Determination of PER-1 and OXA-10-like β-lactamases in Ceftazidime-Resistant *Pseudomonas aeruginosa* Isolates by Molecular Methods

PER-1 ve OXA-10 Benzeri β-laktamazların Seftazidime Dirençli *Pseudomonas aeruginosa* İzolatlarında Moleküler Yöntemler ile Saptanması

Mehtap ÜNLÜ SÖĞÜT,[a]
Tuba YILDIRIM,[b]
Asuman BİRİNCİ,[a]
Belma DURUPINAR[a]

[a]Department of Medical Microbiology, Ondokuz Mayıs University Faculty of Medicine, Samsun
[b]Department of Biology, Amasya University Faculty of Science, Amasya

Geliş Tarihi/*Received:* 14.12.2010
Kabul Tarihi/*Accepted:* 02.08.2011

Yazışma Adresi/*Correspondence:*
Asuman BİRİNCİ
Ondokuz Mayıs University Faculty of Medicine, Department of Medical Microbiology, Samsun, TÜRKİYE/TURKEY
asumanbirinci@yahoo.com

ABSTRACT Objective: *Pseudomonas aeruginosa* is one of the important nosocomial pathogens and resistant to many antibiotics including β-lactams. PER-1 and OXA-10 type extended-spectrum β-lactamases (ESBLs), the major β-lactamases, were identified in *P. aeruginosa*. The aim of this study was to identify PER-1 (blaPER-1) and OXA-10 (blaOXA-10)-like β-lactamases in ceftazidime- resistant nosocomial *P. aeruginosa* strains. **Material and Methods:** The presence of PER-1 and OXA-10 like β-lactamases was investigated by polymerase chain reaction in 50 ceftazidime-resistant *P. aeruginosa* strains isolated from patients hospitalized in various clinics of Ondokuz Mayıs University, School of Medicine between 2007 and 2008. The PER and OXA-10-like β-lactamases were analyzed by restriction fragment length polymorphism (RFLP) and followed by pulsed-field gel electrophoresis (PFGE) for the determination of clonal relationship of the strains. **Results:** The blaPER-1 gene and blaOXA-10 like gene were detected in 23 (46%) and 39 (78%) of the 50 of ceftazidime-resistant *P. aeruginosa* isolates, respectively. In addition, both of the two ß-lactamase genes were also detected in 12 (23%) of the isolates. PER-1 and OXA-10, -11, -14, -16 types were identified by RFLP analysis. Although the PFGE typing results yielded 11 different banding patterns, 74% (n= 37) of the all *P. aeruginosa* strains were included in four main patterns. **Conclusion:** It was concluded that the prevalence of PER-1 and OXA-10 enzymes was common among ceftazidime resistant *P. aeruginosa* isolates. PER-1 and OXA-10 enzymes produced the isolates were being transmitted horizontally as the most of the isolates were clonally related.

Key Words: Beta-lactamases; *Pseudomonas aeruginosa*; molecular researches

ÖZET Amaç: *Pseudomonas aeruginosa* en önemli nozokomiyal patojenlerden biridir ve β-laktamları da kapsayan biçimde birçok antibiyotiğe dirençlidir. PER-1 ve OXA-10 tipinde geniş spektrumlu β-laktamazlar (ESBL'ler), major β-laktamazlar olarak *P. aeruginosa* suşlarında tanımlanmıştır. Bu çalışmanın amacı seftazidime dirençli nozokomiyal *P. aeruginosa* türlerinde PER-1 (blaPER-1) ve OXA-10 (blaOXA-10) gibi β-laktamazları tanımlamaktır. **Gereç ve Yöntemler:** PER-1 and OXA-10 gibi β-laktamazların varlığı 2007 ve 2008 yılları arasında Ondokuz Mayıs Tıp Fakültesi'nin çeşitli kliniklerinde yatan hastalardan izole edilen 50 seftazidime dirençli *P. aeruginosa* türünde PCR ile araştırıldı. PER ve OXA-10 benzeri β-laktamazlar türlerin klonal ilişkisinin saptanması için kesilen parça uzunluk polimorfizmi (RFLP) ile analiz edildi ve pulsed-field jel elektroforezi (PFGE) ile tiplendirildi. **Bulgular:** blaPER-1 geni ve blaOXA-10 benzeri gen seftazidime dirençli 50 P. aeruginosa türünün 23'ünde (%46) ve 39'unda (%78) sırasıyla saptandı. Ek olarak ayrıca her iki β-laktamaz geni izolatların 12'sinde (%23) saptandı. PER-1 ve OXA-10, -11, -14, -16 tipleri RFLP analizi ile tanımlandı. PFGE tipleme sonuçları 11 farklı bantlanma paterni vermesine rağmen tüm *P. aeruginosa* suşlarının %74'ünün (n= 37) dört ana patern içinde olduğu gözlenmiştir. **Sonuç:** PER-1 and OXA-10 enzimlerinin prevalansının seftazidime dirençli *P. aeruginosa* izolatları içinde sık olduğu sonucuna varıldı. İzolatların çoğu klonal olarak ilişkiliyken PER-1 and OXA-10 enzimleri yatay olarak iletilen izolatlar oluşturdular

