TITLE: Virulence potential of multidrug-resistant *Klebsiella pneumoniae* ST307 isolates related to gastrointestinal colonization and bloodstream infection in a leukemia patient.

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ABSTRACT:

Klebsiella pneumoniae is an opportunistic pathogen that can colonize the gastrointestinal tract (GIT) mucosa of humans. Its success in crossing this epithelium and causing extra-intestinal infections seems to be related to virulence and resistance to antimicrobials. High-risk multidrug-resistant *K. pneumoniae* strains, such as those belonging to ST307, often reach the hospital environment causing high mortality infections. We investigated *K. pneumoniae* strains isolated from GIT colonization (strains FZcol-1, FZcol-2 and FZcro-1) and a fatal bloodstream infection (strain HM-1) in a leukemia patient.
Three different genotypes were observed in colonization, being FZcro-1 indistinguishable from infection strain HM-1 genotype. All strains belong to ST307, carry a transferable IncF plasmid containing the $bla_{\text{CTX-M-15}}$ gene, have the same resistome and virulome, and are phenotypically multidrug-resistant. The transfer of this plasmid to the *E. coli* MG1655^{gfp} receptor cell resulted in phenotypic resistance to ceftriaxone, cefepime and gentamicin by the transconjugant bacteria. When comparing the nucleotide sequence of the multidrug-resistant plasmid characteristic of *K. pneumoniae* ST307 with the genome of the strains, we observed partial sequences for FZcol-2 in the region of putative virulence genes. We also generated a phylogenetic tree with the genomes, identifying the same ancestor for all strains; FZcol-1 composing a different branch than the others; and FZcro-1 and HM-1 also occupying different locations. *Galleria mellonella* model of infection revealed distinct virulence potentials, with FZcol-2, FZcro-1 and HM-1 being more virulent than FZcol-1. The association and internalization in eukaryotic cells of Caco-2 intestinal lineage revealed different dynamics, where FZcro-1 seems to be more efficient in this mechanism, followed by HM-1. The growth kinetics of the strains were different in one of the culture media tested, where FZcol-2 showed greater growth rate.
Therefore, we demonstrate how a colonizing strain (FZcro-1) and the infection strain (HM-1) have indistinguishable genotypes, subtle differences in the virulence phenotype, and occupy distinct places in the phylogenetic tree. By identifying different genotypes and phenotypes of the same *K. pneumoniae* sequential type coexisting in the GIT of humans and capable of developing extra-intestinal infections, we indicate the urgency of studies investigating this dynamic to avoid unfavorable clinical outcomes.

Keywords: Colonization, CTX-M-15, infection, *Klebsiella pneumoniae*, ST307.

Development Agencies: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.