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Short communication

The presence of *bla*$_{IMP}$ genes on plasmids DNA isolated from multidrug - resistant *Pseudomonas aeruginosa* strains at University Hospital in Bialystok (Poland) - first report

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Abstract: Resistance to carbapenems is emerging, and it is a great problem to therapeutics. Seven multidrug-resistant (MDR) of *Pseudomonas aeruginosa* strains were isolated from urine and bronchial specimens. All isolates showed resistance to imipenem and meropenem (MIC; ≥16 mg/L). The resistance to carbapenems in two of seven strains was associated with the production of a metallo-β-lactamases. Plasmids DNA probes were used to investigate the presence of genes coding for IMP-type enzymes. PCR experiments revealed that *bla*$_{IMP}$ genes were present in two isolates of *Pseudomonas aeruginosa* (MIC >32 μg/mL for both carbapenems).

Keywords: *Pseudomonas aeruginosa* - Metallo-β-lactamases - Plasmids DNA - PCR detection *bla*$_{IMP}$-like genes

Introduction

Carbapenems, such as imipenem and meropenem, remain one of the best drugs to treat infections caused by *Pseudomonas aeruginosa*. Increasing usage of these drugs and other expanded-spectrum antibiotics has resulted in the development of carbapenem-resistant *P. aeruginosa*. The clinical utility of these antimicrobials is under the thread with the emergence of acquired genes for carbapenemases, particularly those coding metallo-β-lactamases (MBLs). Acquired MBLs expression in gram-negative pathogens is becoming a therapeutic challenge since these enzymes are capable hydrolyzing all β-lactams except the monobactams [5,6].

The genes responsible for the production of MBLs are typically part of an integron structure and carried on transferable plasmids but can also be part of the chromosome [8]. The *bla*$_{IMP}$ genes were often located in *Pseudomonas aeruginosa* rods on largesize plasmids. Acquisition of *bla*$_{IMP}$ genes is not always followed by expression of a high level of resistance to carbapenems, because several isolates carrying the cryptic *bla*$_{IMP}$ genes demonstrated low-level carbapenem resistance [4].

During the past decade, a number of acquired MBLs have been identified in Gram-negative pathogens and were categorized into 2 major types: IMP and VIM [6,12].

IMP-1 was the first acquired carbapenemases detected among *Pseudomonas aeruginosa* in Japan [15]; since them, numerous IMP-type variants have been described in *Pseudomonas aeruginosa* mainly in Asian and some American and European regions [3,9,13].

The aim of this study was to determine the prevalence of blaIMP genes among multidrug-resistant *Pseudomonas aeruginosa* strains isolated from various clinical specimens in the University Hospital in Bialystok (Poland).

In this work we report the first PCR detection of IMP-type plasmid-mediated MBLs genes in two multidrug-resistant isolates of *Pseudomonas aeruginosa*.

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Table 1. Characteristics of multidrug-resistant (MDR) *Pseudomonas aeruginosa* strains.

Strain	Specimen	E-test [b]		MBL E-test	PCR *bla* IMP	MIC (mg/L) of selected antibiotics determined by VITEK automated system [a]									
		MEM	IP			IMI	AZM	FEP	TAX	TAZ	CIP	AN	GM	TZP	TCC
Psa-1	pharyn-geal swab	16	16	-	-	≥16 (R)	≥32 (R)	16 (I)	≥64 (R)	16 (I)	2 (I)	≤2 (S)	2 (S)	≥128 (R)	≥256(R)
Psa-2	bronchial swab	32	32	-	-	≥16 (R)	≥32 (R)	≥32 (R)	≥64 (R)	≥32 (R)	≥4 (R)	≥64 (R)	≥16 (R)	≥128 (R)	≥256 (R)
Psa-3	bronchial swab	24	32	-	-	≥16 (R)	≥32 (R)	≥32 (R)	≥64 (R)	≥32 (R)	≥4 (R)	≥64 (R)	≥16 (R)	≥128 (R)	≥256 (R)
Psa-4	bronchial swab	16	16	-	-	≥16 (R)	≥32 (R)	16 (I)	≥64 (R)	≥32 (R)	≥4 (R)	≥64 (R)	≥16 (R)	64 (S)	≥256 (R)
Psa-5	urine	>32	>32	+	+	≥16 (R)	≥32 (R)	≥32 (R)	≥64 (R)	≥32 (R)	≥4 (R)	≥64 (R)	≥16 (R)	≥128 (R)	≥256 (R)
Psa-6	urine	16	16	-	-	≥16 (R)	≥32 (R)	≥32 (R)	≥64 (R)	≥32 (R)	≥4 (R)	≥64 (R)	≥16 (R)	64 (S)	≥256 (R)
Psa-7	urine	>32	>32	+	+	≥16 (R)	≥32 (R)	≥32 (R)	≥64 (R)	≥32 (R)	≥4 (R)	≥64 (R)	≥16 (R)	≥128 (R)	≥256 (R)

