPs "mistakenly" pick these up (-lactam in particular) to use as building blocks during cell wall formation because the -lactam ring is similar to that of the D-alanine-D-alanine of the N-acetylmuramic acid (NAM) pentapeptide.

The bacterial cell pays the price for this mistake, as acylation of the PBP renders the enzyme (transpeptidases) inactive by suppressing transpeptidation processes, resulting in an accumulation of cell wall precursor units, which activates the cell wall autocatalytic system, ending in cell lysis. [15].

β -lactams impair cell wall production and induce active breakdown of the cell wall, resulting in cell lysis and bug elimination**.**By inhibiting transpeptidases and activating autolysin at the same time,

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2.2. Mechanism of action of penicillin

Penicillin binds to and acylates the active site of the enzyme(s) that crosslinks the cell wall to produce an inactive penicilloyl enzyme. By acting as a structural analog of the acyl-D-alanyl-D-alanine terminal of the nascent bacterial cell wall, This study shows that penicillin acrylates the active site of two penicillin-sensitive enzymes, Bacillus stearothermophilus and Bacillus subtilis D-alanine carboxypeptidases. After covalently tagging with I14Cl penicillin G or trapping an acyl-enzyme intermediate formed from the depsipeptide substrate, L14Cjdiacetyl-I,-lysyl-D-alanyl-D-lactate, active site peptides were created by chemical or enzymatic cleavage of these carboxypeptidases. The penicillin-labeled peptides had identical amino acid sequences. Both penicillin and the substrate were joined in a covalent manner. A serine at position 36 of the B. stearothermophilus carboxypeptidase and the corresponding serine in the B. subtilis carboxypeptidase are linked via an ester linkage to the same active site residue. There was a lot of homology between the two D-alanine carboxypeptidases in the active region. Furthermore, homology between these two enzymes and four known f3-lactamases shows that these two groups of enzymes are evolutionarily related.

2.3. Mechanism of action of cephalosporin

Penicillin binds to and acylates the active site of the enzyme(s) that crosslinks the cell wall to form an inactive penicilloyl enzyme by functioning as a structural analog of the acyl-D-alanyl-D-alanine terminal of the nascent bacterial cell wall. The active site of two penicillin-sensitive enzymes, Bacillus stearothermophilus and Bacillus subtilis D-alanine carboxypeptidases, is acylated by penicillin, according to this study. Active site peptides were generated by chemical or enzymatic cleavage of these carboxypeptidases after covalently tagging with I14Cl penicillin G or trapping an acyl-enzyme intermediate formed from the depsipeptide substrate, L14Cjdiacetyl-I,-lysyl-D-alanyl-D-lactate. The amino acid sequences of the penicillin-labeled peptides were identical. Covalent bonds were formed between penicillin and the substrate. B. stearothermophilus carboxypeptidase serine 36 and the equivalent serine in B. stearothermophilus carboxypeptidase**.**

2.4. Mechanism of action of carbapenems

Meropenem, a carbapenem antibacterial agent, is resistant to hydrolysis by the majority of eta-lactamases found in gram-negative and gram-positive bacteria, including penicillinases and cephalosporinases. [19]. Meropenem kills bacteria by binding to penicillin-

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binding proteins (PBPs) in the bacterial cell wall and inhibiting peptidoglycan cross-linking associated with cell wall synthesis, which results in cell death. [19][20] [21].

Vaborbactam is a non-suicidal, broad-spectrum BATSI that has been intended to be a potent inhibitor of class A serine carbapenemases like KPC, NMC-A, and SME-2, add to other class A (e.g. CTX-M, SHV) and class C (e.g. P99, MIR) -lactamases. [22,23,24]. Vaborbactam has been shown to inhibit the newly discovered class A carbapenemases BKC-1 and FRI-1too, which were discovered in clinical isolates of K. pneumonia and Enterobacter cloacae, respectively. [25]. Vaborbactam does not inhibit carbapenemases of class B (e.g., NDM, VIM) or class D (e.g., OXA-48). [20]. Vaborbactam has no effect on mammalian serine proteases. [19] and possesses no antibacterial activity. [21] [25].Through a novel mechanism of enzyme inhibition, vaborbactam protects meropenem from degradation by serine carbapenemases. This happens when a covalent adduct forms between the catalytic serine residue of β-lactamases and the boron moiety of vaborbactam, simulating the tetrahedral transition state of -lactamase hydrolysis. [19] [22] [26]. Biochemical studies presented that vaborbactam is a slow, tight-binding reversible inhibitor of KPC-2, with a very slow off-rate (enzyme residence time of 992 min) and 1:1 stoichiometry [26] [27]. It was suggested that the slow off-rate of vaborbactam for KPC may play an important role in enhancing the activity of antibacterials against KPC-producing bacteria [20]. Vaborbactam inhibited KPC-2 mediated hydrolysis of nitrocefin with greater potency than tazobactam (> 20-fold) and clavulanic acid (> 500-fold) [19] [27]. Unlike other currently available BLIs (such as tazobactam and avibactam), vaborbactam is not hydrolyzed by KPC [26].

2.5. Mechanism of action βeta lactamase inhibitor

Inhibitors of beta-lactamase work in one of two ways. They could become substrates like the acyl-enzyme, which have a high affinity for the beta-lactamase enzyme but generate sterically unfavorable contacts. They can also function as "suicide inhibitors," permanently inactivating the enzyme by secondary chemical events in the active region. The first mechanism is used by avibactam and relebactam, while the second is used by sulbactam, tazobactam, and clavulanic acid [28].

Resistance to β-lactam antibiotics

Antibiotic resistance is a microorganism's ability to withstand the effects of an antibiotic [29]. Bacteria and other infection-causing microbes have establishedremarkable resistance to antibiotics and other antimicrobial drugs. This is primarily due to increased use and misuse of

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antibiotics for various medical illnesses [30]. Three major mechanisms contribute to bacterial resistance to beta-lactam antibiotics. (i) β-lactamase enzymatic degradation, (ii) PBP target modification results in β-lactam binding loss, and (iii) β-lactam entry and efflux regulation [31].

