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Cent. Eur. J. Med. • 4(2) • 2009 • 156-163
DOI: 10.2478/s11536-008-0090-2

Central European Journal of **Medicine**

Characteristics of *Pseudomonas aeruginosa* isolates from intensive care unit

Research Article

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Received 28 July 2008; Accepted 12 October 2008

Abstract: The study looked at the antimicrobial resistance patterns, serotypes, molecular types, metallo beta-lactamase, and chromosomal beta-lactamase enzymes of P. aeruginosa strains isolated from the patients and the staffs of the intensive care unit. *P. aeruginosa* isolates from the patients as nosocomial pathogens and from the staffs were evaluated for their susceptibilities to the antimicrobials by the disk diffusion and E-test methods. Metallo beta-lactamase enzymes were investigated by E-test, the inducibility of β – lactamase enzymes were detected by the disk antagonism test. Serotyping was performed by slide agglutination method. The *P. aeruginosa* isolates were typed by pulsed field gel electrophoresis. Twenty-five *P. aeruginosa* strains from the patients and three from the staffs were isolated. Fifteen *P. aeruginosa*, eleven of which composed of MDR bacteria, were found in serogroup E, 7 strains in G, 4 strains in B, and 1 strain in serogroup A. In all 12 bacteria in the MDR and serogroup E, metallo beta-lactamase enzyme was found to be positive. And in other 15 strains, except the bacterium which could not be serotyped, chromosomal beta-lactamase was found to be positive. The result of the molecular typing showed PFGE A pattern. In conclusion, a pattern in PFGE which included bacteria from MDR and serogroup E, G which was observed in the *P. aeruginosa* strains which was isolated from the staff's hands and from the 5 patients, and PFGE F pattern were found to be observed the most. Finally, the two different clonal strains were found to be established in the intensive care.

Keywords: *Pseudomonas aeruginosa • Metallo beta-lactamase • Chromosomal beta-lactamase • Pulsed field gel electrophoresis • Serotypes • Intensive care unit*

1. Introduction

Pseudomonas aeruginosa is an opportunistic pathogen that may cause severe invasive diseases in critically ill patients [1]. Because of its ubiquitous nature, ability to survive in moist environments, and innate resistance to many antibiotics and antiseptics, *P. aeruginosa* is a common pathogen in hospitals and particularly in intensive care units (ICUs) [2]. It has become increasingly clear that resistance development in *P. aeruginosa* is multifactorial with mutations in genes encoding porins, efflux pumps, penicillin-binding proteins, and chromosomal β-lactamase, all contributing to resistance to β-lactams, carbapenems, aminoglycosides, and fluoroquinolones [3].

In this study, we aimed to serotype and molecular type in order to put forward the similarity of the nosocomial strains isolated from the patients and the intensive care unit staff and in order to find the antibiotic sensitivity, and the antibiotic resistance mechanism beta-lactamase of the nosocomial pathogen *P. aeruginosa* strains isolated from the intensive care unit in Trakya University Hospital (TUH).

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2. Material and Methods

The study was conducted in a nine month-period. The patients admitted to the intensive care unit of TUH were monitored for nosocomial infections (NIs) by *P. aeruginosa* at all body sites. The study was done via active surveillance method towards target and the everyday service observations and by following the Clinic Microbiology Laboratory records of prospective laboratory data and patients [4]. This study was approved by the Ethical Committee of TUH. The samples of urine, blood, sputum or tracheal aspirate were taken from the patients on the first and the third day of their hospitalization and cultured routinely. Other samples were taken and cultured if there was suspicion of an infection. Hand cultures obtained by using bag broth method [5] and nasal swabs were obtained from all ICU staff every two weeks to determine the transmission of *P. aeruginosa.*

2.1. Organism identification

P. aeruginosa isolates were identified by conventional methods [1] and species identification was confirmed with the Vitek-I system (bioMérieux France) as required.

2.2. Antibiotic susceptibility testing

The isolates were evaluated for their susceptibilities to aztreonam (30 μg), ceftriaxon (30 μg) (Becton Dickinson, USA), piperacillin (100 μg), piperacillin/tazobactam (100/10 μg), ceftazidime (30 μg), cefepime (30 μg), imipenem (10 μg), meropenem (10 μg), ciprofloxacin (5 μg), ofloxacin (5 μg), amikacin (30 μg), gentamicin (10 μg), tobramicin (10 μg), netilmicin (30 μg) (Oxoid, United Kingdom) by the disk diffusion and E-test (AB Biodisk, Norway) methods and evaluated according to Clinical and Laboratory Standards Institute [6,7]. Quality control was performed by testing *P. aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922.

