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Review Article

ROLE OF BETA LACTAMASES IN ANTIBIOTIC RESISTANCE: A REVIEW

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ABSTRACT
Penicillin, the first β-lactam antibiotic was introduced into clinical practice in 1940s. Since then a large number of different β-lactam antibiotics including penicillins, cephalosporins, monobactam and carbapenems have been developed. All of them are structurally related through the presence of a core β-lactam ring. β-lactam antibiotics interfere with the synthesis of bacterial cell wall, they also inhibit the transpeptidases so that cross linking does not take place. The wide use of β-lactam antibiotics has created major resistance problems leading to increased morbidity, mortality and heath care costs. Resistance is most often mediated by lactamases which is produced from both gram negative and positive bacteria. β-lactamases are enzymes responsible for many failures of antimicrobial therapy because of the hydrolysis of β-lactam antibiotics to inert and ineffective agents. β-lactamases are one of the reason behind the bacterial resistance to β- lactam antibiotics. In recent years new varieties of beta lactamases has been detected in increasing rate. Combination of β-lactam antibiotics with β-lactamases inhibitors has been successfully used to control resistance during last two decades. These inhibitors greatly enhance the efficacy of their partner β-lactam antibiotics (amoxillin, ampicillin, piperacillin, and ticarcillin) in the treatment of serious *Entero bacteriaceae* and penicillin resistant *Staphylococcal* infections. The prevalence of clinically relevant β-lactamases from other classes that are resistant to inhibition is rapidly increasing. Lack of effectiveness of currently available β-lactamase inhibitors is a main problem with the combination therapy. This review describes the various β-lactum antibiotics and effect of different β –lactamases that produced by the several bacteria's against the β-lactam antibiotics, their resistance pattern and its effective management with combination therapy.
Keywords: Beta lactam antibiotics, Beta lactamases, Beta lactamase inhibitors, Antibiotic resistance.

INTRODUCTION

β- Lactam antibiotics are a broad class of antibiotics consisting of agents that contain a β-lactam ring in their molecular structure. These include penicillins, cephalosporins, monobactams and carbapenems. Penicillin was the first antibiotic to be used clinically in 1941 by Alexander Fleming, Scottish Bacteriologist. β- lactam antibiotics work by inhibiting cell wall biosynthesis in the bacteria and are the most widely used group of antibiotics. The most widespread cause of resistance to β-lactam antibiotics like penicillin is the production of enzymes called β-lactamases. β- Lactamases are a family of enzymes produced by many Gram positive and Gram negative bacteria that inactivate β-lactam antibiotics by opening the β-lactam ring.[1] Beta lactamases are enzyme that are responsible for many failures of antimicrobial therapy by the hydrolysis of beta lactam ring of these antibiotics.[2] Most strains of *Entero bacteriae* and *Pseudomonas aerogenosa* produce chromosomally determined class1 β-lactamases and these enzymes produce resistance to almost all β-lactams except imipenam and sometimes carbenicillin and tenocillin. Permanent high level enzyme production arises via mutation.[3] Beta-lactamases that protect bacteria from the lethal effects of beta - lactam antibiotics and are of considerable clinical importance. The crystal structure of beta-lactamase from the Gram-positive bacteria *Staphylococcus aureus* PC1 has been determined at 2.5 angstrom resolution. It reveals a molecule of novel topology made up of two closely associated domains. The active site is located at the interface between the domains with the key catalytic residue Ser70 at the amino terminus of a buried helix. The unusual topology of the secondary structure units is relevant to questions concerning the evolutionary relation to the beta-lactam target enzymes of the bacterial cell wall.[4]