Anahtar Kelimeler: Beta-laktamazlar; *Pseudomonas aeruginosa*; moleküler araştırmalar

doi:10.5336/medsci.2010-22180

Turkiye Klinikleri J Med Sci 2011;31(5):1227-35

Pseudomonas aeruginosa is not only one of the most important nosocomial pathogens being responsible for various types of infections including pneumonia and urinary tract infections, but is also notably resistant to many antibiotics including β-lactams.[1-3] Three types of β-lactam resistance have been described to be predominant including production of β-lactamases, loss of outer membrane proteins and up-regulation of efflux pumps.[4] β-lactamases with broad spectrum activity have been reported in *P. aeruginosa*. These enzymes are clavulanic acid-inhibited ESBLs of Ambler class A, metallo of Ambler class B, and expanded-spectrum oxacillinases of Ambler class D.[5,6] Among these enzymes, PER-1 and OXA-10 type extended spectrum β-lactamases (ESBLs) are the major β-lactamases identified in *P. aeruginosa*.[7]

Five types of class A ESBLs (PER-, VEB-, GES-, and IBC-, TEM-and SHV-type) were recently reported in *P. aeruginosa*.[3] Two PER types were described previously. PER-1 was identified first in a *P. aeruginosa* isolate in 1991 recovered in France from a Turkish patient.[1] Poor outcome has been reported as a result of infection caused by PER-1 producers.[4] PER-1 has strong hydrolytic activity against cephalosporin, but like most ESBLs of the TEM and SHV type it cannot hydrolyse carbapenems and cephamycins.[5,8] OXA- type ESBLs, which comprises class D, show extreme sequence variation varying from 16 to 99% between individual enzymes. The ESBLs OXA-11, OXA-14, OXA-16, OXA-17, OXA-19 and OXA-28 are related structurally to OXA-10, whereas OXA-15 and OXA-32 are related to OXA-2.[5,9] The activity of these serin β-lactamases is inhibited weakly by clavulanic acid, unlike that of OXA-18, which is inhibited strongly by clavulanate.[5,10] The epidemiology of these enzymes is unknown, and their isolation may reflect the interest of the research team more than a specific distribution of these expanded-spectrum enzymes.[5]

Several schemes for the molecular typing of *P. aeruginosa* have been proposed to determine the relatedness of nosocomial pathogens. These include PFGE, ribotyping and PCR-based fingerprinting among which PFGE is accepted as the 'gold standard'.[11,12]

In the present study, our aim was to investigate the prevalence of bla_{PER-1} and bla_{OXA-10} related β-lactamase genes in clinical isolates of *P. aeruginosa* strains from patients hospitalized in the Ondokuz Mayıs University School of Medicine during a 12-month period by molecular methods in order to analyze their clonal relationship.

MATERIAL AND METHODS

BACTERIAL STRAINS

All of 571 clinical isolates of *P. aeruginosa* were obtained in the Microbiology Laboratories of Ondokuz Mayıs University School of Medicine, between January 2007 and January 2008. Only 50 clinical isolates of *P. aeruginosa* resistant to beta-lactam and additive inhibitory beta-lactam antibiotics, including ceftazidime, recovered from patients who were hospitalized in various clinics of Ondokuz Mayıs University School of Medicine were included in this study. The isolates were identified using conventional methods (colony morphology, oxidase test and biochemical reactions) and the VITEK automated identification system (BioMérieux, Marcy I'Etoile, France) and were also confirmed by API ID32 GN system (BioMérieux, Marcy I'Etoile, France). The isolates were stored at -80 °C in 15% glycerol until they will used in the study.

ANTIMICROBIAL SUSCEPTIBILITY TESTİNG AND SCREENING FOR ESBLs

Antimicrobial susceptibility testing of 50 clinical isolates of *P. aeruginosa* was performed by disk diffusion method on Mueller-Hinton agar (Oxoid, Hampshire, England) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines using aztreonam (30 μg), and cefepime (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), imipenem (10 μg), meropenem (10 μg), piperacillin (100 μg), piperacillin-tazobactam (100-10 μg).[13] The antimicrobial agents were purchased from Oxoid, Hampshire, England. *P. aeruginosa* ATTC 27853 was used as reference strain.

ESBL production was tested with the double-disc synergy test (DDST) on Mueller-Hinton agar. After overnight culture, test isolates were suspended to the turbidity of a 0.5 McFarland Stan-

dard and used to inoculate a Mueller-Hinton agar plate. After drying, containing discs of ceftazidime, aztreonam, piperacillin and cefepime (30 µg each) were placed 20 or 30mm apart from a disc containing amoxicillin/clavulanic acid (20/10 µg).[14] ESBL production was inferred when the cephalosporin zone was expanded by the clavulanate.