Isolates resistant to three or all four different classes of antimicrobials (ceftazidime, ciprofloxacin, gentamicin and imipenem) were considered multi-drug resistant (MDR) [8].

2.3. Investigation of Metallo beta-lactamase enzymes

Metallo beta-lactamase (MBL) enzymes were investigated by E-test MBL strips (AB Biodisk, Norway) (IP (4 to 256 μg/ml) with IP (1-64 μg/ml)-EDTA (IPE). According to the producer's recommendations, MIC_{IP}/MIC_{IPE} ≥8 was interpreted as being suggestive of MBL production.

2.4. The detection of chromosomal beta-lactamase enzymes

The disk antagonism test was used to detect the inducibility of β –lactamase enzymes [9].

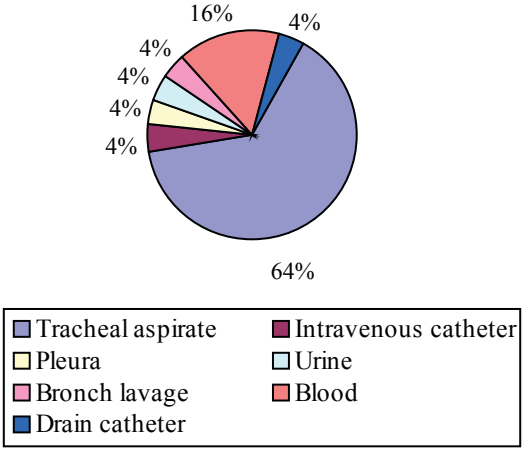
2.5. Serotyping of *Pseudomonas aeruginosa* strains

Serotyping of *P. aeruginosa* strains was performed by slide agglutination method using O-specific commercial anti-sera obtained from Denka Seiken (Denka Seiken, Japan) [10]. All agglutinations were repeated 2 times to assess reproducibility. Strains showing agglutination with saline were coded as auto-agglutinable. Strains reacting with >1 antiserum were referred to as poly-agglutinable. Non-typeable strains were those which did not react with any antiserum [11].

2.6. Molecular typing of *Pseudomonas aeruginosa* strains by pulsed-field gel electrophoresis

The *P. aeruginosa* isolates were typed by pulsed field gel electrophoresis (PFGE) to determine genetic similarity [12]. Bacterial suspension was prepared in 1xTE buffer by bacterial colonies directly from overnight incubated culture on eosin methylene blue agar (Biomérieux, France). Bacterial suspensions (200 μl) were mixed with 200 μl of low-melting-point agarose (1.6% in EET buffer, 45°C) and 8 μl proteinase K (25 mg/ml) (Sigma) and poured into a mold to form agarose plugs (30 min, 4°C). Plugs with immobilized bacteria were incubated overnight at 55°C with 0,5 M EDTA (Sigma), %10 sarcosyl (Sigma) and 100 μg/ml proteinase K. Plugs were washed six times for 30 min each time with 600 μl of $T_{10}E_1$ buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]) and, finally, were washed for 30 min in 1 ml of $T_{10}E_{0.1}$ buffer (10 mM Tris-HCl, 0.1 mM EDTA [pH 8.0]). One-sixth of the plug was cut and washed two times for 30 min each time with 120 μl of restriction buffer M (1x) and was subsequently incubated with 20 U/ml of *Spe*I (Takara, Japan) and 10 μg/ml of bovine serum albumin (Sigma) overnight. The plugs were placed in a 1% agarose (Sigma) gel in 0.5× TBE (45 mM Tris, 46 mM boric acid, 1 mM EDTA). Restriction fragments of DNA were separated with a Gene-Path system (Bio-Rad Laboratories, Hercules, CA, USA). A lambda ladder (Bio-Rad Laboratories) was used as the molecular size marker. The gel was stained with ethidium bromide and visualized with the Gel Doc 2000 system (Bio-Rad Laboratories). The band patterns were interpreted according to the criteria of Tenover *et al.* [13].

Figure 1. The materials from which *Pseudomonas aeruginosa* strains were isolated.



3. Results

Twenty five *P. aeruginosa* strains were isolated from the patients as nosocomial pathogen. Majority (64%) of *P. aeruginosa* strains were isolated from the tracheal aspirate (Figure 1). Also, 3 *P. aeruginosa* strains were isolated from the staff's hands.

