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Review Article

# Bacterial Resistance; A Perspective on Beta-Lactamase Enzymes

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**Received:** November 11, 2019; **Published:** February 10, 2020

## Abstract

Bacteria with resistant genes are spreading fast, from place to place, and genes formerly encoded by the chromosome are now encoded by plasmids, and by lateral transfer can now jump from one bacteria species to a completely new species. Decade of treating bacteria and especially the resistant strains have not brought solace but fear and concern as newer super resistant bacteria are evolving without an equal treatment plan to counter them. Beta-lactamases with their prevalence and increasing types have been successful in raising a worthwhile defense mechanism in bacteria against beta-lactam antibiotics which is the most used group of antibiotics. As a result of the resistance build up to these antibiotics and cost of finding and producing new antibiotics, research has led to a means of overcoming the plague of resistance, hence the introduction of chemical compounds known as inhibitors. Inhibitors work on the premise that beta-lactamase messes up beta-lactam ring of the antibiotic, then the inhibitor messes up the beta-lactamase by deactivation in defense of the antibiotic. They are added to beta-lactam antibiotics to make them more efficient and effective. New antibiotics are seldom produced due to high cost involved, and the saving wall of inhibitors is continually breached by newer version of beta-lactamases. Problem resulting from this failure include increasing cost of treatment, mortalities, and community prone to bacterial resistant scourge. The review summarily highlight the strength of beta-lactamases over beta-lactam antibiotics, and called for pro-active measures to tackle the problem.

***Keywords:*** *Bacteria; Resistance; Plasmid; Beta-lactamase; Antibiotics*

## Introduction

### Bacterial resistance

Bacterial resistance is a mechanism adopted by bacteria to override the cidal and static effects of antimicrobials. Bacterial resistance is a global threat as resistant organisms with evolving genes are found all over the world, even in remote places [1-3]. These organisms are potent with virulent genes leading to onset of severe infections, higher mortalities, and failure of empirical therapy [4]. Microbial resistance is a slow, natural biological process that results from inappropriate use of antibiotics, poor or lack of proper monitoring, self-prescription, fake drug use, antibiotics use as growth promoters in animals and plants, plus natural evolution all promote resistance in microorganisms [5-7].

Resistant microorganisms impacts the patient and community negatively by prolonging duration of illness, exposure to difficult to treat infections, increasing mortality rate and cost of therapy, risks to community and health care workers, and increased economic cost of managing such infections [8-10]. European Centre for Disease Prevention and Control (ECDC) described antimicrobial resistance as

a big threat facing the world as it relates to infectious diseases [11]. Antibiotic resistance by microorganisms could be intrinsic, that is resistance which occurs naturally, or acquired, resulting from mutation in the existing DNA of the microbe or complete acquisition of new DNA particle [1]. Delcour [12] and Martins., *et al*. [13] explained that bacteria acquire resistance through a) alteration of membrane permeability, which involves alteration of lipopolysaccharide composition, change in transport proteins (porins), efflux pump to remove antibiotics, and b) genetic manipulation which could be chromosomal or plasmid mediated [14]. While chromosomal mediated resistance might not be as a result of the presence of antibiotic, plasmid mediated resistance is triggered by antibiotic use which can in turn lead to multiple drug resistance.

