Molecular epidemiology and multiresistance to heavy metals and antimicrobials in environmental Klebsiella variicola lineages

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Klebsiella variicola belongs to the *Klebsiella pneumoniae* species complex and has emerged as a relevant agent in human health, particularly for its association with severe infections that, in the past, were frequently misidentified as *K. pneumoniae*. This species is currently recognized as an opportunistic pathogen with a broad capacity for colonization and infection in various hosts, including humans, animals, insects, and diverse environments, affecting all pillars of One Health. They exhibit a remarkable ability to acquire resistance genes to critical antimicrobials, such as carbapenems, acting as an important reservoir of antimicrobial resistance (AMR). In this context, the recent launch of the National Action Plan for the Prevention and Control of Antimicrobial Resistance (PAN-BR 2026–2030) is noteworthy, proposing to address AMR from a One Health perspective. The present study aimed to evaluate the antimicrobial and heavy metal resistance profiles of four environmental *K. variicola* strains, as well as to investigate the clonal and virulence profiles of these isolates. The strains were isolated from alluvial sediments of a river near an iron mining site in Brazil. The isolates were identified by MALDI-TOF MS and confirmed by bla_{LEN} gene amplification. The antimicrobial susceptibility profile was determined by disk-diffusion on Mueller-Hinton agar, according to the Brazilian Committee on Antimicrobial Susceptibility Testing (BrCAST) guidelines, using 35 antimicrobials. Heavy metal resistance was evaluated by serial microdilution to determine the minimum inhibitory concentration (MIC), testing 10 metals (As, Cd, Pb, Co, Cu, Cr, Fe, Mn, Ni, and Zn), at concentrations between 5.86 and 50,000 ppm. Eleven virulence genes were investigated by PCR (*entB*, *mrkD*, *ycfM*, *kfu*, *ybtS*, *allS*, *rmpA*, *iutA*, *fimH*, K1 and K2). Molecular typing was evaluated by multilocus sequence typing (MLST) (https://mlstkv.insp.mx), analyzing the *leuS*, *pgi*, *pgk*, *phoE*, *pyrG*, *rpoB*, and *fusA* genes. The population structure was analyzed using the goeBURST algorithm, and the phylogeny was reconstructed by Maximum Likelihood with 1,000 bootstrap replications. The results demonstrated that all strains are multiresistant to antimicrobials and tolerant to high metal concentrations, with an MIC of 6,250 ppm for Pb and 2,500 ppm for Mn. All strains carried the virulence genes *entB*, *mrkD*, *ycfM*, *kfu*, and *fimH*. MLST analysis revealed high genetic diversity, identifying STs 371, 187, 772, and 773, with the latter two being novel. Through goeBURST analysis, it was observed that the four isolates showed connectivity with major global clonal complexes. Phylogenetic analysis demonstrated that the isolates cluster into polyphyletic clades containing strains of clinical, animal, and environmental origin. The results highlight environmental *K. variicola* strains with high genetic diversity, multiresistance, and pathogenic potential, demonstrating a role as a reservoir of antimicrobial resistance in different ecological niches. These results emphasize the importance of epidemiological surveillance of environmental isolates and the incorporation of the One Health perspective in addressing antimicrobial

resistance.

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