The MIC values of 25 *P. aeruginosa* strains by E-test method were shown in Table 1. According to the MIC_{50} values, the antimicrobials, in the ranges of susceptibilities, were piperacillin/tazobactam, ceftazidime, imipenem, meropenem, aztreonam, amikacin, and tobramicin. Yet there were not any antimicrobials which were in the ranges of susceptibilities according to the MIC_{90}. The isolates were found to be the most resistant to the ceftriaxone (64%), gentamicin (56%) and piperacillin (56%).

The 28 *P. aeruginosa* strains isolated from the staff and the patients were in 15 different antibiotic pattern. Eleven of the patient isolates and one bacterium from the staff were found to be MDR (Table 2).

After serotyping, 15 *P. aeruginosa* strains were determined as serogroup E, 7 strains as serogroup G, 4 strains as serogroup B, and one of them was determined as serogroup A. Only one strain was non-typeable. There were no auto-agglutinable or poly-agglutinable strains (Table 2).

MBL enzymes and inducible AmpC β-lactamase were determined in 12 and 15 strains, respectively (Table 2). In MDR strains, chromosomal beta-lactamase enzymes could not be identified because they had no zone diameter or were very small.

Six different DNA band patterns were observed in PFGE (Table 2, Figure 2a and 2b). Mostly, A and subtypes of A and F band patterns were observed.
All MDR strains were serogroup E and PFGE pattern A, and all their MBL enzymes were positive. These 12 strains, one of them isolated from the staff, were observed to be in 5 antibiotic patterns.

The second most isolated group according to serotyping and PFGE pattern was serogroup G; and its PFGE pattern was F and the bacteria isolated from 5 patients and 2 staff were in this group. These bacteria were observed to have four different antibiotic patterns and in all MBL enzymes were negative.

Table 1. The MIC (μg/ml) of *Pseudomonas aeruginosa* strains (n=25) against to 16 antimicrobials.

Antimicrobials	Sensitivity Limit≤	Ranges	MIC_{50}	MIC_{90}	Susceptible		Intermediate susceptible		Resistant	
					n	%	n	%	n	%
Piperacillin	64	2->256	4	>256	11	44	-	-	14	56
PTZ*	64/4	2->256	4	>256	14	56	-	-	11	44
Ceftazidime	8	0.5->256	2	64	13	52	0	0	12	48
Cefepime	8	1->32	4	>32	12	48	1	4	12	48
Ceftriaxon	8	4–64	32	>32	2	8	7	24	16	64
Imipenem	4	1->32	2	32	13	52	0	0	12	48
Meropenem	4	0.125->32	0.25	32	14	56	0	0	11	44
Aztreonam	8	2–256	4	32	13	52	3	12	9	36
Gentamicin	4	2->256	4	>256	9	36	2	8	14	56
Amikacin	16	1–256	16	64	13	52	1	4	11	44
Tobramicin	4	0.125–1025	2	256	13	52	0	0	12	48
Netilmicin	8	0.25->256	8	>256	12	48	1	4	12	48
Ciprofloxacin	1	0.125-32	0.25	2	12	48	6	24	7	28
Ofloxacin	2	1->32	>32	>32	12	48	0	0	13	52

**PTZ; piperacillin/tazobactam*

Table 2. The characteristics of *Pseudomonas aeruginosa* isolates.