DETECTION OF PER-1 GENE AND OXA-10-LIKE GENE BY POLYMERASE CHAIN REACTION (PCR)

Total DNA was extracted using a QIA amp DNA mini kit (Qiagen, Hilden, Germany) from the *P. aeruginosa* isolates which were grown on Muller-Hinton agar plates at 36 °C overnight as described by manufacturer. The oligonucleotide primers (Tib Molbiol, Berlin, Germany) for the detection of bla_{PER-1} and bla_{OXA-10}-like genes were used. While PCR amplification of the bla_{PER-1} gene was with primers PERA, 5'-ATG AAT GTC ATT ATA AAA GC-3', and PERD, 5'-AAT TTG GGC TTA GGG CAG AA - 3', yielding a 926 bp product, bla_{OXA-10}-like genes ($bla_{OXA-10, -11, -14, -16, -17}$) with primers OPR1, 5'-GTC TTT CGA GTA CGG CAT TA-3', and OPR2, 5'-ATT TTC TTA GCG GCA ACT TAC-3', yielding a 720 bp product. bla_{PER-1} gene and bla_{OXA-10}-like genes were sought among the isolates by PCR assay according to Aktas et al. Amplification reactions were carried out in final volume of 50 µL and following parameters: 94 ºC for 5 min of initial denaturation; 35 cycles of 95 ºC for 45 sec, 56 ºC for 45 sec, and 72 ºC for 1 min, and a final extension at 72 ºC for 7 min.[5] The MultiBlock PCR System (Thermo, CA, USA) was used for gene amplification. PCR products were loaded onto 2% agarose gel containing 1X TBE and stained with 5 µg/ml ethidium bromide. A 100 bp DNA Ladder Plus (Fermentas, Vilnius, Lithuania) was used to provide molecular size markers, and photographed using a Gel/ChemiDoc XRS system (Bio-Rad Laboratories, CA, USA). Antimicrobial susceptibility testing PER-1 and OXA-10 positive isolates were carried out by microdilution method on Mueller-Hinton broth (Oxoid, Hampshire, England) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The results were interpreted using CLSI criteria for broth dilution.[13]

IDENTIFICATION OF ISOLATES BASED ON BLAPER-1 GENE AND BLA_{OXA-10} GENE BY RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) ANALYSIS

bla_{PER-1}, bla_{PER-2}, and bla_{OXA-10} (discriminates bla_{OXA-11}, bla_{OXA-14}, bla_{OXA-16} from bla_{OXA-10}) gene products were identified by RFLP analysis. PER-1 and PER-2 were digested with 10U of PvuII and StuI also OXA-10 with 10U of PvuII and HaeIII (Fermentas, Vilnius, Lithuania) followed by incubation for 1-16 h at 37°C in 20 µl volumes. The products were separated by electrophoresis in a 3% agarose gel. A 100 bp DNA Ladder Plus was used to provide molecular size markers, and photographed using a Gel/ChemiDoc XRS.

PULSED FIELD GEL ELECTROPHORESIS (PFGE) ANALYSIS

PFGE typing was performed by the method optimized previously by Kayabas et al., with minor modifications.[12] Briefly, *P. aeruginosa* colonies were grown on tryptic soy agar overnight, at 37 ºC. The cells were suspended in 1 ml of Tris-EDTA buffer (100 mM Tris-HCl, 100 mM EDTA pH: 8), and the optical density was adjusted to 1 (λ= 590 nm) in spectrophotometer (WPA UV 1101, Cambridge, U.K). The cells were embedded into (2% wt/vol) low melting point agarose. Whole-cell DNA in the agarose plugs was digested with 20U of SpeI restriction enzyme (Fermentas, Vilnius, Lithuania) for 2 h at 37°C in water bath. The separation of DNA fragments was performed in 1% pulsed-field certified agarose gel (Bio-Rad Laboratories, Nazareth, Belgium) run in 0.5X Tris-borate- EDTA buffer (44.5 mM Tris, 44.5 mM boric acide, 1 mM EDTA, pH: 8.4) by using a CHEF Mapper system (Bio-Rad Laboratories, CA, USA). Fifty µM thiourea was added into the both of the agarose gel and the running buffer. The electrophoresis conditions were set for voltage 6 V/cm at 14°C for 20 h and switch angle of 120° using initial and final pulse times of 5 and 45 seconds, ramped in a linear fashion. After electrophoresis, the gel was stained with ethidium bromide (0.5 µg/ml) and photographed using a Gel/ChemiDoc XRS. PFGE banding patterns were analyzed by Quantity One® Software (Bio-Rad Laboratories, CA, USA) in order to define the pattern, and were compared by unweighted pair group method with arithmetic averages (UPGMA) method with the cut-off value of 85%.

RESULTS

IDENTIFICATION OF STRAINS AND DETERMINATION OF CEFTAZIDIME RESISTANCE

Out of the 571 clinical isolates of *P. aeruginosa*, 166 (30%) were ceftazidime-resistant. Fifty clinical isolates of *P. aeruginosa* resistant to ceftazidime were included in the study. The PFGE-typed clinical isolates of *P. aeruginosa* (n= 50) were resistant to (100%) aztreonam, (100%) cefotaxime, (88%) cefepime, (74%) imipenem, (70%) meropenem, (64%) piperacillin, and (50%) piperacillin-tazobactam. Only 32 (64%) of the 50 isolates were positive in the DDST. Fifty of clinical isolates of *P. aeruginosa* were studied in detail (Table 1).

