

Epidemiological characterization of *P. aeruginosa* isolates of intensive care units in Egypt and Saudi Arabia

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تحديد السمات الوبائية لمعزولات الزائفة الزنجارية في وحدات الرعاية المركزة في مصر وفي المملكة العربية السعودية

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الخلاصة: تهدف هذه الدراسة إلى تحديد سمات المعزولات من الزائفة الزنجارية في وحدتين للرعاية المركزة في مصر وفي المملكة العربية السعودية. وقد حددت أنماط الزائفة الزنجارية المعزولة من المرضى وأيدي العاملين ومن عينات مأخوذة من البيئة، وذلك باستخدام مخطط المضادات الحيوية، و تفاعل إنزيم البلمرة التسلسلي والتعداد التكراري للجراثيم المعوية ERIC-PCR. وتبين من النتائج في مصر أن المعزولات من أنابيب جهاز المص وأنابيب التصريف (A7) وأنابيب التهوية الاصطناعية (A8) ترتبط مع المعزولات من المرضى الذين لديهم نفس أنماط المضادات الحيوية. وفي المملكة العربية السعودية، ترتبط المعزولات من أنابيب جهاز المص (A6) وأنابيب التهوية الاصطناعية (A7) مع المرضى ذوي أنماط المضادات الحيوية ذاتها. وفي مصر ترتبط المعزولات ذات التعداد التكراري للجراثيم المعوية VII و VIII و IX بأنابيب جهاز المص، وأنابيب التهوية الاصطناعية وأحواض التصريف. وفي المملكة العربية السعودية ترتبط المعزولات من المرضى ذوي نماذج التعداد التكراري للجراثيم المعوية VIII و IX بأنابيب جهاز المص وأنابيب التهوية الاصطناعية. وفي مصر وفي المملكة العربية السعودية، أعطى تحديد أنماط التعداد التكراري للجراثيم المعوية مناسب تمييزية أعلى (0.801 مقابل 0.785) مما أعطت مخططات المضادات الحيوية (0.7123 مقابل 0.728). وقد تفوق التعداد التكراري للجراثيم المعوية على مخططات المضادات الحيوية، ويوصي الباحثون باستخدامه في اقتفاء مصادر العدوى.

ABSTRACT This study aimed to characterize *Pseudomonas aeruginosa* isolates in 2 intensive care units in Egypt and Saudi Arabia. *P. aeruginosa* isolates from patients' and staff hands and environmental samples were typed using antibiotyping and ERIC-PCR. In Egypt, isolates from suction apparatus tubing and drainage containers (A7) and AV tubing (A8) were linked to those from patients who had these antibiotypes. In Saudi Arabia, isolates from suction apparatus tubing (A6) and AV tubing (A7) were linked to patients with the same antibiotypes. In Egypt, patients' isolates had ERIC VII, VIII and IX patterns linked to suction apparatus tubing, AV machine tubes and drainage containers. In Saudi Arabia, patients' isolates had ERIC VIII and XI patterns linked to suction apparatus tubing and AV machines. In Egypt and Saudi Arabia, ERIC typing gave higher discriminatory indices (0.801 and 0.785 respectively) than the antibiotyping (0.7123 and 0.728 respectively). ERIC was superior to antibiotyping and should be used in tracing sources of infection.

Caractérisation épidémiologique d'isolats de *Pseudomonas aeruginosa* dans des unités de soins intensifs en Arabie saoudite et en Égypte

RÉSUMÉ La présente étude visait à caractériser des isolats de *P. aeruginosa* dans deux unités de soins intensifs en Arabie saoudite et en Égypte. Des isolats de *P. aeruginosa* prélevés sur les mains du personnel et des patients et des échantillons environnementaux ont été typés par antibiogramme et ERIC-PCR. En Égypte, des isolats prélevés sur les tubulures d'appareils d'aspiration et de respiration artificielle (A8) et sur les cuvettes (A7) étaient liés à ceux prélevés chez les patients ayant ces antibiotypes. En Arabie saoudite, des isolats prélevés sur des tubulures des appareils d'aspiration (A6) et de respiration artificielle (A7) étaient liés à ceux prélevés chez les patients ayant les mêmes antibiotypes. En Égypte, les isolats des patients étaient de types ERIC VII, VIII et IX et correspondaient à ceux des tubulures des appareils d'aspiration et de respiration artificielle et des cuvettes. En Arabie saoudite, les isolats des patients étaient de types ERIC VIII et XI correspondaient à ceux des tubulures des appareils d'aspiration et de respiration artificielle. En Égypte et en Arabie saoudite, le typage ERIC a permis d'obtenir des index de discrimination supérieurs (0,801 et 0,785 respectivement) à ceux de l'antibiogramme (0,7123 et 0,728 respectivement). La méthode ERIC était supérieure à l'antibiotype et devrait être utilisée pour déterminer les sources d'infection.