Enzymatic degradation: the expression of hydrolytic enzymes known as β-lactamases is the single most prominent mechanism of bacterial resistance to β-Lactams. These enzymes recognize and hydrolyze the four-membered -lactam ring, producing an inactive product that no longer inhibits TPs. Resistance is most commonly conferred by mutation of preexisting beta-lactamase enzymes, resulting in an expanded spectrum or targeted specificity of their hydrolytic properties against the aforementioned beta-lactam classes. Many β-lactamases are encoded on mobile genetic elements, resulting in increased transmission and spread to the point where it is now common to find bacterial strains harboring up to eight different β-lactamases, each tailored to inactivate a specific subset of antibiotics [32].

Target modification: Changing the intended target is a common way for bacteria to avoid antibiotic action. This mechanism is so effective that it can be found in any antibiotic class, regardless of mechanism. These target changes are usually caused by genetic mutations in response to selective pressures in the presence of antibiotics. However, in some cases, modified targets may be acquired through genetic exchange. There are numerous examples of target modification among PBPs from various bacterial types. [33].

β-lactam entry and efflux regulation: The third major mechanism causing β-lactam resistance in gram-negative bacteria is reduced drug uptake. β-lactams must enter the periplasmic space to bind PBP targets in the cytoplasmic membrane . Reduced drug uptake can be caused by changes in the porin channels that β-lactams use to cross the outer membrane, or by the presence of efflux pumps that actively extrude β-lactams from the periplasmic space [34]. Furthermore, bacteria have evolved sophisticated genetic mechanisms to adapt to novel beta-lactam antibiotic treatments. Antibiotic resistance mechanisms must be understood in order for future antibiotic treatments of bacterial infections to be successful [35].

β-lactamase enzyme

β-Lactamase: An enzyme that hydrolyzes the β -lactamases ring of antibiotics to render them inactive. Over 200 different varieties of β-lactamases have been identified. Some are specialized for penicillins (penicillinases), cephalosporins (cephalosporinases), or

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carbapenems (carbapenemases), while others have a broad range of activities [36]. Antibiotic resistance is predominantly caused by plasmid-encoded -lactamases in Gram-negative bacteria [37]. The activity of -lactamases, which are enzymes produced by both gram-positive and gram-negative bacteria and hydrolyze the β-lactam amide, causes enzyme-mediated resistance to -lactams [38]. The rising number of β-lactamases discovered, together with the availability of protein, and then nucleotide, sequence information, proved that these enzymes do not represent a single homogenous group but may be classified into various classes[39]. This array of enzymes is classified using two systems: the Bush–Jacoby–Medeiros activity-based system and the Ambler system. [40] [41], based on sequence information. The latter divides β -lactamases into four distinct classes: A, B, C, and D, which are identified by specific sequence motifs but differ fundamentally in their hydrolytic mechanism. There are currently two types of β-lactamases known among the three classes (A, C, and D) of active-site -lactamases: serineβ lactamases (SBLs) and metalloβ-lactamases (MBLs) [42]. One class of these enzymes is famous as serine β-lactamases because they have an essential serine residue in their active site that acts as a nucleophile to attack the β-lactam ring in the first step of the catalytic mechanism, ensuing in the formation of a covalent acyl-enzyme adduct. Historically, serine β-lactamases were known for being highly specialized against penicillins or cephalosporins. [43]. Conversely, since the 1990s, the number of variants known as extended spectrum β -lactamases (ESBLs) has increased significantly, and ESBLs have spread among Enterobacteriaceae and Pseudomonas species. [44]. These enzymes can hydrolyze penicillins, first-, second-, and third-generation cephalosporins, add to aztreonam. They are TEM (named after the patient Temoniera from whom it was isolated), SHV (for sulfhydral variable type 1), CTX-M (because it is active on cefotaxime and was first isolated in Munich), and OXA (oxacillinases) variants. As a result, carbapenems should be reserved as a last-resort antibiotic for the treatment of resistant infections. Nonetheless, genes coding for carbapenemases have spread rapidly in Enterobacteriaceae species and non-fermentative gram-negative bacteria over the last ten years. The most notable carbapenemases are the serine B-lactamases KPC (Klebsiella pneumoniae carbapenemase) and OXA, as well as the metalloβ-lactamases [45]. Unlike ESLs, all β-lactamases can hydrolyze carbapenems and are not inhibited by serine β-lactamase inhibitors [46]. This is due to significant differences in the catalytic mechanisms of these two classes of enzymes. The fact that MBLs are Zn-dependent hydrolases with one or two metal ions in their active site is at the root of the mechanistic differences. The nucleophile in the first step of the mechanism of β -lactam hydrolysis is thought to be a water molecule activated by coordination to one of the Zn (II) ions [47] [48].

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Metallo β-lactamases, such as BcII from Bacillus cereus, are mostly encoded chromosomally in environmental bacteria or opportunistic pathogens. [49]. Metalloβ -lactamase), IMPs (Imipenemase), and, more recently, NDMs (New Delhi Metallo β-lactamase) are clinically relevant metalloβ-lactamases encoded in mobile genetic elements. Despite the fact that various inhibitors have been tested in vitro, no clinical drug has been found to inhibit any of the metallo β-lactamases [50]. These metallo β lactamases have a broad substrate spectrum that includes carbapenems, penicillins, and last-generation cephalosporins. Although they do not hydrolyze aztreonam, they are frequently co-expressed with serine β-lactamases, which reduce the bacteria's sensitivity to this compound [43].

