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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 DH5α 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 (Ki = 0.017 µM) and better acylation efficiency (k+2/K9 = 0.44 µM-1s-1). It forms an acyl-enzyme covalent complex, which is quite stable (k+3 = 0.0057 s-1). Since increasing resistance has been reported against conventional b-lactam antibiotic-inhibitor combinations, we aspire to design a non-b-lactam core containing b-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, it's IC50 (180 nM) is much higher than tazobactam, it has good affinity for CTX-M-15 (Ki = 0.388 µM). This study concludes that ZINC03787097 compound can be used as seed molecule to design more efficient non-b-lactam containing b-lactamase inhibitor that could evade pre-existing bacterial resistance mechanisms.

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