

Biochemical Characterization of CTX-M-15 from *Enterobacter cloacae* and Designing a Novel Non- β -Lactam- β -Lactamase Inhibitor

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Abstract : The worldwide dissemination of CTX-M type β -lactamases is a threat to human health. Previously, we have reported the spread of blaCTX-M-15 gene in different clinical strains of Enterobacteriaceae from the hospital settings of Aligarh in north India. In view of the varying resistance pattern against cephalosporins and other β -lactam antibiotics, we intended to understand the correlation between MICs and catalytic activity of CTX-M-15. In this study, steady-state kinetic parameters and MICs were determined on *E. coli* DH5 α transformed with blaCTX-M-15 gene that was cloned from *Enterobacter cloacae* (EC-15) strain of clinical background. The effect of conventional β -lactamase inhibitors (clavulanic acid, sulbactam and tazobactam) on CTX-M-15 was also studied. We have found that tazobactam is the best among these inhibitors against CTX-M-15. The inhibition characteristic of tazobactam is defined by its very low IC₅₀ value (6 nM), high affinity ($K_i = 0.017 \mu\text{M}$) and better acylation efficiency ($k_{+2}/K_9 = 0.44 \mu\text{M}^{-1}\text{s}^{-1}$). It forms an acyl-enzyme covalent complex, which is quite stable ($k_{+3} = 0.0057 \text{s}^{-1}$). Since increasing resistance has been reported against conventional β -lactam antibiotic-inhibitor combinations, we aspire to design a non- β -lactam core containing β -lactamase inhibitor. For this, we screened ZINC database and performed molecular docking to identify a potential non- β -lactam based inhibitor (ZINC03787097). The MICs of cephalosporin antibiotics in combination with this inhibitor gave promising results. Steady-state kinetics and molecular docking studies showed that ZINC03787097 is a reversible inhibitor which binds non-covalently to the active site of the enzyme through hydrogen bonds and hydrophobic interactions. Though, its IC₅₀ (180 nM) is much higher than tazobactam, it has good affinity for CTX-M-15 ($K_i = 0.388 \mu\text{M}$). This study concludes that ZINC03787097 compound can be used as seed molecule to design more efficient non- β -lactam containing β -lactamase inhibitor that could evade pre-existing bacterial resistance mechanisms.

Keywords : ESBL, non- β -lactam- β -lactamase inhibitor, bioinformatics, biomedicine

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