5. Mechanism of beta lactamase

β -lactamases are enzymes that break down the β -lactam ring. By hydrolyzing the peptide bond of the four-membered beta-lactam ring, beta-lactamase enzymes render beta-lactam antibiotics ineffective. After being inactivated, the bacterium develops resistance to the antibiotic. (fig 1) [51].

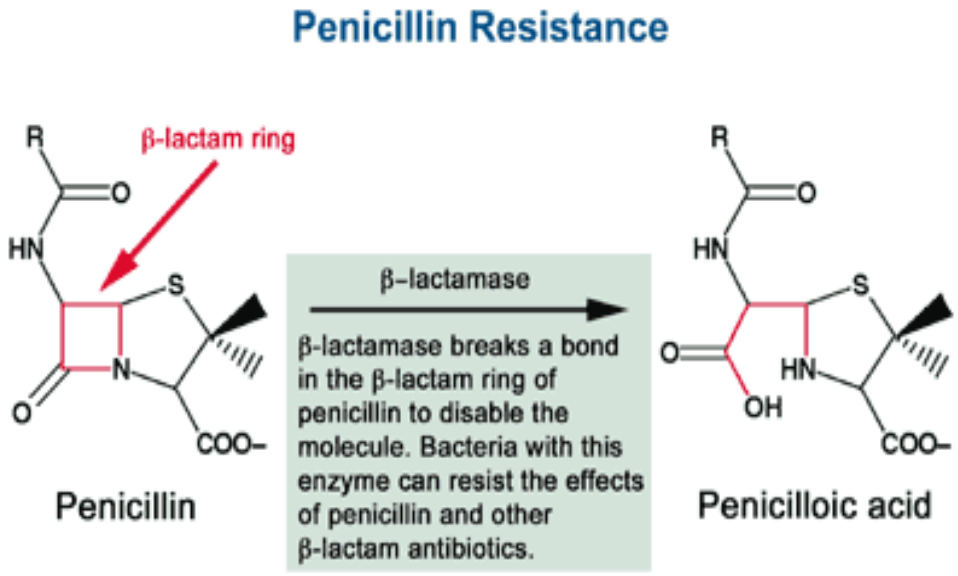

Fig 1: beta lactamase mechanism against penicillin

Ls genes can be transported easily between bacteria since they are situated on mobile genetic elements [52]. They can be encoded on chromosomal or extrachromosomal elements. The generation of beta-lactamases is the most common mechanism of resistance to β -Lactams. [53]. For β -lactamases, there are currently two classification methods in use. -lactamases are classified into class A, C, and D enzymes that employ serine for β -lactam hydrolysis and

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class B metalloenzymes that use divalent zinc ions for substrate hydrolysis based on their amino acid sequence[54].

Active-site serine lactamases are a more immediate concern. The antibiotics are hydrolyzed using the acylation/deacylation pathway [55].

All class A β-lactamases use an active site serine nucleophile to cleave the substrate's β-lactam bond in a two-step acylation-deacylation reaction cycle (figure 2) that leads to overall hydrolysis. The acylation reaction begins with the creation of a precovalent substrate complex (stage 1). The serine hydroxyl executes a general base-catalyzed nucleophilic assault on theβ -lactam carbonyl, which leads to the formation of a transitory acyl-enzyme adduct via a tetrahedral intermediate (step 2). (stage 3). In a typical base-catalyzed assault, the acyl-enzyme adduct (step 3) is attacked by a hydrolytic water molecule to generate a second tetrahedral intermediate (stage 4), which subsequently collapses to form a post covalent product complex (stage 5), from which the hydrolyzed product is released[56].

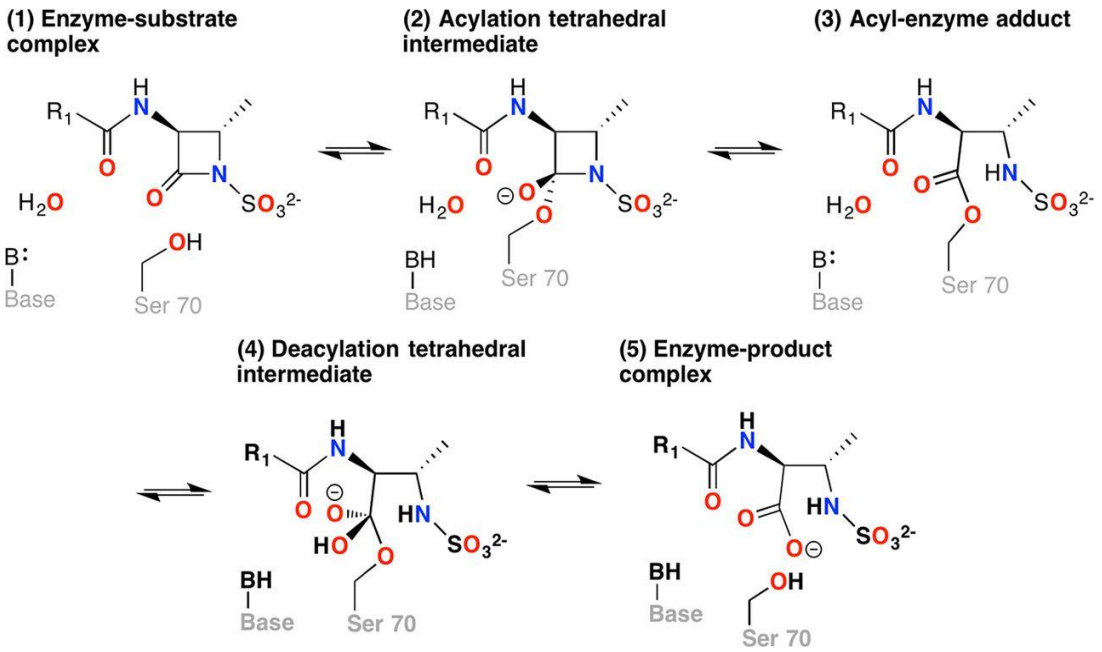

Fig 2: Catalytic cycle of a class A β-lactamase illustrated for a monobactam substrate.

