Chapter 1

Introduction

1.1. Introduction

In Gram negative bacteria lipopolysaccharides are the major components of outer membrane, which comprise of a hydrophobic lipid, a moiety attached to the outer membrane, a non-repetitive hetero-oligosaccharide and O-polysaccharide chains. O-polysaccharide chains, known as O-antigens, extend outwards, and are made up of various repetitive subunits of sugars. O-antigens are the basis of serotypic classification due to their structural variabilities (Caroff and Karibian, 2003). *E. coli* 2443 strain expresses O8-antigen (Meier and Mayer, 1985), whereas *E. coli* K-12 strain does not synthesize O-antigen due to its truncated *rfb* locus or the O-antigen gene cluster (Liu and Reeves, 1994). AGTO2-1K, a genetically modified *E. coli* 2443 strain (rfb_{O8} of *E. coli* 2443 is replaced with $rfb_{k\text{-}12}$ of *E. coli* K-12), is unable to synthesize O8-antigen (Ghosh and Young, 2005).

O-antigens, the outer membrane components, are not essential for the cell survival but possess several biological activities, like serving as receptors for bacteriophage attachment. O-antigens are believed to protect the cells from the action of numerous antibiotics by acting in concert with the outer membrane porins (Allison and Verma, 2003; Caroff and Karibian, 2003). Outer membrane permeation is one of the critical factors influencing antibiotic resistance (Nikaido, 2003). This permeability depends on the architecture and distribution of OM proteins (Omps, e.g. porins), as well as composition of its LPS (Nikaido, 2003), viz., O-saccharide chains (Palomar et al., 1995). Therefore, it is interesting to investigate whether O8-antigen has any role in beta lactam resistance.

Beneath the outer membrane a layer of peptidoglycan (PG) is present, which confers high tensile strength to the bacterial cell wall that resists intracellular pressure of several atmospheres and maintains a specific cellular morphology (Holtje, 1998; Popham and Young, 2003; Ghosh et al., 2008). The final stages of PG synthesis and remodeling are catalyzed by a class of enzymes, that are bound to inner membrane and perform transglycosylase, transpeptidase, DD-carboxypeptidase (DD-CPases), and endopeptidase activities (Holtje, 1998). Due to their ability to bind penicillin, these enzymes are collectively known as penicillin-binding proteins (PBPs). There are

12 known PBPs present in *E. coli*. Based on their migrational mobilities through sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), PBPs are broadly classified as high molecular mass (HMM) and low molecular mass (LMM) PBPs (Goffin and Ghuysen, 1998). Out of 12 PBPs, PBPs 1a or 1b, 2 and 3 are HMM PBPs, and are essential for cell survival. Deletion of other seven PBPs mostly the LMM PBPs (PBPs 4, 5, 6, 7, DacD, AmpC, AmpH) does not affect the cell survival and are called non-essential PBPs (Denome, et al., 1999). All PBPs are target of beta-lactam antibiotics. Beta-lactam antibiotics behave as structural analogue of D-ala D-ala, the substrate of PBPs (Tipper and Strominger, 1965). Alteration in PBPs may affect beta-lactam sensitivity of the cell in many ways, one of which is the decreased affinity to beta-lactam antibiotics (Fontana et al., 1994; Ghosh et al., 1998; Sauvage et al., 2002; Lambert, 2005). The deletion of PBPs also causes the alteration of beta-lactam resistance, such as deletion of PBP4 triggers the overproduction of AmpC beta-lactamase in *Pseudomonas aeruginosa* resulting in increased resistance towards beta-lactams (Moya et al., 2009). There are insufficient evidences depicting the consequence of PBPs deletion on beta-lactam antibiotic sensitivity in *E. coli*. Alteration of beta-lactam sensitivity may arise in PBP deleted mutants due to the lack of targets. Therefore, it is important to study the alteration of beta-lactam sensitivity pattern based upon PBP deletion.

PBPs 5, 6 and DacD are grouped under DD-CPases, which cleave the terminal D-ala residue of muramyl pentapeptide at the final stage of PG maturation and these PBPs share highest identities in their amino acid sequences (Amanuma and Strominger, 1988; Baquero et al., 1996). It is observed that the proteins that share substantial identities might possess similar physiological functions. PBP5 contributes in maintaining the morphology of *E. coli* while PBP6 and DacD do not (Nelson and Young, 2000; Nelson and Young, 2001). It has been reported that PBP5 maintains cell shape through its efficient DD-carboxypeptidase activity, which is weaker for PBP6 (Chowdhury et al, 2010). PBP5 and 6 are expressed at different phases of growth, i.e., PBP5 is expressed during the early logarithmic phase, while PBP6 is expressed during the stationary phase and DacD is expressed in the mid logarithmic phase (Santos et al, 2002). Such differences in temporal expression may explain their different roles in exerting physiological functions. In addition, PBP5 (~35%) and 6 (~15%) together

contribute ~50% of the total PBPs in *E. coli* (Dougherty et al., 1996; Ghosh et al., 2008) and this huge amount of seemingly nonessential PBPs might possibly be involved in more than one physiological function.

PBP1a and 1b are the major bifunctional murein synthases in *E. coli* consisting of two catalytic domains harboring transglycosylase (catalyzes the formation of linear glycan chains of alternating N-acetyl glucosamine and N-acetyl muramic acid) and transpeptidase (catalyzes the formation of cross-link between the peptide side chains of adjacent glycan strands) activities (Holtje, 1998). The transpeptidase activity is inhibited by Penicillins and Cephaolosporins (Spratt and Cromie, 1988; Ramachandran et al., 2006). Upon deletion of PBP1a or 1b, *E. coli* cell can survive in normal laboratory condition and hence, are thought to mutually compensate each other, though certain percentage of cells lyse (Denome et al., 1999). However, the overproduction of inactive variants of PBP1b leads to cell lysis (Meisel et al, 2003).

In *E. coli*, both the PBPs 1a and 1b are reported to be the targets of Cefsulodin (Jacoby and Young, 1991). However, it is not specified which one of them is the preferred target for Cefsulodin and the amount of Cefsulodin that could be most effective in inhibiting these PBPs remains unknown. The effectiveness of Cefsulodin combined with beta-lactams in different ratio has also been observed (Kondo and Tsuchiya, 1981). But, the effect of sub-inhibitory level of Cefsulodin on beta-lactam sensitivity in *E. coli* has not yet been reported.

In this study, using genetic manipulations and subsequent assessments of antibiotic sensitivities, the involvement of O8-antigen and the non-essential PBPs in altering beta-lactam sensitivity is demonstrated. In addition, this work proposes a set of drug combinations involving commonly used beta-lactams that can kill the Gram-negative bacterial population effectively.