Penicillin Binding Proteins: An Insight Into Novel Antibacterial Drug Target

Pallavi Sahare and Archana Moon

Abstract - Penicillin binding proteins (PBP) have been analysed for over 40 years. PBPs are the enzymes that catalyze the synthesis of peptidoglycan in bacteria.The peptidoglycan is made of glycan chains of alternating N-acetyl glucosamine and N-acetylmuramic acid, cross-linked by short stem peptides attached to the N-acetylmuramic acid. Peptidoglycan enables the bacteria to resist the intracellular pressure of several atmospheres and provides shape to the bacterial cell and isreproduced from generation togeneration. Also bacterial cell division requires the biosynthesis of peptidoglycan by PBPs during cell wall elongation and septum formation. These PBPs are divided into three classes based on their functions. The high molecular weight (HMW) PBPs that are divided into class A and class B whichplay bifunctional roles, transpeptidases (the cross-linking between glycan chains)/transglycosylases (polymerization of the glycan strand) and monofunctional transpeptidases, respectively. Some PBPs hydrolyze the last D-alanine of the stem peptide (DD-carboxypeptidation) or hydrolyze the peptide bond connecting two glycan strands (endopeptidation).The low molecular weight (LMW) PBPs are included in Class C. Because of the structural resemblance between PBPs natural substrate, the D-Ala-D-Ala end of the stem peptides and penicillin, the late stage peptidoglycan synthesizing enzymes are sensitive to penicillin with which they form a long-lived acyl-enzyme that impairs their peptidoglycan cross-linking capability. This review article focuses on detailed insight on PBP classification and mechanism, thus opening avenues for an effective and novel antibacterial drug target research and therapy.

Index terms - Penicillin binding proteins, PBPs, drug target, antibacterial.

1 INTRODUCTION

THE pepeptidoglycan is a macromolecule made up of β (1-4) linked N-acetyl glucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) with interlinked glycan chains and peptide bridges. This forms the rigid structural component of the eubacterial cell wall which gives cells osmotic stability and maintains their shapes. The cell wall assembly is catalysed by penicillin binding proteins (PBP), Ghuysen [1]. Many of the final periplasmic steps in the synthesis and maturation of peptidoglycan are performed by the PBPs, enzymes to which β-lactam antibiotics bind covalently, Denome et al [2]. The classical definition of PBP is the protein that targets β-lactam antibiotics. PBPs are the major constituents of the bacterial cell wall and catalyse the final steps in peptidoglycan polymerisation. The target for the β-lactam antibiotics are cell wall synthesizing enzymes that have sensitivity towards penicillin and are easily detected by their ability to bind radiolabelled penicillin. Hence named Penicillin Binding Proteins (PBPs). PBPs are present in all bacteria but vary from species to species in number, size, quantity and affinity for β-lactam antibiotics, Georgopapadakou [3]. PBPs are localised on the outer face of the cytoplasmic membrane, Jacoby [4]. The intrinsic beta lactamase resistance associated with PBP alterations has been found to be an important mechanism in clinical isolates.

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Spratt (1975-77) first observed PBPs in E.coli by PAGE and named them in order of their decreasing molecular mass PBPs 1a (highest molecular weight), 1b, 2, 3, 4, 5 and 6 (lowest molecular weight), Spratt [5, 6]. Five more PBPs have been included in this list later. PBP7 and 8 by Henderson et al (1994-95) [7, 8]; DacD by Baquero et al (1996) [9], AmpC and AmpH by Henderson (1997) [10] and PBP 1c by Schiffer et al (1997) [11].

The PBPs are divided into three categories: Class A, B and C. Class A and B consist of high molecular weight PBPs and class C of low molecular weight PBPs. The peptidoglycan polymerisation activity is known as transglycosylase activity of class A HMW – PBP. The transpeptidase activity of PBPs is well characterized mainly due to its important role in bacterial resistance to β-lactam antibiotics, as described elsewhere [12],[13],[14],[15],[16],[17],[18],[19],[20],[21],[22],[23],[24],[25]. The transpeptidase activity can be characterized by its ability to bind to different β-lactams and hydrolyse analogs of bacterial cell wall stem peptides, as described [13],[14],[15],[16],[17],[18],[19],[20],[21],[22],[23],[24],[25],[26],[27],[28],[29].