Abbreviations: [a]IMI, imipenem; AZM, aztreonam; FEP, cefepime; TAX, cefotaxime; TAZ, ceftazidime; CIP, ciprofloxacin; AN, amikacin; GM, gentamicin; TZP, pipercillin/tazobactam; TCC, ticarcillin/clavulanic acid; [b]MIC (mg/L): MEM, meropenem; IP, imipenem.

Materials and methods

Bacterial strains. Seven carbapenem-resistant strains of *Pseudomonas aeruginosa* used in this study were isolated from clinical samples in the University Hospital in Bialystok (Poland). Bacterial identification was performed using conventional methods and VITEK cards for identification (GNI) in VITEK 1 automated system (bioMérieux, Marcy l'Etoile, France). The identification was repeated by using API 20NE test and ATB system (bioMérieux, Marcy l'Etoile, France).

Antibiotics susceptibility. The VITEK sensitivity test card (GNS-650) was used for determination of the selected antibiotics susceptibility. The following antibiotics were tested: imipenem, amikacin, aztreonam, cefepime, ceftotaxime, ceftazidime, ciprofloxacin, gentamicin, piperacillin/tazobactam and ticarcillin/clavulanic acid. Results were interpreted by Vitek software version SYS R06.06.

The susceptibility to carbapenems was retested using the Etest (AB Biodisk, Solna, Sweden) on Muller-Hinton agar (Oxoid, Basingstoke, UK) according manufacturer's instructions. Resistance to antibiotics was defined according to the Clinical and Laboratory Standards Institute (formerly NCCLS) recommendations [1].

P. aeruginosa ATCC 27853, *Escherichia coli* ATCC 25922, and *Escherichia coli* ATCC 35218 were used as reference strains.

Screening of MBL-producing isolates. MBLs production was screened by the Etest MBL strips (AB Biodisk, Solna, Sweden) technique. Results were considered as positive when the MIC (minmal inhibitory concentration) ratio of imipenem/imipenem plus EDTA was ≥8. The presence of phantom zone (or deformation of imipenem ellipse) was also considered as indicative of MBL production [13].

Plasmids DNA isolation. Plasmids DNA extractions of *P. aeruginosa* isolates were performed with the Plasmid Mini (A&A Biotechnology, Gdynia, Poland) according to the manufacturer's instructions.

Molecular detection of the blaIMP genes. Detection of bla_{IMP} genes was performed using PCR assays with previously described primers [10]. Primer pair was used to amplify a 587 bp fragment of genes encoding the IMP-1 like enzymes. PCR amplification was performed in 25 µl volume with the Cyclone 96 (PEQLAB Biotechnology, GmbH) thermal cycler. The PCR mixtures contained: 25 pmol of each primer, 1× reaction buffer, 2 mM $MgCl_2$, 1 l of dNTPs, 0.5U of Delta 2 DNA polymerase (DNA-Gdansk II, Gdansk, Poland), 2 l of template DNA and ultra pure H_2O to final volume. The cycling parameters of amplification were: initial denaturation at 94°5 C for 2.5 min; denaturation at 94°C for 40 s, annealing at 57°C for 40 s, and extension at 72°C for 60s repeated for 35 cycles. The final elongation step was at 72°C for 4 min.

Detection of PCR products. Products of PCR were analyzed by electrophoresis at 5V/cm for 90 min in 1.8% agarose gel containing 0.5 µg/ml ethidium bromide in TBE buffer and photographed on a UV transilluminator. The sizes of the fragments produced in the amplifications were calculated from their positions relative to the positions of the molecular weight marker.