PER-I AND OXA-10 TYPING

Twenty-three (46%) of the 50 ceftazidime-resistant *P. aeruginosa* isolates were positive by PCR for the bla_{PER-1} gene, 39 (78%) were positive for a bla_{OXA-10}-like gene ($bla_{OXA-10, -11, -14 -16}$), and 12 (23%) were positive for both β-lactamase genes (Table 1). There gene regions of *P. aeruginosa* isolates with positive and negative control strains were shown in Figure 1 and 2. Antibiotic resistance phenotypes according to bla_{PER-1} occurrences were shown in Table 2. This comparison demonstrated no significant differences phenotypes between bla_{PER-1} positive and negative isolates.

PCR-RFLP ANALYSIS

bla_{PER-1} amplification product was not digested with StuI, and bla_{PER-2} amplification product was not digested with PvuII restriction enzyme. Amplification products from PER positive 23 isolates were digested to four fragment (450, 230, 158 and 85 bp) with PvuII and it was assumed that they were the product of bla_{PER-1} gene. Amplification products from 39 OXA-10 positive isolates were digested into two fragments (408 and 312 bp) by PvuII indicating that the alleles were bla_{OXA-10} and two fragments (524 and 196 bp) with HaeIII, it was assumed to be $bla_{OXA-11,}$ bla_{OXA-14} or bla_{OXA-16}.

PFGE ANALYSIS

All 50 ceftazidime-resistant *P. aeruginosa* isolates were genotyped by using PFGE. Figure 1 shows PFGE typing results in a dendrogram. In total, 11 different patterns were identified among isolates. There were four main patterns as shown in the Figure 3. Pattern K consisting of 14 isolates was found to be dominant type in the hospital. Of the 11 isolates in this pattern were from pediatric wards and all of them were positive for $bla_{OXA-11,}$ bla_{OXA-14} or bla_{OXA-16}. Pattern J consisting of 10 isolates was from different types of specimens submitted from various wards. Pattern I consisting of eight isolates was from urology wards and all of them were positive for $bla_{PER-1.}$ Pattern H consisting of six isolates were from various wards and specimen. Other strains were grouped in patterns G (n= 3), E (n= 3), B (n= 2) from various wards. Patterns A, C, D, F each contained single isolates. The results of study were summarized in Table 1.

DISCUSSION

P. aeruginosa is one of the bacterial species most frequently responsible for nosocomial infections and is notably resistant to many antibiotics including β-lactams.[3] Ceftazidime resistance is most often caused by hyper-production of the chromosomal AmpC β-lactamase or acquired ESBLs. Ceftazidime resistance rates of 18-36% have been reported previously in *P. aeruginosa* isolates from hospitalized patients in Turkey.[5,7,15,16] In the present study we found that ceftazidime resistance rate was 30%.

PER-1 is an ESBL conferring high-level ceftazidime. Detection of this ESBL in *P. aeruginosa* and prevention of its spread is important for three reasons: (i) it confers resistance to most β-lactams, including aztreonam and newer antipseudomonal cephalosporins (ie, ceftazidime and cefepime),[5,17,18] (ii) it may be carried on a plasmid that has been transferred in vitro from PER-1 positive *P. aeruginosa* to PER-1 negative strains of the same species,[19] and (iii) unlike other class A β-lactamases of *P. aeruginosa*, PER-1 appeared to be transmissible among different species in Turkey.[17,20]

TABLE 1: Clinical features associated with ceftazidime-resistant *P. aeruginosa* isolates.

