

Thesis Title: *Polyphasic approach to understand the distribution and dynamics of TEM*

β-Lactamase in environmental bacterial populations

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The escalating threat of antimicrobial resistance (AMR), particularly from β-lactam resistant bacteria (BLRB), poses serious public health and environmental challenges, especially in high-density regions like Delhi-NCR. This doctoral research presents a comprehensive, three-phase investigation into the environmental dissemination, molecular diversity, and structural dynamics of TEM-type β-lactamase variants, a key contributor to BLRB-mediated resistance. In the first phase, a computational and data-driven analysis of 474 reported TEM variants was conducted using molecular docking to evaluate their binding interactions with various β-lactam antibiotics and β-lactamase inhibitors. The study revealed that most variants exhibited decreased affinity for penicillin and cephalosporins, while tazobactam showed relatively strong inhibitory potential. Further, by analysing the differential binding energy (ΔBE) of selected TEM mutants against various β-lactam antibiotics and β-lactamase inhibitors, the combination of Aztreonam and Relebactam was identified as the most effective therapeutic strategy, with potential to treat resistant bacterial infections and prevent future AMR outbreaks. In the second phase, environmental surveillance was conducted across 75 diverse sites in Delhi-NCR, including ponds, lakes, Yamuna River, agricultural soil, aquatic weeds, drains, dumping yards, sewage treatment plants (STPs), cow dungs, cow urine and biogas slurry from various gaushalas (Cow shelters), resulting in the isolation of 130 BLRB through culture-based methods. Phenotypic assays and genotypic screening revealed a high prevalence of resistance determinants such as blaTEM, blaCTX, blaSHV, blaOXA, and integrons (int-1 and int-3), with several isolates co-expressing Extended Spectrum β-Lactamases (ESBLs) and Metallo-β-Lactamases (MBLs), and exhibiting Multiple Antibiotic Resistance (MAR) indices ≥ 0.2 . In the third phase, six genetically confirmed blaTEM-positive isolates with elevated resistance profiles were selected for plasmid-based cloning and sequencing. A novel TEM variant, designated TEM(N), was identified in *Escherichia ruysiae* isolated from Okhla Sewage Treatment Plant. Structural modelling and molecular dynamics simulations revealed multiple amino acid substitutions that significantly enhanced resistance to β-lactam antibiotics and inhibitor molecules such as tazobactam, avibactam, and relebactam. Collectively, this study highlights the critical role of environmental reservoirs in harbouring and evolving β-lactamase-

mediated resistance and underscores the need for integrative AMR surveillance and molecular monitoring under the One Health framework to inform effective environmental and public health interventions.