In metallo enzymes, coordination of the substrate and intermediates to the active site metal-ion appears to be involved. [57]. The fact that the enzyme processes alter depending on whether one or two zincs are bound in the active site, which is reliant on the β --lactamase subclass, reflects this variety. [58]. B1 and B3 subclass β -lactamases are known to be catalytically active with a single zinc cofactor, usually in the Zn1 site. [59]The second zinc typically improves catalytic activity [60].A thorough understanding of the mechanism of

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metallo β-lactamase catalysis, including the rate limiting steps and the chemical nature of reaction intermediates for the various enzyme subclasses, will aid in the rational design of inhibitors [61]. Hydrolysis of the di-zinc MBLs, which include the subclasses B1 and B3, is thought to occur via cleavage of the amide bond of the β-lactam ring via attack of a hydroxide ion on the carbonyl carbon. [62]. The carbonyl oxygen connects with Zn1 and the carboxyl group on the 5- or 6-membered fused ring interacts with Zn2 as the β-lactam binds to the metal center. The hydroxide ion, which dwells between the metal ions and is poised to attack the carbonyl carbon, is stabilized by Zn1 and Zn2. The creation of a tetrahedralintermediate [63] is caused by the hydroxide's nucleophilic assault on the carbonyl carbon. Tetrahedral intermediate breakdown and C-N bond cleavage can occur in one of two ways: 1) bond breaking can happen at the same time as nitrogen protonation, or 2) cleavage can happen without nitrogen protonation, resulting in the accumulation of anionic nitrogen intermediates (figure 3) [64].

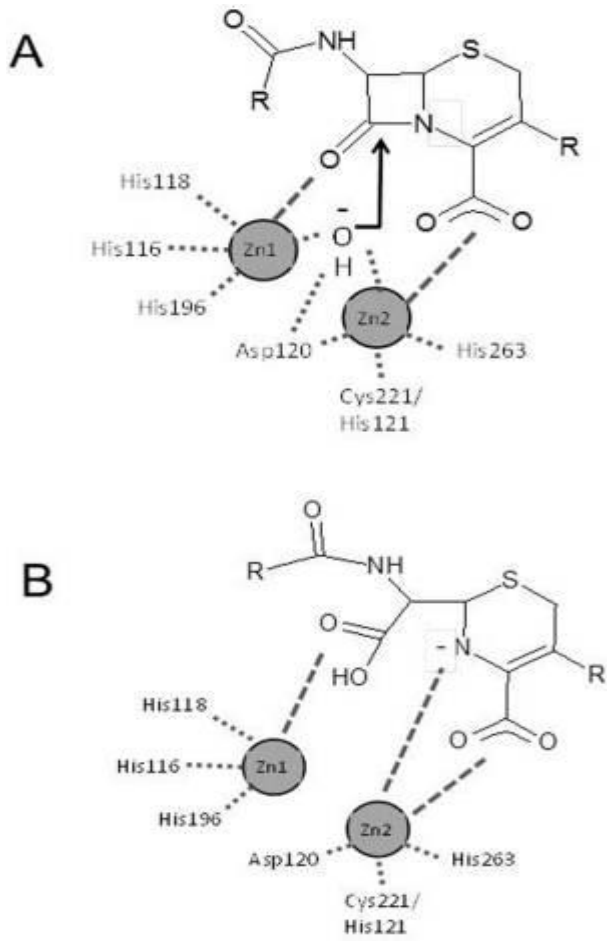

Fig3: Schematic illustration of cephalosporin binding to dizincmetallo-β-lactamase active site (subclasses B1 and B3)

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The B2-lactamases are distinguished from the B1 and B3 enzymes by having just one zinc in the active site and a substrate spectrum that is nearly solely focused on carbapenem hydrolysis. [65]. Although the hypothesized processes differ in some respects, they all share an interaction between Zn2 and the conserved Lys224 residue with the carbapenem β-lactam C-3 carboxyl group (fig 4) [66]. It has been postulated that after the breaking of the C-N bond, an anionic nitrogen stabilized by Zn2 accumulates. [67]. Finally, protonation of the nitrogen has been proposed to occur via a water molecule bound by His118 and Asp120 or via a water bound to Zn2 [68].