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Received: 05/09/11; accepted: 18/01/12

Introduction

The Gram-negative bacterium *Pseudomonas aeruginosa* is frequently associated with hospital-acquired infections in intensive care units (ICUs) [1]. It has been identified as the 2nd most frequent organism causing ventilator-associated pneumonia, the 4th most common causing catheter-associated urinary tract infections, the 5th cause of surgical site infections and the 7th cause of central-line-associated bloodstream infections [2]. *P. aeruginosa* has inherent resistance to many drug classes [3,4]. Moreover, it can acquire resistance via mutations and harbouring integrons with multiple resistance genes such as those coding for metallo- β -lactamases (MBL) that can cleave the most active antimicrobial agents against it (carbapenems) [3].

The source of *P. aeruginosa* in intensive care units (ICUs) can be either endogenous or exogenous; isolates that have unique genotypes are considered as possibly endogenous while those of the same genotype with either patient or environmental samples are considered as possibly exogenous [5]. Optimal control of *P. aeruginosa* outbreaks may require rapid identification and strain differentiation. Traditionally, it has been typed on the basis of its phenotypic characteristics but this technique may lack discriminatory power and stability [6]. Molecular techniques offer a considerable improvement, and can complement phenotypic data to obtain a better understanding of bacterial diversity [7]. Pulsed field gel electrophoresis is commonly employed and has achieved widespread recognition as the gold standard for *P. aeruginosa* deoxy-ribonucleic acid (DNA) typing [8,9]. However, this method is limited by its technical complexity, expense and prolonged turnaround times for results [7]. As an alternative, repetitive-element-based polymerase chain reaction (rep-PCR) assays that utilize primers targeting highly-conserved

repetitive sequence elements in the bacterial genome have shown considerable potential as DNA typing tools in the laboratory [10]. One of such groups of repetitive elements is the enterobacterial repetitive intergenic consensus (ERIC) sequences which are common to Gram-negative enteric bacteria [11,12]. The products of ERIC-PCR, with chromosomal DNA of different bacterial strains, were found to generate very characteristic patterns when separated on agarose gels. Thus, it has been proposed that ERIC-PCR is a useful method to fingerprint bacterial genomes [13].

This work was designed to elucidate the epidemiology of *P. aeruginosa* isolates in ICUs in both Egypt and Saudi Arabia and to identify MBL-producing isolates. It also aimed to compare typing methods by antibiogram and ERIC-PCR for their discriminatory power and compatibility.

Methods

This study was conducted in the Department of Medical Microbiology and Immunology, Faculty of Medicine, University of Zagazig, Egypt and the Department of Medical Microbiology, King Abdulaziz University, Saudi Arabia in the period from January 2009 to December 2010. This study was approved by the ethics committees of Zagazig University Hospital and King Abdulaziz University.

Study sample

A total of 270 samples were collected from patients, staff hands and the environment from surgical adult ICUs of both hospitals:

- 180 samples were collected from patient sources, including 30 sputum, endotracheal aspirate, blood, wound exudate, burn exudate and urine specimens.
- 30 samples were collected from staff hands at midday, by which time staff

members had been in contact with patients for several hours. For each staff member 30 mL of sterile nutrient broth was poured into a sterile plastic bag and the hands were massaged in the broth for 30 s [14].

- 60 environmental samples were taken throughout the ICU, including water taps, faucet handles, drainage containers, mops, manual resuscitation bags, AV tubing, suction apparatus tubing, air condition outlets and antiseptic solutions. Surfaces were swabbed with sterile cotton swab sticks and fluid samples were pipetted using sterile disposable plastic pipettes.

