

Colistin-resistant *Klebsiella pneumoniae* in hospital and urban effluents within a "One Health" context

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Wastewater and environmental surveillance have emerged as effective strategies for monitoring the dissemination of antimicrobial resistance, as these environments act as reservoirs and convergence points for clinically relevant bacteria. In this context, *Klebsiella pneumoniae* stands out, as it is classified as a critical priority pathogen, particularly when resistant to carbapenems and third-generation cephalosporins. This scenario becomes even more concerning when associated with resistance to colistin, a last-resort antimicrobial in clinical practice. Thus, the environmental circulation of these microorganisms represents a significant public health risk. This study aimed to investigate the presence of colistin-resistant *K. pneumoniae* strains in hospital and urban effluents. A total of 500 mL samples of hospital (n = 14) and urban (n = 5) effluents were collected biweekly between August and November 2024, covering the five Planning Areas of the city. Samples were concentrated and enriched in BHI broth supplemented with ceftazidime, meropenem, and/or colistin, followed by plating on selective media. Isolates were identified by MALDI-TOF. *K. pneumoniae* isolates were subjected to phenotypic screening by broth microdilution in Mueller-Hinton medium, using BrCAST breakpoints for colistin. Isolates colistin-resistant were screened for the presence of *mcr* genes (*mcr*-1 to *mcr*-12) by multiplex PCR. The *mcr*-positive strains were further investigated for genes associated with carbapenemase and β -lactamase production by qPCR-HRM. A total of 46 *K. pneumoniae* isolates were recovered, of which 39% (18/46) were from urban effluents and 61% (28/46) were from hospital effluents, distributed across four of the five Planning Areas of the municipality: PA1 (n = 5), PA2 (n = 3), PA3 (n = 26), and PA4 (n = 12). Of these, 43,5% (20/46) strains exhibited resistance to colistin, including 25% (5/20) from urban effluents and 75% (15/20) from hospital effluents. Within the resistant isolates, only 10% (2/20) the strains harbored colistin resistance genes, both identified as *mcr*-10, and both recovered from hospital effluents. The qPCR-HRM analysis revealed that these isolates also carried *bla*_{TEM} and *bla*_{KPC}, suggesting the potential co-occurrence of resistance to carbapenems, cephalosporins, and colistin. These findings demonstrate the presence of *K. pneumoniae* harboring multiple resistance determinants in hospital effluents, including genes associated with carbapenemases, β -lactamases, and colistin resistance. As a next step, these isolates will undergo whole-genome sequencing to further characterize resistance mechanisms and investigate the presence of clinically relevant clones. From a One Health perspective, these results reinforce the importance of environmental surveillance as a complementary tool for monitoring antimicrobial resistance and mitigating the spread of clinically important microorganisms.



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