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Genotypic Characteristics of Clinical and Non-Clinical Isolates of *Pseudomonas aeruginosa:* Distribution of Different antibiogram Profiles and Molecular Typing

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Abstract

The present study aims to characterize *Pseudomonas aeruginosa* isolates in three hospitals in Hadhramout, Yemen. Two hundred samples were collected from patients, health workers staff hands and environmental samples and *P. aeruginosa* isolates were typed using antibiotyping and ERIC-PCR. The results showed that eleven yielded ERIC genetic patterns (PI-PXI) and size of amplified DNA bands size approximately ranged from 150-200 bp to 450-500 bp per sample, the highest rate of isolates from genetic patterns (ERIC-PCR) PI and PII was 23.5%. ERIC-PCR typing results showed horizontal transmission from patient to patient who had the same genetic patterns, isolates from patients linked to all isolates from environmental sources and staff hand samples which had the same genetic patterns, that indicated there was direct relationships between them. About 16 Antimicrobial Resistance Profile (ARP) including resistance ranged from 5 to 13 antimicrobial. Resistance pattern A1 (48.2%) isolates was the more frequent. Antibiogram typing showed a link between strains isolated from patients and environmental sources, but failed to show a direct relationships between patients and staff hands samples. ERIC-PCR typing was a more precise molecular technique than antibiogram methods and should be used for monitoring and determination of the sources of infection.

Keywords: Nosocomial Infection, *Pseudomonas aeruginosa*, Genetic Pattern, Antibiogram, ERIC-PCR.

1. Introduction

Nosocomial infections occur worldwide and affect both developed and resource-poor countries. Infections acquired in health care settings are among the major causes of death and increased morbidity among hospitalized patients (Tikhomirov, 1987). Pseudomonas aeruginosa is a ubiquitous bacterium and considered the fourth most commonly isolated nosocomial pathogen of all hospital-acquired infections (Todar, 2008).

It is widespread in natural environments and considered an opportunistic secondary pathogen for humans that is capable of causing a major nosocomial infections and broad spectrum of infections such as urinary tract, burn, respiratory tract, meningitis, chronic otitis media and otitis externa, pseudomonal endocarditis, septicemia, etc. (Singh et al., 2006; Yang et al., 2011). There is a high mortality rate associated with this pathogen, especially in immunocompromised patients, and excessive mortality and morbidity associated with inefficient empirical therapy, leading to complications during treatment (Estahbanati et al., 2002).

Typing of strains is essential for determining the epidemiology of nosocomial infections and aiding in the design of pathogen control methods (Pal et al., 2010). P. aeruginosa has been typed based on its phe¬notypic characteristics, such as serotyping, biotyping, bacteriophage typing, pyocin typing, and antimicrobial susceptibility testing (Pitt, 1988). Determination of antimicrobial profiles is another typing method used frequently as a supplemental epidemiological tool for strain differentiation of P. aeruginosa (Jamasbi and Proudfoot, 2008).

Due to the plasticity of the phenotypic characteristics, molecular techniques (which are inherently more stable than phenotypic characteristics) have gained popularity for strain differentiation and epidemiological studies of many organisms. Molecular techniques, such as Pulsed-Field Gel Electrophoresis (PFGE) and DNA fingerprinting, have been used for P. aerugi¬nosa typing in recent years (Hernández et al., 1997).

In Yemen, few studies on prevalence, biochemical identification and antibiotics sensitivity test for P. aeruginosa from clinical specimens were reported (El-Souny and Magaam, 2009; Yacin, 2009).

The present study was done the first time in Yemen to identify the bacteria to their molecular level and to detect the epidemiology and source of nosocomial infections by genotyping using molecular techniques and antibiogram.

Molecular typing of strains is essential to determine the epidemiology of nosocomial infections and aiding in the design and monitoring of pathogen control methods (Pal et al., 2010)..

2. Materials and Methods

The present study was done with cooperation betweenMicrobiologydivision–Biology Department- Faculty of Science- Sana'a University, Yemen and the Clinical Genomics Center- Faculty of Medicine, Alexandria University, Egypt.

The samples were collected and cultivated in Departments of Medical Microbiology in the National Center for Central Public Health Laboratories of Hadhramout, Yemen.

2.1. Study Samples

The samples were collected from patients, who stayed three days in hospitals before specimen's collection, staff hands and the environment samples that during the period from November 2013 to May 2014."

A total of 200 samples were collected were distributed as the following:

- 140 samples were taken from patients admitted to all hospitals, the source of the clinical isolates included wound and burn exudates, urine, sputum, blood, CSF, throat swab and ear secretions using sterile swabs. Blood samples were cultivated (2cc blood/ 10 ml brain heart infusion) then incubated at 37oC for 7–14 days. Subcultures were done every 48 hrs on blood agar plates (Mansour et al., 2013).
- 15 samples were collected from health workers staff hand swab at midday, by which time staff members had been in contact with patients for several hours.
- 45 environmental samples were collected from various sites of Intensive Care Units (ICU), Neonatal Intensive Care Unit (NICU), pediatric unit, endoscopy unit, Operation Theatres (OT) and delivery room, including suction apparatus tubing, respirators (artificial ventilation tubing), air condition outlet, endoscopes, antiseptic solutions and water tap. Surfaces were swabbed with sterile moisture cotton swab sticks and fluid samples were pipetted using sterile disposable plastic pipettes. All swab specimens were carried to microbiology laboratory by using cetrimide agar medium.

