ORIGINAL RESEARCH

Molecular epidemiology of *Pseudomonas aeruginosa* in University Clinical Center of Kosovo

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Background: *Pseudomonas aeruginosa* is an important opportunistic pathogen. It is frequently resistant to many commonly used antibiotics and develops easily resistant forms. Colonization with this organism often precedes infection, and its prevention is, therefore, critical. There is no information on molecular epidemiological investigation of outbreaks caused by *P. aeruginosa* in Kosovo.

Materials and methods: The present investigation was carried out to enlighten molecular epidemiology of *P. aeruginosa* in University Clinical Center of Kosovo (UCCK) using pulsed-field gel electrophoresis (PFGE). During our study period, 80 isolates of *P. aeruginosa* were included. The overall antimicrobial susceptibility pattern showed a high level of resistance against aminoglycosides and the lowest against carbapenems. Forty isolates of *P. aeruginosa* were subjected to genotyping, of whom 31 (77.5%) were male patients and nine (22.5%) were female patients.

Results: The most common diagnosis upon admission was polytrauma, sepsis, and coma cerebri. Majority of the patients were in mechanical ventilation (76.2%). Bacterial isolates were most frequently recovered from respiratory tract specimens (60%) and wounds (22.5%). Majority of the samples were recovered from intensive care unit (ICU) (47.5%). The length of ICU stay was higher compared to patients from other units. Genotype analysis of *P. aeruginosa* isolates identified seven distinct PFGE patterns, with the predominance of PFGE clone A (40%) and PFGE clone N (12.5%). All of these isolates were indistinguishable. The appearance of the indistinguishable genotypes supports the possibility of a cross and horizontal transmission of *P. aeruginosa* due to insufficient preventive measures.

Conclusion: The results emphasize the need for strict infection control measures to prevent the nosocomial transmission of *P. aeruginosa* in our hospital.

Keywords: genotyping, *P. aeruginosa*, pulsed-field gel electrophoresis, nosocomial infection, ICU

Introduction

Pseudomonas aeruginosa is one of the most frequent and severe causes of acute nosocomial infection, mainly affecting immunocompromised patients or those admitted to the intensive care unit (ICU).[1,2]

This organism has a remarkable ability to acquire antibiotic-resistant genes, to persist in the hospital environment, and to spread easily from patient to patient.[3] Standard antibiotic regimens against *P. aeruginosa* are increasingly becoming ineffective due to the rise in drug resistance.[4] Strain typing by traditional phenotypic methods is an important part of epidemiological surveillance but may lack discriminatory power and stability. Molecular techniques offer a considerable improvement and can complement phenotypic data to obtain a better understanding of bacterial diversity.[5] Several

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molecular typing schemes have been described to differentiate between the isolates and clonal groups of *P. aeruginosa*. Among them, the main typing method with a broader application is pulsed-field gel electrophoresis (PFGE) known as gold standard genotypic technique.[6] This is particularly important in endemic and epidemic nosocomial outbreaks of bacterial infections to improve their management.[7]

The infection rates for nosocomial infections and their pathogens differ significantly between different types of ICU corresponding to different risk structures of the patients.[8]

P. aeruginosa contributes to 11% of all nosocomial infections, which result in high mortality and morbidity rates.[9] Although the prevalence of nosocomial infections rates in different European countries is reported to be between 3.5 and 12%, the prevalence of nosocomial infections in Kosovo is high (17.4%).[10,11]

Knowledge regarding species, strains, and clones of *P. aeruginosa* which circulate in Kosovo hospitals is lacking. The present study was undertaken to understand the clonal relationship of *P. aeruginosa* among strains isolated from inpatients in our hospital.

Materials and methods
Hospital setting and patients
The study was conducted in the Department of Microbiology of the National Institute of Public Health of Kosovo. Laboratory diagnosis of microbiological samples and susceptibility testing was performed in the Laboratory of Nosocomial Infection and Antimicrobial Resistance in Department of Microbiology. The bacterial isolates selected for the present study included 40 isolates of *P. aeruginosa* from 40 patients hospitalized in different units of University Clinical Center of Kosovo (UCCK), the only tertiary care center in Kosovo with 2,100 beds, during the study period of 12 months, in 2014. Only monomicrobial samples containing *P. aeruginosa* were included in the study.