Isolation and identification of *P. aeruginosa*

Blood samples were used for inoculating blood culture bottles (Egyptian Diagnostic Media) then incubated at 37 °C for 7–14 days. Subcultures were done every 48 h on blood agar plates. Sputum, endotracheal aspirate, wound exudate, burn exudate and urine specimens were cultured on *Pseudomonas* cetrimide agar (Oxoid). *Pseudomonas* spp. isolates were subcultured on nutrient agar plates at 37 °C for 24 h to identify the growth characters. For analysis of staff hand specimens, a loopful sample of the broth was cultivated. Environmental samples were cultured in nutrient broth to dilute any disinfectants present and to encourage growth of low organism numbers. After 24 h incubation, subcultures were made onto *Pseudomonas* cetrimide agar plates and incubated at 37 °C. Any growth was identified as *P. aeruginosa* if it has the following criteria: characteristic colony morphology, grape-like odour, exopigments production [15], Gram-negative motile bacilli, positive oxidase test (Oxoid code BR0064A), growth at 42 °C [16], gelatin liquefaction test positive [17] and red butt and slant of triple sugar–iron medium [18].

Antibiogram

Antibiotic resistance testing was done by the Kirby–Bauer disk diffusion

method according to Clinical Laboratory Standards Institute guidelines [19]. The following antibiotics (Oxoid) were used: piperacillin 100 µg, ceftazidime 30 µg, cefepime 30 µg, aztreonam 30 µg, imipenem 10 µg, meropenem 10 µg, colistin 25 µg, amikacin 30 µg, gentamycin 10 µg, tobramycin 10 µg and ciprofloxacin 5 µg. Screening for MBLs was performed by the imipenem-EDTA combined-disk test as described by Yong et al. [20].

PCR methods

DNA extraction

All strains were freshly cultured on nutrient agar before DNA extraction using the QIAamp DNA mini kit (Qiagen GmbH) according to the manufacturer's instructions. The extracts were then kept at -20 °C until use.

ERIC-PCR typing

ERIC-PCR typing was performed as reported previously [21] using PCR-GOLD Master-Mix Beads (Bioron, The Enzyme Company). The following materials were added to each PCR bead: 50 ng of DNA template, 10 pmole of each primer (Bioneer™ Corporation); ERIC1R (5' ATG TAA GCT CCT GGG GAT TCA C 3') and ERIC2 (5' AAG TAA GTG ACT GGG GTG AGC G 3') [22] and deionized distilled water to a final volume of 50 µL. These were mixed well by automatic pipette, followed by brief centrifugation to collect the contents at the bottom of the tube. For the negative control reaction, all components of PCR reaction were added to the bead except for DNA to exclude any source of contamination. The gene segments were amplified using a DNA thermal cycler (Biometra). The reaction conditions were as follows: initial denaturation at 94 °C for 1 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 52 °C for 45 s and DNA chain extension at 72 °C for 2 min, and a final extension at 72 °C for 10 min.

DNA detection by agarose gel electrophoresis

Amplification products and DNA molecular weight marker (GeneRuler™ 1 kb Plus*) were detected by using agarose gel electrophoresis [23]. These were visualized on a UV transilluminator (Biometra) and photographed.

Statistical analysis

All data were tabulated and then processed using SPSS, version 12.0. Qualitative variables were expressed by percentages and compared using the chi-squared test or Fisher exact test when appropriate. A *P*-value < 0.05 was considered statistically significant. The numerical discriminatory index, which is a measure of the discriminatory ability of the typing methods was calculated according to Hunter [24].

Results

Isolation rate of *P. aeruginosa*

Table 1 shows that in Egypt and Saudi Arabia, *P. aeruginosa* was isolated from 32.8% and 30.0% of patient samples respectively. In Egypt, the highest isolation rate was from burn exudates (66.7%), while in Saudi Arabia, it was from endotracheal aspirates (36.7%). There was a statistically significant difference between data from Egypt and Saudi Arabia only in the rate of isolation of *P. aeruginosa* from burn exudates (*P* = 0.02).

The isolation rate of *P. aeruginosa* from environmental samples in Egypt was 25.0% overall and was highest (57.1%) from suction apparatus tubing (Table 1). In Saudi Arabia, it was 23.3% overall and was highest from suction apparatus tubing and artificial ventilation machine tubes (42.9%).

Table 1 shows that 10.0% and 6.7% of staff hand samples from Egypt and Saudi Arabia respectively were culture positive

Risk factors for acquiring *P. aeruginosa*

Table 2 shows the univariate analysis of risk factors for acquiring *P. aeruginosa* infection based on analysis of patient samples in Egypt and Saudi Arabia. There were statistically significant difference as regards age ≥ 60 years (*P* = 0.04 in Egypt; *P* < 0.001 in Saudi Arabia), length of hospital stay ≥ 7 days (*P* < 0.001 in both countries), cancer in Egypt only (*P* = 0.01), surgery (*P* < 0.001 in both countries) and use of antibiotics ≥ 2 days (*P* = 0.009 in Egypt; *P* < 0.001 in Saudi Arabia).