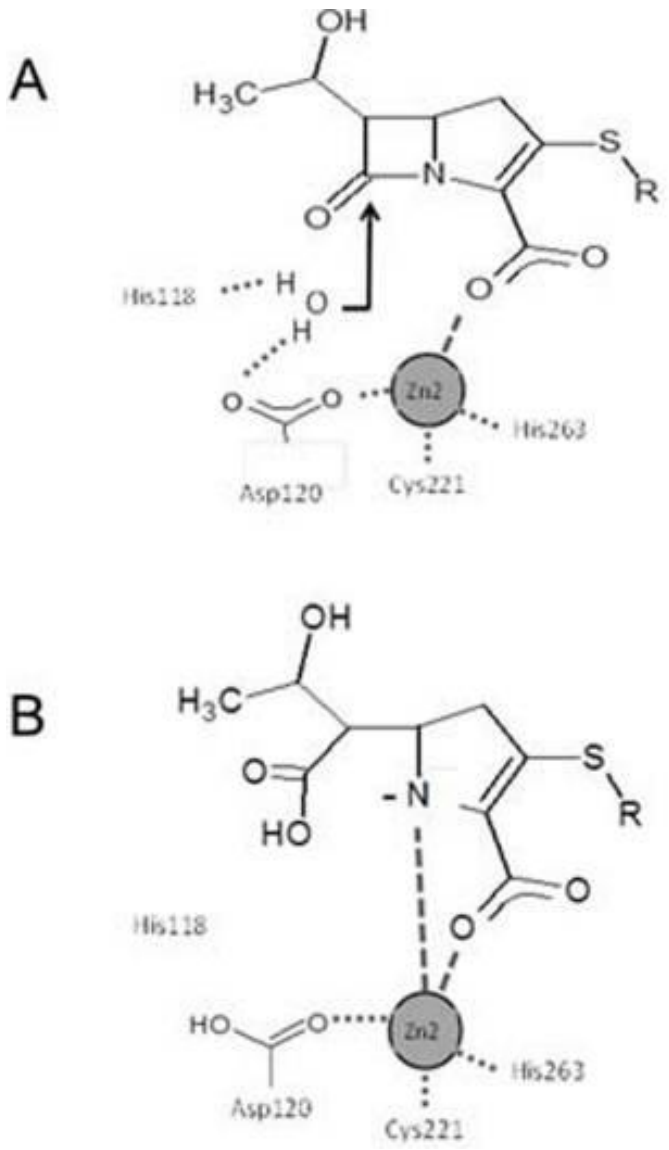

Fig4: Carbapenem substrate and anionic intermediate binding to monozincmetallo-β-lactamase active site (subclass B2)

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6. Extended Spectrum Beta-lactamases

Extended spectrum beta-lactamases (ESβLs) are enzymes that hydrolyze extended spectrum cephalosporin and are produced by some bacteria. As a result, beta-lactam antibiotics including ceftazidime, ceftriaxone, cefotaxime, and oxyimino- monobactam are ineffective against them. [69]. ESβLs are a fast developing class of β-lactamases that can hydrolyze third-generation cephalosporins and aztreonam but are blocked by clavulanic acid. Mutations that modify the amino acid structure surrounding the active site of these β-lactamases are commonly derived from the TEM-1, TEM-2, or SHV-1 genes.This broadens the range of β-lactam antibiotics that can be hydrolyzed by these enzymes. A growing number of ESLs that are not of the TEM or SHV lineage have recently been described. The presence of ESβLs has significant clinical implications. [70]. ESβL producer strains are sensitive to carbapenems and cephamycine. ESBLs are normally inhibited by clavulanic acid and tazobactam. Gram-negative bacteria, particularly enterobacteriacea and Pseudomonas aeruginosa, contain ESβLs. [71]. TEM-1 is the most usually encountered beta-lactamase. It is estimated that the presence of TEM-1 is responsible for more than 90% of E.coli ampicillin resistance. [72]. Penicillin and first-generation cephalosporins can be hydrolyzed by TEM-1.The first derivative of TEM-1 is TEM-2, which has a single amino acid replacement [73].The difference between beta-lactamase enzymes is the amino acid substitution that produces lactamases, which was first described in K. pneumoniae.SHV-1 is thought to be responsible for plasmid-mediated ampicillin resistance in bacteria that harbor it.The substitution of amino acids alters the structure and activity of enzymes. [69]. In defined amino acid positions, substitutions are more common in TEM, SHV, and OXA enzymes.The combination of altered amino acids results in different beta-lactamase enzyme phenotypes with varying ability to hydrolyze 3rd generation cephalosporin and increases resistance to beta-lactamase inhibitors [74].The usage of various oxamino –beta –lactam antibiotics, as well as first generation cephalosporin and penicillin, has been observed to cause changes in ESβLs [75].Because beta-lactam antibiotics were overused, ESβLs producer strains were chosen.These strains have different phenotypes and cause changes in porins like Omp.[76] to develop resistance to cephamycins and other antimicrobials [77]. The plasmids are in charge of ESβL production, as well as other genes that show resistance to aminoglycosides and cotrimoxazole. [78]. Quinolone resistance was found to be more prevalent in strains that produce ESβL, though the mechanism of co-resistance is unknown. [79]. ESβLs are becoming increasingly important in the medical field. This is due to their ability to make the strains resistant to cephalosporins, the workhorse hospital antibiotics used as first line

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antibiotics for many illnesses.Any delay in identifying and treating the severe infections caused by ESβL producers would increase morbidity and mortality.Because ESBL producer strains frequently exhibit multidrug resistance, including resistance to amino glycosides and fluoroquinolones, therapeutic options for these strains are limited.As a result, it is clear that the prevalence of ESβLs producers is increasing, and the ESβLs producer strains are causing higher levels of morbidity, mortality, and health-care-related costs.Extended-spectrum β-lactamase (ESβL) producers were identified using Kaplan–Meier curves for hospital contacts (A) and household contacts (B) (B). "ESβL transmission" refers to contacts who have the same ESβL strain as the index patient, whereas "ESβL no transmission" refers to contacts who have ESβL carriage but do not have the same strain as the index patient (according to the definition given in the Methods). ESβL-producing Escherichia coli and Klebsiella pneumoniae are categorized together. The differences between the curves were calculated using the log-rank test. ESβL stands for extended-spectrum β-lactamase, and Kp stands for Klebsiella pneumonia [80]. Ec stands for Escherichia coli, ESL stands for extended-spectrum β-lactamase, and Kp stands for Klebsiella pneumonia [81-82].