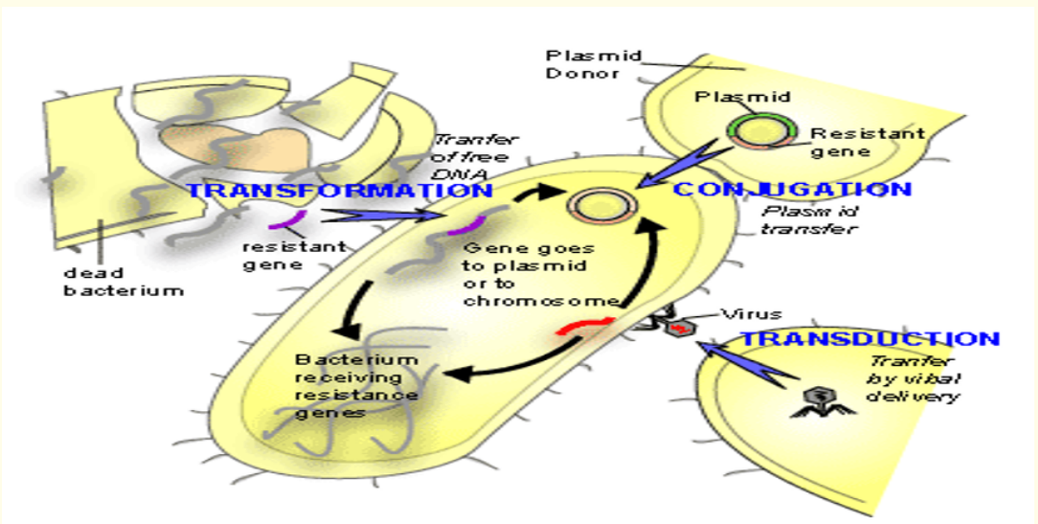


***Figure 1:*** *Mechanisms of resistant gene transfer in bacteria.*

## Beta-lactams

Antibiotics exert their actions on bacteria cell by either inhibiting cell wall, protein (bonding 50S or 30S ribosomal subunits), and nucleic acid synthesis, by altering cell membrane function (binding sterols or inhibiting ergosterol synthesis), or by alteration of cell metabolism [15-17]. Beta-lactams are a complex class of antibiotics made up of the penicillins, cephalosporins, monobactams, and carbapenems. They are well tolerated and act by inhibiting cell wall synthesis thus bringing about cell lysis making them the most prescribed antibiotics [18,19]. Beta lactam interact specifically with the d-alanyl-d-alanine carboxypeptidase-transpeptidase to alter cell wall [18,20].

Beta-lactams are the largest bacterial cell wall synthesis inhibitor with a central square shaped ring shared by all classes of the antibiotics to which are attached different side chains.

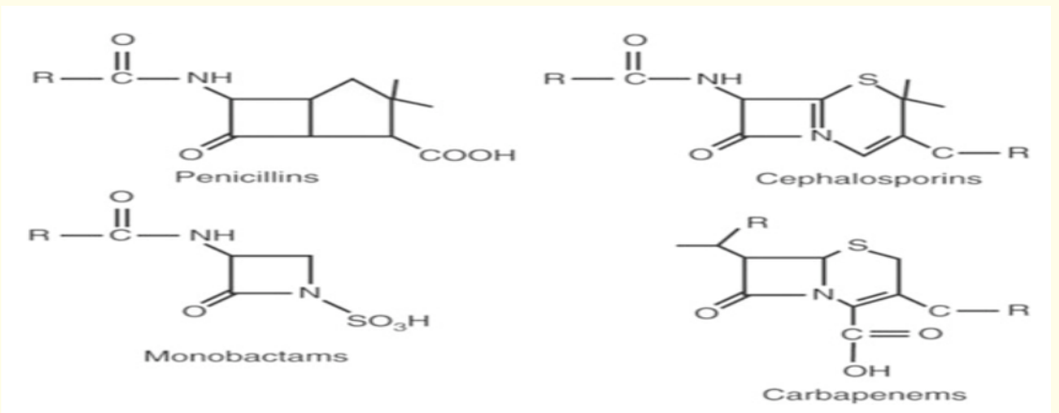


***Figure 2:*** *Structure of the beta-lactam family.*

## Beta-lactam subgroups

1. Penicillins: Divided into four sub-types:
    a. Natural penicillins also known as penicillinase sensitive penicillins such as Penicillin G and Penicillin V.
    b. Antistaphylococci or penicillinase resistant penicillin such as nafcillin, oxacillin, methicillin, dicloxacillin. These group of penicillin have bulky R-group side chains [21].
    c. Aminopenicillins, which have amino side chain, examples are ampicillin and amoxicillin.
    d. Extended spectrum (antipseudomonal) penicillins such as piperacillin and ticarcillin.
2. Cephalosporins: The cephalosporins are divided into five generations of antibiotics:
    a. 1st Generation cephalosporins have large $R^1$ side chain which confer protection against beta-lactamases. Examples are cefazolin, cephalexin, cephalothin, cefadroxil.
    b. 2nd Generation cephalosporins have different side group which confer potency to the antibiotic. Examples are cefotetan, cefoxitin, cefuroxime, cefaclor.
    c. 3rd Generation cephalosporins are better at penetration into body tissues. Examples are cefotaxime, ceftazidime, ceftriaxone.
    d. 4th Generation cephalosporins have activity against bacteria resistant to 3rd Generation antibiotics [22]. Example is cefepime.
    e. 5th Generation cephalosporins have increased affinity for penicillin binding protein 2a (PBP2a) that mediate methicillin resistance in Staphylococci.
3. Monobactams: Monobactam antibiotics possess monocyclic β-lactam ring and bind only the penicillin binding protein 3 (PBP3) [15]. An example is aztreonam.
4. Carbapenems are mostly used in treating bacteria with multidrug resistance (MDR). Carbapenems labelled as the last line of treatment against stubborn bacterial infections binds PBP of most bacteria species and they are known to be resistant to beta-lactamases. Examples are imipenem, meropenem, doripenem and ertapenem.