Antibiotic resistance

Table 3 reveals that antibiotic resistance rates of clinical isolates in Egypt and Saudi Arabia were highest to aztreonam (96.6% and 98.1%), followed by cefepime (76.3%) and tobramycin (67.8%) in Egypt, and followed by meropenem (72.2%) and ceftazidime (70.4%) in Saudi Arabia. On the other hand, the resistance rate of environmental isolates was 100% to aztreonam and ceftazidime in Egypt and ceftazidime and cefepime in Saudi Arabia (Table 3). Colistin was the only antibiotic to which nearly all strains were sensitive.

Table 4 shows the frequency of MBL-producing imipenem-resistant strains was 16/29 (55.2%) in Egypt and 36/54 (66.7%) in Saudi Arabia from clinical isolates, was 2/3 (66.7%) from environmental strains and was not detected in staff hand strains in either Egypt or Saudi Arabia. These differences were statistically significant (*P* < 0.001) in the same country.

Antibiotyping

Tables 5 and 6 shows the antibiotyping of isolates, showing 9 antibiotype patterns in Egypt and 8 in Saudi Arabia, ranging from pattern A1, which was sensitive to all tested antibiotics to pattern A9 and A8 which were resistant to all tested antibiotics except colistin. In

Table 1 Isolation rate of *Pseudomonas aeruginosa* strains from samples obtained from patients, the environment and staff hands in surgical adult intensive care units in Egypt and Saudi Arabia

Source	No. of samples	<i>P. aeruginosa</i> isolates				<i>P</i> -value
		Egypt		Saudi Arabia		
		No.	%	No.	%	
<i>Patient samples</i>						
Burn exudates	30	20	66.7	11	36.7	0.02
Wound exudates	30	11	36.7	10	33.3	0.78
Blood	30	9	30.0	5	16.7	0.22
Urine	30	8	26.7	9	30.0	0.77
Endotracheal aspirate from patients with ventilator associated pneumonia.	30	6	20.0	11	36.7	0.15
Sputum	30	5	16.7	8	26.7	0.34
Total	180	59	32.8	54	30.0	0.57
<i>Environment samples</i>						
Suction apparatus tubing	7	4	57.1	3	42.9	1.00
Drainage container	7	3	42.9	2	28.6	1.00
Artificial ventilation tubing	7	2	28.6	3	42.9	1.00
Mops	6	2	28.6	1	20.0	1.00
Air condition outlet	5	1	20.0	2	28.6	1.00
Manual resuscitation bags	7	1	20.0	2	28.6	1.00
Faucet handles	7	1	20.0	1	20.0	1.00
Water tap	7	1	20.0	0	0.0	1.00
Antiseptic solutions	7	0	0.0	0	0.0	1.00
Total	60	15	25.0	14	23.3	0.83
<i>Staff hand samples</i>						
Total	30	3	10.0	2	6.7	1.00

Egypt, there were epidemiological relationships between suction apparatus tubing and drainage containers (A7) and artificial ventilation (AV) machine tubes (A8) and patients who had A7 and A8 antibiotypes; however, there was no direct link between isolates from staff hands (A1 and A2) and patients. In Saudi Arabia, there were relationships between antibiotypes of suction apparatus tubing (A6) and AV machine tubes (A7) and patients where they had A6 and A7 antibiotypes but there was no direct link between isolates from staff hand (A3 and A4) or patients.

ERIC-PCR typing

P. aeruginosa isolates were typeable by ERIC-PCR and yielded 15 and 12 ERIC patterns from Egypt and Saudi Arabia respectively, with 4 to 11 bands in Egypt (Figure 1) and 3 to 9 bands

in Saudi Arabia (Figure 2). The size of amplified DNA bands ranged from 110 bp to 1535 bp.

From ERIC-PCR typing methods MBL-producing strains showed epidemiological relationships among 3 sites in Egypt: suction apparatus tubing, AV tubing and drainage container (ERIC VII, VIII and IX genetic patterns respectively). In Saudi Arabia, we found links to suction apparatus tubing and AV machine sites (ERIC VIII and XI genetic patterns respectively).

On calculating the discriminatory index of both typing methods (antibiotype typing and ERIC), we found that ERIC typing gave a higher discriminatory index in Egypt and Saudi Arabia (0.801 and 0.785 respectively) than the antibiogram (0.712 and 0.728 respectively) (Table 7).