Mechanism of Action of Beta Lactam Antibiotics

The cell wall of bacteria contains peptidoglycan. The cell wall is a rigid outer layer unique to bacterial species. It completely surrounds the cytoplasmic membrane, maintains cell shape and integrity and prevents cell lysis from high osmotic pressure (Figure 1). The cell wall is composed of a complex cross linked polymer of polysaccharides and polypeptides like peptidoglycan. The polysaccharide contains alternating amino sugars, N-acetyl glucosamine and N-acetyl muramic acid. A 5- amino- acid peptide is linked to the N-acetyl muramic acid sugar. This peptide terminates in D-alanyl- D- alanine. Penicillin- binding protein is an enzyme that removes the terminal alanine in the process of forming a cross link with a nearby peptide.[5] The β- lactams inhibit the final transpeptidation by forming covalent bond with penicillin- binding proteins that have transpeptidase and carboxypeptidase activities thus preventing formation of the cross links. The final bactericidal action is the inactivation of an inhibitor of autolytic enzymes in the cell wall, which leads to the lysis of the bacteria. (Figure 2) Some tolerant organisms have defective autolytic enzymes and are inhibited but not lysed in the presence of drug.[6]

Mode of Resistance

Resistance to penicillins and other β-lactams is due to one of four general mechanisms like; inactivation if antibiotic by β-lactamase, modification of target Penicillin – Binding Protein (PBPs), impaired penetration of drug to target PBPs and efflux. B-lactamase production is the most common mechanism of resistance. B-lactamases produced by

Staphylococcus aureus, Haemophilus sp. and *Escherichia coli* are relatively narrow in substrate specificity preferring penicillins to cephalosporins. Other β-lactamases like AmpC β-lactamase produced by *Pseudomonas aeruginosa* and *Enterobacter sp,* and ESBLs hydrolyze both cephalosporins and penicillins. Carbapenems are highly resistant to hydrolysis by penicillinases and cephalosporinases but they are hydrolysed by metallo-β-lactamase and carbapenemases. Altered target PBPs are the basis of methicillin resistance in *Staphylococci* and of penicillin resistance in *pneumococci* and *Enterococci.* These resistant organisms produce Penicillin- Binding Protiens (PBPs) that have low affinity for binding β-lactam antibiotics; consequently they are not inhibited except at relatively high drug concentrations. Resistance due to impaired penetration of antibiotic to target PBPs occurs only in gram-negative species because of their impermeable outer cell wall membrane which is absent in gram-positive bacteria. β-lactam antibiotics cross the outer membrane and into enter gram-negative organisms via outer membrane protein channels (porins). Absence of proper channel or down regulation of its production can greatly impair drug entry into the cell. Poor penetration alone is usually not sufficient to confer resistance because enough antibiotic eventually enters the cell to inhibit its growth. However this barrier can become important in the presence of a β-lactamases even a relatively inactive one as long as it can hydrolyze drug faster than it enters the cell. Gram-negative organisms also may produce an efflux pump which consists of cytoplasmic and periplasmic transport some β-lactam antibiotics from the periplasm protein componenets that efficiently back across the outer membrane.[7] B-lactamases of *Entero bacteriaceae* are the most important against β-lactam drugs. Two types of β-lactamase can confer resistance against third generation cephalosporins. Chromosomally mediated β-lactamases are inducible and are not inhibited by clavulanic acid. Resistance due to their enzyme is non transferable. The second type of enzyme is plasmid-medited β-lactamases which are inhibited by clavulanic acid. These enzymes are more important clinically. These can be tranferd between various species of *Entero bacteriaceae.* These enzymes are called as Extended Spectrum Beta Lactamases (ESBLs). ESBLs can confer resistance against all β-lactam drugs except carbapenems and cephamycins.[8]

Different Types

Most common types of β-lactamases include: ESBLs, Ampc s, SHV, TEM-, OXA etc.