## Mechanism of action of beta-lactams

Beta-lactam antibiotics are bactericidal agents as they bring about the loss of bacterial cell wall integrity which leads to lysis and subsequent death. Beta-lactams inhibit synthesis of peptidoglycan layer by binding to transpeptidase enzyme responsible for cross-linking the peptidoglycan chain [19]. Cross-linking of the peptidoglycan layer ensures maintenance of cell integrity and bacterial shape, which prevent lysis by high osmotic pressure within the cell.

The peptidoglycan is a polymer of N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) cross-linked at the NAM end by four amino acids (L-alanine (A), D-glucose (G), L-lysine (L), and D-alanine (A)). Synthesis of the polymer is carried out by the addition of five amino acids to NAM and NAG after which it is transported across the cell membrane. Transpeptidase enzyme (a penicillin binding protein PBP) removes the fifth and terminal amino acid (D-alanine) and links the fourth D-alanine to L-lysine of another NAM thus forming a lattice work. Beta-lactams being a structural analog of the amino acid substrate, covalently bond the transpeptidase active site and so blocking cross-linking in the cell wall. PBPs are enzymes located in the periplasmic space between the peptidoglycan layer and the cell membrane. Transpeptidases which are also cytoplasmic protein and membrane bound are involved in the final stage of peptidoglycan synthesis and the main target of beta-lactams.

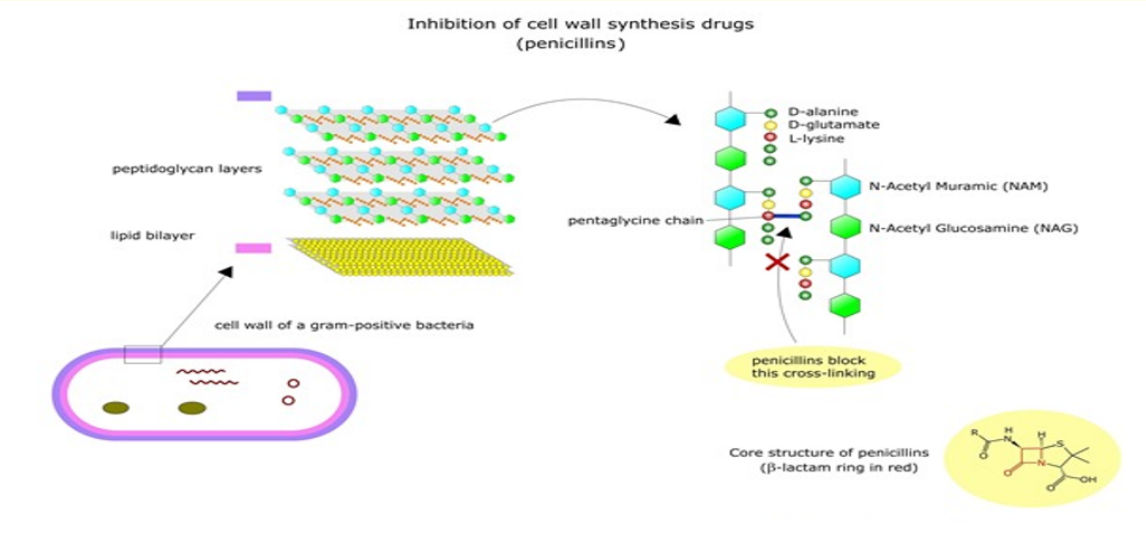


***Figure 3:*** *Mechanism of action of beta-lactam on bacterial cell wall.*

## Resistance to beta-lactams

Beta-lactams are the most used antibiotics as they are well tolerated and exerting their actions on their precise target [20,23]. Bacteria has evolved several mechanisms of resistance to beta-lactam antibiotics as a result of exposure to antibiotics over time [24,25]. The exigency of survival and natural selection lead to resistance mechanisms imbued in the organisms.

