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Paper Poster Session

Deciphering carbapenem resistance

Characterization of class 1 integrons carrying blaVIM-2 and blaVIM-4 gene cassette in Pseudomonas aeruginosa isolated in the University Clinical Hospital of Bialystok (northeastern Poland)

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Background: The worldwide spread of acquired metallo-beta-lactamases (MBLs) in Gram negative bacilli has become a great concern. The *bla*_{VIM} gene is carried on mobile gene cassettes inserted into class 1 integrons having the potential to move from one bacterium to another. The aim of this study was to genetically examine carbapenem-resistant (producing MBLs) *Pseudomonas aeruginosa* isolates with similar integrons.

Material/methods: A total of five nonduplicate clinical isolates of *Pseudomonas aeruginosa* with MBLs gene were collected from hospitalized patients in University Hospital in Bialystok (Poland) during October 2012 to December 2013. Identification and susceptibility to antibiotics was performed using VITEK 2 System and Etest. PCR analysis was performed using primers specific for *bla*_{VIM}, *bla*_{IMP} and *bla*_{NDM} genes and class 1, 2 and 3 integrons. Sequence analysis were made to identify the metallo-beta-lactamase. Integron structures were determined by sequencing PCR products amplified with 5' and 3' conserved sequence primers. All strains were genotyped by multilocus sequence typing (MLST) using the genes *acsA*, *aroE*, *guaA*, *mutL*, *nuoD*, *ppsA* and *trpE*, and by pulsed-field gel electrophoresis (PFGE) with *Xba*I.

Results: Sequence analysis of PCR products detected the presence of *bla*_{VIM-2} in four isolates and *bla*_{VIM-4} in one isolate. Clonal analysis by PFGE showed five different profiles. Multilocus sequence typing analysis of *Pseudomonas aeruginosa* isolates identified five new sequence types (Table 1).

Table 1.

Isolates	Allelic profile							ST	Integron accession number
	<i>acsA</i>	<i>aroE</i>	<i>guaA</i>	<i>mutL</i>	<i>nuoD</i>	<i>ppsA</i>	<i>trpE</i>		
Psa02	99	89	62	8	9	4	7	New	AM08740 In249
Psa11	99	89	62	8	45	6	7	New	AJ585042 In239

Psa21	63	89	New	4	45	4	3	New	not assigned
Psa22	63	89	96	4	45	4	3	New	EU912537 In196
Psa23	New	39	New	57	1	2	48	New	AJ295229 In630

* ST, sequence types

** interpretation profile according <http://pubmlst.org/paeruginosa>

By the PCR mapping of integrons we found the presence of five different gene cassette arrays (Psa02, *aacA4-bla_{VIM-2}*; Psa11, *aacA4-bla_{VIM-4}*; Psa21, *bla_{VIM-2}-aacA4-aadA1*; Psa22, *bla_{VIM-2}-aacA4-aacA40-bla_{OXA-2}* and Psa23, *bla_{VIM-2}-aacA4-aadA1-bla_{OXA-1}*).

Conclusions: All of the *bla_{VIM}*-carrying isolates of our study harboured class 1 integrons. We observed large diversity of *bla_{VIM}* gene cassette among the tested isolates. This report indicates that genetically different isolates of *Pseudomonas aeruginosa* (producing MBLs) may be the cause nosocomial infection in northeastern Poland.