

Characteristics of Carbapenem-Resistant *Pseudomonas aeruginosa* Strains in Patients with Ventilator-Associated Pneumonia in Intensive Care Units

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Summary. The aim of this study was to determine the characteristics of carbapenem-resistant *Pseudomonas aeruginosa* (*P. aeruginosa*) strains and 5-year changes in resistance in a tertiary university hospital.

Material and Methods. The study included 90 and 101 randomly selected *P. aeruginosa* strains serotyped in 2003 and 2008, respectively. The standardized disk diffusion test and E-test were used to determine resistance to antibiotics. *P. aeruginosa* strains were considered to have high-level resistance if a minimum inhibitory concentration (MIC) for imipenem or meropenem was $>32 \mu\text{g/mL}$. To identify serogroups, sera containing specific antibodies against O group antigens of *P. aeruginosa* were used. *P. aeruginosa* isolates resistant to imipenem or/and meropenem were screened for metallo- β -lactamase (MBL) production by using the MBL E-test.

Results. Comparison of the changes in resistance of *P. aeruginosa* strains to carbapenems within the 5-year period revealed that the level of resistance to imipenem increased. In 2003, 53.3% of *P. aeruginosa* strains were found to be highly resistant to imipenem, while in 2008, this percentage increased to 87.8% ($P=0.01$). The prevalence of MBL-producing strains increased from 15.8% in 2003 to 61.9% in 2008 ($P<0.001$). In 2003 and 2008, carbapenem-resistant *P. aeruginosa* strains were more often resistant to ciprofloxacin and gentamicin than carbapenem-sensitive strains. In 2008, carbapenem-resistant strains additionally were more often resistant to ceftazidime, cefepime, aztreonam, piperacillin, and amikacin than carbapenem-sensitive strains. MBL-producing *P. aeruginosa* strains belonged more often to the O:11 serogroup than MBL-non-producing strains (51.7% vs. 34.3%, $P<0.05$). A greater percentage of non-MBL-producing strains had low MICs against ciprofloxacin and amikacin as compared with MBL-producing strains.

Conclusions. The results of our study emphasize the need to restrict the spread of O:11 serogroup *P. aeruginosa* strains and usage of carbapenems to treat infections with *P. aeruginosa* in the intensive care units of our hospital.

Introduction

It has been proved that early appropriate antimicrobial therapy in patients with nosocomial infections may have a major impact on mortality, length of stay, emergence of resistant strains, and overall health care costs. Treatment of serious infections in an intensive care unit requires an empirical strategy providing broad-spectrum coverage to a wide range of suspected pathogens, such as *Pseudomonas aeruginosa* (*P. aeruginosa*) (1). Infections with multi-drug-resistant *P. aeruginosa* has been recognized as a growing problem in clinical settings, and the occurrence of infections due to strains that are resistant to almost all commercially available antibacterial

drugs has become a rather common event. The carbapenems (meropenem [MEM] and imipenem [IMP]/cilastatin) represent a realistic option for initial empirical therapy in many serious nosocomial infections because of their broad spectrum of activity and the continued susceptibility of difficult-to-treat and antibiotic-resistant pathogens to these agents (2). However, resistance to carbapenem is being observed more frequently among non-fermenting bacteria, such as *P. aeruginosa* and *Acinetobacter* spp. Data from many European centers show an increasing resistance of *P. aeruginosa* strains to carbapenems, conditioned by beta-lactamase synthesis (3–5). Increasing resistance to carbapenems mediated by metallo- β -lactamase (MBL) is a cause for concern because MBL-producing *P. aeruginosa* strains have been reported to be important causes of nosocomial infections, and it adversely affects

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clinical outcomes and adds to treatment costs (6). Other mechanisms of the resistance of *P. aeruginosa* strains to carbapenems, such as the production of AmpC, extended-spectrum β -lactamases, or Toho-1-type β -lactamases, can be involved as well, but the overall rates of morbidity and mortality among patients infected with MBL-producing strains are high (7). Early detection of MBL-producing strains is important for clinicians for the selection of appropriate antimicrobial agents. During the last years, the prevalence of carbapenem-resistant *P. aeruginosa* strains in the tertiary hospital of our university has increased from 10% to 40% (data not published). Such a high rate prompted us to study the characteristics of carbapenem-resistant *P. aeruginosa* strains and 5-year changes in resistance at our hospital and to present the guidelines in the future in order to decrease such a resistance.