## Porin channels

Porins are proteins that are located in the outer membrane of Gram-negative bacteria. They function to form a water-filled pore through the membrane, from the exterior to the periplasm, which is a region located between the outer and inner membranes. The porin channel allows the diffusion of small hydrophilic (water-loving) molecules through to the periplasm. The size of the diffusing molecule depends on the size of the channel. Mutated porins that decrease entry of drug into the cell wall is common to Gram negative bacteria. The porin could have an altered pore to frustrate entry of smaller metabolites or very small opening to sieve out large molecules [26].

## Efflux pump

Efflux pumps in bacteria transport antibiotics out of the cell, and in the process prevent access to intracellular organelles.

## Altered targets

The penicillin binding proteins could be mutated in bacteria and hence hinder binding to the antibiotic.

## Degrading enzymes

The enzymes known as penicillinases are made up of the different beta-lactamases which split the amide bond of the lactam ring.

## Mechanism of altered penicillin binding protein in gram positive bacteria

Gram positive bacteria mostly employ the altered PBP mechanism in resistance to beta-lactams. While altered PBP may be unique to a particular pathogen (PBP2a for methicillin-resistant *Staphylococcus aureus*, PBP2x for penicillin-resistant *Streptococcus pneumo-*

*nia*), beta-lactam antibiotics differ in affinity to PBPs. Rice [1] concluded that resistance in Gram positive bacteria is by PBPs binding to beta-lactams with low affinity. Penicillin binding proteins are a group of enzymes to include transpeptidases, carboxyl peptidases, and endopeptidases involved in the formation of the peptidoglycan layer in bacteria. PBPs are encoded by a gene found in the plasmid, and the transfer of the element between strains ensures resistance [27]. Variations has also been reported in the size of the genetic element brought about by genome with particular gene from the genomic island coding for a particular PBP whose active site does not bind beta-lactam antibiotic [28]. Some other Gram positive bacteria such as *Streptococcus pneumonia* and *Neisseria gonorrhoeae* are capable of picking naked DNA of resistant strains with mosaic genes from the environment, and homologous transformation becomes part of the host genome, after which is codes and alters the PBP leading to resistance in such bacteria [29,30].

## Beta lactamases

The use of beta-lactams as antibiotics is grossly hampered by a group of deactivating enzymes collectively known as beta-lactamases [34-36]. Beta-lactamases hydrolyse the beta-lactam ring and bring about decarboxylation to release carbon (IV) oxide and in the process deactivate the antibiotic [37]. The enzyme has broad specificity against the beta-lactam antibiotics as it has large active site to bind large substrate, and R154A point mutation for destabilizing omega loop in cephalosporins, and reverse inhibition effected by some inhibitors [34,38,39]. Inappropriate and reckless use of beta-lactams over the years had led to the production of these inactivating enzymes as described by Zeng and Lin [40]. The enzymes create efficient, prevalent, and threatening defense mechanisms of resistance to organisms sensitive to beta-lactam antibiotics [1,20,40].

Over 900 different types of beta-lactamases have been listed and classified according to their amino acid sequence and functional properties [15,41]. The classification based on amino acid sequencing was proposed by Ambler in 1980 into classes A, B, C and D.

Gram negative bacteria inhibit the activity of beta-lactams by expressing beta-lactamases encoded by plasmid or bacteria chromosome. Production of beta lactamase in Gram positive bacteria is inducible and the enzyme is released into the extracellular environment before beta-lactam enters the cell. In Gram negative bacteria, production is constitutively and the enzyme is retained in the periplasmic space where at low concentrations, it is still potent against the antibiotic.

The first set of beta-lactamases were TEM-1 and SHV-1 which were narrow spectrum penicillinases resistant to ampicillin. Point mutation opens up the enzymes thus extending their resistance spectrum to cephalosporins [31] and subsequently CTX-M (natural cephalosporinases and carpenemases emerged [32,33].

