TITLE: CARBAPENEM RESISTANT *Enterobacter cloacae* COMPLEX CLINICAL ISOLATES: DIVERSITY OF CLONES, VIRULENCE AND RESISTANCE GENES, AND bla_{KPC-2}-HARBORING PLASMIDS.

AUTHORS: ANDRADE, L.K.; SILVA, C.R.; BARROSO, M.V.; BENFATI, L.R. CASELLA, T.; NOGUEIRA, M.C.L.

INSTITUTION: FACULDADE DE MEDICINA DE SÃO JOSÉ DO RIO PRETO – FAMERP, SÃO JOSÉ DO RIO PRETO, SP (AVENIDA BRIGADEIRO FARIA LIMA, 5416, CEP 15090-000, SÃO JOSÉ DO RIO PRETO – SP, BRAZIL)

ABSTRACT:

The emergence of the *Enterobacter cloacae* complex (ECC) as important nosocomial pathogens is a global challenge to the treatment of serious infections in hospitalized patients. These bacteria can easily become resistant to a diversity of antimicrobial drugs by the acquisition of plasmids carrying resistance genes. Despite the worldwide emergence of KPC-2 ECC, associated to high mortality, information about plasmids and clones carrying bla_{KPC-2}, and virulence, is scarce. Hereby, we present the characterization of seven KPC-2-producing ECC from various clinical samples of patients admitted to a Brazilian tertiary hospital, from April, 2017 to March, 2019. Isolates were whole genome sequenced. The PBRT 2.0 kit (DIATHEVA) was used to identify plasmids carrying $bla_{KPC-2.}$ Sizes were estimated by the S1-PFGE and southern blot-hybridization. Biofilm formation and cytotoxicity on Vero cells were tested. *In silico* analysis identified six isolates as *E. hormaechei* (ST114 - 3 isolates; ST184, ST269 and ST1261 - 1 isolate each), and one as *E. cloacae* (ST84). The $bla_{CTX-M-15}$, bla_{OXA-1}, $bla_{CMH-3,}$ bla_{ACT-7} and bla_{ACT-16} genes were the main determinants of resistance to third-generation cephalosporins; and *aac(6')-Ib-cr*, *qnrB1*, and *oqxAB* to quinolones. A diversity of aminoglycoside modifying enzymes were associated to resistance to amikacin and gentamicin, while no isolates presented 16S-methyltransferases. No isolates presented resistance to polymixins. The bla_{KPC-2}-plasmids were IncM-70 kb (ECC35), IncN2-48.5 kb (ECC36), IncM-60 kb (ECC71), IncN2-40 kb (ECC75), IncQ1-15 kb and IncFII-100 Kb (ECC69), IncQ1-15 kb and IncFII-180 kb (ECC86), IncHI2-210 kb (ECC90). The presence of two bla_{KPC-2-}plasmids in different isolates was remarkable finding. All isolates presented virulence genes associated to adhesion, iron uptake, production of capsule, and secretion systems. Noteworthy, genes codifying for type 6 secretion systems (T6SS), enterotoxins, urease, and superoxide-dismutase were also detected. All isolates showed moderate or strong biofilm production, and ECC36, ECC86 and ECC90 were cytotoxic. Different plasmids were responsible for bla_{KPC-2} spreading among multidrug-resistant ECC, and pathogenicity may result of various virulence determinants. Further studies, including more isolates and deeper analysis of genotypic and phenotypic features are necessary to expand knowledge about this intriguing group of bacteria.

Keywords: virulence, plasmid, antibiotic resistance, *Enterobacter cloacae*

Development Agency: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES