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ANTIMICROBICI

MOLECULAR CHARACTERIZATION OF CARBAPENEM RESISTANCE DETERMINANTS OF PSEUDOMONAS AERUGINOSA MULTI-DRUG RESISTANT STRAINS

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INTRODUZIONE

The new cephalosporin ceftolozane in combination with tazobactam is one of the few new antibiotics that can be active against multi-drug resistant P. aeruginosa. Thus, the spread of carbapenem-resistant and the emergence of ceftolozane/tazobactam resistant P. aeruginosa is a big public health concern.
The aim of the study was to characterize 53 P. aeruginosa strains isolated from purulent material to identify the molecular mechanism(s) responsible for their resistance to carbapenems and/or ceftolozane/tazobactam .

METODI

All the strains used in this study were identified by the MALDI TOF.
The antibiotics susceptibilities test were performed by using either the Broth Microdilution and E- test mehod.
Phenotypic testing methods have been joined by PCR techniques for the molecular characterization of the most frequent carbapenemase genes (blaKPC, blaIMP, blaVIM, blaNDM and blaGES).
OprD gene presence/absence was investigated by PCR and in case of presence amplicons were sequenced.
Efflux pumps involvement was investigated by phenotypic test using antimicrobial susceptibilities test in presence of CCCP.
In order to highlight the overproduction of Amp C enzyme, which is a Class C β-lactamase, a phenotypic test was performed based on the determination of the minimum concentration inhibiting growth using Ceftazidime in the presence and absence of Cloxacillin,

RISULTATI

The molecular characterization revealed that only 4 out of 53 strains harbour carbapenemases: 3 strains with bla_{VIM} and one strain with bla_{GES}.,
We have characterized all our strains for Opr D. 30% of strains have no amplification of OprD gene, suggesting a deletion of this. Sequences of OprD amplicons of the other strains reveal the presence of different mutations other than IS insertion or deletion that can be responsible of carbapenem resistance
Efflux pumps involvement in the carbapenems resistance by phenotypic test was very low (3 strains).
Other than strains harbouring metallo-beta-lactamase, Ceftalozane/tazobactam resistance was present at low level in other 8 strains that were confirmed to have an involvement of AmpC enzyme.

CONCLUSIONI

Molecular analysis revealed that carbapenem resistance of P. aeruginosa is combinatory and is not given by a single resistance mechanism, even in our strains OprD plays a very important role. For this reason and because of the high heterogeneity of carbapenem resistance, and the emergence of ceftolozane-tazobactam resistance in P. aeruginosa it is important to monitor the multi-resistant strains constantly to avoid the onset of new nosocomial epidemics.