Number of isolates	Clinics	Specimen	DDST	Ceftazidime MIC (mg/L)	PER-1	OXA-10	PFGE
13	Pediatrics	TA	+	128-R	-	+	K
19	Pediatrics	TA	+	512-R	-	+	K
24	Pediatrics	C	-	512-R	-	+	K
26	Pediatrics	TA	+	256-R	-	+	G
31	Pediatrics	U	-	128-R	-	+	K
33	Pediatrics	TA	+	64-R	-	+	F
34	Pediatrics	S	+	256-R	-	+	K
38	Pediatrics	TA	-	≥1024-R	-	+	K
39	Pediatrics	C	+	512-R	-	+	K
40	Pediatrics	W	+	128-R	+	+	K
41	Pediatrics	TA	-	256-R	-	+	K
43	Pediatrics	U	+	512-R	-	+	J
44	Pediatrics	TA	+	64-R	-	+	K
45	Pediatrics	C	-	128-R	-	+	K
1	Urology	U	+	≥1024-R	+	-	I
10	Urology	C	+	512-R	+	-	I
11	Urology	U	-	128-R	+	+	J
12	Urology	U	-	256-R	-	+	H
15	Urology	U	+	256-R	+	+	I
23	Urology	TA	+	≥1024-R	-	+	A
27	Urology	W	-	128-R	-	+	C
28	Urology	U	+	64-R	+	+	H
3	Urology	U	+	512-R	+	-	I
35	Urology	U	+	≥1024-R	+	+	J
4	Urology	W	+	≥1024-R	+	-	I
47	Urology	U	-	128-R	+	-	I
6	Urology	U	-	128-R	+	-	I
9	Urology	U	-	256-R	+	+	I
14	Intensive care	TA	+	512-R	+	+	G
17	Intensive care	U	+	256-R	+	-	B
22	Intensive care	TA	+	128-R	+	+	J
25	Intensive care	PF	-	64-R	+	+	H
42	Intensive care	TA	-	256-R	+	-	J
49	Intensive care	S	+	64-R	-	+	B
8	Intensive care	CSF	+	128-R	+	-	E
2	Internal medicine	U	+	≥1024-R	+	-	H
30	Internal medicine	C	-	256-R	-	+	H
46	Internal medicine	U	-	256-R	+	+	K
48	Internal medicine	B	+	512-R	-	+	E
36	Cardiovascular surgery		-	128-R	+	+	K
37	Cardiovascular surgery		+	256-R	+	+	J
5	Cardiovascular surgery		+	64-R	-	+	K
16	Plastics surgery	W	+	128-R	-	+	D
18	Plastics surgery	W	-	64-R	-	+	E
20	General surgery	W	+	256-R	-	+	H
50	Neurology	S	+	256-R	+	-	J
32	Neurosurgery	U	+	512-R	-	+	J
21	Orthopedics	W	-	64-R	-	+	J
7	Otorhino laryngology	W	+	≥1024-R	-	+	G
29	Thoracic surgery	TA	+	64-R	-	+	J

U; urine, W; wound, TA; tracheal aspirate, CSF; cerebrospinal fluid, C; catheter, PF; pleural fluid, S; sputum, B; blood, MIC; minimum inhibitory concentration, PFGE: Pulsed field gel electrophoresis.

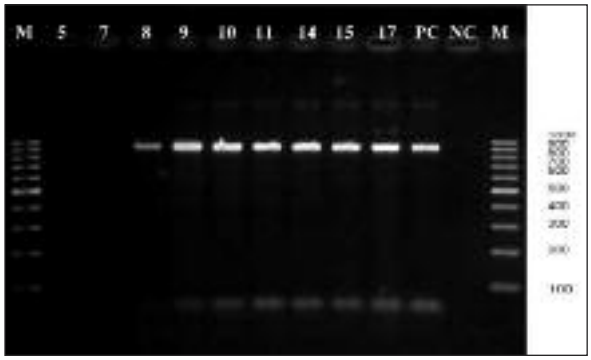

FIGURE 1: Images of 960 bp *PER*-1 gene regions of *P. aeruginosa* isolates.
5,7: Negative strains, 8,9,10,11,14,15,17: Positive strains, PC: Positive control strain, NC: Negative control strain, M: Marker.

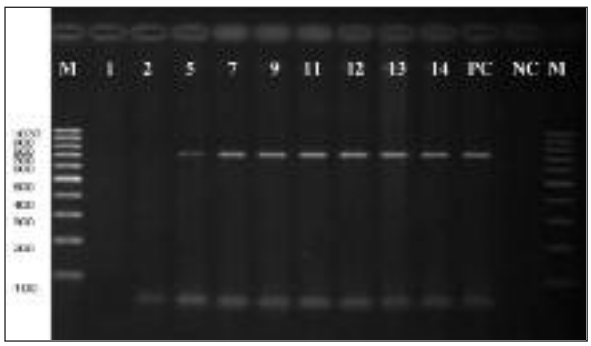

FIGURE 2: Images of 720 bp *OXA*-10 gene regions of *P. aeruginosa* isolates.
1,2: Negative strains, 5,7,9,11,12,13,14: Positive strains, PC: Positive control strain, NC: Negative control strain, M: Marker.

TABLE 2: Antibiotic resistance phenotypes of bla_{PER-1}, positive and negative isolates.

Antimicrobial agent	PER (+) R rate (%)	PER (-) R rate (%)
CAZ	100	100
TZP	39.1	59.2
FEP	95.6	81.4
PRL	60.8	66.6
CTX	100	100
ATM	100	100
IPM	69.5	77.7
MEM	69.5	70.3

CAZ: ceftazidime, TZP: piperacillin-tazobactam, FEP: cefepime, PRL: piperacillin, CTX: cefotaxime, ATM: aztreonam, IPM: imipenem, MEM: meropenem.

Studies conducted in Turkey indicated that PER-1 production rates were high among ceftazidime resistant *P. aeruginosa* strains. Kolaylı et al., reported the PER-1 presence in 55.4% of ceftazidime-resistant *P. aeruginosa* strains, isolated in intensive care units (ICUs) of seven university hospitals.[7] In another study, 86% ceftazidime-resistant ICU (Turkey)-isolated strains of *P. aeruginosa* were found to be PER-1 positive.[5] Erac and Gulay found the percentage of PER-1 of ceftazidime-resistant *P. aeruginosa* strains as 46.2%.[21] In the present study, the percentage of PER-1 of ceftazidime resistant *P. aeruginosa* strains was found as 46%, similar to other studies.