References

1. Neuhauser, M. M., & Danziger, L. H. (2005). β-lactam antibiotics. In Drug interactions in infectious diseases (pp. 255). Humana Press.
2. Gandhi, K., Sharma, R., Gautam, P. B., & Mann, B. (2020). Chemical Quality Assurance of Milk and Milk Products. Springer. (pp. 202).
3. Zango, U. U., Ibrahim, M., & Shawai, S. A. A. (2019). A review on β-lactam antibiotic drug resistance. MOJ Drug Des Develop Ther, 3(2), page 52.
4. Bennett, J. E., Dolin, R., & Blaser, M. J. (2014). Mandell, douglas, and bennett's principles and practice of infectious diseases: 2-volume set (Vol. 2) (pp. 246). Elsevier Health Sciences.
5. Thakuria B, Lahon K. The Beta Lactam Antibiotics as an Empirical Therapy in a Developing Country: An Update on Their Current Status and Recommendations to Counter the Resistance against Them. J Clin Diagn Res. 2013 Jun;7
6. Pandey, N., & Cascella, M. (2020). Beta lactam antibiotics. StatPearls [Internet].
7. Long AJ, Clifton IJ, Roach PL, Baldwin JE, Rutledge PJ, Schofield. CJ. Structural studies on the reaction of isopenicillinNsynthase with the truncated substrate analogues delta-(L-alpha-aminoadipoyl)-L-cysteinyl-glycine and delta-(L-alpha-aminoadipoyl)-L-cysteinyl-D-alanine. Biochemistry 2005; 44:6619–6628

8. Zango, U. U., Ibrahim, M., & Shawai, S. A. A. (2019). A review on β-lactam antibiotic drug resistance. MOJ Drug Des Develop Ther, 3(2), page 53
9. Massova I, Mobashery S. Kinship and diversification of bacterial penicillin-binding proteins and beta-lactamases. Antimicrob. Agents Chemother. 1998;42:1–17.
10. Moellering Jr, R. C., Eliopoulos, G. M., & Sentochnik, D. E. (1989). The carbapenems: new broad spectrum β-lactam antibiotics. Journal of Antimicrobial Chemotherapy, 24(suppl_A), 1-7.
11. Jeon, J. H., Lee, J. H., Lee, J. J., Park, K. S., Karim, A. M., Lee, C. R., Jeong, B. C., & Lee, S. H. (2015). Structural basis for carbapenem-hydrolyzing mechanisms of carbapenemases conferring antibiotic resistance. International journal of molecular sciences, 16(5), 9654–9692.
12. Bennett, J. E., & Mandell, G. L. (2005). Mandell, Douglas, and Bennett's principles and practice of infectious diseases. In Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases (pp. 1726-1726).
13. Massova I, Mobashery S. Kinship and diversification of bacterial penicillin-binding proteins and beta-lactamases. Antimicrob. Agents Chemother. 1998;42:1–17.
14. S. C. Parija, Textbook of Microbiology & Immunology, Elsevier Health Sciences, 2014.
15. A. Zapun, C. Contreras-Martel, and T. Vernet, “Penicillin-binding proteins and β-lactam resistance,” FEMS Microbiology Reviews, vol. 32, no. 2, pp. 361–385, 2008.
16. [Tipper, D. J.& Strominger, J. L. (1965) Proc. Nati. Acad. Sci. USA 64,1133-11381.
17. Sauvage E, Kerff F, Terrak M, Ayala JA, Charlier P. The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. FEMS Microbiol Rev. 2008 Mar;32(2):234-58. [PubMed]
18. Sarkar P, Yarlagadda V, Ghosh C, Haldar J. A review on cell wall synthesis inhibitors with an emphasis on glycopeptide antibiotics. Medchemcomm. 2017 Mar 01;8(3):516-533. [PMC free article] [PubMed].
19. Hecker SJ, Reddy KR, Totrov M, et al. Discovery of a cyclic boronic acid beta-lactamase inhibitor (RPX7009) with utility vs class A serine carbapenemases. J Med Chem. 2015;58(9):3682–92.
20. Lomovskaya O, Sun D, Rubio-Aparicio D, et al. Vaborbactam: spectrum of beta-lactamase inhibition and impact of resistance mechanisms on activity in Enterobacteriaceae. Antimicrob Agents Chemother. 2017;61(11):e01443-17.
21. The Medicines Company. VABOMERE™ (meropenem and vaborbactam): US prescribing Information. 2018.

22. Zhanel GG, Lawrence CK, Adam H, et al. Imipenem-relebactam and meropenem-vaborbactam: two novel carbapenem-beta-lactamase inhibitor combinations. Drugs. 2018;78(1):65–98.
23. Cho JC, Zmarlicka MT, Shaeer KM, et al. Meropenem/vaborbactam, the first carbapenem/beta-lactamase inhibitor combination. Ann Pharmacother. 2018;52(8):769–79.
24. Lomovskaya O, Tsivkovski R. Vaborbactam (RPX7009) plus meropenem is active against the newly discovered BKC-1 and FR-1 carbapenemases [abstract no. P1289]. In: 26th ECCMID. 2016.
25. Castanheira M, Rhomberg PR, Flamm RK, et al. Effect of the β-lactamase inhibitor vaborbactam combined with meropenem against serine carbapenemase-producing Enterobacteriaceae. Antimicrob Agents Chemother. 2016;60(9):5454–8.
26. US Center for Drug Evaluation and Research. Clinical microbilology/virology review(s). 2017.
27. Tsivkovski R, Griffith DC, Hecker S, et al. Biochemical characterization of the beta-lactamase inhibitor RPX7009 [abstract no. F-849 plus poster]. In: 52nd ICAAC. 2012.
28. Drawz SM, Bonomo RA. Three decades of beta-lactamase inhibitors. Clin Microbiol Rev. 2010 Jan;23(1):160-201. [PMC free article] [PubMed].
29. Gaude, G. S., & Hattiholli, J. (2013). Rising bacterial resistance to beta-lactam antibiotics: Can there be solutions?. Journal of Dr. NTR University of Health Sciences, 2(1), 4.
30. Kumarasamy KK, Toleman MA, Walsh TR, et al. Emergence of anew antibiotic resistance mechanism in India, Pakistan, and the UK:A molecular, biological, and epidemiological study. Lancet Infect Dis.2010;10:597–602.
31. King, D. T., Sobhanifar, S., & Strynadka, N. C. (2017). The mechanisms of resistance to b-lactam antibiotics. Handbook of Antimicrobial Resistance. New York: Springer. page 182
32. Bush K (2013) Proliferation and significance of clinically relevant beta-lactamases. Ann N YAcad Sci 1277:84–90
33. Lambert PA (2005) Bacterial resistance to antibiotics: modified target sites. Adv Drug Deliv Rev 57:1471–1485
34. Rossolini, G. M., Arena, F., & Giani, T. (2016). Mechanisms of antibacterial resistance. fourth edition. Pages 1181-1196.e1,