Material and Methods

Patients and Bacterial Strains. This study was carried out at the 2000-bed tertiary-care teaching Hospital of the Lithuanian University of Health Sciences (HLUHS). According to the data of the Laboratory of Microbiology at the HLUHS, a total of 145 *P. aeruginosa* strains in 2003 and 151 in 2008 were isolated from lower respiratory tract specimens (bronchial or bronchoalveolar lavage) of patients treated for ventilator-associated pneumonia in the intensive care units. The study included 90 *P. aeruginosa* strains serotyped in 2003 and 101 *P. aeruginosa* strains serotyped in 2008, which were randomly selected. *P. aeruginosa* strains were identified according to the standard methodology described previously (8).

Antimicrobial Susceptibility, Interpretive Criteria, and Detection of Metallo- β -Lactamase. All *P. aeruginosa* strains were screened for resistance to carbapenems (IMP and MEM) by the routine disk diffusion test with antibiotic-containing disks (BBL, USA) according to the Clinical and Laboratory Standards Institute (CLSI) recommendations (9). Every case of resistance to carbapenems determined by disk diffusion test was confirmed using the E-test (AB Biodisk, Solna, Sweden). *P. aeruginosa* strains were defined as resistant to carbapenems if a minimum inhibitory concentration (MIC) for IMP or MEM was ≥ 16 $\mu\text{g}/\text{mL}$ by the E-test; intermediate resistant, if the MIC was 6–12 $\mu\text{g}/\text{mL}$; and sensitive, if the MIC was ≤ 4 $\mu\text{g}/\text{mL}$. *P. aeruginosa* strains were considered to have high-level resistance if the MIC for IMP or MEM was > 32 $\mu\text{g}/\text{mL}$.

P. aeruginosa isolates resistant to IMP or/and MEM were screened for the presence of MBL phenotype by using the IMP/EDTA E-test (MBL E-test) (AB Biodisk, Solna, Sweden). The phenotypic E-tests were performed by following the CLSI

recommendations for the E-test and manufacturer's instructions (AB Biodisk, Solna, Sweden). The MIC ratio of IMP/IMP-EDTA of ≥ 8 was interpreted as being indicative of MBL production.

To investigate the resistance level of MBL-producing and non-MBL-producing carbapenem-resistant strains to antibiotics, the MICs of ciprofloxacin, amikacin, and piperacillin/tazobactam against *P. aeruginosa* strains of both groups were determined. The MIC for each bacterial strain was interpreted according to the CLSI criteria. Standard *P. aeruginosa* ATCC 27853 strain was used as a control strain.

Serogroup Detection. To identify serogroups, sera containing specific antibodies against O group antigens of *P. aeruginosa* were used (Bio-Rad, France). Serogroups were denominated by Arabic numerals from O:1 to O:16 according to the classification of the ICSB Subcommittee on Pseudomonas and Related Organisms and identified using the agglutination method according to the methodology approved by the manufacturer of specific antibodies (Bio-Rad, France).

Statistical Analysis. Statistical analysis was conducted using the SPSS (Statistical Package for Social Sciences, Microsoft Inc., USA) software, version 12.0 for Windows. While analyzing differences in frequency, nonparametric statistical criterion χ^2 and Fisher exact test were used. The differences between the groups were considered significant if P was < 0.05 .

Results

In 2003, of the 90 clinical *P. aeruginosa* isolates included in this study, 19 (21.1%) were resistant or intermediate resistant to carbapenems (IMP or/and MEM). In 2008, there were more carbapenem-resistant or intermediate resistant *P. aeruginosa* strains as compared with 2003 (41.6%, 42/101; $P=0.002$). Comparison of the changes in resistance of *P. aeruginosa* strains to carbapenems within the 5-year period revealed that the level of resistance to IMP increased, but that to MEM did not change. In 2003, 53.3% of *P. aeruginosa* strains were found to be highly resistant to IMP, while in 2008, this percentage increased to 87.8% ($P=0.01$) (Fig.).

All *P. aeruginosa* strains that were found to be resistant to at least one of carbapenems using the disk diffusion test were screened for MBL activity. In 2008, a considerable increase in the proportion of carbapenem-resistant MBL-producing *P. aeruginosa* strains was observed as compared with 2003 (61.9%, 26/42, and 15.8%, 3/19, respectively; $P<0.001$).

The percentages of carbapenem-resistant *P. aeruginosa* strains resistant to ciprofloxacin and gentamicin were significantly greater than those of carbapenem-sensitive *P. aeruginosa* strains both in 2003 and 2008.

