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A review on β-lactam antibiotic drug resistance

Abstract

β-lactam antibiotics constitutes a broad class of antibiotic agents that contain β-lactam ring in their molecular structure these agents includes cephalosporins, monobactams, penicillins and carbapenems. These are the most widely used antibiotics which act by inhibiting the synthesis of the bacterial cell wall such activity leads to the lysis and death of the bacteria. Due to the wide applications of these antibiotics bacteria have developed resistance mechanism against these antibiotics which is usually mediated by the enzymes β-lactamases, it hydrolyses β-lactam ring of the β-lactam antibiotics rendering it inactive. Recent studies have revealed that the combination of β-lactam antibiotics with β-lactamase inhibitors can be used to successfully overcome the effect of β-lactamases. This review discussed the mechanism of β-lactam antibiotic activity, the mechanisms of β-lactam antibiotic resistance and how to overcome the effect of the β-lactamases.

Keywords: beta-lactamases, beta-lactam, penicillin-binding protein, Penicillin, amp-genes

Volume 3 Issue 2 - 2019

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Received: December 31, 2018 | **Published:** April 05, 2019

Introduction

The concept that involves the use of chemicals to alleviate disease date back to the ancient Egypt One of the major significant advances in medicine is the development of antibiotics.[1] Antibiotics have saved many lives and continue to be the main therapy for infections related to bacteria. Penicillin G was the first of beta-lactam developed which lead the search for the synthesis of additional derivatives. The quest gave result to the beta-lactam antibiotics in clinical application today.[2] the class of broad-spectrum antibiotics that consist of all antibiotic agents with beta-lactam ring in their structures is called β-lactam antibiotics. It includes penicillin derivatives, monobactams, cephalosporin and carbapenems.[3]

β-lactam antibiotics act by inhibiting the bacterial cell wall biosynthesis; they are the most available antibiotics which treat a number of bacterial infections. For having a global positive impact on health by treating bacterial infections, penicillin and other β-lactam antibiotics are arguably considered the most important drugs ever.[4] A broad spectrum of bacteria can be killed by β-lactams and its toxicity to humans is very low this implies that, the resistance to β-lactam antibiotics is severe threat,[5] bacteria and other infection causing microbes are remarkably developed several ways to become resistant to antibiotics and other antimicrobial drugs. This is as a result mainly of increase use and misuse of the antibiotics in different medical illnesses.[6] nowadays, about. It was reported that 70% of the bacteria causing infections in hospitals are resistant to at one or more of the commonly used drug, some bacteria are found to be resistant to almost all antibiotics that are approved and can be treated only by some drugs that are potentially toxic. There have been reports which are documented about the alarming increase in bacterial antibiotic resistance which cause community acquired infections, examples include the *staphylococci* and *pneumococci* which are major causes of disease and mortality.[7] high prevalence of bacterial resistance to various pathogens such as *Acinetobacter*, *Proteus*, *E.coli*, *Klebsiella* and *Pseudomonas*.[8]

Accumulate evidence also proved that bacteria could pass resistance genes between strains and species. *Staphylococci* genes of antibiotic-resistance are carried on plasmids which will be exchanged with enterococcus, bacillus and Streptococcus making it possible for acquiring additional genes and gene combinations. Organisms that are resistant to treatment with many drugs are known as multiple drug resistant.[9] Examples of multiple drug resistant organisms include: Extended-spectrum beta-lactamases (ESBLs) which show resistance to monobactams and cephalosporins. Penicillin-resistant *Streptococcus pneumonia* (PRSP) the enzymes of ESBL are plasmid mediated enzymes that have the capability to hydrolyze and inactivate a wide variety of beta lactams, including, third generation aztreonam, cephalosporins and penicillins.[10]

β-lactam antibiotic resistance however has become a major health care issue. The reactions that involve the cleavage of the β-lactam ring of the antibiotic by β-lactamases of bacteria is the primary mechanism of β-lactam resistance.[11] the cell wall of bacteria consists of Peptidoglycan which is a giant polymer of repeated chains of disaccharides joined by peptide bridges. The joining results from a transpeptidation reaction catalysed by enzymes which are inhibited by β-lactams. The enzymes responsible for the assembly of peptidoglycan are known as PBPs2 or penicillin-binding proteins. They consist of penicillin-binding domain which generally catalyses the transpeptidation reaction, but can also act as a endopeptidase or carboxypeptidase in some cases Figure 1.[12]

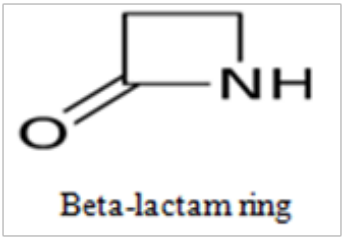

Figure 1 Some clinically important β-lactams.