Isolate No	Patient No	Isolation date	Source*	Antibiotic susceptibilities¢Ó														Antibiotic patterns	MDR¢Ó	Serotypes	PFGE patterns	Metallo B-Lactamase	Chromosomal B-Lactamase¡×
				PRL	PTZ	CAZ	FEP	CRO	IPM	MEM	ATM	GN	AK	TOB	NET	OFX	CIP						
1	1	21.4.2003	Tr.asp.	R	R	R	R	R	R	R	I	R	I	R	R	R	I	1	MDR	E	A	+	
2	11	12.5.2003	Tr.asp.	S	S	S	I	R	S	S	S	I	S	S	S	S	S	2		A	B	_	+
3	15	12.6.2003	Tr.asp.	S	S	S	S	S	R	S	S	S	S	S	S	R	R	3		NTi«	C	_	
4	25	2.6.2003	Tr.asp.	R	S	S	S	S	S	S	S	R	S	R	S	S	S	4		E	A	_	+
5	37	6.7.2003	Tr.asp.	R	R	R	R	R	R	R	R	R	R	R	R	R	I	5	MDR	E	A	+	
6	54	1.8.2003	Pleura	R	R	R	R	R	R	R	R	R	R	R	R	R	I	5	MDR	E	A	+	
7	51	4.8.2003	IV Cath.	R	R	R	R	R	R	R	R	R	R	R	R	R	I	5	MDR	E	A	+	
8	51	4.8.2003	Blood	R	R	R	R	R	R	R	R	R	R	R	R	R	I	5	MDR	E	A	+	
9	59	15.8.2003	Tr.asp.	R	R	R	R	R	R	R	R	R	R	R	R	R	R	6	MDR	E	A	+	
10	83	2.10.2003	Blood	R	R	R	R	R	R	R	R	R	R	R	R	R	R	6	MDR	E	A	+	
11	89	8.10.2003	Tr.asp.	R	S	S	S	R	S	S	S	R	S	S	I	S	S	7		E	A1	_	+
12	90	14.10.2003	Tr.asp.	S	S	S	S	I	S	S	S	S	S	S	S	S	S	8		B	D	_	+
13	90	14.10.2003	Blood	S	S	S	S	I	S	S	S	S	S	S	S	S	S	8		B	D	_	+
14	97	21.10.2003	Tr.asp.	S	S	R	R	R	S	S	R	S	R	S	S	S	S	9		B	E	_	+
15	106	3.11.2003	Tr.asp.	S	S	S	S	I	S	S	S	S	S	S	S	S	S	8		B	D1	_	+
16	108	10.11.2003	Tr.asp.	R	S	S	S	R	S	S	S	R	S	S	R	S	S	10		E	A2	_	+
17	107	25.11.2003	Tr.asp.	S	S	S	S	I	S	S	S	S	S	S	S	S	S	8		G	F	_	+
18	108	13.11.2003	Catheter	S	S	S	S	I	S	S	S	S	S	S	S	S	S	8		G	F	_	+
19	108	12.11.2003	Blood	S	S	S	S	I	S	S	S	S	S	S	S	S	S	8		G	F	_	+
20	109	25.11.2003	Tr.asp.	S	S	S	S	I	S	S	S	I	S	S	S	R	I	12		G	F	_	+
21	107	2.12.2003	B.Lav.	S	S	S	S	R	S	S	S	S	S	S	S	S	S	11		G	F	_	+
22	124	29.12.2003	Tr.asp.	R	R	R	R	R	R	R	R	R	R	R	R	R	R	6	MDR	E	A	+	
23	131	28.1.2004	Tr.asp.	R	R	R	R	R	R	R	R	R	R	R	R	R	R	6	MDR	E	A	+	
24	134	17.2.2004	Urine	R	R	R	R	R	R	R	I	R	R	R	R	R	R	13	MDR	E	A	+	
25	134	20.2.2004	Tr.asp.	R	R	R	R	R	R	R	R	R	R	R	R	R	R	6	MDR	E	A	+	
26	Staff	24.10.2003	Hand	R	R	R	R	R	R	R	R	I	S	R	R	R	I	14	MDR	E	A	+	
27	Staff	16.11.2003	Hand	S	S	S	S	I	S	S	S	I	S	S	S	S	S	15		G	F	_	+
28	Staff	16.11.2003	Hand	S	S	S	S	I	S	S	S	S	S	S	S	S	S	8		G	F	_	+

**Tr. Asp; tracheal aspirate, IV. Cath.; intravenous catheter, B. lav; bronch lavage.*
¢Ó IPM; imipenem, CRO; ceftriaxone, PTZ; Piperacilline/tazobactam, MEM; meropenem, OFX; ofloxacin, CAZ; ceftazidime, TOB; tobramicin, CIP; ciprofloxacin, FEP; cefepime, GN; gentamicin, AK; amikacin, PRL; piperacillin, ATM; aztreonam, NET; netilmicin.
R; resistant, I; intermediate susceptible, S; susceptible.
¢Ó; MDR; multidrug resistant
i×β lactamase; beta lactamase
i«NT; Non-typeable

Three of the other nine bacteria were in serogroup E; and according to PFGE, they had A, A1, and A2 patterns; however, there were no MBL enzymes and they had different antibiotic patterns and they were not MDR. 4 were in serogroup B; and according to PFGE, two had D, one had D1, and the other had E pattern. A bacterium was in serogroup A; and according to PFGE, it had B pattern. A bacterium which could not be typed via serotyping had C pattern according to PFGE; and the antibiotic pattern, in which only this bacterium included, was 3 (Table 2).