Discussion

In spite of significant changes in the spectrum of organisms causing nosocomial infections in ICUs, *P. aeruginosa* has held a nearly unchanged position as an important pathogen [2]. In Egypt and Saudi Arabia, the rate of isolation from patient samples was 32.8% and 30.0% respectively. This in agreement with rates reported in Egypt by Ashour et al. (30.0%) [25] and Gad et al. (24.0%) [26] and in Saudi Arabia by Al Johani et al. (30.6%) [27]. In Egypt, the highest isolation rate from patient samples was from burn exudates (66.7%). This in accordance with Gad et al. (72.0%) [24] and may be attributed to inappropriate infection control measures to burn patients. On the other hand, in Saudi Arabia, isolation was highest from endotracheal aspirates

Table 2 Univariate logistic regression analysis of risk factors for infection by *Pseudomonas aeruginosa* isolated from samples obtained from patients in surgical adult intensive care units in Egypt and Saudi Arabia

Variable	Egypt (n = 180)				P-value	Saudi Arabia (n = 180)				P-value
	Infected (n = 59)		Non-infected (n = 121)			Infected (n = 54)		Non-infected (n = 126)		
	No.	%	No.	%		No.	%	No.	%	
Sex										
Female	24	40.7	51	42.1	0.85	20	37.0	41	32.5	0.55
Male	35	59.3	70	57.9		34	63.0	85	67.5	
Age (years)										
12–20	7	11.9	20	16.5		6	11.1	18	14.3	
20–40	26	44.1	68	56.1		19	35.2	75	59.5	
40–60	18	30.5	28	23.1		19	35.2	31	24.6	
≥ 60	8	13.5	4	3.3	0.04	10	18.5	2	1.6	< 0.001
Length of hospital stay (days)										
≥ 7	55	93.2	39	32.2	< 0.001	50	92.6	63	50.0	< 0.001
Comorbid conditions										
Diabetes mellitus	16	27.1	26	21.5	0.4	10	18.5	20	15.9	0.66
Cardiac disease	10	16.9	36	29.8	0.06	16	29.6	42	33.3	0.11
Cancer	9	15.3	4	3.3	0.01	5	9.3	9	7.1	0.76
Trauma	16	27.1	27	22.3	0.47	18	33.3	26	20.6	0.06
Surgery	30	50.8	26	21.5	< 0.001	39	55.7	24	19.0	< 0.001
No. of antibiotics used										
≥ 2	36	61.0	49	40.5	0.009	45	64.2	53	42.1	< 0.001

and burn exudates (36.7%) and this is in agreement with Al Johani et al. (43.0%) [25]. The univariate logistic regression analysis showed that the risk factors for *P. aeruginosa* infection were age group ≥ 60 years, hospital stay ≥ 7 days, cancer, surgery and use of antibiotics ≥ 2 days. These findings are consistent with those of other studies [28–30].

In the current study the environmental sampling in Egypt and Saudi Arabia showed that 25.0% and 23.3% of the samples were positive, which is slightly higher than a previous study in Egypt (19.5%) [26]. This reflects the fact that *P. aeruginosa* is ubiquitous in the hospital environment. The isolation rate was the highest from suction apparatus tubing in Egypt (57.1%) and from both suction apparatus tubing and AV machine tubes from Saudi Arabia (42.9%). This may be explained by the failure of sterilization of suction apparatus tubing

and inadequate application of standard precautions for infection control.

On the other hand, 10.0% and 6.7% of staff hands in this study were culture-positive in Egypt and Saudi Arabia respectively, which is higher than the rate reported by Crivaro et al. in Italy (3.5%) [31]. The higher rate of isolation from staff hands in this study could be due to lack of compliance of health care workers to hand-washing practices.

In Egypt antibiotic resistance rates were highest to aztreonam (96.6%), followed by cefepime (76.3%) and tobramycin (67.8%). Kamel et al., also in Egypt, reported that isolates were completely resistant to tobramycin and gentamicin and were sensitive to amikacin (68%), imipenem and meropenem (52%) and ciprofloxacin (36%) [32]. This difference could be attributed to the different rate of use of these antibiotics in different localities. In Saudi Arabia we found antibiotic resistance

rates were also highest to aztreonam (96.6%), but followed by meropenem (72.2%) and ceftazidime (70.4%). This is in agreement with a study by Al Johani et al. in Saudi Arabia who found that the resistance significantly increased after 2007, especially for carbapenem (34% in 2004 to 74% in 2009), ceftazidime (31% in 2004 to 36% in 2009) and ciprofloxacin (33% to 51%) [27]. The difference between Egypt and Saudi Arabia in antibiotic resistance patterns was not statistically significant in our study, presumably due to the different rates of use of antibiotics in different countries.

