Phenotypic assessment of multiple antibiotic resistance among polymyxin B-resistant Klebsiella spp. from hospital wastewater

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Antimicrobial resistance is among the top ten global health threats, with bacterial resistance representing a challenge of the 21st century. Hospital wastewater has gained attention as a potential reservoir of multidrug-resistant (MDR) bacteria. Polymyxin B (PB), a last-resort antimicrobial, is widely used to treat severe infections caused by carbapenem-resistant Gram-negative bacteria, including *Klebsiella pneumoniae*. This study aimed to evaluate the resistance profile of PB-resistant *Klebsiella* spp. isolated from hospital wastewater. Sewage samples from a tertiary hospital were cultured on MacConkey agar supplemented with PB (2 µg/mL) for selective isolation. A total of 25 *Klebsiella* spp. isolates were identified phenotypically. PB resistance was confirmed by minimum inhibitory concentration (MIC) using the broth microdilution method. Antimicrobial susceptibility testing was performed by disk diffusion against 17 clinically relevant antibiotics, with interpretation based on BrCAST criteria. As expected, all isolates were resistant to PB, with MIC values ranging from 4 to >64 µg/mL. Four isolates exhibited resistance to all tested antibiotics. High resistance rates were observed for amoxicillin-clavulanate (92%) and ampicillin-sulbactam (88%). Intermediate-to-high resistance frequencies were detected for piperacillin-tazobactam, cefepime, and aztreonam (64%); ceftazidime, cefotaxime, and tobramycin (72%); and ertapenem and norfloxacin (68%). Lower resistance rates included ciprofloxacin and gentamicin (60%), levofloxacin (56%), amikacin, trimethoprim-sulfamethoxazole, and chloramphenicol (52%), imipenem (48%), and meropenem (32%). The mean multiple antibiotic resistance (MAR) index was 0.63 (SD ± 0.36), indicating a high MDR profile. Data dispersion of MAR index was substantial (Q1 = 0.28; Q3 = 0.94; IQR = 0.67), reflecting marked heterogeneity among isolates, with coexistence of low- and extremely high-resistance phenotypes. These findings reinforce hospital wastewater as a critical reservoir of MDR bacteria and highlight the environmental dissemination of clinically relevant resistance. The detection of PB-resistant *Klebsiella* spp. with elevated MAR indices supports the One Health perspective, emphasizing the interconnectedness of human and environmental health. Integrating microbiological surveillance of hospital effluents into epidemiological monitoring is essential to address antimicrobial resistance through coordinated, intersectoral strategies.

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