Extended Spectrum Beta Lactamases (ESBLs)

Extended spectrum β- lactamases (ESBLs) are mutant enzymes with a wide range of activity than their parent molecules. They hydrolize third and forth generation cephalosporins and aztreonam but do not affect the second generation cephalosporins and remains susceptible to β-lactamase inhibitors. The most common plasmid-mediated β-lactamases in *Entero bacteriaceae* are TEM-1, TEM-2 and SHV-1. Classical ESBLs are derived from TEM and SHV enzymes whereas non-classical ESBLs are derived from enzymes other than TEM and SHV. Classical ESBLs are primarily found in *E. coli* and *Klebsella* species. They differ from their parent enzymes only by 1-4 amino acids. Non-classical ESBLs are less common than classical ESBLs. It includes CTX-M and OXA.

AmpCs

They are not inhibited by β-lactamase inhibitors that are normally repressed and are produced at low levels. Plasmid-mediated AmpCs are also inducible.[9] Two mechanisms responsible for Ampc activity in *E. coli* are mutations in Ampc promoter and attenuator regions resulting in Ampc over expression and acquisition of plasmid-carried Ampc genes.[10]

Sulfhydryl Variant (SHV)

The most prominent β-lactamases produced by *Entero bacteriacea* are the Sulfhydryl Variant (SHV) family. The first reported SHV had a narrow spectrum of activity. Derivatives of SHV-1 have been evolved due to the accumulation of point mutations at the active site of the enzyme. These derivatives have an extended spectrum of activity which is capable of inactivating third-generation cephalosporins.[11]

Plasmid – Encoded Transposable Element Beta lactamases (TEM-1)

It is one of the most well known in producing antibiotic resistance. It confers resistance to penicillins and early cephalosporins. It is commonly found in gram negative bacteria. Almost 90 % of ampicillin resistance in *E. coli* is due to the production of TEM-1. It is mostly found in *E. coli* and *K. pneumoniae.* By opening the active site to beta-lactam substrates enhances the susceptibility of the enzyme to beta lactamase inhibitors like clavulanic acid. Currently 140 TEM-type enzymes are identified.[12]

Oxacillinases (OXA)

The OXA-type (oxacillin hydrolyzing) enzymes are produced by *Entero bacteriaceae* and *P. aerugenosa.* They pose resistance against amino and ureidopenicillin and high level hydrolytic activity against cloxacillin, oxacillin and methicillin. Clavulanic acid strongly inhibits the activity of this enzyme. They belong to ambler class-D and thus posses an active serine site as classes A and C beta lactamases.[13]

Management

β-lactamase inhibitors

These agents structurally resemble β-lactam antibiotics but do not possess any significant antimicrobial action. They bind irreversibly to the catalytic site of susceptible β-lactamases (particularly penicillinase) to prevent hydrolysis of penicillins. These are generally effective against plasmid-mediated β-lactamases which are responsible for transferred drug resistance such as those produced by methicillin-sensitive *Staphylococcus aureus, H. influenzae, H. ducreyi, E. coli, Klebsiella pneumoniae, Proteus mirabilis, Neisseria gonorrhoeae, Salmonella species* and *Shigella species.* These are generally ineffective against chromosomally mediated β-lactamases found in *Enterobacter, Pseudomonas aeruginosa, Citrobacter* and *Serrratia* and organisms producing inducible extended spectrum β-lactamases. Currently three β-lactamase inhibitors are available; Clavulanic acid, Sulbactam and Tazobactam. Clavulanic acid is combined with amoxicillin, sulbactam with ampicillin, and tazobactam with piperacillin and are available as fixed dose combinations.[14]

Clavulanic acid

It is isolated from *Streptomyces clavuligerus.* It is only orally absorbed. It is a potent inhibitor of many β-lactamases, including those found in *E. coli* (plasmid mediated), *K.*

aerogenes, Proreus mirabilis and *S. aureus.* It has a β-lactam ring but lacks antibacterial activity of its own. It is a progressive inhibitor, initially revesible by binding with β-lactamase but becomes covalent later and inhibition increases with time. It is also called as suicide inhibitor as it gets inactivated after binding to enzyme. It permeates the outer layers of the cell wall of gram negative bacteria and inhibits the periplasmically located β-lactamase. It has rapid oral absoption and bioavailability of 60 % and can also be injected.[15] Amoxicillin and clavulanic acid is an effective combination in the treatment of urinary tract infection caused by amoxicillin resistant organisms and also used in the treatment of respiratory tract infections. Nausea, vomiting, diarrhoea and skin rashes being the most frequent adverse effects of this combination