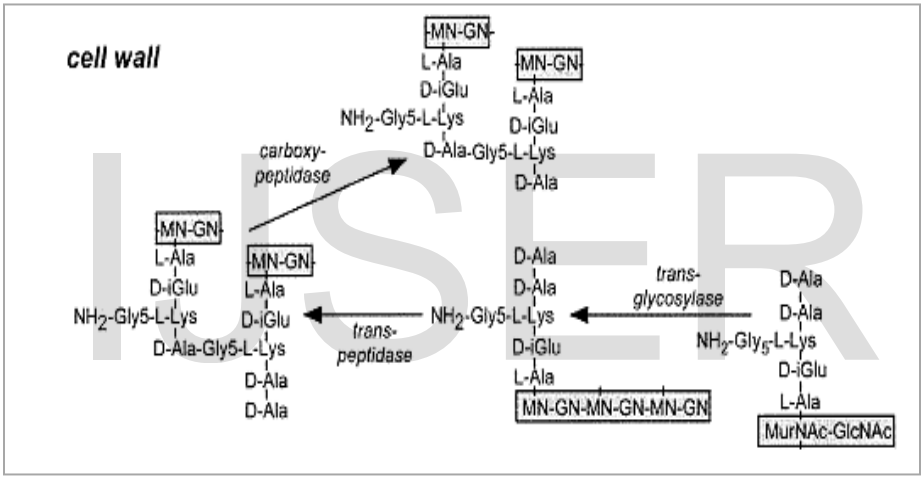


Fig 1 Biosynthetic reactions of cell wall assembly

2 CLASSIFICATION OF PBPs

HMM PBPs are multimodular PBPs responsible for peptidoglycan polymerization and insertion into pre-existing cell wall. Their topology consists of a cytoplasmic tail, a transmembrane anchor and two domains joined by a b-rich linker located on the outer surface of the cytoplasmic membrane where peptidoglycan synthesis takes place. The different types of HW-PBPs are 1a, 1b, 2, 3a and 3b [30].

Depending on the structure, sequence similarity and the catalytic activity of their N-terminal domain, they belong either to class A or class B PBPs. The C-terminal penicillin- binding (PB) domain of both classes has a transpeptidase activity, catalyzing peptide cross-linking between two adjacent glycan chains [31].

I. Class-A PBPs include a single trans-membrane segment sometimespreceded by a short N-terminal cytoplasmic region and two extracellular domains. The first extracellular domain carries the glycosyltransferase activity and is responsible for the polymerisation of the glycan strand. The C-terminal region constitutes the penicillin binding domain that catalyses transpeptidation [30].

Class A PBPs promote both the polymerization of glycan from its disaccharide precursor, i.e., the successive addition of MurNAc (-L-Ala–D-isoGlu–L-Lys–D-Ala–D-Ala)-GlcNAc to C55–PPMurNAc(- L-Ala–D-isoGlu–L-Lys–D-Ala–D-Ala)-GlcNAc, and the transpeptidation (cross-linking) of wall peptides. The latter reaction results in the