As regards screening for MBLs production in imipenem-resistant strains in Egypt and Saudi Arabia, the high resistance was consistent with an Egyptian study, which found that 10/31 (32.3%) of strains were MBL-positive [33]. In Saudi Arabia, Al-Agamy et al. found that the prevalence of resistance

Table 3 Antibiotic resistance rates of *Pseudomonas aeruginosa* isolates from samples obtained from patients, the environment and staff hands in surgical adult intensive care units in Egypt and Saudi Arabia

Source/Antibiotic	Resistant isolates				P-value
	Egypt		Saudi Arabia		
	No.	%	No.	%	
<i>Patient samples</i>	<i>(n = 59)</i>		<i>(n = 54)</i>		
Colistin	0	0.0	0	0.0	1.00
Imipenem	29	49.2	36	66.7	0.06
Meropenem	30	50.8	39	72.2	0.02
Gentamycin	31	52.5	29	53.7	0.90
Amikacin	32	54.2	30	55.6	0.88
Piperacillin	34	57.6	33	61.1	0.70
Ceftazidime	37	62.7	38	70.4	0.38
Ciprofloxacin	38	64.4	28	51.9	0.17
Tobramycin	40	67.8	36	66.7	0.89
Cefepime	45	76.3	35	64.8	0.18
Aztreonam	57	96.6	53	98.1	0.93
<i>Environment and staff hand samples</i>	<i>(n = 18)</i>		<i>(n = 16)</i>		
Colistin	0	0.0	0	0.0	1.00
Gentamycin	10	55.6	10	62.5	0.68
Amikacin	11	61.1	11	68.8	0.64
Tobramycin	12	66.7	11	68.8	0.89
Imipenem	12	66.7	13	81.2	0.44
Piperacillin	13	72.2	12	75.5	0.83
Meropenem	13	72.2	12	75.5	0.83
Ciprofloxacin	14	77.8	10	62.5	0.45
Cefepime	16	88.9	16	100.0	0.48
Ceftazidime	18	100.0	16	100.0	1.00
Aztreonam	18	100.0	14	87.5	0.21

to carbapenem was 34%, and 22% of them produced MBLs [34]. ICUs are epicentres of antibiotic resistance and the principal sources of outbreaks of multi-resistant bacteria. The nongenetic causes of antibiotic resistance include

excessive consumption of antibiotics by the population. More than 80% of admitted patients in Egypt are prescribed antibiotics and in many cases without documented proof of infection and this exerts a selective pressure on bacteria

[35] and/or horizontal dissemination [36]. Genetic causes of antibiotic resistance are mutation, over-expression of efflux pumps, loss or modification of porins, and acquired extended-spectrum β -lactamases [37].

Table 4 Frequency of metallo- β -lactamases-(MBLs)-producing imipenem-resistant *Pseudomonas aeruginosa* isolates from samples obtained from patients, the environment and staff hands in surgical adult intensive care units in Egypt and Saudi Arabia

Source	MBL-producing imipenem-resistant <i>P. aeruginosa</i> strains				<i>P</i> -value
	Egypt		Saudi Arabia		
	No.	%	No.	%	
Patient samples	16/29	55.2	36/54	66.7	0.30
Environment samples	2/3	66.7	2/3	66.7	1.00
Staff hand samples	0/3	0.0	0/2	0.0	1.00
Total	18/35	51.4	38/59	64.4	0.21
<i>P</i> -value	< 0.001		< 0.001		