Activity against β-Lactamases

Clavulanic acid is an effective inhibitor against β-lactamases of the Richmond types II, III, IV and V. Some isolated type I β-lactamases are inhibited by clavulanic acid only at high concentration of drug with pre-incubation, so synergy with β-lactam antibiotics does not occur against cephalosporinases of Richmond type I containing intact cells.

Antibacterial activity

Clavulanic acid has only weak antibacterial activity against most of the organisms but the addition of clavulanic acid to amoxicillin will increases the susceptibility to amoxicilln in organisms like amoxicillin resistant *Stephylococcus aureus, Entero bacteriaceae,* Amoxicillin resistant *Haemoehilus influenzae* and *Neisseria gonorrhoeae, Bacteroides fragilis* etc .The usual adult dose of drug in the treatment of uncomplicated infections of moderate severity is 375 mg (amoxicillin 250 mg and clavulanic acid 125 mg) or 312.5 mg (amoxicillin 250 mg and clavulanic acid 62.5 mg) 8 hourly, twice this dose may be required in more severe and complicated infectious diseases.[16]

Sulbactam

It is a semi synthetic β-lactamase inhibitor. It is combined with certain β-latam antibiotics to extend their activity against bacteria that are resistant to the antibiotics. It is highly active against class II-V but is poorly active against class I β-lactamase. It is 2-3 times less potent than clavulanic acid. It does not induce chromosomal β-lactamases while clavulanic acid can induce some of them. Its combination with ampicillin is effective against β-lactamase producing resistant strains and will extend the antibacterial activity of ampicillin and also increases the susceptibility of many sensitive strains.