Beta-lactam antibiotics

The structures of penicillin consist of a thiazolidine ring connected to a beta-lactam ring, which is attached to a side chain. All penicillins are derived from 6-Amino-penecillinic acid; the various penicillins differ in their side chain structure.

Penicillins are divided into natural and semi-synthetic ones. Natural penicillins are extracted from the cultural solution of penicillia. Prototype is penicillin G which is PH sensitive and effective against Gram- positive cells susceptible to penicillinase.

Semi-synthetic penicillins are produced by growing penicillium in culture so that only the nucleus is synthesised. R group are attached in lab or grow penicillium, extract natural penicillin, remove the R group and attach wanted R group. This group of penicillins have broader spectrum they are effective against Gram- negative cells and they are not resistant to Penicillinase.

The cephalosporins are a class of β-lactam antibiotics originally derived from the *fungus Acremonium*, which was previously known as "*Cephalosporium*".

Cephalosporins are derivatives of 7-amino-cephalosporanic acids and are closely related in structure to Penicillin. They have beta-lactam ring. They are relatively stable in dilute acid and are highly resistant to penicillinase. All cephalosporins are active against most Gram-positive cocci, the first generation include cephalothin, cefazolin, cephalexin est. they have stronger effect against Gram-positive bacteria than Gram-negative bacteria, the second generation antimicrobial acation against Gram-negative bacteria is increased. The third generation has broadest effect against gram-negative and lowest activity against Gram-positive bacteria. The fourth generations are extended-spectrum agents with similar activity against Gram-positive organisms as first-generation cephalosporins, Fourth-generation cephalosporins are zwitterions that can penetrate the outer membrane of Gram-negative bacteria.[13] They also have a greater resistance to β-lactamases than the third-generation cephalosporins Figure 2 and Figure 3.

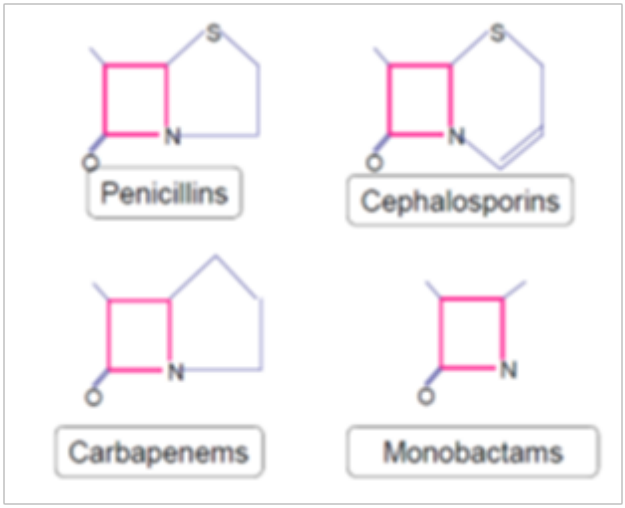

Figure 2 Mechanisms of action of β-lactam antibiotics.

The mechanism of action of beta-lactam antibiotics is usually by inhibiting the enzyme responsible for the bacterial cell wall synthesis.[13] the stability of cell wall is essential for the shape and protection of the cell in hostile and hypertonic environment the cell wall is comprised of two alternating units which are the N-acetylmuramic acid (NAM) and N-acetyl glucosamine (NAG), these two units are linked together by enzyme transglycosidase. Pentapeptide is attached to each NAM unit which includes D-alaine-D-alanine. The cross-link between the two Dalanine of two NAM is catalysed by PBP. The cross-linked between the adjacent glycans gives the rigidity of the cell wall.[14,15] The ring of beta-lactams antibiotics is similar to the pentapeptide's D-alaine-D-alanine of N-acetylmuramic acid, because of this similarity the penicillin binding proteins uses beta-lactam as building blocks for the synthesis of cell wall instead of NAM Pentapeptide.[16] This result in the acylation of the enzyme PBP subsequently rendering the enzyme incapable of catalyzing further transpeptidation reactions.[17] when this reaction comes to a halt, Peptidoglycans autolysis commence which result to the compromises of the integrity of the cell wall and increase its permeability .thus the beta-lactam mediated activity (inhibition) causes the lyses of the cell and the death of the bacteria.[18]