Genotyping of the samples was performed in the Faculty of Veterinary Medicine, Saints Cyril and Methodius University of Skopje, Skopje, Macedonia. Clinical specimens included tracheostomy tube, endotracheal aspirate, wound, blood culture, pleural punctuate, and thoracic drain. Data collected from medical charts of the patients with *P. aeruginosa* infection or colonization included age, gender, number of patient-days in hospital, underlying diseases or conditions, susceptibility pattern, and clinical outcome.

Microbiological methods
P. aeruginosa strains were collected from clinical specimens by using standard methods, isolated in blood-agar and MacConkey agar plates. Identification was performed by VITEK 2 Compact (bioMerieux, Marcy l'Etoile, France). From a 24-hour plate culture of *P. aeruginosa*, a suspension was made in the CRYOBANK™ medium. The tube was mixed by shaking and inverting to allow the organism in the suspension to coat the beads. Using a sterile syringe, the CRYOBANK medium was removed and the tube was placed in a –70°C freezer. Afterward, the samples were transported to Macedonia.

Antimicrobial susceptibility
Antimicrobial susceptibility testing was performed by the disk-diffusion tests on Mueller-Hinton agar, using antibiotic disks. Results were interpreted as susceptible, intermediate, or resistant according to the criteria recommended by The European Committee on Antimicrobial Susceptibility Testing (EUCAST).[12] The following antimicrobials were tested: ceftazidime 10 μg, cefepime 30 μg, ciprofloxacin 5 μg, norfloxacin 10 μg, amikacin 30 μg, tobramycin 10 μg, gentamicin 10 μg, piperacillin–tazobactam 30 μg, imipenem 10 μg, and meropenem 10 μg. Colistin Etest (bioMerieux) was performed in isolates expressing resistance toward abovementioned antimicrobials.

Molecular typing by PFGE and dendrogram analysis
Genotyping of all *P. aeruginosa* isolates by PFGE was performed according to US Centers for Disease Control and Prevention's (CDC) highly standardized PFGE protocols for Gram-negative rods with some minor modifications.[13] Bacterial suspensions were prepared from individual bacterial colonies directly obtained from cultures after overnight incubation on nutrient agar at 37°C. The suspensions were adjusted to an OD of 2.5 McFarland in EDTA–saline buffer (75 mmol/L NaCl and 25 mmol/L EDTA). The cell suspension was mixed with an equal volume of 2% low-melting point SeaKem Gold Agarose and was allowed to solidify in a 100 μL plug mold. The agarose plug was incubated for 24 hours at 37°C in 500 μL of lysis buffer (6 mmol/L Tris–HCl [pH 7.6], 0.1 mol/L EDTA, 1 mol/L NaCl, 0.5% Brij® 58, 0.4% sodium deoxycholate, 0.5% sodium lauryl sarcosine, and 1 mg/mL lysozyme). Next, the lysis buffer was replaced with 500 μL of proteinase K buffer (1% sodium lauryl sarcosine, 0.5 mol/L EDTA, and 50 μg/mL proteinase K) and this solution was incubated overnight at 56°C. The plugs were then washed four times for 30 minutes at 4°C with 10 mL of Tris–EDTA buffer. One-third of a slice of each plug was cut and incubated at 37°C for 4 hours with 30 U

of SpeI in the restriction buffer. NA restriction fragments were separated by PFGE using a CHEF MAPPER apparatus (Bio-Rad Laboratories Inc., Hercules, CA, USA) at 12°C, 6 V/cm, for 30 hours, with a time switch of 1–50 seconds. A *Salmonella* serotype Branderup strain (H9812) ladder (Bio-Rad Laboratories Inc.) restricted with XbaI was used as a universal size marker. The gel was stained with ethidium bromide and visualized with the Gel-Doc system (Bio-Rad Laboratories Inc.). According to the interpretative criteria of Tenover et al,[14] isolates were classified as indistinguishable, closely related, possibly related, or different. Indistinguishable isolates (no band differences) and closely related isolates (2–3 band differences) were considered to be the same genotype, while possibly related and different isolates (4–6 and >7 band differences, respectively) were considered different genotypes.[15]

Statistical analysis

Statistical analysis was performed using SPSS Version 22 (IBM Corporation, Armonk, NY, USA). The data were analyzed in terms of the mean, percentage, SD, and chi-squared test. A statistically significant difference was considered for $P < 0.05$ at 95% CI.