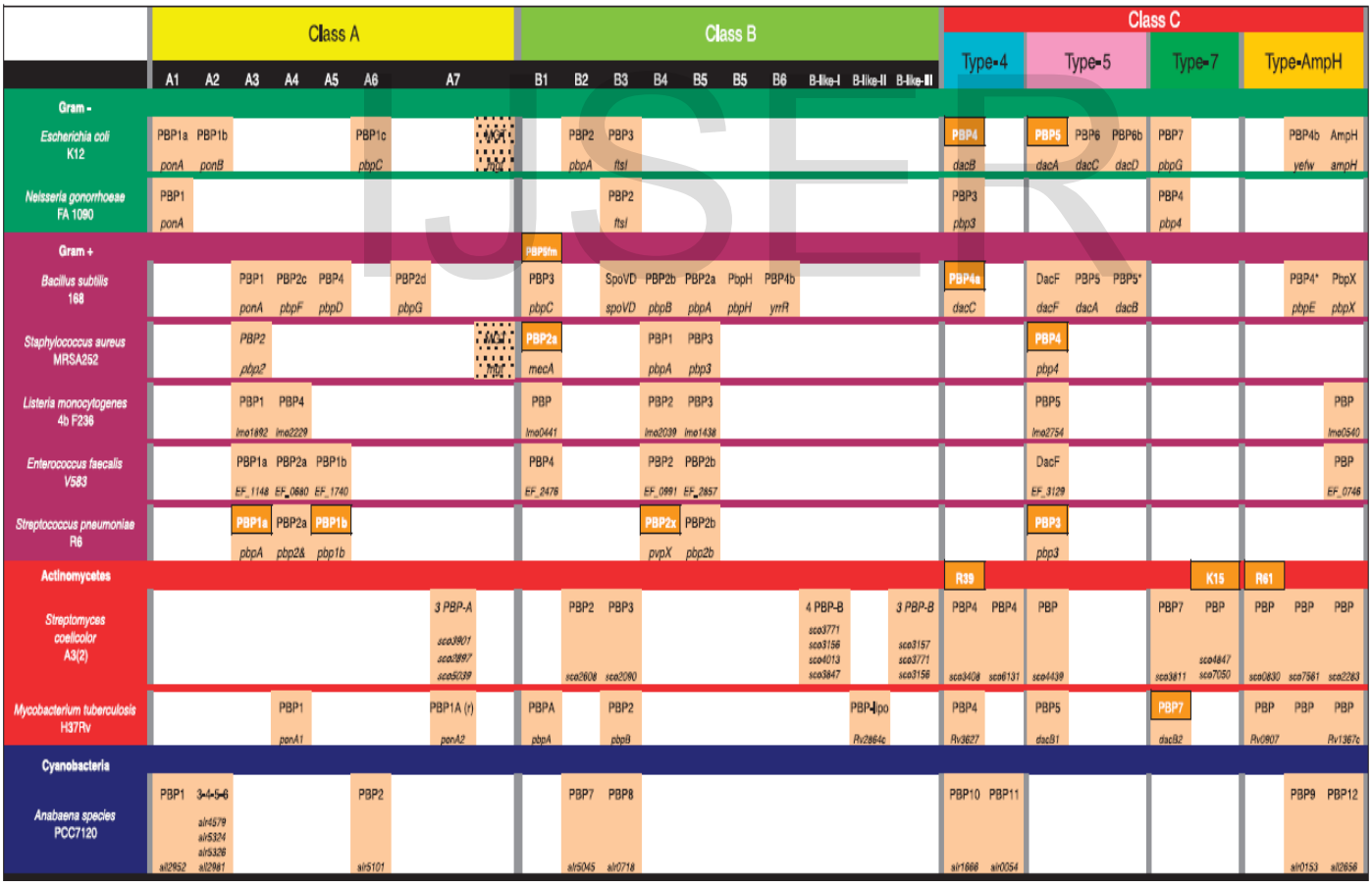
proteolytic removal of the D-Ala at the C-terminal end of the pentapeptide and the formation of a new amide bond between the amino group of the cross bridge and the carbonyl group of D-Ala at position 4. This reaction is the target of penicillin and other β-lactam antibiotics which mimic the structure of D-alanine – D-alanine. After cleavage, the β-lactam ring continues to occupy the active site serine residue of PBPs, thereby inhibiting PBPs [12].

II. Class-B PBPs consist of a transmembrane anchor,N-terminal domain and C-terminaltranspeptidase penicillin-binding domain. The N-terminal domain is believed to play a role in cell morphogenesis by interacting with other proteins involved in the cell cycle [31].

III. Low molecular weight PBP constitutes the third group. As a whole, LMW PBPs are frequently described with the general term of class C PBPs, sometimes with C1, C2 and C3 as subdivisions [31].These consist mainly of penicillin-binding domain with a small additional C-terminal domain, which is anchored to the plasma membrane either through a transmembrane segment or an amphipathic helix presumably lying on the lipid bilayer. LMW PBPs have either DD-carboxypeptidase or DD-endopeptidase activity [30].

Seven low-molecular-weight PBPs are now known: PBPs 4, 5, 6, 7, DacD, AmpC, and AmpH. Although AmpC and AmpH belong to the family of class C β-lactamases, these two proteins are referred to as PBPs because both enzymes bind covalently to at least one radioactively labelled β-lactam [10].

TABLE 1: CLASSIFICATION OF PBPS31



PBPs for which the X-ray structure has been determined are highlighted in orange.

2.1 SUBCLASSES OF PBPS

By sequence alignment, class A PBPs can be grouped inat least seven subclasses (Table-1). The subclasses A1 and A2 group Gram-negative PBPs, whereas subclasses A3, A4, and A5 form three clusters of Gram-positive PBPs. Subclass A6 contains unusual PBPs such as *Escherichia coli* PBP1c and *Anabaena* sp. PBP2. The three *Streptococcus coelicolor* class A PBPs are grouped with the penicillin-resistant *M. tuberculosis* PBP1a in subclass A7 [31].

The class B PBPs are grouped in five subclasses. *Staphylococcus aureus* PBP2a and *Enterococcus faecium* PBP5 are in the subclass B1 and all the PBPs in this subclass are supposed to show a low affinity for penicillin [31]. The subclass B2 contains an elongase complex specific to PBP2 type of Gram-negative bacteria (e.g. *Escherichia coli* PBP2), whereas the subclass B3 contains divisome specific to PBP3type of Gram-negative bacteria (e.g. *Escherichia coli* PBP3) [31]. *Streptococcus pneumoniae* PBP2x is part of subclass B4 together with other enzymes involved in division of Gram-positive bacteria [31], and subclass B5 contains enzymes from Grampositive not directly involved in septation. Subclasses B-like I, II and III are defined by Goffin & Ghuysen (2002) and contains PBPs from *mycobacteria, streptomycetes* and related bacteria. The overall fold of class B PBPs is made of a complex N-terminal domain coupled to the C-terminal transpeptidase domain. In the transpeptidase domain, the residue following motif 3 [KTG(T/S)] is, with very few exceptions, an alanine in class B PBPs [KTG(T/S)A] while it is a threonine or a serine in class A PBPs [KTG(T/S)(T/S)], and this might be related to their specific role as class A or class B PBPs [31].