Figure 2a. Pulsed field gel electrophoresis patterns of A and subtips of A. Lane M; DNA molecular marker, Lane 1; isolate no 1, Lane 2; isolate no 4, Lane 3; isolate no 5, Lane 4; isolate no 6, Lane 5; isolate no 7, Lane 6; isolate no 8, Lane 7; isolate no 9, Lane 8; isolate 10, lane 9; isolate no 11, lane 10; isolate no 22, lane 11; isolate no 23, lane 12; isolate no 24, lane 13; isolate no 25, lane 14; isolate no 26.

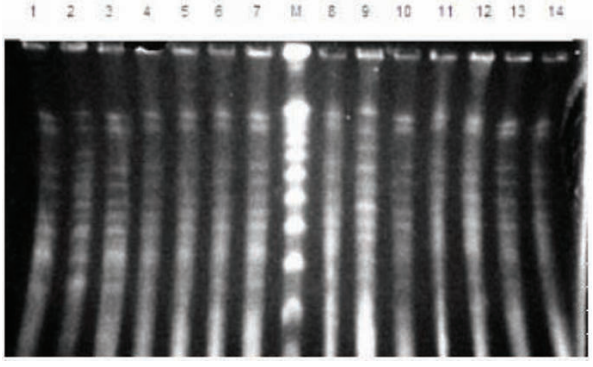
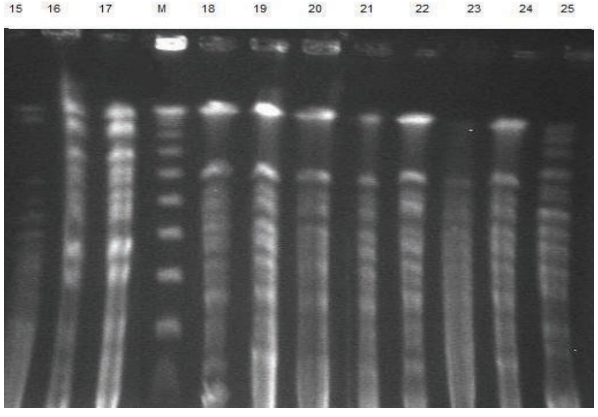


Figure 2b. Pulsed field gel electrophoresis patterns of the other strains. Lane M; DNA molecular marker, Lane 15; isolate no 3, Lane 16; isolate no 12, Lane 17; isolate no 13, Lane 18; isolate no 17, Lane 19; isolate no 18, Lane 20; isolate no 19, Lane 21; isolate no 20, Lane 22; isolate no 21, Lane 23; isolate no 27, Lane 24; isolate no 28, Lane 25; isolate no 16.



4. Discussion

Resistance rates are higher in *P. aeruginosa* isolates from cystic fibrosis patients and the patients in ICUs. And also resistance to β-lactams is more frequent in isolates from hospitalized patients than from the community [14].

MDR *P. aeruginosa* (MDR-PA) isolates are more common among ICU patient isolates than among non-ICU inpatient isolates. As in our university, outbreaks of MDR-PA inside and outside ICUs are an increasingly reported problem in hospitals [8]. 12 (42.9%) of all *P. aeruginosa* isolated in our hospital ICU were MDR-PA, and most of them were isolated from tracheal aspirates and were related with LRTIs.

Active penicillins against *P. aeruginosa* are azlocillin, carbenicillin, mezlocillin, piperacillin, and ticarcillin. Piperacillin is more effective than ticarcillin and karbenicillin. Resistance to piperacillin usually occurs during treatment [15]. By adding tazobactam, a beta-lactamase inhibitor, sensitivity may be increased [15]. In our study, 15 of the *P. aeruginosa* were resistant to piperacillin and while resistant to piperacillin, there were 3 strains susceptible to piperacillin/tazobactam. 12 of these 15 strains were MDR and serogroup E, 3 were not MDR, however, were again serogroup E. Chromosomal beta-lactamase of these 3 non-MDR origins was positive. In the others, no interaction was observed between the disks due to resistance. In the MDR origins, possibly there were OXA enzymes that were responsible for plasmid interagency resistance, and they were not affected from beta-lactamase inhibitors [16]. That the OXA enzymes were plasmid interagency showed the importance of having hard cautions in order to prevent expansion.