Ethical approval

Approval of this study was given by the ethics committee of the Faculty of Medicine, University of Pristina, Kosovo (approval reference number 1853). Informed consent was not needed for this study, because the samples were collected from patients as a part of routine diagnostic care.

Results

During our study period, a total of 80 *P. aeruginosa* isolates were obtained. An analysis of antimicrobial resistance rates showed that *P. aeruginosa* was resistant to ceftazidime 31 (38.8%), cefepime 21 (26.3%), amikacin 51 (63.7%), gentamicin 62 (77.5%), tobramycin 44 (55%), ciprofloxacin 23 (28.7%), norfloxacin 24 (30%), and piperacillin–tazobactam 29 (36.3%). Resistance to imipenem 14 (17.5%) and meropenem 10 (12.5%) was low compared with other antimicrobials.

Forty isolates from 40 patients (31 males and nine females) hospitalized in the UCCK were subjected to genotyping. Only one isolate of patients was enrolled in the study. Patients age' was ranged from 0 to 82 years (mean age 36.7 years, median age 35.5 years). Isolates were more frequently recovered from tracheostomy tubes (n=15), endotracheal aspirate (n=9), wound (n=9), pleural punctate

(n=3), drain swab (n=2), and blood culture and central venous catheter (CVC) one each.

The most common diagnoses were polytrauma (n=8), sepsis (n=5), coma cerebri (n=3), appendicitis acuta (n=3), fasciitis necrosis (n=3), meningoencephalitis (n=2), and pleural effusion (n=2). Clinical characteristics of patients and respective PFGE patterns are presented in Table 1. PFGE A and N suggest the cross-contamination. PFGE N represents strains isolated during February, March, and April, while the larger group, PFGE A, with 16 strains of *P. aeruginosa*, was isolated during a 6-month period, July to December. All of them were indistinguishable strains as shown in Figure 1.

The length of stay ranged from minimum 4 days to maximum 126 days, with a median time of 25.5 days. During the stay, 12 patients died. Majority of the samples were recovered from ICU (n=19).

Genotypic analysis of *P. aeruginosa* isolates from patients identified seven major PFGE clusters that contained PFGE patterns A–N. Of these, we identified 16 indistinguishable strains that belonged to PFGE A. They were found in ICU (n=12), post-ICU (n=2), and neurosurgery and plastic surgery unit one each. PFGE F had three indistinguishable strains, two from pulmonology and one from ICU. PFGE K with two indistinguishable strains was found in abdominal surgery and orthopedics unit and PFGE N had five indistinguishable strains, all of which were isolated in ICU. Other PFGE patterns were possibly related and, therefore, were considered different genotypes. PFGE profiles of *P. aeruginosa* strains isolated from UCCK are shown in Figure 1.

Discussion

P. aeruginosa is one of the important agents of nosocomial and health care-associated infections and responsible for lung, urinary tract, surgical site infections, and sepsis.[16]

Thus, many outbreaks of nosocomial infections due to *P. aeruginosa* have been reported, especially in ICUs, burn wound units, and cancer centers.[17] Critically, ill patients are at high risk for getting the hospital-acquired (nosocomial) infections, as evidenced by several studies.[18]

Nosocomial infection accounts for 7% in developed countries and 10% in developing countries.[19] Severe underlying diseases and invasive diagnostic and therapeutic procedures used in ICUs have been demonstrated to predispose patients to severe infections.[20]

There are many causes for high rate of nosocomial infections in Kosovo. Main factor remains the lack of support and implementation of prevention and control policies

Table 1 Clinical and PFGE data of the patients with *Pseudomonas aeruginosa* isolates

