Microbiologia Clínica e Infecção Hospitalar – Resistência e mecanismos de resistência a antimicrobianos (fenotípica e molecular)

TITLE: COMPARISON OF DATABASES FOR ANTIMICROBIAL RESISTANCE GENES PREDICTION IN *Klebsiella pneumoniae* CLINICAL ISOLATES

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ABSTRACT:
Emerging technologies for rapid identification of resistance determinants, such as whole genome sequencing, propose a shift from traditional antimicrobial susceptibility testing toward analysis of genetic elements. Here we report a comparison of available databases for antimicrobial resistance genes prediction in *K. pneumoniae* (Kp) isolated from a tertiary-care teaching hospital, located in Curitiba, Paraná, Brazil. We selected six multidrug resistant *K. pneumoniae* isolates to genome sequencing. AST was performed for 15 antimicrobial agents by agar dilution. PCR was used to detect β-lactam resistance genes. PFGE was carried out to discriminate strains. Kp isolates were sequenced on a Illumina HiSeq 2000 platform using the RAPID method. Paired-end (PE) reads assembly were performed using Velvet. Chromosomal and plasmid contigs were manually inspected and separated based on BLASTn results. Chromosomal contigs were scaffolded using SSPACE. Plasmid Inc groups prediction was performed using PlasmidFinder. *In silico* multilocus sequence typing was defined by MLST. Antibiotic resistance genes were predicted by four published databases: ARDB, CARD, ResFinder and SRST2, also one unpublished database: GeneFinder. Both GeneFinder and SRST2 use a mapping approach from PE reads to detect resistance genes. GeneFinder looks also for mutations in selected chromosomal targets that confer resistance to fluoroquinolone, rifampicin and colistin, further indicates of potential inactivation of genes by insertion sequences. Our isolates belong to CC11 (ST11 and ST437). We found a wide resistance genes repertoire. Resistance genes *bla*$_{KPC-2}$, *bla*$_{CTX-M2}$, *bla*$_{CTX-M15}$, *bla*$_{TEM-1}$, *bla*$_{OXA-1}$, *bla*$_{OXA-2}$, aac(3')-IId, aac(6')-Ib-cr, aph(3')-Ia, ant(2'')-Ia, ant(4')-Ia, aadA and *qnrB1* were variedly distributed among the isolates and presumably contributed to the diverse MDR profiles. It was noted a divergence in identification of aminoglycoside modifying enzymes and β-lactamase variants, when comparing the databases. The *bla*$_{KPC-2}$ genes were found to be located within Tn4401b and to be associated with plasmids of similar Inc groups. In general, we observed a great match between databases. It is very important to choose a database that is properly updated. In fact, expanding use of DNA sequencing together with new bioinformatics databases and algorithms, make important advances enabling rapid antimicrobial resistance surveillance for public health management and improved clinical outcomes.

Keywords: *Klebsiella pneumoniae*, multidrug resistance, antimicrobial resistance genes databases

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