All of the MDR-PAs we isolated included metallo-beta-lactamase. These origins were resistant to imipenem and meropenem. Because the metallo-beta-lactamase enzyme hydrolyzes the cephalosporin and carbapenems, the bacteria which have this enzyme are expected to be resistant these antibiotics. However, the MBL enzyme is ineffective to aztreonam [17]. In our study, two of the MDR-PAs which included MBL were semi-sensitive to aztreonam, the other origins were resistant. So, in those enzymes there may be PER-1 enzyme and OXA type enzyme as well as MBL; or extensive AmpC may occur [18]. Vahapoglu *et al.* [19] stated that in the *P. aeruginosa* strains isolated from the hospitals in Turkey, there were PER-1 enzyme; and it is pointed out that it was responsible from the increase in death in nosocomial infections. Lee *et al.* [20] reported the sputum and urine were found to be the main sources of MBL-producing isolates as in our study. For this reason, whether carbapenem resistant bacteria include MBL enzyme or not should be investigated, the MBL producing bacteria should be prevented from expanding.

In the isolated *P. aeruginosa*, resistance to aminoglycoside was around 50%. The number of the origins sensitive to amikacin was higher while they were resistant to the other. In the other studies, as in our findings, the bacterium resistant to aminoglycoside is usually observed to be resistant to the other aminoglycoside except amikacin [21,22]. All MDRs were resistant to the four aminoglycoside. And if we accept the intermediate susceptible as resistant, resistance

to ciprofloxacin, ofloxacin, and carbapenems was detected. So, in those, extensive MexXY may occur, or these origins may include one of the AAC, ANT, APH enzymes which changes aminoglycoside with the other resistance mechanisms [23]. Another possibility is that aminoglycoside in the chromosomes of these origins may be activated with mutation by resistance gene *aphA* [15].

Except the *P. aeruginosa* isolated from the mucus of the patients with cystic fibrosis, that their serotyping is repeatable is important in epidemiologic studies [24]. In our study, as in the other studies, according to International Antigenic Typing Scheme (IATS), O6 (G serogroup/ Denka Seiken) and O11 (E serogroup/ Denka Seiken) serotypes were highly observed [25,26]. Similar to our study, there are studies pointing out origins which cannot be typed via serologic methods [11]. In our study, an origin which could not be serotyped was assessed separately because it was different from the others as antibiotype (type 3). Generally, the strains isolated from the same patient have the same serotype and same antibiotic pattern (Patient no 51, 90 in Table 2). When the antibiotic sensitivity of origins from the same patient with the same serotype but with different antibiotic pattern was investigated, the isolated origins were first observed to be intermediate susceptible; later, they became resistant (Patient no 107, 134 in Table 2). However, the origins isolated from the patient 108's catheter and blood cultures were identical, and the ones isolated from the tracheal aspirates were different from these. In the same serotype and the same antibiotic patterns isolated from different patients, strains including the same enzymes may be accepted to be carried from one patient to the other. One of the three *P. aeruginosa* isolated from the ICU staff's hand cultures was serogroup E, the other two were group G; and the strain typed as group E was MDR like the ones isolated from the patients. These findings make us think that the bacteria might have been carried to the patients via the hands of the staff. When the isolation dates considered, it was seen that same serotypes were isolated and the G serotype strains isolated from the patients were isolated from the staff synchronously. *P. aeruginosa* isolated between July and October was serogroup E and MDR-PA. Although it was not isolated from the patients between 2nd October and 29th December and 22 days after 2nd October, MDR-PA in serogroup E was isolated from one of the staff. And this showed that, although not in patients, this bacterium did exist in the ICU flora. At the end of December, that infections formed by MDR-PAs were again begun to be seen supports this thought.

Serious pseudomonas infections are generally accepted to be treated with two antibiotics with different mechanisms and with synergic effect [27]. Although their susceptibilities allow using two beta-lactams, it should not be preferred.

In our hospital ICU, in combine empiric treatment, mostly amikacin and piperracillin/tazobaktam, amikacin and 3rd generation cephalosporins, aminoglycoside and carbapenems, aminoglycoside and quinolones were preferred. The reason why resistance with aminoglycosides and quinolones were higher might be that these antibiotics were used ever so often. However, the amikacin/tazobactam combination used in empiric treatment is a correct preference for the origins other than MDR-PA origins. MDR bacteria were resistant to most of the antibiotics tried and were intermediate susceptible to some. In order to decide the antibiotics that are going to be used in the treatment of the infections formed with these bacteria, synergy tests may be beneficial. The important thing is to prevent the growth and expansion of the resistant strains. When MDR-PA is isolated, patients should surely be isolated.