Class B PBPs play an important role in the resistance to β-lactams in many bacteria. One mechanism in some Gram-positive bacteria is the presence of an endogenous or acquired penicillin-resistant PBP that can take over the transpeptidase function of all other PBPs, like *Staphylococcus aureus* PBP2a and *Enterococcus faecium* PBP5. Strikingly a similar PBP in *L. monocytogenes* (lmo0441) is responsible for the resistance to monobactamsand some cephalosporins but not penicillin [31]. There is generally one or zero type-4 class C PBP in bacteria. Of note is the absence of this type of protein in Grampositivecocci like *staphylococci, enterococci, streptococci* or *Listeria*. *Streptococcus coelicolor* and some *cyanobacteria* are the only presumably known bacteria possessing two type-4 PBPs. Type-4 PBPs are not anchored into the cytoplasmic membrane via a transmembrane helix (Gittins*et al.,* 1994; Harris *et al*., 2002; Stefanova*et al.,* 2003) [31]. The groups of type-5 and type-7 class C PBPson the basis of the presence in type-5 PBPs of a C-terminal domain that was shown in *Escherichia coli* to be essential for the correct functioning of the PBP5 (Nelson & Young, 2001) [31]. The PBP5 C-terminal domain ends with an amphipathic helix that associates PBP5 to the cytoplasmic membrane. The truncated PBP5, lacking the C-terminal domain, is soluble (like PBP7) and its production leads to cell lysis (Nelson & Young, 2001). With the exception of *Staphylococcus aureus* PBP4, type-5 PBPs are strict DD-carboxypeptidases unable to catalyze a transpeptidation reaction (Matsuhashi et al., 1979) [31]. They play a major role in the control of cell diameter and correct septum formation. *Escherichia coli* PBP7 and PBP5 have homologous sequences. The structure of PBP7 differs from that of PBP5 by the absence of the C-terminal domain and the amphipathic helix that anchors PBP5 to the cytoplasmic membrane.The structure of type-AmpH PBPs is mainly based on the structure of the DD-peptidase of *Streptomyces* R61 (R61)(Fre`re, 2004). The active site lies at the interface of the two subdomains and the first motif SxxK is similar to other PBPs. The conserved serine of the second motif (SxN) is, in type- AmpH PBPs, replaced by a tyrosine and this is the main feature that distinguishes the enzymes of this family [31].

3 MECHANISM

PBPs are characterised by the presence of penicillin-binding (PB) domain common to the ASPRT protein family (active site serine penicillin recognising enzymes which includes class A and C β-lactamases). The PB domain is made of two sub domains, a five stranded β-sheet covered by three α-helices and all-helical domain. The active site lies at the interface of these two subdomains [31]. The penicillin binding domain contains 3 specific motifs SXXK, (S/Y) XN and (K/HXS/T) G. The topology of these β-lactamases is shared with the penicillin binding domain of PBPs [30]. PBPs share a common DD-peptidase activity, whether a DD-transpeptidase, a DD-carboxypeptidase or a DD-endopeptidase activity (Ghuysen, 1991; Goffin & Ghuysen, 1998) [31].

The carboxypeptidation and transpeptidation reactions catalyzed by PBPs follow a three-step mechanism:

- The rapid and reversible formation of a non-covalent Henri–Michaelis complex between the enzyme and a peptidoglycan stem pentapeptide [L-Ala-g-D-Glu-A2pm (or L-Lys)-D-Ala-D-Ala], called the donor strand.
- It is followed by the attack of the active serine on the carbonyl carbon atom of the C-terminal D-Ala- D-Ala peptide bond, leading to the formation of an

acyl-enzyme intermediate and the concomitant release of the C-terminal D-Ala (acylation).

- The final step (deacylation) consists of either hydrolysis, with release of the shortened peptide (carboxypeptidation), or cross-link formation with a second peptidoglycan stem peptide called the acceptor strand (transpeptidation). The DD-endopeptidase activity of PBPs consists of the hydrolysis of the cross-bridge resulting from the DD-transpeptidase activity [31].