The OXA-10 derivates all had increased resistance to β-lactams, especially to ceftazidime. Ceftazidime-resistance is correlated with hydrolytic activity. OXA- 10 derivates (OXA-11,-14,-16,-17) were first reported in *P. aeruginosa* strains from Turkey and have been found frequently among *P. aeruginosa* strains.[5,18,19] Similarly, we found that 78% of ceftazidime-resistant *P. aeruginosa* strains were positive for OXA-10 and also OXA-11,-14,-16 except OXA-17.

The results of the present study indicated that PER-1 was present in 46%, and OXA-10 in 78% of the *P. aeruginosa* isolates, with 23% producing both β-lactamase genes. The collection in this study represents only ceftazidime-resistant isolates. According to some of the previous studies, *P. aeruginosa* isolates with a ceftazidime-resistant and piperacillin susceptible phenotype may be suspected of carrying the bla_{PER-1} gene since PER-1 shows poor activity against piperacillin.[5,22] In the present study, resistance to piperacillin and other major antibiotics was found to be extremely high similar to previous studies in Turkey.[5,7] Other resistance mechanism might also be present. No significant differences were found in antibiotic resistance phenotypes between bla_{PER-1} positive and negative isolates, as also reported in another report.[7] The double disk synergy test may fail to detect strains that produce PER-1 and OXA-10 derived enzymes, and only 32 (64%) of the 50 isolates had positive double disk synergy test DDST in our study similar to other studies.[5,23]

P. aeruginosa has emerged as an important nosocomial pathogen and frequently colonizes in hospitalized patients at rates exceeding 50%, and

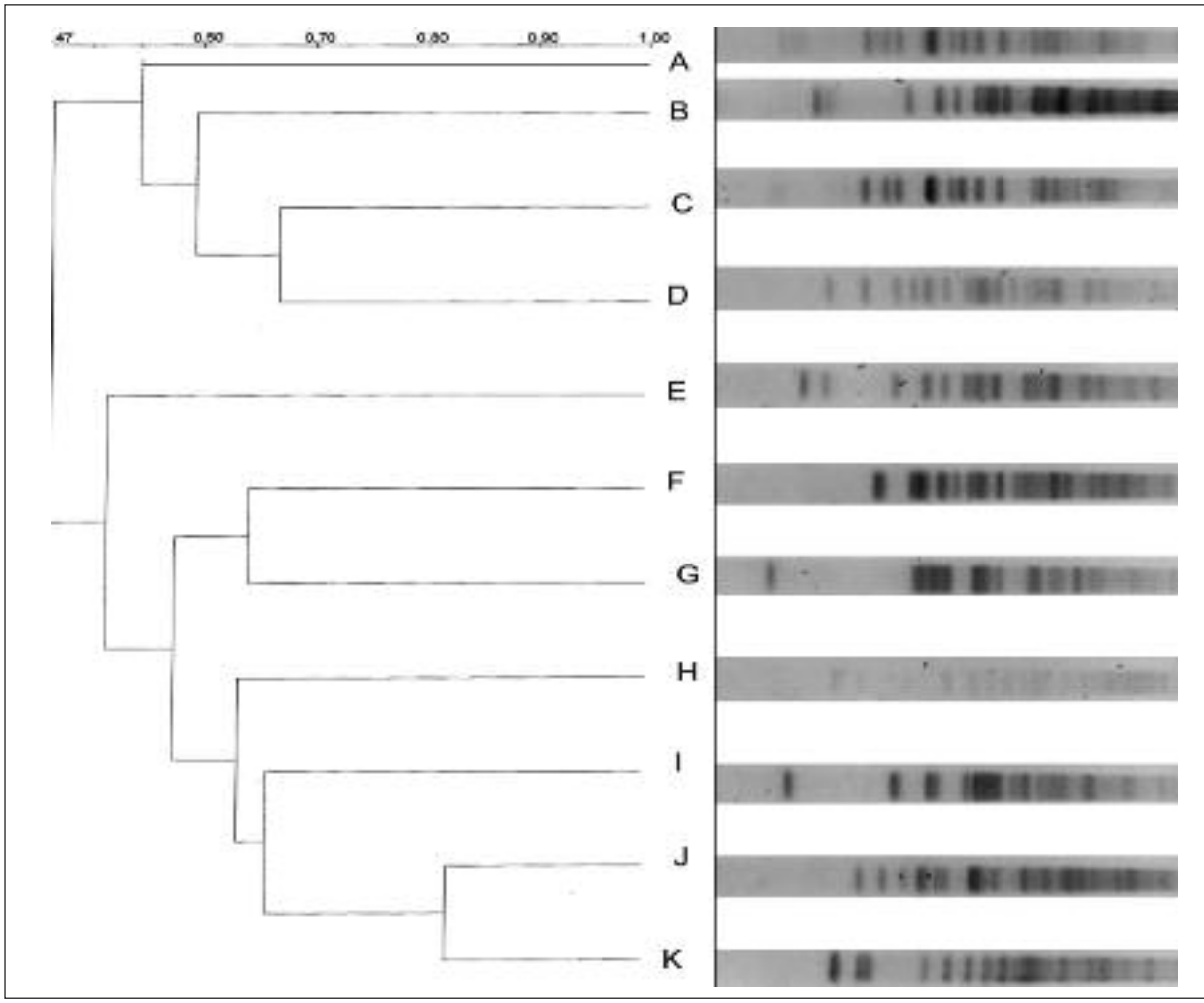

FIGURE 3: Presentation of clonal association of P. aeruginosa strains by dendrogram of pulsed field gel electrophoresis results.