Results and discussion

Production of MBLs represents an important mechanism of resistance to carbapenems and other β-lactams among *P. aeruginosa* rods. Resistance to carbapenems in nosomical populations of *P. aeruginosa* in Poland have been increasing in recent years and indicated clearly those MBLs have started to constitute a countrywide problem [9,11].

All of *P. aeruginosa* isolates tested in this study was carbapenem-resistant, but the levels of resistance were diverse (Table 1). A comparison of MICs of 2 MBLs-positive (MIC, >32 mg/L for imipenem and

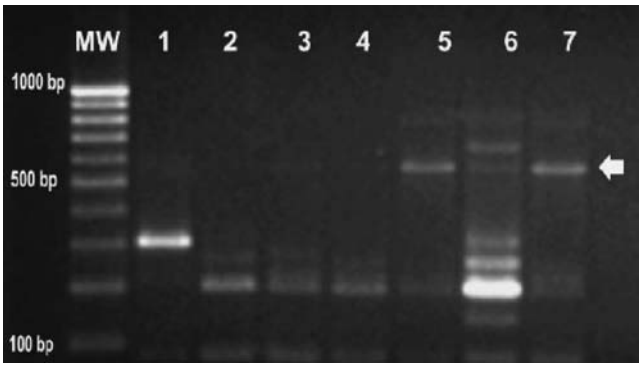

Fig. 1. PCR detection of bla_{IMP} genes (587bp) in *Pseudomonas aeruginosa* isolates. Lanes MW, DNA molecular size marker (M100-1000 bp, DNA-Gdansk II, Gdansk, Poland); Lanes 1, strain *Psa-1*; 2, *Psa-2*; 3, *Psa-3*, 4, *Psa-4*; 5, *Psa-5*; 6, *Psa-6*; 7, *Psa-7*.

meropenem) isolates with five MBLs-negative (MIC, 16-32 mg/L) isolates suggested presence different mechanisms resistance to carbapenems. Mechanisms low-level resistance to carbapenems (MIC, 832 mg/L) in *P. aeruginosa* are often associated with reduced uptake as a results loss of the OprD porin combined with de-repression of the chromosomal AmpC β-lactamase gene or by over-expression of an efflux pump system [5,11].

Only one isolate (*Psa-1*) was susceptible to aminoglycoside antibiotics, intermediate to cefepime, ceftazidime and ciprofloxacin. Two (*Psa-4* and *Psa-6*) of MBLs-negative was susceptible to piperacillin with tazobactam (MIC, 64 mg/L).

Screening by the Etest MBL strips showed possibility to production of MBLs in two (*Psa-5* and *Psa-7*) isolates. The same isolates were found to be positive in PCRs for the presence of the bla_{IMP} gene (Fig.1).

In our study bla_{IMP} gene was carried on a plasmids DNA. The two of bla_{IMP} - positive *P. aeruginosa* strains were detected in different patients in urine.

Broad-spectrum β-lactams usually administrated to inpatients through drip infusion, and they are mostly excreted in urine without being dissolved modified. Since these agents tend to be highly condensed in urine and bacteria surviving in the urinary tract must acquire high-level resistance to these antibiotics.

Despite the low number of isolates, this study is the first report of the presence of IMP-type carbapenemases in Poland. Further investigations are necessary to gain a better understanding of epidemiology and genetic background of these enzymes (study ongoing).

Long-term hospitalisation, indwelling urinary catheters, and long-term antibiotic use (in particular of carbapenems) are the possible risk factors for colonization and/or infection such pathogens as MBLs-positive *Pseudomonas aeruginosa*. In absence of novel antibiotics for the treatment of infections caused by multidrug-resistant gram-negative bacteria in the near future, uncontrolled spread of MBLs producers may lead to treatment failures with increased morbidity and mortality. Appropriate therapeutic protocols and a regular screening/monitoring system should be established to prevent the wider spread of this worrisome resistance determinant.

Understanding the epidemiology and mechanisms for dissemination of MBL-producing strains is an essential step to control these phenomena. In Europe, although infections caused by VIM-producing *P. aeruginosa* have been observed for almost a decade, little is still known about the epidemiological relation between IMP-producing isolates from different regions.

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