TITLE: *K. PNEUMONIAE* ST11 CLONE HARBORING *BLA*$_{KPC-2}$ IN INCN CONJUGATIVE PLASMID AND *BLA*$_{NDM-1}$ IN INCC PLASMID CAUSED AN OUTBREAK IN A TEACHING HOSPITAL

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ABSTRACT:
Resistance mechanisms are emerging and spreading globally, threatening the ability to treat common infectious diseases. Carbapenems are a powerful group of broad-spectrum antibiotics used as a last resort against multidrug-resistant bacterial infections. The most common resistance mechanism to these antibiotics is the production of carbapenemases, which are enzymes with the highest spectrum/potential among β-lactamases capable of hydrolyzing practically all β-lactams. In this study, we characterized four isolates belonging to an outbreak of a multi-drug resistant (MDR) *Klebsiella pneumoniae*. The isolates were typed by DNA macro-restriction followed by pulsed-field gel electrophoresis revealing the existence of one pulsotype, with BHKPC93 having one band difference, considered a closely related subtype. BHKPC93 and BHKPC104 were isolated from central catheter tip and urine, respectively, and were representatives of each subtype for the whole genome sequence by Illumina and Nanopore technologies. The two isolates harbored 20 resistance genes, including the *bla*$_{KPC-2}$ and *bla*$_{NDM-1}$ genes. *bla*$_{KPC-2}$ gene was in the Tn*4401* in a ~56 Kbp conjugative IncN plasmid, and *bla*$_{NDM-1}$ gene was in a ~102 Kbp IncC plasmid along with other five resistance genes. Although the *bla*$_{NDM-1}$ plasmid has no relaxase, it has not been clarified whether it is mobilizable or not. *In vitro* conjugation assays to the *E. coli* J53 receptors resulted in the *bla*$_{KPC}$ transference but not *bla*$_{NDM-1}$ with a conjugation rate of approximately 2 x 10^{-5} transconjugants/recipient with no change in the recipient strain fitness cost. The minimum inhibitory concentration (MIC) of meropenem/imipenem against BHKPC93 and BHKPC104 was 128/64 and 256/128 mg/L, respectively. Although the MICs of meropenem and imipenem against *E.* coli J53 transconjugants carrying the *bla*$_{KPC}$ gene were 2 mg/L, considered susceptible by BrCAST, there was a substantial MIC increment from 0.06 and 0.125 mg/L, respectively. In conclusion, we characterized the MDR ST11 *K. pneumoniae* clone carrying *bla*$_{KPC-2}$ and *bla*$_{NDM-1}$ genes and demonstrated that only conjugation of *bla*$_{KPC}$ occurred *in vitro* to *E. coli*, keeping its susceptible status, which could contribute to the "silent" dissemination of antimicrobial resistance genes.

Keywords: Carbapenemases; *bla*$_{KPC}$; *bla*$_{NDM}$; *Klebsiella pneumoniae* ST11; Conjugation

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