N	ID	Gender	Age (years)	Diagnosis	Sample	Sensitivity	Unit	Length of stay/days	Outcome	PFGE pattern
1	1196	M	45	Sclerosis multiplex	TT	CPM	ICU	82	Died	A
2	863	M	34	Coma cerebri	TT	IMI, MRP	Post-ICU	7	Died	A
3	2065	M	68	Polytrauma	TT	CAZ, TOB, IMI	ICU	39	Transferred	A
4	2152	M	47	Vulnus sclopetarium	W	CAZ, TOB, CIP	Neurosurgery	21	Transferred	A
5	794	M	24	Polytrauma	TT	CAZ, AK, CIP, IMI	ICU	21	Transferred	A
6	1212	M	66	Cerebrovascular accident	TT	CPM	Post ICU	63	Died	A
7	2198	F	19	Polytrauma	EA	IMI, MRP	ICU	67	Transferred	A
8	2043	M	71	Status postarrest cardiac	TT	CPM, TOB, IMI	ICU	99	Died	A
9	2166	M	21	Fractura cervicalis C4–C5–C6	TT	CAZ, IMI,	ICU	117	Died	A
10	1982	M	78	Polytrauma	TT	CPM, IMI, PTZ	ICU	63	Died	A
11	510	M	52	Coma cerebri	EA	TOB, IMI	ICU	30	Transferred	A
12	869	M	33	Coma cerebri	TT	CAZ, MER	ICU	13	Transferred	A
13	937	M	51	Appendicitis acuta gangrenosa perforata	TT	CAZ, MER	ICU	4	Died	A
14	939	M	21	Vulnus sclopetarium	CVC	AK, MER	ICU	4	Transferred	A
15	949	M	41	Defectuous cutis region inguinales	W	AK, CIP, IMI, PTZ	Plastic surgery	21	Recovered	A
16	1022	M	50	Appendicitis acuta gang perf	TT	CAZ, TOB, CIP, IMI	ICU	14	Died	A
17	1984	M	10	Polytrauma	EA	IMI, MRP	ICU	53	Transferred	B
18	455	F	0	RDS, sepsis	EA	CPM, CIP, IMI	Neonatology	53	Died	C
19	2117	M	57	Status febrilis	BC	CPM, TOB, CIP, IMI	Hematology	7	Transferred	D
20	666	M	22	Meningoencephalitis	EA	IMI, MRP	Infectious disease	123	Transferred	E
21	2125	M	18	Meningoencephalitis	TT	CPM, TOB, CIP, IMI	Infective	126	Died	E
22	443	M	26	Effusio pleuralis	AP	IMI, MRP	Pulmonology	25	Recovered	F
23	598	M	66	Effusio pleuralis	PP	CPM, TOB, CIP, IMI	Pulmonology	26	Recovered	F
24	2292	F	55	Fasciitis necrosis	PP	CPM, TOB, CIP, IMI	ICU	73	Transferred	F
25	1260	F	28	Phlegmon	W	CAZ, AK, MRP	Gyn/Obs	15	Recovered	G
26	599	M	5	Status febrilis	W	CPM, TOB, CIP, IMI	Pediatrics	10	Recovered	H
27	900	M	0	Sepsis	EA	CIP	Neonatology	28	Recovered	I
28	2247	F	0	Respiratory distress syndrome, coronary insufficiency	EA	CIP, IMI, PTZ	Neonatology	20	Died	J
29	2312	M	0	Sepsis	W	CIP, IMI, PTZ	Neonatology	10	Recovered	J
30	816	F	46	Appendicitis acuta	DS	AK, CIP, IMI	Abdominal surgery	14	Recovered	K
31	815	F	32	Polytrauma	W	AK, CIP, IMI	Orthopedics	21	Transferred	K
32	2091	F	0	Sepsis	EA	IMI, MRP	Neonatology	22	Recovered	M
33	1248	M	0	Sepsis	EA	CAZ, CIP, MRP	Neonatology	7	Recovered	M
34	499	M	82	Fasciitis necrosis	W	CAZ, CIP, IMI, PTZ	Vascular surgery	28	Recovered	L
35	303	M	35	Abdomenis acuta	DS	CS	ICU	27	Transferred	N
36	819	M	57	Carcinoma vesica urinaria	W	CPM, PTZ	ICU	16	Transferred	N
37	371	M	45	Polytrauma	TT	IMI, MRP	ICU	84	Transferred	N
38	353	M	44	Hemorrhagia epiduralis	TT	IMI, MRP	ICU	85	Died	N
39	1266	F	36	Polytrauma	TT	CPM, IMI, MRP	ICU	12	Transferred	N
40	673	M	82	Fasciitis necrosis	W	CS	Plastic surgery	28	Recovered	O