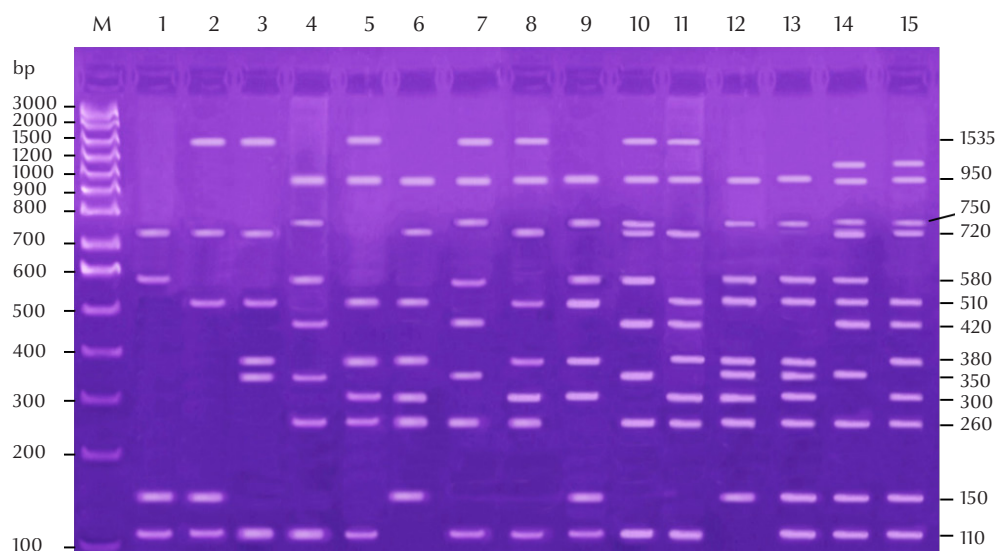


Figure 1 Different 15 enterobacterial repetitive intergenic consensus (ERIC)-PCR patterns of *Pseudomonas aeruginosa* strains isolated from samples obtained from patients, the environment and staff hands in Egypt. ERIC genotypes of staff hands were ERIC I to III, environmental isolates were IV to IX and XII to XV and patient isolates belong to ERIC VII to XI

Evidence-based prevention strategies targeting specific pathogens should be based on a thorough knowledge of their epidemiology, reservoirs in the ICU and modes of transmission [38]. However, isolation of *P. aeruginosa* per se is not sufficient to determine the epidemiological importance of the site of isolation, and typing techniques are

needed. The numerical discriminatory index for antibiotyping in Egypt and Saudi Arabia gave a low D index (0.712 and 0.728 respectively). This can be explained by the small number of types defined by it (9 antibiotypes in Egypt and 8 Saudi Arabia) and the heterogeneous distribution of the isolates in the groups (22 out of 77 isolates were

present in one group in Egypt). This is in agreement with Freitas and Barth who declared that the low discriminatory power of susceptibility tests was not surprising since the power of a method was determined by the number of types defined by it and the relative frequencies of these types [39]. The D index for the ERIC method demonstrated that in

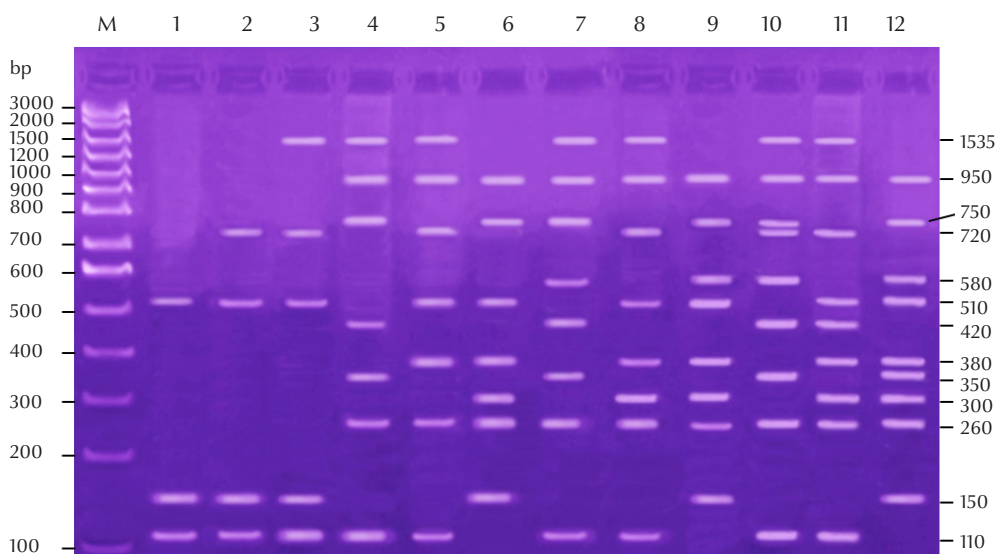


Figure 2 Different 12 enterobacterial repetitive intergenic consensus (ERIC)-PCR patterns of *Pseudomonas aeruginosa* strains isolated from samples obtained from patients, the environment and staff hands in Saudi Arabia. ERIC genotypes of staff hands were ERIC IV to V, environmental isolates were I to III and VI to XI and patient isolates belong to ERIC VII to XII