It is known that the hospital infection factors are carried from patient to patient and from environment to patient via staff's hands and the tools used. In order to determine whether these is clonal distribution or not, the bacteria should be typed. For this purpose, antibiotic typing, serotyping, and molecular typing methods can be used. Antibiotic typing fails especially with bacteria which have multi-resistance. In this study, 15 different patterns were observed among 28 bacteria. With multi-resistant bacteria, especially PFGE has a high distinguishing power; however, it cannot be applied in every laboratory and it requires hard work. Serotyping can easily be applied in every laboratory via commercial kits. In this study, 11 from the patients and one from the staff, in total 12 MDR-PA were observed in the same serogroup and with the same PFGE pattern. Also, another group of bacteria, five from the patients and 2 from the staff, was observed to be in the same serogroup and with the same PFGE pattern. In the cases that molecular typing cannot be applied, serotyping may provide rapid information about the existence of clonal distribution.

At the end of the typing methods, two different clonal *P. aeruginosa* strains were found in our hospital. It is concluded that glove use and hand wash trainings should often be repeated and supervised.

Acknowledgements

This study was granted by Trakya University Scientific Research Projects (TUBAP 544).

References

[1] Kiska D.L., Gilligan P.H., Pseudomonas, In: Muray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH (Eds.), Manual of Clinical Microbiology. 8th ed., ASM Pres, Washington, 2003, 1970-1980

[2] Pollack M., Pseudomonas aeruginosa, In: Mandell GL, Bennett JE, Dolin R (Eds), Principles and Practice of Infectious Diseases. 4th ed., Churchill Livingstone, New York, 1995, 1980-2003

[3] Hancock R.E.W., Resistance mechanisms in Pseudomonas aeruginosa and other nonfermentative gram-negative bacteria, Clin. Infect. Dis., 1998, 27 (Suppl), 93-99

[4] Gaynes R.P., Horan T.C., Surveillance of nosocomial infections, In: Mayhall CG (ed), Hospital Epidemiology and Infection Control.1st ed. Lippincott Willams and Wilkins, Philadelphia, 1996, 1017-1037

[5] Strausbaugh L.J., Sewell D.L., Tjoelker R.C., Heitzman T., Webster T., Ward T. et al., Comparison of three methods for recovery of yeasts from hands of health-care workers, J. Clin. Microbiol., 1996, 34, 471–473

[6] Clinical and Laboratory Standards Institute (CLSI), Performance Standards for Antimicrobial Disk Susceptibility Tests, CLSI document M2-A9, (2006)

[7] Clinical and Laboratory Standards Institute (CLSI), Performance Standards for Antimicrobial Susceptibility Testing,CLSI document M100-S16 (2006)

[8] Karlowsky J.A., Draghi D.C., Jones M.E., Thornsberry C., Friedland I.R., Sahm D.F., Surveillance for antimicrobial susceptibility among clinical iosalates of Pseudomonas aeruginosa and Acinetobacter baumannii from hospitalized patients in the United States, 1998 to 2002, Antimicrob. Agents. Chemother., 2003, 47, 1681-1688

[9] Akata F., Otkun M., Teker B., Karabay O., Öğütlü A., Tuğrul M. et al., Frequencies of extended spectrum and chromosomal beta-lactamases in nosocomial gram-negative bacteria, Turkish Journal of Infection, 1997, 11, 256-259

[10] Goto S., Tsuji A., Oguri T., Kobayashi I., Nishida M., Yabuuchi E., Present situation of serotyping of Pseudomonas aeruginosa in Japan and correlation among three kinds of commercially available serotyping kits, J. Infect. Chemother., 1999, 5: 201-205

[11] Hernàndez J., Ferrús M.A., Hernàndez M., Owen R.J., Arbitrary primed PCR fingerprinting and serotyping of clinical Pseudomonas aeruginosa strains, FEMS Immun. Med. Microbiol., 1997, 17, 37-47

[12] Speijer H., Savelkoul P.H.M., Bonten M.J., Stobberingh E.E., Tjhie J.H.T., Application of different genotyping methods for Pseudomonas aeruginosa in a setting of endemicity in an intensive care unit, J. Clin. Microbiol., 1999, 37, 3654–3661