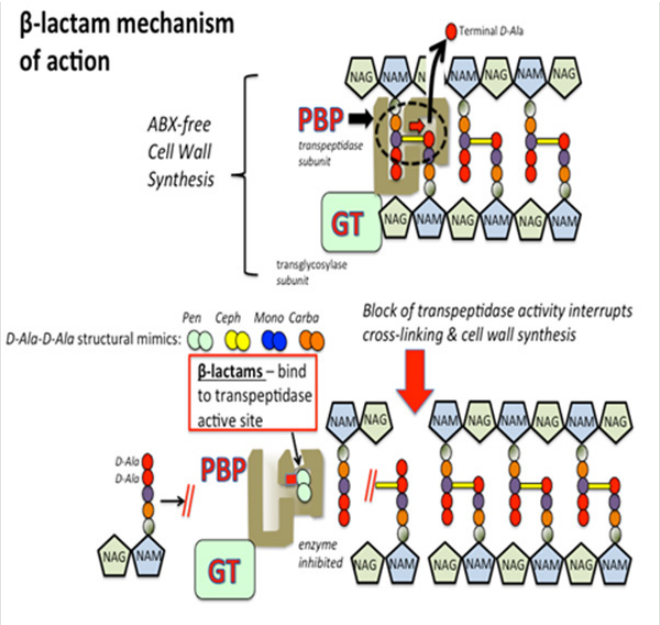

Figure 3 Mechanism of action of β-lactam.

Physiological analysis of β-lactam effect

It was discovered by Gardner that bacteria forms filaments when treated with low concentration of penicillin.[19] this discovery support early investigation which indicated that penicillin interferes with the maintenance of the cell shape of the cell. Results from subsequent studies by Duguid using different concentration of penicillin proved the interference of penicillin in cell division and maintenance of the integrity of the bacterial cell.[20]

Biochemical analysis of β-lactam effect

Park and Johnson gave the first biochemical clue about the penicillin action site.[21,22] they observed the accumulation of novel uridine peptides in the cytoplasm of *S. aureus* after being treated with penicillin. Subsequent investigation by Park and Stominger revealed that the amino acids and sugars of the accumulated peptides were similar to those of the cell wall of bacteria. This observation suggested that the accumulated uridine peptides in the cytoplasm were the precursors of cell wall accumulated as a result of inhibition of cell wall biosynthesis by penicillin.[23]

Biophysical analysis of β-lactam effect

In 1949, radioactive penicillin was used to study the specific site of action on the cell wall of bacteria.[24–27] it was observed that penicillin bind to a target which was termed penicillin binding component PBC and the complex formed was penicillin-PBC complex.[25] penicillin binds to its target via covalent bond. The complex was subjected to SDS-PAGE and the PBC resolved into various proteins of molecular weight that ranges between 40-90 KDa.[28] these proteins were

Citation: Zango UU, Ibrahim M, Shawai SAA, et al. A review on β-lactam antibiotic drug resistance. *MOJ Drug Des Develop Ther.* 2019;3(2):52–58. DOI: 10.15406/mojddt.2019.03.00080

termed PBPs and were given numbers according to their descending molecular weight. The concentration of the proteins, their numbers, molecular weight and sensitivity to β-lactam antibiotics varies from one specie to another.[29]

Genetic analysis

PBP1 functions involve the elongation of the cell wall. PBP1-cephaloridine is an agent that act against PBP1 resulting in the inhibition of cell wall elongation.[30] There are two distinct components of PBP1 which are PBP1a and PBP1b. PBP1a gene was mapped to MrcA or ponA. Mutant strain lacking ponA/MrcA appeared to grow normally but show slow rate of β-lactam induced lysis.[31] PBP1a catalysis the polymerization of glycan subunits.[32] while PBP1b is responsible for transglycosylase and DD-transpeptidase enzymatic reaction.[33,34]

PBP2 was the first to be discovered due to its ability to specifically bind with mecillinam.[35] its binding with mecillinam causes changes in *E.coli* shape from rod to ovoid.[36] PBP2 is the major protein involves in the maintenance of cell shape; its inhibition by β-lactams can cause destruction of the cell shape and inhibition of division.[37–38]