Table 1. Resistance of Carbapenem-Resistant *Pseudomonas aeruginosa* Strains to Various Antibiotics

Antibiotic	2003			2008		
	Carbapenem-Resistant Strains N=19	Carbapenem-Sensitive Strains N=71	P	Carbapenem-Resistant Strains N=42	Carbapenem-Sensitive Strains N=59	P
Ciprofloxacin	11 (78.5)	2 (2.8)	0.0001	26 (61.9)	14 (23.7)	0.001
Ceftazidime	2 (10.5)	3 (4.5)	0.260	12 (28.6)	2 (3.4)	0.002
Cefepime	2 (10.5)	2 (2.9)	0.177	8 (19.0)	1 (1.7)	0.019
Piperacillin	8 (42.1)	15 (21.1)	0.080	12 (28.6)	5 (8.5)	0.038
Piperacillin/ tazobactam	2 (10.5)	9 (14.5)	0.554	7 (16.7)	2 (3.4)	0.085
Aztreonam	8 (42.1)	15 (21.1)	0.08	20 (47.6)	5 (8.5)	0.001
Gentamicin	10 (52.6)	8 (12.9)	0.001	16 (38.1)	5 (8.5)	0.002
Amikacin	2 (10.5)	2 (2.8)	0.177	7 (16.7)	1 (1.7)	0.003

Values are number (percentage).

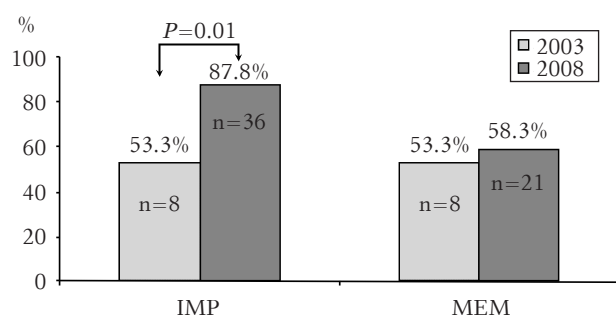


Fig. Distribution of *Pseudomonas aeruginosa* strains highly resistant to imipenem (IMP) and meropenem (MEM) in 2003 and 2008

Table 2. Distribution of Carbapenem-Resistant *Pseudomonas aeruginosa* Strains by O Serogroups

O Serogroup of <i>Pseudomonas aeruginosa</i>	Carbapenem-Resistant <i>Pseudomonas aeruginosa</i> Strains		
	MBL-Producing N=29	Non-MBL-Producing N=32	Total N=61
1	5 (17.3) ^b	4 (12.5)	9 (14.8)
2	—	2 (6.3)	2 (3.3)
4	—	1 (3.1)	1 (1.6)
6	5 (17.3) ^b	4 (12.5)	9 (14.8)
7	—	1 (3.1)	1 (1.6)
11	15 (51.7) ^a	11 (34.3) ^b	26 (42.6)
12	1 (3.4) ^b	2 (6.3)	3 (4.9)
Nontypable	3 (10.3) ^b	7 (21.9)	10 (16.4)

Values are number (percentage). $P < 0.05$, a compared with b.

Moreover, in 2008, carbapenem-resistant *P. aeruginosa* strains were resistant to ceftazidime, cefepime, aztreonam, piperacillin, and amikacin more often than carbapenem-sensitive *P. aeruginosa* strains (Table 1).

Serogroups of carbapenem-resistant *P. aeruginosa* strains were identified. *P. aeruginosa* strains be-

longed to 7 out of the 16 O serogroups: O:1 (14.8%, 9/61); O:2 (3.3%, 2/61), O:6 (14.8%, 9/61), O:11 (42.6 %, 26/61), O:12 (4.9%, 3/61), O:4 (1.6%, 1/61), and O:7 (1.6%, 1/61) serogroups. In 16.4% of cases (10/61), *P. aeruginosa* strains did not agglutinate with specific serogroup serums; therefore, the

Table 3. Minimum Inhibitory Concentrations of Ciprofloxacin, Amikacin, and Piperacillin/Tazobactam Against MBL-Producing and Non-MBL-Producing *Pseudomonas aeruginosa* Strains

	Ciprofloxacin			
	MIC range, $\mu\text{g/mL}$	0.032–1 $\mu\text{g/mL}$	1.5–3 $\mu\text{g/mL}$	4–64 $\mu\text{g/mL}$
MBL+, n=29	0.75–64	5 (17.2)*	2 (6.9)	22 (75.9)
MBL-, n=32	0.032–32	15 (46.9)*	6 (18.7)	11 (34.4)
Interpretation		Sensitive	Intermediate	Resistant
	Amikacin			
	MIC range, $\mu\text{g/mL}$	1.5–16 $\mu\text{g/mL}$	32 $\mu\text{g/mL}$	64–256 $\mu\text{g/mL}$
MBL+, n=29	4–64	19 (62.5)*	2 (6.9)	8 (27.6)
MBL-, n=32	1.5–32	31 (96.9)*	1 (3.1)	0
Interpretation		Sensitive	Intermediate	Resistant
	Piperacillin/tazobactam			
	MIC range, $\mu\text{g/mL}$	$\leq 64/4 \mu\text{g/mL}$	—	128/4–256/4 $\mu\text{g/mL}$
MBL+, n=29	1–256	23 (79.3)	—	6 (20.7)
MBL-, n=32	0.5–128	29 (90.6)	—	3 (9.4)
Interpretation		Sensitive	—	Resistant

Values are number (percentage). MBL, metallo- β -lactamase.