[13] Tenover F.C., Arbeit R.D., Goering R.V., Mickelsen P.A., Murray B.E., Persing D.H., et al., Interpreting Chromosomal DNA restriction patterns produced by Pulsed-Field Gel Electrophoresis: Criteria for bacterial strain typing, J. Clin. Microbiol., 1995, 33, 2233-2239

[14] Henwood C.J., Livermore D.M., James D., Warner M. and the Pseudomonas study group, Antimicrobial susceptibility of Pseudomonas aeruginosa: results of a UK survey and evaluation of the British Society for Antimicrobial Chemotherapy disk susceptibility test, J. Antimicrob. Chemother., 2001, 47, 789-799

[15] Swenson J.M., Hindler J.A., Peterson R.L., Antimicrobial agents and susceptibility testing. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH (Eds.), Manual of Clinical Microbiology. 8th ed. ASM Pres, Washington, 2003, 1178-1195

[16] Hall L.M.C., Livermore D., Gur D., Akova M., Akalın H.E., OXA-11, an extended-spectrum variant of OXA-10 (PSE-2), beta-lactamase from Pseudomonas aeruginosa, Antimicrob. Agents. Chemother., 1993, 37, 1637-1644

[17] Walsh T.R., Toleman M.A., Poirel L., and Nordmann P., Metallo-Lactamases: the quiet before the storm? Clin. Microbiol. Rev., 2005, 18, 306–325

[18] Pournaras S., Maniati M., Petinaki E., Tzouvelekis S., Tsakris A., Legakis N.J. et al., Hospital outbreak of multiple clones of Pseudomonas aeruginosa carrying the unrelated metallo-beta-lactamase gene variants blaVIM-2 and blaVIM-4, J. Antimicrob. Chemother., 2003, 51, 1409-1414

[19] Vahaboglu H., Coskunkan F., Tansel O., Oztürk R., Sahin N., Koksal I. et al., Clinical importance of extended-spectrum beta-lactamase (PER-1 type)-producing Acinetobacter spp, and Pseudomonas aeruginosa strains, J. Med. Microbiol., 2001, 50, 642-645

[20] Lee K., Lee W.G., Uh Y., Ha G.Y., Cho J.,Chong Y. et al., VIM and IMP-type metallo-beta-lactamase-producing Pseudomonas spp. and Acinetobacter spp. in Korean hospitals, Emerg. Infect. Dis., 2003, 9, 868-871

[21] Gales A.C., Jones R.N., Turnidge J., Rennie R., Ramphal R., Characterization of Pseudomonas aeruginosa isolates: occurance rates, antimicrobial susceptibility patterns, and molecular typing in

the Global SENTRY Antimicrobial Surveillance Program, 1997-1999, Clin. Infect. Dis., 2001, 32(Suppl 2), 146-155

[22] Shawar R.M., Macleod D.L., Garber R.L., Burns J.L., Stapp J.R., Clausen C.R. et al., Activities of tobramycin and six other antibiotics against Pseudomonas aeruginosa isolates from patients with cystic fibrosis, Antimicrob. Agents. Chemother., 1999, 43, 2877–2880

[23] Wolter D.J., Smith-Moland E., Goering R.V., Hanson N.D., Lister P.D., Multidrug resistance associated with mexXY expression in clinical isolates of Pseudomonas aeruginosa from a Texas hospital, Diag. Microbiol. Infec. Dis., 2004, 50, 43–50

[24] The International Pseudomonas aeruginosa Typing Study Group, A multicenter comparison of methods for typing strains of Pseudomonas aeruginosa predominantly from patients with cystic fibrosis, J. Infect. Dis., 1994, 169, 134-142

[25] Tassios P.T., Gennimata V., Spaliara-Kalogeropoulou L., Kairis D., Koutsia C., Vatopoulos A.C. et al., Multiresistant Pseudomonas aeruginosa serogroup O11 outbreak in an intensive care unit, Clin. Microbiol. Infect., 1997, 3, 621-628

[26] Vale T.A., Gaston M.A., Pitt T.L., Subdivision of seroypes of Pseudomonas aeruginosa with monoclonal antibodies, J. Clin. Microbiol., 1988, 26, 1779-1782

[27] Wu Y.L., Scott E.M., Po A.L.W., Tariq V.N., Ability of azlocillin and tobramycin in combination to delay or prevent resistance development in Pseudomonas aeruginosa, J. Antimicrob. Chemother., 1999, 44, 389-392