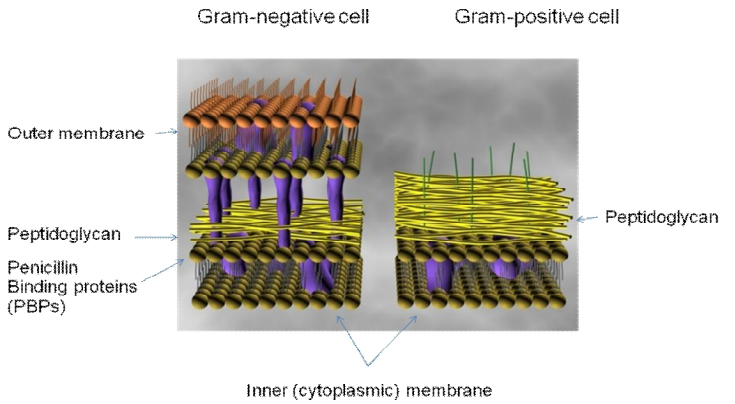

Figure 1: Structure of bacterial cell wall

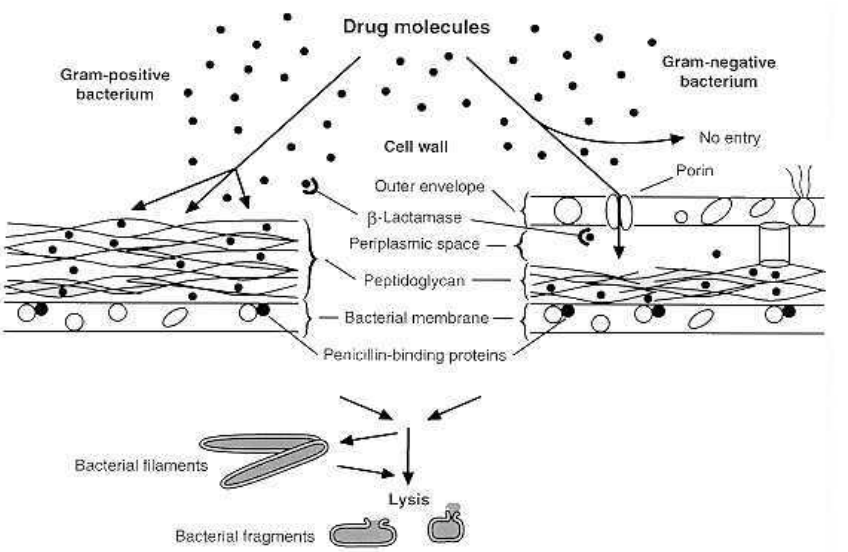

Figure 2: Mechanism of action of β-lactam antibiotics

It is poorly absorbed after oral administration therefore ampicillin-sulbactam is parenterally administered. Sulbactam- ampicillin compound sultamicillin has been developed which is well absorbed after oral administration. Multiple dose therapy with ampicillin- sulbactam is clinically well effective in the treatment of various infections like UTI, infections of skin and soft tissue, bones and joints, respiratory tract, ears, nose and throat as well as intra abdominal and obstetrics and gynecological infections and septicaemia. Single intra muscular doses of ampicillin- sulbactam with probenecid are therapeutically well effective in gonorrhoea and infections produced by ampicillin resistant *Neisseria gonorrhoeae*. The main adverse reactions of combination therapy are pain at the site of injection which can be minimized by diluting the intramuscular dose with lignocaine. The other side effects include diarrhea and phlebitis. Sulbactam plus ampicillin in a 1: 2 concentration ratio is available as a combination for parenteral administration. The recommended adult therapeutic dose of the combination is 1.5 – 12 g daily (0.5 - 4 g sulbactam plus 1 – 8 g ampicillin) in divided doses every 6 to 8 hours. In case of less severe infections the interval between doses may be increased to 12 hours. For children, infants and neonates the recommended therapeutic dose of the combination is 150 mg/kg/day (sulbactam 50 mg/kg/day plus ampicillin 100 mg/kg/day).[17]

Tazobactam

Its action is similar to sulbactam. Its pharmacokinetics matches with piperacillin with which it has been combined for using severe infections like peritonitis, pelvic, urinary and respiratory infections caused by β-lactamase producing bacilli. The combination is not active against piperacillin-resistant *Pseudomonas.*[18] Piperacillin-tazobactam is a beta lactam / beta lactamase inhibitor combination with a broad spectrum of antibacterial activity that includes gram positive and negative aerobic and anaerobic bacteria. This combination retains its *in-vitro* activity against broad spectrum beta lactamase. The spectrum of antibacterial activity includes Gram-positive and Gram -negative aerobic and anaerobic bacteria. But it is not active against isolates of gram negative bacilli harboring AmpC beta lactamases. It has been found to be effective for the treatment of patients with intra-abdominal infections, skin and soft tissue infections, lower respiratory tract infections, complicated urinary tract infections, gynecological infections and more recently, febrile neutropenia. It has an excellent safety and tolerability profile.[19]

CONCLUSION

Antibiotics have always been considered as one of the wonder discovery of the 20th century. Antibiotic resistance is a serious and growing phenomenon in contemporary medicine and has emerged as one of the pre-eminent public health concerns of the 21st century. Every time antibiotics are used in any setting, bacteria evolve by developing resistance. This process can happen with alarming speed. Antimicrobial resistance has cast a shadow over the medical miracles we take for granted, undermining every clinical and public health program designed to contain infectious diseases worldwide. Limited access to medical care and effective treatments, the common practice of self-medication, and the availability of counterfeit drugs have exacerbated drug resistance in the developing world. These drugs are a precious, limited resource—the more we use antibiotics today, the less likely we are to have effective antibiotics tomorrow.

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