The serine of the SXXK motif is central to the catalytic mechanism. The nucleophilic attack of O- of the serineon the carbonyl D-ala amino acid of the stem peptide results the removal of last D-ala and formation of covalent acyl-enzyme complex between the donor stem peptide and the protein. The carbonyl group of the D-ala now forms an ester linkage with the active site serine then undergoes nucleophilic attack from primary amine linked in various ways to the third residue of the second acceptor stem peptide. This second reaction forms the peptide bond between the two stem peptides and regenerates a free active site serine (transpeptidation Fig 1) [31].

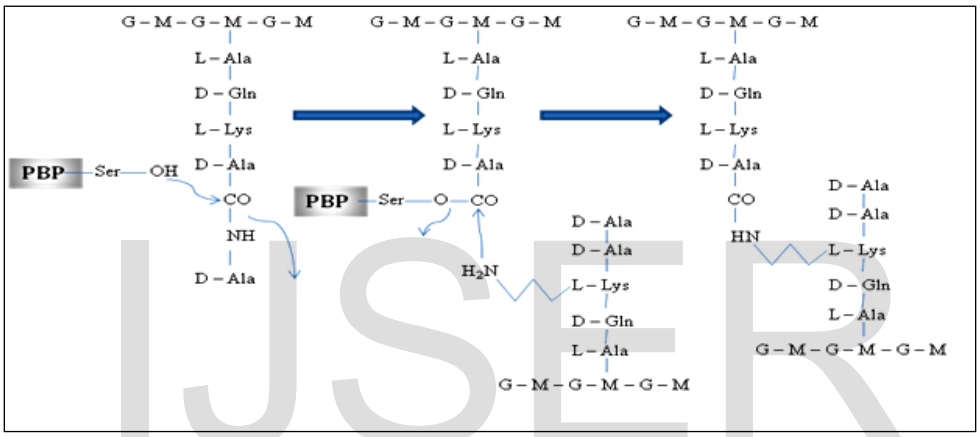


Fig 2 Catalysis by transpeptidation: Fragments of glycan strands are represented by chains of hexagons standing for the hexoses N-acetyl glucosamine (G) and N-acetyl muramic acid (M). The donor pentapeptide is depicted on the upper strand and acceptor in lower.

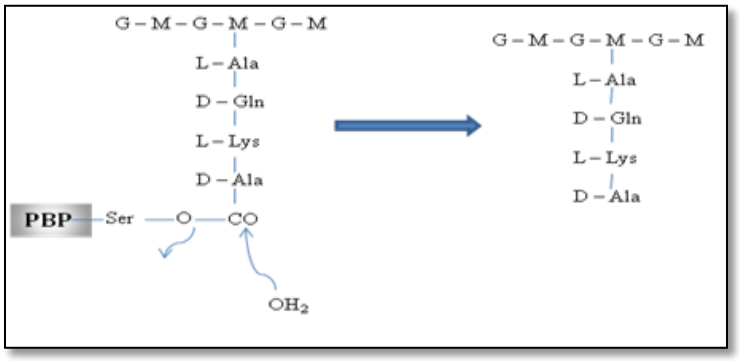


Fig3 Catalysis by DD-carboxypeptidase

In DD-carboxypeptidases, the acyl enzyme intermediate is hydrolysed (Fig 3). The serine in the active site of PBPs attacks the carbonyl of the β-lactam ring. This opens the ring and formsa covalent acyl-enzyme complex. This complex is hydrolysed very slowly, thus effectively preventing the active site serine from engaging in further reactions. The β-lactamases differ in that they react with β-lactams but not D-ala-D-ala dipeptides, and the hydrolysis of this acyl-enzyme complex is extremely fast, thus releasing an active enzyme and inactive compound [31].

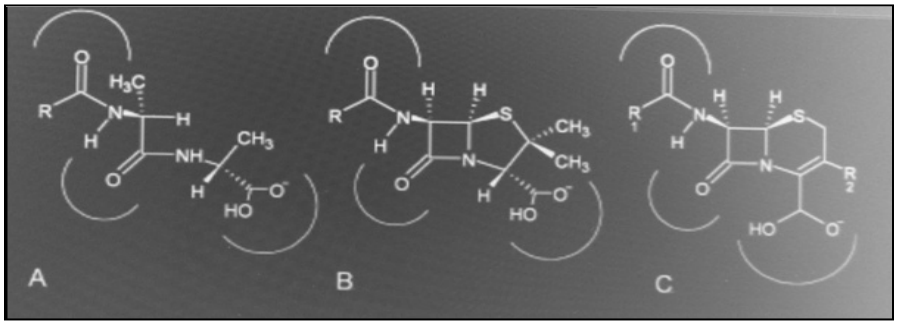


Fig 4 Structural similarity between beta lactam and natural substrate of PBP.(A) N-acetyl D-alanyl D-alanine peptide (B) penicillin backbone (C) cephalosporin backbone.