35. Heesemann J. (1993). Resistenzmechanismen gegen Betalaktamantibiotika [Mechanisms of resistance to beta-lactam antibiotics]. Infection, 21 Suppl 1, S4–S9.
36. Murray, Patrick R., author.Medical microbiology / Patrick R. Murray, Ken S. Rosenthal, Michael A. Pfaller ; consultant, JMI Laboratories.—8th edition.page 163,164.
37. Min Tao, Li Zhang, Yinlong Guo. Dual-Channel Enzymatic Inhibition Measurement (DEIM) Coupling Isotope Substrate via Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry. Journal of the American Society for Mass Spectrometry 2018.
38. Bush K. Past and present perspectives on beta-lactamases. Antimicrob. Agents Chemother. 2018.
39. Naas T., Oueslati S., Bonnin R.A., Dabos M.L., Zavala A., Dortet L. Beta-lactamase database (BLDB)—structure and function. J. Enzyme Inhib. Med. Chem. 2017;32:917–919.
40. Bush K., Jacoby G.A., Medeiros A.A. A functional classification scheme for beta-lactamases and its correlation with molecular-structure. Antimicrob. Agents Chemother. 1995;39:1211–1233.
41. Ambler R.P. The structure of beta-lactamases. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 1980;289:321–331.
42. Massova I., Mobashery S. Kinship and diversification of bacterial penicillin-binding proteins and beta-lactamases. Antimicrob. Agents Chemother. 1998;42:1–17.
43. Fisher, J.F.; Meroueh, S.O.; Mobashery, S. Bacterial resistance to beta-lactam antibiotics: Compelling opportunism, compelling opportunity. Chem. Rev. 2005, 105, 395–424.
44. Paterson, D.L.; Bonomo, R.A. Extended-spectrum beta-lactamases: A clinical update. Clin. Microbiol. Rev. 2005, 18, 657–686.
45. Nordmann, P.; Naas, T.; Poirel, L. Global Spread of Carbapenemase-producing Enterobacteriaceae. Emerg. Infect. Dis. 2011, 17, 1791–1798.
46. Crowder, M.W.; Spencer, J.; Vila, A.J. Metallo-beta-lactamases: Novel weaponry for antibiotic resistance in bacteria. Acc. Chem. Res. 2006, 39, 721–728.
47. Llarrull, L.I.; Tioni, M.F.; Vila, A.J. Metal content and localization during turnover in B. cereusmetallo-beta-lactamase. J. Am. Chem. Soc. 2008, 130, 15842–15851.
48. Tioni, M.F.; Llarrull, L.I.; Poeylaut-Palena, A.A.; Martí, M.A.; Saggu, M.; Periyannan, G.R.; Mata, E.G.; Bennett, B.; Murgida, D.H.; Vila, A.J. Trapping and characterization

of a reaction intermediate in carbapenem hydrolysis by B. cereus metallo-beta-lactamase. J. Am. Chem. Soc.2008, 130, 15852–15863.

49. Lim, H.M.; Pène, J.J.; Shaw, R.W. Cloning, nucleotide sequence, and expression of the Bacillus cereus 5/B/6 beta-lactamase II structural gene. J. Bacteriol. 1988, 170, 2873–2878.
50. Lauretti, L.; Riccio, M.L.; Mazzariol, A.; Cornaglia, G.; Amicosante, G.; Fontana, R.; Rossolini, G.M.Cloning and characterization of blaVIM, a new integron-borne metallo-beta-lactamase gene from a Pseudomonas aeruginosa clinical isolate. Antimicrob. Agents Chemother. 1999, 43, 1584–1590.
51. Majiduddin, F. K., Materon, I. C., & Palzkill, T. G. (2002). Molecular analysis of beta-lactamase structure and function. International journal of medical microbiology
52. Ambler RP (1980) The structure of beta-lactamases. Philosophical Transactions of the Royal Society of London Series B: Biological Sciences 289: 321–31. pmid:6109327
53. Long, S. S., Prober, C. G., & Fischer, M. (2017). Principles and practice of pediatric infectious diseases E-Book. Elsevier Health Sciences. (page 1424)
54. Bush, K., & Jacoby, G. A. (2010). Updated functional classification of beta-lactamases. Antimicrobial agents and chemotherapy, 54(3), 969–976.
55. WALEY, S.G. (1992). 13-lactamase: mechanism ofaction. In The Chemistry of 13-Lactams (M.I.Page, Ed.), pp. 198-228. Chapman & Hall, London.
56. Vandavasi, V. G., Langan, P. S., Weiss, K. L., Parks, J. M., Cooper, J. B., Ginell, S. L., & Coates, L. (2017). Active-site protonation states in an acyl-enzyme intermediate of a class A β-lactamase with a monobactam substrate. Antimicrobial agents and chemotherapy, 61(1).
57. Waley SG. β-lactamase: mechanism of action. In: Page MI, editor. In: The Chemistry of β-Lactams; 1992; London, UK. Blackie; pp. 198–228.
58. Palzkill, T. (2013). Metallo-β-lactamase structure and function. Annals of the New York Academy of Sciences, 1277, 91.
59. Carfi A.Pares S.Duee E.Galleni M.Duez C. Frere J.-M.Dideberg O. EMBO J. 1995; 14: 4914-4921
60. Rasia, R. M., & Vila, A. J. (2002). Exploring the role and the binding affinity of a second zinc equivalent in B. cereus metallo-β-lactamase. Biochemistry, 41(6), 1853-1860.
61. Wang Z, Fast W, Benkovic SJ. On the mechanism of metallo-β-lactamase from Bacteroides fragilis. Biochemistry. 1999;38:10013–10023.