PBP3 – mutant *E.coli* strains lacking PBP3 proteins when isolated and cultured at temperature of 30°C.[35] appeared slightly longer than the parental strain. At an increase restrictive temperature to 42°C cell division ceased but, there was continuous increase in cell density. This suggested that DNA replication and cell growth were not affected in the absence of PBP3 at restrictive temperature. It also proves that PBP3 it is essential cell division protein.[39] Other Supporting evidence for its vital role in cell division came from the use of piperacillin, PBP3- specific β-lactam antibiotics and, furazlocillin.[40]

PBP4–strains of *E.coli* lacking the penicillin- sensitive activities of DD-endopeptidase and DD-carboxypeptidase1b showed a loss of PBP4.[41,42] This mutation was mapped and the gene was located at 68min on the E. coli map which is dacB gene.[42] However, the PBP4 overexpression showed an increase in DD-endopeptidase and DD-carboxypeptidase, which has no effect transpeptidation reaction.[43] PBP4 was demonstrated as the only PBP of E. coli that possessed DD-endopeptidase activity.[44]

PBP5 membrane-bound proteins which catalyzes a transpeptidase reaction and have a weak penicillinase activity.[45] the gene which is encoding PBP5 was mapped to dacA.[46]

PBP6–the gene that encode for this protein is the dacC shares up to 62% sequence with PBP5.[47] PBP6 and PBP5 catalyze identical reactions but, PBP5 shows higher specific activity than PBP6 toward uncross-linked peptidoglycan.[48] Deletion of dacC had no effect on cell morphology and growth rate.[49] However, strains lacking PBP6 showed a very slight increase in antibiotic sensitivity.[49]

PBP7 and PBP8 these are characterized more recently than the other PBPs. PBP8 is a product of PBP7 as result of OmpT proteolytic reaction.[50] PBP8 increased expression is usually associated with the increased ceftazidime and cephaloridine resistance.[51] Both PBP7 and PBP8 are soluble periplasmic proteins that are peripherally associated with the membrane. Encoding gene of was narrowed to 47.8 and 48min on the *E. coli* chromosome and pbpG encode for PBP7.[52]

Resistance to β-lactam activity

There are four major ways bacteria avoid the bactericidal effect of beta-lactams

Altered Penicillin-binding proteins that exhibit relatively low affinity toward beta-lactam antibiotics some examples are the PBP 2_(PBP2a) of Staphylococcus aureus and PBP 2x of *Streptococcus pneumoniae*.[53] penicillins are unable to inactivate these PBPs because they are relatively resistant to it and they can assume the functions of other PBPs after their deactivation. Diminished or completely lack of expression of outer membrane proteins (OMP) in gram-negative bacteria. In order to acquire access to PBPs, beta-lactam have move through porin channels in the outer membrane, decrease expression of OMPs limits the of certain beta-lactams from entry into the periplasmic space of gram-negative bacteria, therefore restrict its access to PBPs on the inner membrane. Resistance to Imipenem in *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* can arise from the loss of OmpK36 and OMP D2, respectively.[54–56] It was reported that the resistance to meropenem and Imipenem in some isolate of multidrug resistant *Acinetobacter* baumannii to is associated with the loss of the CarO OMP.[57,58] insertion of some sequence to porin encoding genes or its mutation can lead to the production of proteins with reduce functions and subsequently decrease the diffusion of beta-lactam into the cell.[59] it is believed that the destruction of porin alone is not sufficient enough for acquiring resistance phenotype. This mechanism is usually coupled with the expression of beta-lactamases.[59,60]

Efflux pumps it is a part of intrinsic resistance or acquired resistance phenotype. Efflux pumps have the capability to export various substrates from the periplasmic part of the cell to the surrounding environment.[61] these pumps are the determinant of multidrug resistance in various Gram-negative bacteria especially *P. aeruginosa*. The decrease in the organism outer membrane permeability in combination with the upregulation of the mexA-mexB-OprD can contribute to decreased susceptibility to various beta-lactam antibiotics including Cephalosporin, penicillin, tetracycline, quinolones and Chloramphenicol.[62–65]