TABLE 2 FUNCTIONAL MECHANISMS OF DIFFERENT CLASSES OF PBPS

PBP Class	Gene	Catalytic function	Essential role
1	*mrcA, B*	transpeptidases-transglycosylases [31].	Cell elongation, Growth of rod shaped bacteria
2	*pbpA*	Transpeptidases [31],[32].	Cell shape, cell elongation
3	*FtsI*	Divisome protein	Septal peptidoglycan synthesis, cell division
4	*dacB*	Carboxypeptidase, endopeptidase	cleave crossbridges between two glycan strands [31]
5	*dacA*	carboxypeptidase	Cell diameter and morphology [31], [45] Cleaves the terminal D-Ala-D-Ala bond
6	*dacC*	carboxypeptidase	cell separation, peptidoglycan maturation or recycling [31]
7	*pbpG*	endopeptidase	Cleave cross-bridges between two glycan strands [31]
AmpH	*ampH*	β-lactamase [39],[40],[41],[42],[43],[44].	peptidoglycan recycling
AmpC	*ampC*	β-lactamase [39],[40],[41],[42],[43],[44].	Peptidoglycan hydrolase
DacD	*dacD*	carboxypeptidase	Cell separation, peptidoglycan maturation or recycling [31]

4 PBP BASED β-LACTAM RESISTANCE

The β-lactams are potent, broad spectrum antibacterial agents of low toxicity towards eukaryotes. The antibiotic susceptibility towards β-lactam varies among bacterial species. This is due to the combined effect of binding of PBPs to the target, stability to β - lactamase and in gram negative bacteria, the outer membrane permeability[3]. The inhibition of PBPs produces an imbalance in cell wall metabolism resulting in cell lysis or growth inhibition.

4.1 E.coli

To synthesize or modify peptidoglycan cell wall, *Escherichia coli*maintain 12 penicillin binding proteins (PBPs), most of which have no demonstrable physiological purpose [33]. The high-molecular-weight PBPs are bifunctionaltransglycosylase/ transpeptidases or monofunctionaltranspeptidases which synthesize and incorporate individual peptidoglycan strands into the mureinsacculus [34], [35], [36], [37]. These HMW PBPs are three class A PBPs (PBP1a, PBP1b and PBP1c) and two class B PBPs (PBP2 and PBP3). PBP1a and PBP1b are the major transpeptidases-transglycosylases. Deletion of one of them is not lethal for the bacteria (Suzuki *et al*., 1978; Denome*et al*., 1999; Meberg*et al*., 2001) [31]. Two class B PBPs of *Escherichiacoli* are monofunctional transpeptidases. PBP2

isinvolved in the 'elongase', a dynamic protein complexspecific to cell elongation while PBP3 is a major protein ofthe divisome, the cell division complex [31].

The seven LMW PBPs (PBPs 4, 5, 6, and 7, DacD, AmpC, and AmpH) are involved in cell separation, peptidoglycan maturation or recycling [31].These are subdivided into four enzymatic classes: three monofunctional DD-carboxypeptidases, PBP 5, PBP 6, and DacD; one bifunctional DD-carboxypeptidase/DD-endopeptidase, PBP 4; one DD-endopeptidase, PBP 7; and two class C β -lactamases, AmpC and AmpH[39],[40],[41],[42],[43],[44]. It has been reported that PBP 5 helps maintain normal cell wall morphology and diameter in E. coli [45]. PBP4 and PBP7 are two endopeptidases that cleave cross bridgesbetween two glycan strands. PBP5 is the major carboxypeptidase and it only cleaves the terminal D-Ala-D-Ala bond, making the stem peptide unavailable for transpeptidation (Spratt &Strominger, 1976). PBP6 and PBP6b both have sequences homologous to PBP5 and their activity is like PBP5, that of a strict carboxypeptidase. Both PBP4b and AmpH have a sequence close to the paradigmatic *Streptomyces* R61 DD-peptidase (Henderson *et al*., 1997; Vega & Ayala, 2006). The role of AmpH is associated with peptidoglycan recycling [31].

4.2 S.aureus

Staphylococcus aureus is a Gram-positive coccus. It incorporates peptidoglycan at the division site and has no elongase complex (Pinho&Errington, 2003; Zapun*et al*., 2008). *S. aureus* normally produces four PBPs [47] (Table-1). Its unique class A PBP localizes to the septum. Strains of *S. aureus* susceptible to β-lactam antibiotics have two class B PBPs (Pinho*et al*., 2000; Pereira *et al*., 2007), but resistant strains have acquired an additional PBP with a low sensitivity to β-lactams (Zapun*et al*., 2008). The gene *mecA* encodes PBP2a that is responsible for giving methicillin resistance to *S. aureus* [46].