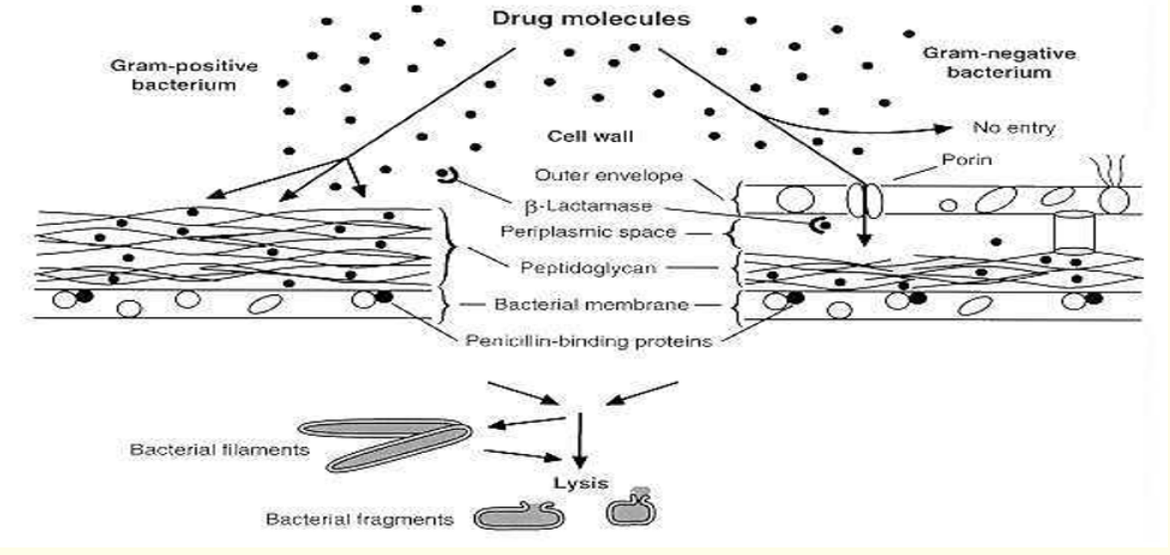


***Figure 4:*** *Mechanisms of resistance to beta-lactam antibiotics in Gram positive and negative cell walls.*

## Ambler structural classification of beta-lactamases

1. **Class A:** Enzymes in this class are plasmid mediated with interspecies lateral transfer of genetic elements (plasmid) and could be chromosomally encoded. They are active serine enzymes with broad and extended spectra of activity. They hydrolyse penicillins and destroy the third generation cephalosporins forming penicilloyl-o-ser covalent intermediate. An example is TEM-1.

2. **Class B:** They are chromosomally mediated inducible enzymes also known as metalloenzymes [42]. Enzymes in this class have broad spectrum of activity with one or two Zn co-factors with which they activate a water molecule and catalyse hydrolysis of carbapenems, penicillins, and cephalosporins [15,43]. The enzymes do not form penicilloyl-o-ser covalent intermediate which makes them susceptible to activities of some drugs, though Hinchliffe., *et al*. [44] reported their capacity to hydrolyse most beta-lactams and resist actions of the available beta-lactamase inhibitors. Metalloenzymes are further sub-divided into Class B1, B2 and B3. Enzymes in the 3 sub-class have two binding sites. B1 and B3 have 3 His residue on their protein ligand at Zn 1 site, while B2 has one of its His residue replaced with Asn [45].

3. **Class C:** Beta-lactamase enzymes are encoded by genes on chromosome, and they use serine active site in covalent hydrolysis of beta-lactam. Major activity is against cephalosporins and forms penicilloyl-o-ser covalent intermediate after catalysis. AmpC is an example of an active serine enzyme in class C, inducible and gene expressing it is not easily transferable.

4. **Class D:** These are plasmid mediated inactivating enzymes. They are active serine beta-lactamase enzymes with narrow to broad spectrum of activity [46].

Beta-lactamases apart from building resistance for bacteria against beta-lactam antibiotics has become a global threat to therapy and so increasing disease burden by promoting multiple drug resistance and extended spectrum factors, and so creating super pathogenic microorganisms [25,47]. The need to check continued proliferation of beta-lactamases led to a means of overcoming the plague of resistance, hence the introduction of chemical compounds known as inhibitors.

