TITLE: MOLECULAR EPIDEMIOLOGY AND ANTIBIOTIC RESISTANCE TRENDS IN CLINICAL ISOLATES OF *PSEUDOMONAS AERUGINOSA* FROM RIO DE JANEIRO: IMPORTANCE OF MUTATIONAL MECHANISMS OVER THE YEARS (1995-2015)

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ABSTRACT:

The emergence of Multidrug-Resistant (MDR) and Extensively Drug-Resistant (XDR) *Pseudomonas aeruginosa* has become a great concern worldwide. This phenotype has increased in recent years as a consequence of acquisition of resistance genes or mutational processes. To evaluate the resistance mechanisms and clonal dissemination over the years, 88 isolates recovered in Rio de Janeiro from 1995 to 2015 were studied. The antimicrobial susceptibility were evaluated by disk diffusion method and E-test to imipenem. The hydrolysis of imipenem was detected by colorimetric assay (Carba NP). Carbapenemases genes (*bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{NDM}$, *bla*$_{SPM}$, *bla*$_{KPC}$, *bla*$_{GES}$, *bla*$_{OXA-48}$), genes of resistance to aminoglycosides (*armA, rmtA, rmtB, rmtC, rmtD* e *npmA*), fluoroquinolones (*qnrA, qnrB, qnrC* and *qnrS*) and mutations in the *oprD* (Resistance to imipenem), *mexT* (Regulator of the efflux pump MexEF-OprN) and *gyrA* genes (Resistance to fluoroquinolones) were surveyed by PCR and sequencing. Molecular typing was performed using the PFGE. Over the years studied, the MDR phenotype was replaced by XDR, with significant increase in resistance to carbapenems, fluoroquinolones and aminoglycosides. All isolates were susceptible to polymyxin. Carbapenemases and *rmtD* genes (*bla*$_{KPC}$, n=5, *bla*$_{SPM}$, n=3 and *rmt*D, n=5) were found in isolates recovered since 2007, however all KPC-producing isolates were recovered in 2015. All correctly identified by Carba-NP. Mutations in the *oprD* gene was observed in 30 of 33 isolates analyzed (24 carbapenem-resistant and 9 susceptible). However, analyzes with the PROVEAN tool revealed 59,4% (n=19) with mutations that resulted in modification of protein. Most of them were isolated since 2006. Among fluoroquinolones-resistant isolates, 21 of the 27 isolates tested showed mutation in the QRDR of *gyrA*. Regarding the *mexT* gene, was observed deletion of 8 nucleotides at the beginning of the gene in 25 out of 26 isolates analyzed. By PFGE, a great variability of clonal groups was observed mainly in the last 10 years of the study, the same period in which higher antimicrobial resistance was evidenced. The present study shows the evolution of antimicrobial resistance in *P. aeruginosa* over 20 years in Rio de Janeiro, although resistance has increased, no prevalent clone of XDR phenotype has been found. This study highlights to the important role of selective pressure and mutational processes in the development of resistance in *P. aeruginosa*.

Keywords: *Pseudomonas aeruginosa,* antimicrobial resistance, mutational mechanisms, clonal diversity.

Development Agency: CAPES, CNPQ.