Staphylococcus aureus has only one LMM PBP, which is of type-5, but unlike Escherichia coli PBP5, Staphylococcus aureus PBP4 has a transpeptidase activity necessary to achieve the high degree of cross-linking observed in the peptidoglycan of staphylococci (Wyke*et al*., 1981) [31].

After the spread of *S. aureus* strain that were resistant to penicillin through the acquisition of a β-lactamase, the semi synthetic β-lactam methicillin was introduced which was not degraded by β-lactamases. Soon, a methicillin resistant strain was isolated called MRSA (methicillin resistant *S. aureus*, 1960) and found exhibit resistance to all β-lactam antibiotics along with others. The *S. aureus* has been found to resist β-lactams in three ways- by using β-lactamases to degrade the antibiotic, by lowering the affinity of its endogenous PBPs for β-lactam and most dangerously through the recruitment of an additional PBP that is unaffected by β-lactam [30].

4.3 Enterococci

The intrinsic resistance to β-lactam is a characteristic of *enterococci*. *Enterococcus faecalis* and *Enterococcus faecium* are two species which cause important human health problems, particularly nosocomial infection.

Enterococci morphologically resemble *streptococci*, which may be related to the fact they share the same set of three classes A and two class B HMW PBPs. However the intrinsic resistance property results from the additional sixth HMW PBP5 (Duez*et al*., 2001; Zapun*et al*., 2008) that has transpeptidase activity [31]. It was found that the mutants hypersensitive to penicillin were found to lack PBP5 expression. As in most Gram- positive cocci, *enterococci* have one type-5 LMM PBP (Kharroubi*et al*., 1989). *Enterococcus faecalis* also possesses a type-AmpH PBP, similar to the PBPX of *B. Subtilis* [31].

The wide range of elevated levels of resistance was found to arise from two mechanisms: increased expression of PBP5 and mutation of PBP5 that further decreases affinity for beta lactam. Several point mutations in PBP5 correlated with the low affinity for β-lactam and high resistance. Modification in PBP5 has been shown to be associated with high level ampicillin resistance in *Enterococcus faecium* [48].

In *Enterococcus hirae*, the mutation responsible for the resistance phenotype has been mapped in a genetic element (*psr*) which is located 1 kb upstream of the pbp5 gene and which negatively controls the expression of this gene [49].The acquisition of high-level ampicillin resistance in *Enterococcus faecium* has been found to be associated with the overproduction of a PBP 5 with a reduced β-lactam affinity [50].

4.4 *Streptococcus*

The infection caused by multidrug resistant *Streptococcus pneumoniae* has been increasing worldwide at an alarming rate.Theβ-lactam resistance in *S. pneumoniae* is due to the modified version of their own PBPs that are inefficiently acylated by β-lactams [30].

Streptococcus pneumoniae has three class A PBPs (Hoskins et al., 1999), two class B PBPs and one type-5 class C PBP (Morlot et al., 2005). The five high-molecular-massPBPs (PBP1a [79.7 kDa], PBP1b [89.6 kDa], PBP2x [82.3kDa], PBP2a [80.8 kDa], and PBP2b [82.3 kDa]) and onelow-molecular-mass PBP (PBP3 [45.2 kDa]) [51],[52],[53],[54],[55],[56],[57],[58],[59].The high-molecular-mass PBPs are made up of an N-terminal hydrophobic region, a central penicillin-binding domain, and aC-terminal domain. The active site of transpeptidase activity isformed by three conserved amino acid motifs, SXXK, SXN and KT(S)G. These motifs occur at amino acid positions 370 to373, 428 to 430, and 557 to 559 in PBP1a, at positions 337 to340, 395 to 397, and 547 to 549 in PBP2x, and at positions 385to 388, 442 to 444, and 614 to 616 in PBP2b [58], [59]. The changes in these motifs or in the positions flanking these motifs are associated with low-affinity variants of the PBPs. These changes are the results of point mutations in strains or recombination of PBP genes with PBP genes of other *pneumococci* or of viridians *streptococci* to form mosaic genes. Specifically, decreased affinity of PBP1a, -2x, and-2b for β-lactams has been reported to play an important part in resistance [56],[60],[61],[62],[63],[64]. Alterations in the conserved motifs in PBP2b are associated with resistance to penicillin [55], and alterations in PBP2x mediate low-level resistance tocephalosporins [53], [55], [60], [65]. Additional alterations in PBP1 arise penicillin G MICs to >1 μg/ml and cefotaxime MICs to>0.5 μg/ml [60],[66],[67],[68].