* $P < 0.05$.

serogroup was not identified. Among the 61 carbapenem-resistant *P. aeruginosa* isolates tested, MBL-producing *P. aeruginosa* strains belonged more often to O:11 serogroup (51.7%, n=15) compared with other serogroups and non-MBL-producing *P. aeruginosa* strains ($P<0.05$) (Table 2).

The MICs against 29 MBL-producing and 32 non-MBL-producing carbapenem-resistant *P. aeruginosa* strains were determined. A greater percentage of non-MBL-producing strains had low MICs against ciprofloxacin and amikacin as compared with MBL-producing strains (Table 3).

Discussion

The emergence of resistance to carbapenems in *P. aeruginosa* is a serious concern. The prolonged use of imipenem/meropenem for the treatment of nosocomial infections can favor the development of resistance to other antibiotics (10). The increasing prevalence of strains possessing resistance to carbapenems still remains a major issue in the management of hospitalized patients. In this study, a high rate of increased resistance among *P. aeruginosa* strains isolated from respiratory tract specimens of patients treated at the ICUs was documented. Susceptibility testing revealed that carbapenem-resistant *P. aeruginosa* strains were highly resistant to other antibiotics: ciprofloxacin, ceftazidime, piperacillin, and gentamicin. Trouillet et al. have reported that ventilator-acquired pneumonia (VAP) episodes due to piperacillin-resistant strains were more frequently associated with prior fluoroquinolone administration than were VAP episodes due to piperacillin-susceptible strains. The results of this study highlighted the major role of fluoroquinolones in the emergence of multidrug-resistant *P. aeruginosa* responsible for ventilator-associated pneumonia (11). In contrast, a study by Mueller et al. demonstrated that the use of fluoroquinolones was not a risk factor for nosocomial colonization or infection with *P. aeruginosa* isolates resistant to both fluoroquinolones and imipenem (12). Our findings suggest that the resistance level of *P. aeruginosa* to imipenem increased during the 5-year period. Our previous studies that compared the data on defined daily doses per every 100 occupied bed-days during the period 2004–2007 revealed a significant increase in meropenem use, but a decrease in ofloxacin use (13). Other studies have shown that prolonged exposure to carbapenems and colistin independently predicted pandrug-resistant *P. aeruginosa* pneumonia (14). Many factors

play a role in the acquisition of multidrug-resistant (MDR) *P. aeruginosa*; previous exposure to quinolones and carbapenems is also recognized as an important contributor to the acquisition of MDR *P. aeruginosa* (15).

In the present study, 29 *P. aeruginosa* isolates were found to produce MBLs. MBL-producing *P. aeruginosa* strains were resistant to multiple antibiotics belonging to different structural families, and this makes our study more relevant from a clinical point of view. The majority of MBL-producing clinical *P. aeruginosa* isolates in our study were resistant to ciprofloxacin. It was determined that a greater percentage of non-MBL-producing strains had low MICs against ciprofloxacin and amikacin as compared with MBL-producing strains.

Our results in this study remind us that MBL-producing *P. aeruginosa* has become a serious clinical and therapeutic problem in our hospital. Other studies demonstrated that almost all MBL-producing organisms were highly resistant to extended-spectrum cephalosporins and beta-lactamase inhibitor combinations (16).

The present study has demonstrated a considerable increase in the percentage of *P. aeruginosa* strains belonging O:11 serogroup in our hospital in 2008. Our previous study revealed that there were no imipenem-resistant strains among *P. aeruginosa* strains belonging to the O:11 serogroup in 2003, while in 2008, even 47.5% of imipenem-resistant strains were assigned to the O:11 serogroup (17). The data reported by Patzer and Dzierzanowska showed that in Poland, *P. aeruginosa* strains resistant to aminoglycosides and beta-lactams more often belonged to the O:12 serogroup (18), while in Greece, *P. aeruginosa* strains of the O:11 serogroup were more frequently resistant to different antibiotics (19). Increasing resistance rates of *P. aeruginosa* to carbapenems in our hospital are associated not only with an increase in carbapenem use, but also with the spread of MDR O:11 serogroup *P. aeruginosa* strains.

Conclusions

The results of our study emphasize the need to restrict the spread of O:11 serogroup *Pseudomonas aeruginosa* strains and usage of carbapenems to treat infections with *Pseudomonas aeruginosa* in the intensive care units of our hospital.

Statement of Conflict of Interest

The authors state no conflict of interest.

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