Abbreviations: AK, amikacin; BC, blood culture; CAZ, ceftazidime; CIP, ciprofloxacin; CPM, cefepime; CS, colistin; CVC, central venous catheter; DS, drain swab; EA, endotracheal aspirate; F, female; Gyn/Obs, gynecology and obstetrics; ICU, intensive care unit; IMI, imipenem; M, male; MRP, meropenem; PFGE, pulsed-field gel electrophoresis; PTZ, piperacillin/tazobactam; TOB, tobramycin; TT, tracheostomy tubes; W, wound.
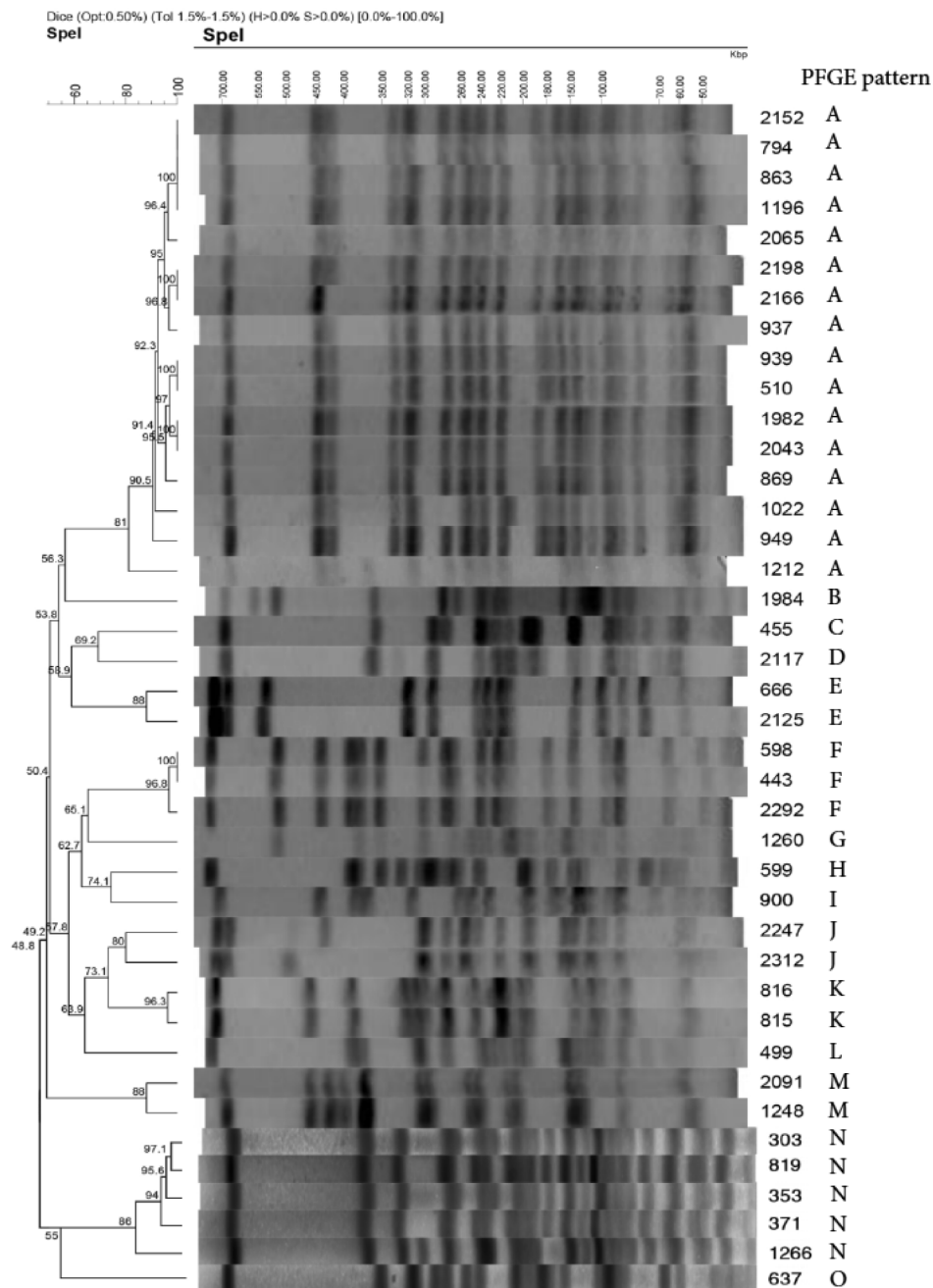
Figure 1 Dendrogram depicting 40 representative isolates of *P. aeruginosa*.
Abbreviation: *P. aeruginosa*, *Pseudomonas aeruginosa*; PFGE, pulsed-field gel electrophoresis.

and protocols regarding this issue and limited human and financial resources.