Beta-lactamases were also summarily classified by Bush-Jacoby-Medeiros into the following classes of antibiotics.

| Ambler Class | Bush-Jacoby-Medeiros group | Active Site | Enzyme Type | Host Organisms | Substrates |
|---|---|---|---|---|---|
| A | 2b, 2be, 2br, 2c, 2e, 2f | Serine | Broad spectrum β-lactamases (TEM, SHV), ESBL (TEM, SHV, CTX-M), Carbapenemases (KPC, GES, SME) | Enterobacteriaceace and nonfermenters | Ampicillin, cephalothin, Penicillins, 3rd generation cephalosporins, All β-lactams |
| B | 3 | Zinc-binding thiol group | Carbapenemases (VIM, IMP) | Enterobacteriaceace and nonfermenters | Cephamycins, 3rd generation cephalosporins |
| C | 1 | Serine | AmpC cephamycinases (AmpC) | Ecterobacter species, Citrobacter species | Cephamycins, 3rd generation cephalosporins |
| D | 2d | Serine | AmpC cephamycinases (CMY, DHA, MOX, FOX, ACC), Broad spectrum β-lactamases (OXA), ESBL (OXA), Carbapenemases (OXA) | Enterobacteriaceace and nonfermenters | Oxacillins, Ampicillin, cephalothin, Penicillins, 3rd generation cephalosporins, All β-lactams |

***Table 1:*** *Modified Ambler and Bush-Jacoby-Medeiros classification schemes*

## Inhibitors

Inhibitors are administered along with the antibiotics, and they binds the betalactamase enzyme so that the beta-lactam antibiotic can locate the transpeptidase responsible for peptidoglycan synthesis and deactivate it thus bringing about cell wall lysis as a result of turgor pressure. Production of inhibitors was the result of resistance build up by bacteria against the limited treatment options available for handling infection cases. Inhibitors are combined with beta-lactam with easily displaced bonds such as penicillin and amoxicillin so as to restore and expand the spectrum of activity of such beta-lactam antibiotics though such inhibitor might have no antimicrobial activity [48]. Augmentin is made of amoxicillin and clavulanic acid, Unasyn (ampicillin and sulbactam) and Timentin is made of ticarcillin and clavulanic acid. Inhibitors could be naturally produced, synthetically or semi-synthetically produced.

## Types of Inhibitors

1. Reversible inhibitors: Also known as product inhibitors. Penilloic acid and penicilloic acid products of hydrolysis and decarboxylation reaction act as reversible inhibitors

2. Clavulanic acid: Is a natural, orally absorbed inhibitor with no antimicrobial activity. It is also known as suicide inhibitor as it inactivate beta-lactamase enzyme through series of reactions at the enzyme's active site [26]. The inhibitor induces chromosomal beta-lactamase and deactivates class A beta-lactamase.

3. Mechanism based inhibitors: They are substrate analogs and shares the beta ring structure. They are active against most class A beta-lactamase [47]. Sulbactam, tazobactam, sulfenimines all belong to this type of inhibitors. While sulbactam is semi-synthetic with activities against class B beta-lactamases, tazobactam has broad spectrum of activity [49].

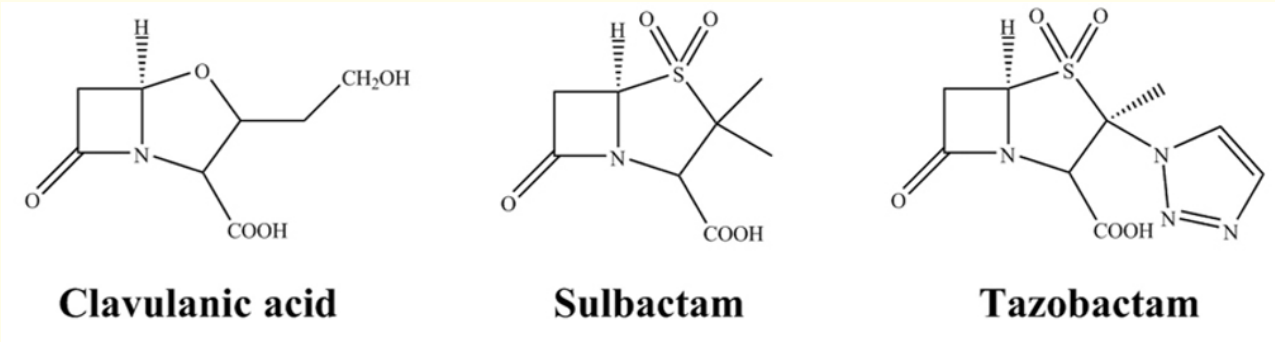


***Figure 5:*** *Examples of inhibitors.*

Most inhibitors used in therapy presently have no activity against cephalosporins, while metallo beta-lactamases structural diversity of their active site make it difficult to produce an effective inhibitor [44]. These inhibitors have the beta-lactam ring which renders them susceptible to deactivation by beta-lactamase. Mutation of the porin channels also reduces entry of drug into the cell wall [34]. Other problems with inhibitors include over production of beta-lactamases, amino acid substitution which leads to reduced affinity of the enzyme for mechanism based inhibitors [20].