colonization often presages invasive infection.[24] Its high intrinsic resistance to antimicrobials and ability to develop multidrug resistance poses serious therapeutic problems.[24,25] Investigations of *P. aeruginosa* clones and resistance patterns are particularly useful in patient management and maintenance of infection control procedures.[26] International epidemiologic surveillance requires reliable techniques that can differentiate unrelated strains from clonally related ones. PFGE has been accepted as a "gold standard" for confirming relatedness among *P. aeruginosa* strains.[26,27] In the present study, PFGE typing results of 50 *P. aeruginosa* strains yielded 11 different banding patterns. There were four main patterns which comprised 74% (n= 37) of the total *P. aeruginosa* strains. The first pattern (K) consisted of 13 isolates which were positive for $bla_{OXA-11,}$ bla_{OXA-14} or bla_{OXA-16}. This pattern was probably endemic as it derived from various specimens submitted from pediatric patients, suggesting nosocomial spread in pediatric wards. Pattern I (n= 8) were isolated from urine (n= 6), catheter (n= 1), wound (n= 1), taken from urology wards and all of the isolates in this pattern were positive for $bla_{PER-1.}$ Pattern H and J consisted of 16 isolates were from various wards and specimens. These patterns were may be endemic in our hospital. This may reflect cross-infection or acquisition from a common or point source. However, no conclusion can be

drawn regarding the source of infection for the reason that environmental specimens were not available for testing.

CONCLUSION

The results of the present study show that isolates of *P. aeruginosa* producing PER-1 and OXA-10 β-lactamases are encountered frequently in our hospital, and that their clonal diversity and high prevalence indicate a considerable potential for nosocomial spread among patients. In conclusion, it can be mentioned that efforts should be focused on tracing the source of infections to be able to control nosocomial infections and design strategies to diminish the nonspecific use of broad spectrum of antibiotics in the hospitals.

