Epidemiological Assessment, Molecular Characterization And *in-silico* Analysis Of Colistin Resistance Genes In Clinical Isolates Of Pseudomonas Aeruginosa From Khyber Teaching Hospital, District Peshawar

Abstract

Pseudomonas aeruginosa is opportunistic pathogen responsible for a variety of hospital-acquired infections, particularly in immunocompromised and critically ill patients. The current study aimed to screen, identify, and assess the epidemiological distribution and antimicrobial susceptibility of P. aeruginosa isolates from 3,189 clinical specimens collected over a six-month period. Using selective media such as Blood Agar, MacConkey Agar, and Cetrimide Agar, a total of 384 isolates were confirmed as *P. aeruginosa* through biochemical tests and molecular identification via PCR amplification of the *OrpL* gene. The highest prevalence was found from male patients (62.5%) and predominantly from the 41–60 years age group, with the highest isolation rates found in wound and pus samples (38%), followed by urine samples (27%). Antimicrobial susceptibility testing revealed a broad spectrum of resistance patterns across the isolates. Carbapenems and polymyxins exhibited the highest efficacy, with imipenem and meropenem showing 94.8% and 91.6% sensitivity, respectively. *β*-lactam antibiotics, such as piperacillin–tazobactam (96.4% resistance) and cefoperazone–sulbactam (83.1% resistance), displayed the highest resistance rates. Colistin and polymyxin B remained highly effective, with 99% sensitivity among all isolates. This highlights the continued relevance of colistin as a last-resort antibiotic in treating P. aeruginosa infections. The age-wise distribution of the isolates revealed which showed higher rates of infection in middle-aged and elderly patients. The pathogen was most isolated from hospitalized patients (74.5%), particularly in surgical wards, ICUs, and burn units, supporting its role in nosocomial infections. The Minimum Inhibitory Concentration (MIC) for colistin and polymyxin B was low, indicating high susceptibility, with MIC_{50} and MIC_{90} values of 1 µg/mL for both polymyxins. In contrast, carbapenems exhibited a broader range of MICs, with imipenem and meropenem showing moderate resistance rates. The prevalence of antibiotic-resistant genes was examined, identifying genes such as PhoQ(36%), PhoQ (44%), PmrA (18%), and mcr-1 (8%), with a higher occurrence of PmrB (54%) and oprD (87.5%) genes. These findings highlight the complex genetic mechanisms underlying P. aeruginosa resistance to polymyxins and carbapenems. Further, the study correlated the presence of these resistance genes with phenotypic resistance in urinary, sputum, blood, and wound isolates, showing broad-spectrum resistance in specific clinical specimens. Mutational analysis revealed significant nucleotide substitutions in the PmrA, PmrB, PhoQ, and PhoQ genes, with *mcr*-1 also exhibiting mutations that could potentially impair colistin resistance. Additionally, protein structure analysis of the mutated PhoPQ, PmrB, OprD, and MCR-1 proteins through docking studies confirmed strong binding affinities of various ligands, suggesting possible therapeutic targets for overcoming resistance. This study investigates the molecular docking interactions of the ANP ligand with PhoQ, PhoQ, and PMR B receptors in *P. aeruginosa*, focusing on colistin resistance mechanisms. The ANP ligand was analyzed for its physicochemical properties, pharmacokinetic parameters, and drug-likeness. Molecular exhibited favorable characteristics for bioavailability, with a molecular weight of 572.36 g/mol, moderate flexibility, and an acceptable hydrophilicity-lipophilicity balance. Despite some violations of Lipinski's and Veber's rules, the ligand showed a bioavailability score of 0.52, suggesting potential for drug development. Pharmacokinetic predictions indicated moderate gastrointestinal absorption, no blood-brain barrier penetration, and minimal drug-drug interaction risks. Molecular docking studies revealed that the ANP ligand strongly interacts with PhoQ and PhoQ, two key regulatory

proteins involved in colistin resistance. The docking results with PhoQ highlighted stable hydrogen bond interactions, particularly at amino acid residues ASN 119, GLN 121, and ARG 118, suggesting that ANP may inhibit the transcriptional activity of PhoQ, disrupting resistance pathways. Similarly, interactions with PhoQ receptor residues such as ILE 428, ASN 389, and TYR 393 demonstrated stable binding, supporting the potential of ANP in targeting PhoQ in colistin-resistant strains. Further, docking of the ANP ligand with the mutant PhoQ receptor showed a stronger binding profile, with interactions at GLU 271 and GLN 277, indicating more robust inhibitory potential in the mutant form. The PMR B receptor docking also revealed significant hydrogen bond interactions with ASP 37, indicating a strong affinity. In contrast, the mutant PMR B receptor exhibited an even more complex interaction profile, with additional hydrogen bond formations and pi-pi stacking interactions, suggesting a higher binding affinity for the ligand. Molecular docking studies were conducted to explore the interactions of ligands with membrane proteins OprD, MCR1, and their mutant forms, focusing on their potential to disrupt antimicrobial resistance mechanisms in Pseudomonas aeruginosa. The interaction of Hydroxy-ethyloxy-tri (ethyloxy)octane (C8E) with OprD revealed stable hydrogen bonding with ASP 295 and ARG 410, contributing to a strong binding affinity. The molecular docking results indicated favorable binding poses for C8E, with low RMSD values and high interaction energy, suggesting its potential as a therapeutic candidate targeting OprD. Similarly, interactions between the UE1 ligand and mutant OprD showed significant hydrogen bonds with key residues, such as ASP 268, ARG 104, and GLY 101, enhancing stability and affinity. Furthermore, the UE1 ligand demonstrated notable hydrogen bonding and pi-H stacking interactions with MCR1 proteins, indicating its strong binding potential. The mutant *mcr*1 receptor exhibited stronger and more diverse interactions with UE1 compared to the wild-type, with key residues such as ASN 16 and THR 406 contributing to enhanced stability. The RMSD and E_Place values for the mutant receptor revealed a more stable complex, supporting the therapeutic potential of UE1 in targeting MCR1 mutations.