Table 5 The 9 different antibiotypes of *Pseudomonas aeruginosa* strains isolated from samples obtained from patients, the environment and staff hands in a surgical adult intensive care unit in Egypt

Antibiotype	Amikacin	Aztreonam	Cefepime	Ceftazidime	Ciprofloxacin	Colistin	Gentamycin	Imipenem	Meropenem	Piperacillin	Tobramycin
A1	S	S	S	S	S	S	S	S	S	S	S
A2	R	R	S	S	S	S	S	S	S	S	R
A3	S	R	R	R	S	S	S	S	S	S	R
A4	S	R	R	S	R	S	S	R	S	S	S
A5	R	R	R	R	S	S	R	S	S	S	S
A6	S	R	R	R	R	S	R	S	S	R	S
A7	R	R	S	R	R	S	R	S	R	R	R
A8	R	R	R	R	R	S	S	R	R	R	R
A9	R	R	R	R	R	S	R	R	R	R	R

S = sensitive; R = resistant.

Table 6 The 8 different antibiotypes of *Pseudomonas aeruginosa* strains isolated from samples obtained from patients, the environment and staff hands in a surgical adult intensive care unit in Saudi Arabia

Antibiotype	Amikacin	Aztreonam	Cefepime	Ceftazidime	Ciprofloxacin	Colistin	Gentamycin	Imipenem	Meropenem	Piperacillin	Tobramycin
A1	S	S	S	S	S	S	S	S	S	S	S
A2	S	R	S	S	R	S	S	S	S	R	R
A3	S	R	S	S	R	S	R	R	S	S	R
A4	S	R	R	R	R	S	S	S	S	R	S
A5	R	R	R	R	R	S	S	S	R	S	S
A6	R	R	R	R	R	S	R	S	S	S	R
A7	R	R	R	R	R	S	R	S	S	R	R
A8	R	R	R	R	R	S	R	R	R	R	R

S = sensitive; R = resistant.

Table 7 Numerical discriminatory index of enterobacterial repetitive intergenic consensus (ERIC) and antibiogram in Egypt and Saudi Arabia

Variable	Egypt			Saudi Arabia		
	No. of different types	No. of strains belonging to most numerous type	Numerical discriminatory index	No. of different types	No. of strains belonging to most numerous type	Numerical discriminatory index
ERIC	15	26	0.801	12	22	0.785
Antibiogram	9	22	0.723	8	18	0.728

Egypt and Saudi Arabia, ERIC typing gave a higher D index (0.801 and 0.785 respectively).

Sharing of certain ERIC types between patient strains may be explained by horizontal transmission from patient to patient, probably from the hands of health care workers or environmental sources. Although *P. aeruginosa* was isolated from the hands of nursing staff, both typing methods failed to show a direct link with strains isolated from patients. Other studies revealed that staff hands play an important role in its spread in the adult ICU [40] and neonatal ICU [31].

As regards *P. aeruginosa* isolated from environmental sites in our study, 3 sites in Egypt—suction apparatus tubing, AV machine tubes and drainage container—had direct epidemiological relationships with patients. Evacuation

of suction apparatus fluid into drainage containers is a possible link that could explain this relationship. In agreement with our study, Yorioka et al. in Japan found that *P. aeruginosa* was one of the main contaminant bacteria isolated from suction tubes [41]. In Saudi Arabia, 2 sites (suction apparatus tubing and AV machine tubes) had a direct epidemiological relationships with patients. On the other hand, in our study in Egypt and Saudi Arabia, MBL-producing strains were epidemiologically linked to suction apparatus tubing, AV machine tubes and drainage containers, while in Saudi Arabia they linked epidemiologically to suction apparatus tubing and AV machines. This finding can be explained by the fact the MBL genes are harboured in mobile genetic elements and integrons [42]. The same argument can be used to

explain why some non-MBL-producing strains shared the same genotype as MBL-producing strains. Also, MBL-producing strains may have originated from the non-MBL strains that acquired the genes through some mobile genetic elements [43].

In summary, this study adds to the evidence that strain typing using the ERIC method is superior to antibiotyping and can increase the efficiency of infection control procedures by determining the patient sources of infections that need to be eliminated and the environmental sources of *P. aeruginosa* that may receive less stringent action. Evidence was also provided that stricter antibiotic prescribing policies and resistance surveillance programmes are needed in Egypt and Saudi Arabia to assist in implementing infection control measures.

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