In our study, the majority of isolates were from patients hospitalized in ICU (47.5%), and the highest number of isolates was from respiratory specimens (65%) and wounds (22.5%). Among all Gram-negative bacilli responsible for nosocomial infections, *P. aeruginosa* was the second most prevalent isolate with 18.1%. Other most frequent isolates

were *Acinetobacter* spp. (25%), *Escherichia coli* (15.5%), and *Enterobacter* spp. (10.4%), and others were less than 10%. Previous prevalence studies in Kosovo showed that *P. aeruginosa* was 23.8%, *Acinetobacter* spp. was 15.1%, *Klebsiella* spp. was 12.9%, and *Citrobacter* spp. was 11.9%.[10]

Similar to our findings, another study showed that the Gram-negative bacteria responsible for the primary infections were *Klebsiella pneumoniae* (30% of ICU-acquired

Gram-negative infections), *Acinetobacter baumannii* (20%), *E. coli* (20%), and *P. aeruginosa* (17%) and, in 13% of cases, the infections were caused by other Gram-negative bacteria. The infection sites were mostly the respiratory tract (60%).[21]

Data from many authors demonstrate that gentamicin, amikacin, imipenem, and ciprofloxacin are considered potent agents in the treatment of infections caused by *P. aeruginosa*.[22] We found that carbapenems were the most active antibiotic, with a resistance rate of 15 and 20.4% for imipenem and meropenem, respectively. Gentamicin was the least active agent (resistance rate, 77.7%). Similar to our results, in a study in Turkey, resistance rates against imipenem and meropenem from carbapenem groups were determined as 15 and 20%, respectively.[23] Another study showed that, among the aminoglycoside group, gentamicin showed highest resistance (51.92%) and minimal resistance was observed with other aminoglycosides such as amikacin (29.8%) and tobramycin (29.8%).[24] In our study, the resistance toward amikacin and tobramycin was higher (63 and 55%, respectively). *P. aeruginosa* exhibits the highest rates of resistance for the fluoroquinolones, with resistance to ciprofloxacin and levofloxacin ranging from 20 to 35%.[25] We found similar results for ciprofloxacin and norfloxacin resistance rate ranging from 28 to 30%.

The resistance pattern of *P. aeruginosa* may vary among hospitals and even between the wards within given hospital, depending on the antibiotic used in the treatment.[26]

In our study, antibiotic susceptibility testing of genotyped isolates demonstrated that genetically related isolates had different sensitivity patterns, which is consistent with another study.[27]

Molecular epidemiological studies have an essential role in the management of infections by determining the routes of pathogen transmission.[28] Typing of bacterial isolates by PFGE is a considerable help in the control and prevention of hospital infections. Colonization and infection of patients vary according to the compliance of health care workers to infection control measures, to the contamination of the environment, and probably also to the biology of the pathogen (intrinsic factors).[29] Intrinsic factors predispose patients to Healthcare Associated Infections (HAIs). The higher likelihood of infection is reflected in vulnerable patients who are immunocompromised because of age (neonate, elderly), underlying diseases, severity of illness, immunosuppressive medications, or medical/surgical treatments. Patients with alterations in cellular immune function, cellular phagocytosis, or humoral immune response are at increased risk of infection and the ability to combat infection.[30]

Endogenous colonization was defined as colonization occurring with a strain of *P. aeruginosa* that had not previously been isolated from another patient. Exogenous colonization or cross-colonization was defined as colonization with a strain of *P. aeruginosa* with similar PFGE typing results to that of isolates from another patient.[15]

Although the present study had limitations in the number of isolates tested genetically, it contributes to the knowledge in regard to *P. aeruginosa* and its transmission rate in UCCK.