Substitution of amino acid residue at different positions especially position 69, 130, 244, 275, 276 etc confers resistance to some antibiotics and deactivation of the inhibitor combined with it [50-54]. Amino acid substitution at any of the different position leads to

reduced affinity of the inhibitor to the substrate (enzyme). Ripoll., *et al*. [55], Thomas., *et al*. [56], Sun., *et al*. [57] and Aumeran., *et al*. [58] variously concluded that mutation of amino acid at position Ser130Gly confers resistance on some Class A beta-lactamases, and molecular changes have been documented as being responsible for resistance recorded against clavulanic acid [59]. Evasion by over-production of beta-lactamases, and deactivation of inhibitors is a source of concern in the clinical setting presently. Supposedly effective and metallo beta-lactamase inhibitors have toxicity challenges making them inappropriate for use, coupled with the problem of genes chromosomally mediated moving into plasmid calling for concern and concerted effort from everybody [8,60,61].

### What next?

Continued dependence on beta-lactam, the most widely used antibiotics in therapy and other antibiotics continued use in agriculture drastically increased cases of bacteria resistance; though evidences abound that resistance were recorded in bacteria even when not exposed to antibiotics [62,63]. Increasing number of extended spectrum beta-lactamases (ESBL) cases globally has been attributed to over-dependence on antibiotics as no alternative option is presently available [15]. Deactivation and suppression of the beta-lactam+inhibitor combinations by newly discovered beta-lactamases especially class metallo beta-lactamases calls for concern as they spread resistance factors through genes encoded in mobile genetic elements and create and spread new infections [47,64,65].

Problem of multidrug resistance labelled by evolution, mutation, and other such factors threatens effective treatment as a particular bacteria can harbour many PBPs thus frustrating activity of inhibitors and treatment and so increase cost of managing such infections [66,67]. Production of a universal beta-lactamase inhibitor is presently encouraged with the hope it will handle cases with a single isolate harbouring many penicillinases [1,4,68]. Emerging inhibitor-resistant beta-lactamases are frustrating treatment and increasing risks in organ transplant procedure, surgery, and immunosuppressive chemotherapy [63,68].

World Health Organization reported that patients with pathogens with resistant factors are at more risk of associated negative outcomes as treatment will no longer be effective in some of such patients. This has led to WHO updating treatment guideline for infections such as gonorrhoea, chlamydia infection, and syphilis, urinary tract infection caused by resistant *E. coli*, MDR-TB and pneumonia. The negative outcomes have been variously described to include death, new infections, complications, treatment failure, cost of treatment, time spent at health facility [69-75].

## Conclusion

The Centre for Disease Control and Prevention classified some resistant bacteria as serious threat (Extended-spectrum beta-lactamase ESBL) and others as urgent threat (Carbapenem-resistant enterobacteriaceae CRE) due to the threat they pose to human health [76], while Lau., *et al*. [3], Suzuki., *et al*. [77] and Liferaeka., *et al*. [78] reported from their findings that ESBL is presently a global pandemic. They are plasmid encoded beta-lactamases that inactivates 3rd Generation cephalosporins, penicillins and narrow spectrum cephalosporins. The present challenge calls for every hand to be on deck. Complication in therapy of community and hospital acquired infections that results from bacterial strain with plasmid encoding resistant genes harboring other antibiotic resistance gene which confer MDR is a red signal for something to be urgently done [1]. This review reiterates the need to have better understanding of how inhibitor deactivate beta-lactamase enzymes, the molecular basis underlying beta-lactamases binding to inhibitors, and production of super inhibitors with the capacity to inhibit multiple or different types of beta-lactamases to be encouraged as beta-lactamase inhibitor combinations are highly pathogen-focused. Sagar., *et al*. [34] reported the formulation of new beta-lactamases inhibitors with record success in pre-clinical trials, though further trials are needed before been fit for human therapy.

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