Beta-lactamases Production Bacteria produce enzymes known as Beta-lactamases that hydrolyze the beta-lactam ring subsequently the beta-lactam antibiotic is rendered inactive before it get to the PBP target. because of the structural relation that beta-lactamases shares with PBP, it bind, acylate and also use water molecules to hydrolyze and inactivate beta-lactam share with PBPs allows these enzymes to bind, acylate, and use a strategically located water molecule to hydrolyze and thereby inactivate the beta-lactam.[66] in gram-negative bacteria the most important resistance mechanism is the inactivation of beta-lactams by beta-lactamases. It has been reported that there are over 530 beta-lactamase enzymes (K. Bush, 9th International Congress on beta-Lactamases, Leonessa, Italy). beta-lactamases contains either serine residue or metal ion In their active site, betalactamases with a serine residue (Ambler classes A, C, D) and metal ion Zn^{2+} (Ambler class B) that attack beta-lactam ring and break the amide bond in the ring Figure 4.[67–69]

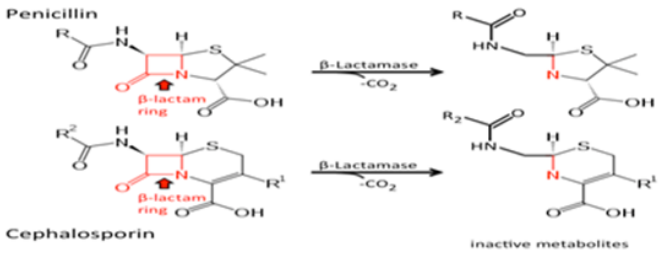

Figure 4 Mechanism of beta-lactam resistance.

Serine β-lactamases have serine as an active-site which is used to hydrolyze the ring of β-lactam in β-lactam antibiotics. The serine

β-lactamases are classified based on sequence similarity into three classes, A, C, and D.[70–72] which are all related to the DD peptidases.[73]

Amber class A

This class was first observed in *E.coli* in 1963 and was termed TEM; it was named after the person from whom it was isolated. This class of enzyme exhibit a level of susceptibility to many commercially available β-lactamase inhibitors like Clavulante, Sulbactam and Tazobactam.[74,75] other members of this class including VH5, PER and SHV were also reported.[76] SHV-1 and TEM-1 have almost 68% sequence homology and can be found in *E.coli*, K. Pneumoniae and other pathogens responsible for various infections. TEM-t and SHV-1 confers resistance to Ampicillin and Piperacillin.[77]

Ambler class B

These enzymes contain an enzymes a small number Zn^{2+} this class one of the atoms of Zinc in inactivation cephalosporins and penicillins of are MBLs that use one of two zinc (Zn^{2+}) atoms for inactivating penicillins and cephalosporins. However, their activity can be inhibited by chelating agents (EDTA) but not by sulfones or clavulanic acid. IMP-1 was the first to be discovered in this class form *P. aeruginosa*. Varieties of genetic element such as plasmid, integron were found to have the bla genes encoding.[78]

Ambler class C

Enzymes in this class are active against cephalosporins, therefore sometimes called cephalosporinases.[79] their genes are encoded in the chromosome and are mostly synthesized by Gram-negative bacteria. The sequences of these enzymes that are known are highly conserved.[80] The cephalosporin-hydrolyzing chromosomal β-lactamase of his class in *P. aeruginosa* are encoded by ampC (PA4110), which was cloned and sequenced.[81]

Amber class D

The enzymes in this class are capable of degrading isoxazolyl β-lactams like methnicillin and oxacillin. Thus they are also called oxacillinases.[82] however their activity is inhibited by clavulanic acid.[83]

Overcoming β-lactamases

There are basically two ways to overcome the effect of hydrolytic activity of beta-lactamases. The first principle involves getting molecules that inactivate or inhibit beta-lactamases. Sulbactam, clavulanic acid and tazobactam-lactamase are the three inhibitors that are used in the clinical application. All of these three compounds share similar structures with penicillin. Some of the features of these compounds include high affinity for β-lactamases, each of these compounds occupies the active site relatively longer than β-lactams and undergoes different reaction chemistry and they are also poorly hydrolyzed by the enzyme.[84–86] therefore, β-lactamase inhibitors are also called "suicide inhibitors" because they get trapped by the beta-lactamase. This phenomenon has been the subject of research by academic laboratories and some pharmaceutical companies.[87–93] synthesis of compounds by substituted sulfones, cephem and penem gives optimism that new inhibitors of β-lactamase will be found.[94] The Recent research studies that are being carried out to elucidate the mechanistic details of beta-lactamase inhibition of deacylation-deficient beta-lactamases will surely advance the knowledge of the chemistry of inactivation.[95]

