Abstract

Gram-negative bacteria are covered by three distinct layers, namely, outer membrane or OM (outer most), peptidoglycan layer or PG (middle) and cytoplasmic membrane or CM (inner). After entering through the OM porin channels, beta-lactam antibiotics inhibit the crosslink formation among the PG monomers by acting as the substrate analogues to the enzymes that polymerize peptidoglycan. These enzymes are known as Penicillin-binding proteins (PBPs) that share the namesake by virtue of their binding ability to the beta-lactams. PBPs are classified as high molecular mass (HMM) and low molecular mass (LMM) based on their electrophoretic mobility. Only the HMM PBPs are involved in the PG crosslink formation and generally termed as essential PBPs. Apart from the porins of OM and the essential PBPs present on CM, there are certain auxiliary components of OM and CM that affect the sensitivity to several antibiotics, especially beta-lactams, viz., O-antigens of OM, DD-carboxypeptidases (non-essential PBPs), etc. The role of O-antigens in beta-lactam sensitivity was established by using O8-antigen possessing *Escherichia coli* 2443 strain, it's genetically modified counterpart and the O-antigen deprived CS109 where the presence of O8-antigens apparently sensitized the cells to Penicillins by 2–4 fold. Next, to understand the role of so called DD-carboxypeptidase PBPs (PBP5 and PBP6), the individual deletion mutants were created. Deletion of PBP5 sensitized the *E. coli* cells by 4–8 fold towards a wide range of beta-lactam antibiotics. However, deletion of PBP6, which bears ~50% identity with PBP5, could not sensitize the *E. coli* cells. Ectopic complementation of PBP5 by the cloned gene from a plasmid was able to reverse the lost resistance in PBP5 mutants, indicating the involvement of PBP5 in maintaining an intrinsic beta-lactam resistance in *E. coli* cells. Similarly, the ectopic complementation of PBP5 reversed the lost beta-lactam sensitivity in PBP5 and 6 double deletion mutant whereas the same of PBP6 could not, indicating that PBP5 and PBP6 behave differently in maintaining intrinsic beta-lactam resistance. In another study, PBP1b mutants were found 8–32 folds more sensitive to the Cephalosporins than their respective parents, but PBP1a mutant could not alter the resistance. The ectopic expression of PBP1b restored the lost resistance whereas the same of PBP1a did not. Therefore, PBP1b inactivation coupled with inhibition of other PBPs would be a good choice for designing a combination therapy where a PBP1 specific inhibitor (e.g., Cefsulodin) could serve as a key component. Surprisingly, use of Cefsulodin in sub-inhibitory concentration (4 μg ml^{-1}) in combination with other beta-lactams mimicked the similar effects as observed for PBP1b deletion. The usage of Cefsulodin in sub-inhibitory concentration (4 μg ml^{-1}) combining with other beta-lactams effectively sensitized bacterial species other than the laboratory mutants of *E. coli*. Therefore, through this study it is now evident that the auxiliary membrane components are apparently involved in maintaining intrinsic beta-lactam resistance and inactivating them through combination therapy might increase the effectiveness of the commonly use beta-lactam agents.

Keywords:
Escherichia coli, Beta-lactam sensitivity, O-antigen, Penicillin-binding protein, Cefsulodin