

TITLE: Pattern of virulence genes in clinical carbapenemase-producing *Klebsiella pneumoniae* isolates from Brazil

AUTHORS: Caio Augusto Martins Aires^{a,b}; Polyana Silva Pereira^a; Natacha Ferreira Pereira^a, Cláudio Marcos Rocha-de-Souza^a; Marise Dutra Asensi^a; Ana Paula D'alincourt Carvalho-Assef^a

INSTITUTIONS:

^a Laboratório de Pesquisa em Infecção Hospitalar (LAPIH), Instituto Oswaldo Cruz—FIOCRUZ, Rio de Janeiro, Brazil.

^b Departamento de Ciências da Saúde, Universidade Federal Rural do Semi-Árido (UFERSA), Mossoró, Brazil.

ABSTRACT:

Klebsiella pneumoniae is an opportunistic pathogen which causes a variety of infections that can be potentiated by carbapenemase production. In addition, *K. pneumoniae* possesses different virulence factors that contribute to its pathogenicity including lipopolysaccharide, capsular polysaccharide, adhesions, and siderophores. This study intended to detect virulence genes in carbapenemase-producing *K. pneumoniae* from Brazil. This study included 110 carbapenemase-producing *K. pneumoniae* isolates collected from different sources of hospitalized patients from 2009 to 2014 from 14 Brazilian states. The isolates were sent to the laboratory by voluntary demand from Brazilian healthcare services to investigate carbapenemase genes by multiplex PCR. The isolates were identified by conventional biochemical methods. To identify genetic similarities, the isolates were submitted to Multi-locus Sequence Typing (MLST). The virulence factor-encoding genes *cf29a*, *ycfM*, *mrkD*, *fimH*, (adhesion), *entB*, *iron*, *kfu*, *ybtS* (siderophores), *magA* (hypermucoviscous phenotype), *allS* (allantoin metabolism) were searched by PCR. The selection included isolates positive for *bla*_{KPC} (76), *bla*_{NDM} (12), and *bla*_{OXA-370} (22) recovered from hospitals located in the states of Alagoas (1), Amazonas (1), Ceará (3), Federal District (15), Espírito Santo (9), Goiás (4), Maranhão (5), Minas Gerais (6), Mato Grosso (1), Paraíba (2), Pernambuco (9), Rio de Janeiro (46), Rio Grande do Sul (3), and Santa Catarina (5); comprising all geographical regions of the country. The isolates were collected in 2009 (1), 2010 (36), 2011 (20), 2012 (3), 2013 (30), and 2014 (20) from samples of rectal swabs (68), blood (18), urine (12), catheter (6), and others (6). The MLST revealed 42 STs, with a prevalence of STs 16 (21%), 11 (15%), 437 (10%), 37 (8%), and 340 (5%). The research for virulence determinants identified *entB* (100%), *fimH* (100%), *ycfM* (100%), *mrkD* (97%), *ybtS* (54%), *kfu* (17%), and *allS* (3%). The *iron*, *magA* and *cf29a* genes were not detected. Comparing the gene detection and the carbapenemase production, we found 67% (8/12) association between *kfu* and NDM; and 74% (56/76) association between *ybtS* and KPC. Thus, the identification of virulence markers in carbapenemase-producing *K. pneumoniae* could elucidate aspects related to

colonization, evolution to infection and the successful dissemination of such pathogens; and may be used in the future for the development of new specific therapeutic targets.

KEYWORDS: *Klebsiella pneumoniae*; Virulence; Carbapenemase.

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