Streptococcus coelicolor has 21 PBPs: three Class A, nine Class B, and nine class C PBPs. The time schedule of their expression is unknown *[31].*

4.5 *Neisseria*

N. meningitidis and *N. Gonorrhoeae*are the pathogens that have acquired reduced susceptibility to penicillin via two routes: the modification of at least one chromosomally encoded PBP or the production of plasmid encoded β-lactamase.

The Gram-negative *Neisseria gonorrhoeae* has only four PBPs. PBP1 is analogous to *Escherichia coli* PBP1a (Ropp et al., 2002) and PBP2 is homologous to *Escherichia coli* PBP3 (Spratt & Cromie, 1988; Dowson et al., 1989; Zhang & Spratt, 1989) [31]. PBP2 is encoded by *penA* genes. The cell wall of strains with altered *penA* alleles has a greater amount of unprocessed pentapeptides, suggesting that the transpeptidase and/or Carboxypeptidase activity of low affinity PBP2 is modified [30]. *Neisseria gonorrhoeae* incorporates new peptidoglycan through its divisome complex (Giesbrecht et al., 1976). The absence of an elongase complex is coherent with its coccoıd shape. *Neisseria gonorrhoeae* PBP3 and PBP4 have sequences similar to *Escherichia coli* PBP4 and PBP7, respectively (Stefanova et al., 2003, 2004) [31].

4.6 Bacillus subtilis

Bacillus subtilis is the model organism for sporulating Gram-positive bacteria. Most of its 16 PBPs have been extensively studied for their role in vegetative peptidoglycan synthesis and in sporulation. *Bacillus subtilis* has four Class-A PBPs. PBP1 is part of the cell division machinery and is required for the efficient formation of the asymmetric sporulation septum (Scheffers & Errington, 2004). Among the six Class B PBPs, PBP2b is the cell division specific class B transpeptidase (Daniel et al., 2000). PBP4a is the equivalent of *Escherichia coli* PBP4 and PBP5 is the major carboxypeptidase. Two other carboxypeptidases similar to PBP5 are present in *B. subtilis*, PBP5 and dacF. PBP5 is required for proper spore cortex synthesis (Popham et al., 1995) and dacF regulates the degree of cross-linking of spore peptidoglycan (Popham et al., 1999). PBP4 and PBPX, two class C PBPs of AmpH-type, are supposed to be involved somehow in sporulation (Scheffers, 2005). Of note is the absence of type-7 PBP in *B. subtilis* [31].

4.7 Others

Mycobacterium tuberculosis produces two class A PBPs, two class B PBPs and a lipoprotein sharing some motifs with the class B PBPs (Goffin&Ghuysen, 2002). Six class C PBPs, one type-4, one type-5, one type-7 and three

putative type- AmpH, complete the set of PBPs of *M. tuberculosis* [31]. The *cyanobacterium anabaena* sp. PCC7120 has 12 PBPs. Six class A PBPs, two class B PBPs, two type-4 PBPs and two type-AmpH PBPs (Lazaro et al., 2001; Leganes et al., 2005). Strikingly, *Anabaena sp*. is devoid of type-5 (and type-7) class C PBP, a property shared by all cyanobacteria (Leganes et al., 2005) [31]. Most resistant clinical isolates of *H. influenza* evade the action of β-lactams by producing a β-lactamase. However the number of beta lactamase negative Ampicillin resistant (BLNAR) strains is rising. BLNAR strains were found to express PBPs with reduced reactivity towards penicillin [30]. *Listeria monocytogenes* has six PBPs (Guinane et al., 2006), including two class A PBPs (PBP1 and PBP4), three class B PBPs (PBP2, PBP3 and lmo0441), and one class C PBP of type-5 (PBP5) (Vicente et al., 1990; Korsak et al., 2005a; Zawadzka-Skomial et al., 2006) [31].

Discussion

The penicillin binding proteins are the enzymes essential for the growth and propagation of bacteria. It is broadly seen that the alterations /mutations in PBPs are one of the reason for the emergence of multidrug resistance in pathogenic bacteria. This is a cause of alarm for dealing with infections. Persistent infections bring higher mortality. Resistance to antibiotics prescribed to tackle bacterial infections and antimicrobials drugs is spreading at an alarming rate. The treatment for many infectious diseases is now reliant on just one or two drugs. Hence, the need to investigate the allelic differentiation and mutations of PBPs becomes central to research. So that novel drugs, herbal drugs with good efficacy combined with lesser or no side effects with goodness of cost effectivity can be used for treatment of difficult to treat MDR bacterial infections.

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