Typing methods for discriminating different bacterial isolates of the same species are essential epidemiological tools in the prevention and control of infection. Highly discriminatory techniques, refined over the past decade, include PFGE, chromosomal restriction fragment length polymorphism analysis, random amplified polymorphic DNA analysis, multilocus sequence typing (MLST), and arbitrarily primed PCR fingerprinting. Random amplification of polymorphic DNA and arbitrarily primed PCR is based on the parallel amplification of a set of fragments by using short arbitrary sequences as primers (usually 10 bases) that target several unspecified genomic sequences. The main drawback of the RAPD method is its low intra-laboratory reproducibility since very low annealing temperatures are used. Repetitive-element PCR (rep-PCR) is based on genomic fingerprint patterns to classify bacterial isolates. The main limitation of rep-PCR combined with electrophoresis using traditional agarose gels is that it lacks sufficient reproducibility, which may result from variability in reagents and gel electrophoresis systems. MLST is based on the principles of phenotypic multilocus enzyme electrophoresis (MLEE), which relies on the differences in the electrophoretic mobility of different enzymes present in a bacterium. For most of the multi-virulence-locus sequence typing (MVLST) approaches, additional research is needed. This should involve different and larger sets of isolates, and the results should also be correlated with conventional epidemiological data in order to validate the applicability of MVLST for epidemiological typing.

PFGE is one of the older methods for the molecular characterization and comparison of microorganisms including bacteria. The success of PFGE results from its excellent discriminatory power and high epidemiological concordance. Unfortunately, although widely used, PFGE suffers from several limitations. The method is technically demanding, labor intensive and time consuming.[3,31,32]

In our study, PFGE as an epidemiological tool has enabled us to compare and determine genetic relationship of these isolates. PFGE A with 16 isolates has been the largest obtained group; the majority of them were from ICU, two

were from post-ICU, one was from neurosurgery unit, and one was from plastic surgery unit. All of these isolates were indistinguishable. Majority of the samples for this pattern were obtained from the respiratory system, wound swabs, and CVC. This close genetic relationship among isolates suggests cross-contamination as a result of the transfer of patient or the movement of health care personnel between these units.

Another pattern found in ICU during the study period was PFGE N. These isolates were also indistinguishable. PFGE patterns A and N were parts of more than one outbreak during our study period.

Based on our study results, isolates with the higher genetic relationship are isolated in the same unit, especially for ICU, compared to isolates from other units that expressed diversity. The isolation of the same bacteria from patients in the same unit may be of help to detect an outbreak.[33]

Similar to our results, genetically indistinguishable isolates of *P. aeruginosa* were obtained from 19 infected or colonized ICU patients in another study also.[34] Cross-colonization seems to play an important role in the general spread of *P. aeruginosa* in ICUs.[35]

Majority of the patients, in whom indistinguishable *P. aeruginosa* isolates were found, were on mechanical ventilation (16 [76.2%]) and spontaneous ventilation (5 [23.8%]). This demonstrates that invasive devices could contribute to spreading of this clone in ICU. Several studies found that all patients infected with genetically similar strains were under the artificial ventilation. ICU stay in addition to the prolonged use of mechanical ventilation and surgical interventions may have contributed to the colonization/infection with *P. aeruginosa*.[18,36] A study in Spain found that the larger clone involving 79.6% of isolates was a possible endemic clone predominant in ICU.[37] In our study, length of hospitalization has varied from 4 days (lowest) to 117 days (highest). Length of stay, when compared, for endemic and nonendemic strains was 42.33 and 36.26 days, respectively. Prolonged stay can play an important role in spreading these strains between patients. It accounts as a major risk factor for serious health issues leading to death. About 75% of the burden of these infections is present in developing countries.[9] Based on the study findings, nosocomial infections and *P. aeruginosa* colonization have persisted for months and clonally related strains usually displayed different sensitivity to antibiotics.

Conclusion

The appearance of the indistinguishable genotypes, isolated from different samples from patients within the same ward, supports the possibility of a cross and horizontal transmission of *P. aeruginosa* due to insufficient preventive measures. In addition, prudent antimicrobial use and strict infection control measures in ICU are necessary to prevent and decrease the infection rate in ICU patients. However, as no environmental or staff sampling was performed, the source of contamination could not be assessed and further investigation should be performed.

Disclosure

The authors report no conflicts of interest in this work.

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