REFERENCES

1. Empel J, Filczak K, Mrówka A, Hryniewicz W, Livermore DM, Gniadkowski M. Outbreak of Pseudomonas aeruginosa infections with PER-1 extended-spectrum beta-lactamase in Warsaw, Poland: further evidence for an international clonal complex. J Clin Microbiol 2007; 45(9):2829-34.
2. Pagani L, Mantengoli E, Migliavacca R, Nucleo E, Pollini S, Spalla M, et al. Multifocal detection of multidrug-resistant Pseudomonas aeruginosa producing the PER-1 extended-spectrum beta-lactamase in Northern Italy. J Clin Microbiol 2004;42(6):2523-9.
3. Lee S, Park YJ, Kim M, Lee HK, Han K, Kang CS, et al. Prevalence of Ambler class A and D beta-lactamases among clinical isolates of Pseudomonas aeruginosa in Korea. J Antimicrob Chemother 2005;56(1):122-7.
4. Szabó D, Szentandrássy J, Juhász Z, Katona K, Nagy K, Rókusz L. Imported PER-1 producing Pseudomonas aeruginosa, PER-1 producing Acinetobacter baumanii and VIM-2-producing Pseudomonas aeruginosa strains in Hungary. Ann Clin Microbiol Antimicrob 2008;7:12.
5. Aktaş Z, Poirel L, Salcioğlu M, Ozcan PE, Midilli K, Bal C, et al. PER-1- and OXA-10-like beta-lactamases in ceftazidime-resistant Pseudomonas aeruginosa isolates from intensive care unit patients in Istanbul, Turkey. Clin Microbiol Infect 2005;11(3):193-8.
6. Weldhagen GF, Poirel L, Nordmann P. Ambler class A extended-spectrum beta-lactamases in Pseudomonas aeruginosa: novel developments and clinical impact. Antimicrob Agents Chemother 2003;47(8):2385-92.
7. Kolayli F, Gacar G, Karadenizli A, Sanic A, Vahaboglu H; Study Group. PER-1 is still widespread in Turkish hospitals among Pseudomonas aeruginosa and Acinetobacter spp. FEMS Microbiol Lett 2005;249(2):241-5.
8. Danel F, Hall LM, Gur D, Akalin HE, Livermore DM. Transferable production of PER-1 beta-lactamase in Pseudomonas aeruginosa. J Antimicrob Chemother 1995;35(2):281-94.
9. Danel F, Hall LM, Duke B, Gur D, Livermore DM. OXA-17, a further extended-spectrum variant of OXA-10 beta-lactamase, isolated from Pseudomonas aeruginosa. Antimicrob Agents Chemother 1999;43(6):1362-6.
10. Philippon LN, Naas T, Bouthors AT, Barakett V, Nordmann P. OXA-18, a class D clavulanic acid-inhibited extended-spectrum beta-lactamase from Pseudomonas aeruginosa. Antimicrob Agents Chemother 1997;41(10): 2188-95.
11. Johnson JK, Arduino SM, Stine OC, Johnson JA, Harris AD. Multilocus sequence typing compared to pulsed-field gel electrophoresis for molecular typing of Pseudomonas aeruginosa. J Clin Microbiol 2007;45(11):3707-12.
12. Kayabas U, Bayraktar M, Otlu B, Ugras M, Ersoy Y, Bayindir Y, et al. An outbreak of Pseudomonas aeruginosa because of inadequate disinfection procedures in a urology unit: a pulsed-field gel electrophoresis-based epidemiologic study. Am J Infect Control 2008;36(1): 33-8.
13. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically; approved standard. 7th ed. CLSI Document. Wayne, PA: Clinical and Laboratory Standards Institute; 2006. M7-A7.
14. Jiang X, Zhang Z, Li M, Zhou D, Ruan F, Lu Y. Detection of extended-spectrum beta-lactamases in clinical isolates of Pseudomonas aeruginosa. Antimicrob Agents Chemother 2006; 50(9):2990-5.
15. Günseren F, Mamikoğlu L, Oztürk S, Yücesoy M, Biberoğlu K, Yuluğ N, et al. A surveillance study of antimicrobial resistance of gram-negative bacteria isolated from intensive care units in eight hospitals in Turkey. J Antimicrob Chemother 1999;43(3):373-8.
16. Gonlugur U, Bakici MZ, Ozdemir L, Akkurt I, Icagasioglu S, Gultekin F. Retrospective analysis of antibiotic susceptibility patterns of respiratory isolates of Pseudomonas aeruginosa in a Turkish University Hospital. Ann Clin Microbiol Antimicrob 2003;2:5.
17. Luzzaro F, Mantengoli E, Perilli M, Lombardi G, Orlandi V, Orsatti A, et al. Dynamics of a nosocomial outbreak of multidrug-resistant Pseudomonas aeruginosa producing the PER-1 extended-spectrum beta-lactamase. J Clin Microbiol 2001;39(5):1865-70.
18. Vahaboglu H, Ozturk R, Akbal H, Saribas S, Tansel O, Coşkunkan F. Practical approach for detection and identification of OXA-10-derived ceftazidime-hydrolyzing extended-spectrum beta-lactamases. J Clin Microbiol 1998; 36(3):827-9.
19. Danel F, Hall LM, Livermore DM. Laboratory mutants of OXA-10 beta-lactamase giving ceftazidime resistance in Pseudomonas aeruginosa. J Antimicrob Chemother 1999;43(3): 339-44.
20. Vahaboglu H, Oztürk R, Aygün G, Coşkunkan F, Yaman A, Kaygusuz A, et al. Widespread detection of PER-1-type extended-spectrum beta-lactamases among nosocomial Acinetobacter and Pseudomonas aeruginosa isolates in Turkey: a nationwide multicenter study. Antimicrob Agents Chemother 1997;41(10): 2265-9.
21. Eraç B, Gülay Z. Molecular epidemiology of PER-1 extended spectrum beta-lactamase among gram-negative bacteria isolated at a tertiary care hospital. Folia Microbiol (Praha) 2007;52(5):535-41.
22. Docquier JD, Luzzaro F, Amicosante G, Toniolo A, Rossolini GM. Multidrug-resistant Pseudomonas aeruginosa producing PER-1 extended-spectrum serine-beta-lactamase and VIM-2 metallo-beta-lactamase. Emerg Infect Dis 2001;7(5):910-1.

23. Yong D, Shin JH, Kim S, Lim Y, Yum JH, Lee K, et al. High prevalence of PER-1 extended-spectrum beta-lactamase-producing Acinetobacter spp. in Korea. Antimicrob Agents Chemother 2003;47(5):1749-51.

24. Thong KL, Lai KS, Ganeswrie R, Puthucheary SD. Pulsed-field gel electrophoresis of multidrug-resistant and -sensitive strains of Pseudomonas aeruginosa from a Malaysian hospital. Jpn J Infect Dis 2004;57(5):206-9.

25. Köhler T, Epp SF, Curty LK, Pechère JC. Characterization of MexT, the re-gulator of the MexE-MexF-OprN multi-drug efflux system of Pseudomonas aeruginosa. J Bacteriol 1999; 181(20): 6300-5.

26. Yetkin G, Otlu B, Cicek A, Kuzucu C, Durmaz R. Clinical, microbiologic, and epidemiologic characteristics of Pseudomonas aeruginosa infections in a University Hospital, Malatya, Turkey. Am J Infect Control 2006;34(4):188-92.

27. Goering RV. Pulsed-field gel electrophoresis. In: Persing DH, Tenover FC, Versalovic J, Tang YW, Unger ER, Relman DA, et al. eds. Molecular Microbiology: Diagnostic Principles and Practice. 1st ed. Washington, DC: American Society for Microbiology; 2004. p.185-96.