TITLE: ANTIMICROBIAL RESISTANCE AND VIRULENCE OF GRAM-NEGATIVE ESKAPE PATHOGENS ISOLATED IN BRAZIL

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ABSTRACT:

Gram-negative ESKAPE pathogens (*Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp) are bacteria of increasing concern due to their ability to cause nosocomial infections and its high acquisition rates of antimicrobial resistance genes. This study investigated the antibiotic susceptibility and the *in vivo* virulence of *K. pneumonia* P1298, *A. baumannii* P3380, *P. aeruginosa* P2307, and *E. cloacae* P2224. All the strains belong to the Collection of Reference Bacteria on Health Surveillance (CBRVS) and were kindly provided by the Fundação Oswaldo Cruz (FIOCRUZ). We tested strain susceptibility to 12 antibiotics as proposed by the Clinical & Laboratory Standards Institute (2018) and we employed the *Galleria mellonella* larvae model to evaluate strain virulence. *K. pneumonia* P1298 was resistant to amoxicillin-clavulanate and ampicillin (penicillins). *A. baumannii* P3380 and *P. aeruginosa* P2307 were susceptible to all of the tested antibiotics. *E. cloacae* P2224 was resistant to cefazolin (1st generation cephalosporins), ceftriaxone (3rd generation cephalosporins), amoxicillin-clavulanate and ampicillin (penicillins), and tetracycline (tetracyclines). For virulence assessment, the strains were cultivated in LB agar plates at 37 °C and grown in LB broth to an optical density corresponding to ~ 10^8 CFU/ml. Then, 5 ml of the bacterial suspension was centrifuged at 5,000 rpm for 5 min at 4 °C and re-suspended in 0.5 ml of PBS buffer (~ 10^9 CFU/ml) and serially diluted to inoculate *G. mellonella* larvae at final concentrations of 10^7 to 10^0 CFU/larvae. The larvae were incubated at 37°C and monitored for 96 h. *K. pneumoniae* concentrations superior to 10^5 CFU/larvae resulted in 100% mortality in 24 h. LT_{50}-time taken to kill 50% of the larvae for 10^4 CFU was 48 h. Similarly, *A. baumannii* concentrations superior to 10^6 CFU/larvae resulted in 100% mortality in 24 h, and LT_{50}-time taken to kill 50% of the larvae for 10^4 CFU was 24 h. In contrast, 10^1 CFU/larvae of *P. aeruginosa* resulted in 100% mortality in 24 h and 10^0 CFU/larvae resulted in 10% mortality after 96h. For *E. cloacae*, 10^7 CFU/larvae resulted in 100% mortality after 24h, and LT_{50}-time taken to kill 50% of the larvae for 10^5 CFU was 24 h. These results are in accordance with previous findings of virulent Gram-negative ESKAPE strains and highlight the *G. mellonella* killing assay as a simple method to assess bacterial virulence.

Keywords: surveillance, ESKAPE pathogen, multidrug-resistant, antimicrobial

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