TITLE: VIRULENCE-ASSOCIATED CHARACTERISTICS OF CARBAPENEMASE-PRODUCING *Pseudomonas aeruginosa*: PHENOTIPIC PRODUCTION AND DETECTION OF RELATED GENES

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ABSTRACT:
Pseudomonas aeruginosa is among the leading causes of severe nosocomial infections, particularly affecting critically ill and immunocompromised patients. The versatility of this bacteria to infect a plethora of human tissue may be a consequence of its ability to produce a wide variety of both cell-associated and extracellular virulence factors.The aim of this study was to determine the production of virulence factors and the presence of virulence genes in previous characterized carbapenemase-producing *P. aeruginosa* isolated from patients hospitalized at Hospital Universitário de Londrina. Seventy *P. aeruginosa* isolates (63 SPM-1, 3 KPC-2, 2 IMP-16, 1 VIM-1 and 1 VIM-7) recovered from different specimes were studied. Detection of haemolysin and lecithinase production was performed by spotting fresh cultures on 5% sheep blood agar medium and 2.5 % yolk agar, respectively. The protease activity (caseinase and gelatinase) was determined using 15% soluble casein agar and 3% gelatin as substrate. For the aesculin hydrolysis the medium containing Fe^{3+} citrate was utilized. All plates were incubated for 24 h at 37°C. The ability of producing biofilm was examined by crystal violet microtiter plate assay. Eight virulence genes encoding for elastase (*las*B), exoenzymes (*exo*S, *exo*Y, *exo*U), haemolytic phospholipase C (*plc*H), exotoxin A (*tox*A), neuraminidase (*nan*I) and the autoinducer synthase (*las*I), involving in quorum sensing (QS) were investigated by PCR. The pore forming toxins and enzymes were expressed in variable proportions: haemolysin (98.6%), caseinase (94.3%), lecithinase (58.6 %) and gelatinase (82.6%), indicating the ability of these strains to induce tissue lesions and disseminate the infection. Variable rates of adherence were obtained to the inert substratum: 41.4%, 27.1% and 31.4% of isolates were considered strong, moderate and weak biofilm producers, respectively. The most frequent virulence genes detected were the *exo*T and *las*I (100%). The genes codifying for phospholipase and elastase were found in 97.1% and 95.7% of isolates, respectively. Rates of 95.7%, 92.8% and 5.7% were detected for Exotoxins A, S and U. All isolates coproduced more than three type of virulence factors revealing their easy to adapt to the microenvironment encountered within the host by modulating the expression of these genes.

Keywords: *Pseudomonas aeruginosa,* carbapenemases, virulence factors

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