The second principle involves getting a new beta-lactam antibiotic that possesses great affinity for the β-lactamases and cannot be hydrolyzed by the PBP, or poorly hydrolyzed by it. This has been the original rationale behind extended-spectrum carbapenems or cephalosporins. Common example of this principle is the development of compounds such as doripenem and ceftobiprol**.** Ceftobiprole is an "anti-MRSA cephalosporin" which demonstrates very high affinity for PBP2, it is active against gram-negative bacteria possessing betalactamases and resistant to penicillinase of *S. aureus* and is.[96] Doripenem is a modified carbapenem with sulfamoylaminomethyl substituted pyrrolidylthio group at the C2 position and 1-beta-methyl group, which shows very high activity against *Acinetobacter* spp, *P. aeruginosa* and Burkholderia cepacia. [97]

Sulbactam

Sulbactam known as a semi synthetic substance capable of inactivating β-lactamases though it is not as potent as Clavulanic acid it shows high activity against class ii-iv and displays relatively low action against class1β-lactamase. The combination of sulbactam with some antibiotics tends to increase their activity against antibiotic resistant bacteria for example; the antibacterial activity of ampicillin will be extended and becomes more effective when it is combining with sulbactam. A compound was developed containing sulbactam-ampicillin known as sultamicillin was found clinically effective in treatment of various infections such as those of skin and soft tissues as well as many other infections. it was also reported that a single dose of ampicillin-sulbactam administered intra-muscularly with probenecid had therapeutic effect against infections of neisseria gonorrhae which is an ampicillin resistant.[98]

Tazobactam

Piperacillin combined with tazobactam was first prepared in 1993 in the United state. piperacillin is known to have antibiotic activity against gram-negative and gram-positive as well as aerobes and anaerobes.[99] piperacillin-tazobactam combination act as a good β-lactamase inhibitor with broad spectrum of antibacterial activity in both gram-negative and gram-positive bacteria. But such combination has no inhibition effect against isolates of gram-negative bacillus having AmpC β-lactamase. Piperacillin-tazobactam combination is reported to be effective for treatment of various infections including intra-abdominal infections.[100]

Clavulanic acid

Ticarcillin-clavulanate was the first combination β-lactam β-lactamase inhibitor developed in 1985 for parenteral administration. It increases the inhibitory activity against -lactamase-producing *staphylococci*, *Proteus* spp, H. influenzae, *Pseudomonas* spp, *Klebsiella* spp *Providencia*, and *E. coli*.[101] the combination of amoxicillin to clavulanic acid increases the organism susceptibility to amoxicillin like amoxicillin resistant *Haemoehilus influenza* and *Neisseria gonorrhea*.[102]

Conclusion

Since b-lactam antibiotics introduction into clinical field more than 60years ago, beta-lactam antibiotics have been the major source of antimicrobial therapy. The mechanism of action of beta-lactam antibiotics is usually by inhibiting the enzyme responsible for the bacterial cell wall synthesis subsequently resulting in the lysis and death of the bacteria. Unfortunately, bacteria have developed resistance to β-lactam antibiotics through a defense mechanisms to protect themselves against the effect of the antibiotics by Altered Penicillin-binding proteins that exhibit relatively low affinity toward beta-lactam antibiotics, diminished or completely lack of expression

Citation: Zango UU, Ibrahim M, Shawai SAA, et al. A review on β-lactam antibiotic drug resistance. *MOJ Drug Des Develop Ther.* 2019;3(2):52–58.
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of outer membrane proteins (OMP) in gram-negative bacteria, Efflux pumps it is a part of intrinsic resistance or acquired resistance phenotype and by beta-lactamases Production which plays the major role in resistance mechanism by hydrolyzing the beta-lactam ring subsequently the beta-lactam antibiotic is rendered inactive before it get to the PBP target. This review have shown how the β-lactamase activity can be overcome which is by two principles, the first involves getting molecules that inactivate or inhibit beta-lactamases. molecules like sulbactam, clavulanic acid and tazobactam .the second principles involves getting a new beta-lactam antibiotic that possesses great affinity for the β-lactamases and cannot be hydrolyzed by or poorly hydrolyzed by β-lactamases.

Acknowledgment

None.

Conflicts of interests

Authors declare that there is no conflict of interest.

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