62. Park H, Brothers EN, Merz KM. Hybrid QM/MM and DFT investigations of the catalytic mechanism and inhibition of the dinuclear zinc metallo-β-lactamase CcrA from Bacteroides fragilis. J. Amer. Chem. Soc. 2005;127:4232–4241
63. Crowder MW, Spencer J, Vila AJ. Metallo-β-lactamases: Novel weaponry for antibiotic resistance in bacteria. Acc. Chem. Res. 2006;39:721–728.
64. Wang Z, Fast W, Benkovic SJ. On the mechanism of metallo-β-lactamase from Bacteroides fragilis. Biochemistry. 1999;38:10013–10023.
65. Wommer S, Rival S, Heinz U, Galleni M, Frere JM, Franceschini N, Amicosante G, Rasmussen B, Bauer R, Adolph HW. Substrate-activated zinc binding of metallo-β-lactamases: physiological importance of mononuclear enzymes. J. Biol. Chem. 2002;277:24142–24147.
66. Simona F, Magistrato A, Dal Peraro M, Cavalli A, Vila AJ, Carloni P. Common mechanistic features among metallo-β-lactamases. A computational study of Aeromonas hydrolphila CphA enzyme. J. Biol. Chem. 2009;284:28164–28171
67. Wu S, Xu D, Guo H. QM/MM studies of monozinc β-lactamase CphA suggest that the crystal structure of an enzyme-intermediate complex represents a minor pathway. J. Amer. Chem. Soc. 2010;132:17986–17988
68. Garau G, Bebrone C, Anne C, Galleni M, Frere JM, Dideberg O. A metallo-β-lactamase enzyme in action: Crystal structures of the monozinc carbapenemase CphA and its complex with biapenem. J. Mol. Biol. 2005;345:785–795.
69. Bradford, P. A. (2001). Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clinical microbiology reviews, 14(4), 933-951.
70. Paterson, D. L., & Bonomo, R. A. (2005). Extended-spectrum β-lactamases: a clinical update. Clinical microbiology reviews, 18(4), 657-686.
71. Nordmann, P., & Guibert, M. (1998). Extended-spectrum beta-lactamases in Pseudomonas aeruginosa. The Journal of antimicrobial chemotherapy, 42(2), 128–131.
72. Livermore DM. 1995. Beta-lactamases in laboratory and clinical resistance. Clin Microbiol Rev, 8, 557-584.
73. Du Bois, S. K., Marriott, M.S., and Amyes, S.G. (1995). TEM- and SHV- derived extended-spectrum beta- lactamases: relationship between selection, structure and function. J. Antimicrob. Chemother, 35, 7–22.
74. Winokur P.L, Brueggemann, D.L. DeSalvo, Hoffmann,L., Apley, M. D., Uhlenhopp, E. K., Pfaller, M.A., and Doern, G. V. (2000). Animal and human multidrug - resistant,

cephalosporin-resistant Salmonella isolates expressing a plasmid-mediated CMY-2 AmpC betalactamase. Antimicrob Agents Chemother, 44,2777-2783.

75. Blazquez, J., Morosini, M.I., Negri M.C., and Baquero, F. (2000). Selection of naturally occuring extended- spectrum TEM ß-lactamase variants by fluctuating ß- lactam pressure. Antimicrob Agents Chemother; 44,2182-2184.
76. Bradford, P. A., Cherubin, C. E., Idemyor, V., Rasmussen,B. A., and Bush, K. (1994). Multiply resistant Klebsiella pneumoniae from two Chicago hospitals: identification of the extended-spect rum TEM-12 and TEM-10 ceftazidime-hydrolyzing beta-lactamases in a singleisolates. Antimicrob Agents Chemother, 38, 761-766.
77. Villa, L., Pezzella, C., Tosini, F., Visca, P., Petrucca, A., andCarattoli, A. (2000). Multiple-antibiotic resistance mediated by structurally related IncL/M plasmids carrying an extended spectrum beta-lactamase gene and class 1 integron. Antimicrob Agents Chemother, 44, 2911-2914.
78. Paterson, D. L., Mulazimoglu, L., Casellas, J.M., Ko, W.C., Goossens, H., Von Gottberg, A., Mohapatra, S., Trenholme, G. M., Klugman, K. P., McCormack, J. G., and Yu, V. L. (2000). Epidemiology of ciprofloxacin resistance and its relationship to extended-spectrum beta-lactamase production in Kelbsiella pneumonia isolates causing bacteremia. Clin Infect Dis, 30, 473-478.
79. Shakib, P., Ghafourian, S., Zolfaghary, M. R., Hushmandfar, R., Ranjbar, R., and Sadeghifard, N. (2012). Prevalence of OmpK35 and OmpK36 porin expression in beta-lactamase and non-betalactamase- producing Klebsiella pneumoniae. Biologics, 6, 1-4.
80. Ghafourian, S., Sadeghifard, N., Soheili, S., & Sekawi, Z. (2015). Extended spectrum beta-lactamases: definition, classification and epidemiology. Curr Issues Mol Biol, 17(1), 11-22.
81. Alsafee, Bassam Abdulhussein Hasan. "Synthesis, Identification and Antibactrial Activity of Some New Heterocyclic Compounds Contaning 1, 3, 4-Thiadiazole and 1, 3, 4-Oxadiazole Bearing Schiff Base." *Iraqi National Journal Of Chemistry* 17.2 (2017).
82. Jabir, Najim A. "Ruta Graveolens (Hurb Of Grace)–Anti Bacterial Effects, A."