2.2. Isolation, Purification and Preservation of Pseudomonas aeruginosa

All specimens were cultured on the cetrimide agar which is considered the selective media to isolate P. aeruginosa strains and incubated at 37oC for 24 hrs (Fazeli et al., 2012). P. aeruginosa colonies were purified by streaking and sub-culturing on Nutrient agar plates until pure cultures were obtained, then transferred in glycerol and stored at -20oC (Guessas and Kihal, 2004).

2.3. Antibiogram

Antibiotic susceptibility tests were carried out by the Kirby- Bauer disk diffusion technique according to Clinical Laboratory Standard Institute guidelines (CLSI, 2014). The following antibiotics (OXOID and HIMEDIA) were used: Amikacin (AK 30 µg), Aztreonam (AT 30 µg), Ceftazidime (CAZ 30 µg), Gentamicin (GEN 10 µg), Imipenem (IPM 10 µg), Piperacillin (PI 100 µg), Piperacillin/ tazobactam (PIT 100/10 µg), Ciprofloxacin (CIP 5µg), Ceftriaxone (CRO 30 µg), Ampicillin (AMP 10 µg), Cefotaxime (CTX 30 µg), Tobramycin (TOB 10 µg), Chloramphenicol (C 30 µg) and Erythromycin (E 15 µg). The test medium was Mueller-Hinton agar was used for growing the lawn of culture of the strains (Rajput et al., 2012).

2.4. Molecular Technique

2.4.1. DNA Extraction

A single colony of each isolate was selected and subcultured aerobically on blood agar at 37 °C for 24 hrs. DNA extract was obtained by suspending a loopful of the bacteria in 50 µl of lysis solution in 0.5 ml Eppendorf tubes. An even distribution of the bacterial pellet and adequate turbidity was obtained by 5 minutes vortexing of the suspension.

Lysates were diluted 10 times by adding them to 450 µl of sterile distilled water; vortex was done for 2 minutes, and finally, centrifugation for 5 minutes at 5000 rpm. Five µl of the supernatant were used for PCR (Potrykus et al., 2014).

2.4.2. Polymerase Chain Reaction Technique

Typing by Enterobacterial Repetitive Intergenic Consensus PCR (ERIC-PCR) (PCR TECHNE Company) was carried out as follows. Bacterial DNA was amplified with each primer (Invitrogen; primer1 (Q4255F06), primer2 (Q4255FO7)): ERIC1R (5' to 3') (ATG TAA GCT CCT GGG GAT TCA C) and ERIC2 (5' to 3') (AAG TAA GTG ACT GGG GTG AGC G) using a standard reaction mixture (1.5 mM MgCl2, 2 mM primer, 2.5 U of AmpliTaq, and 100 ng of template DNA) [Invitrogen; PCR Super Mix (Cat. No. 10572-014)].

The reaction conditions were as follows: 1 cycle of initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 58°C for 60 s, and DNA chain extension at 72°C for 45 s, and a final extension at 72°C for 10 min.

Amplicons were separated by electrophoresis on 1.4% agarose gels and stained with ethidium bromide (Biotium, 2007).

2.4.3. DNA Detection by Agarose Gel Electrophoresis

Amplicons and DNA molecular weight marker (Gene Ruler 100 bp) were separated by electrophoresis (HVD Vertriebs- GmbH) on 1.4 % agarose gels prepared in Tris-borate EDTA buffer and stained with 10µg/ml of ethidium bromide incorporated in the gel.

The gel was subjected to electrophoresis at 80 volts for 1 hr. The gel was observed over an ultraviolet transilluminator (SCIE-PLAS), and the presence or absence of bands in a gel lane was determined by visual inspection.

2.5. Statistical Analysis

Statistical analyses were performed using the statistical Package for Social Science (SPSS for windows, version 16) (SPSS Inc., Chicago, IL, U.S.A) were expressed by percentages and compared using the chi-squared test and ROC curve. The difference was regarded significant when ($P< 0.05$) and non significant when ($P>0.05$).

3. Results

3.1. Sample Type and Isolation Rate of P. aeruginosa

Our results showed that P. aeruginosa where found and isolated from the patients, health workers and environmental hospital samples and the total of the positive samples were 56 (28%) out of 200.

3.2. Distribution of P. aeruginosa Isolates

The number of positive isolated from patients was 42 (30%) and the highest isolation rate was (60%) from wounds and burns patients followed by sputum (26.7%), urine (22.2%), throat swabs(20%), ear secretions(18.8%), CSF(18.2%) and the lowest rate from blood(16.6%).

The isolation rate of P. aeruginosa from hospital environmental samples was (26.7%). The highest rate of isolated was from suction apparatus tubing 50% where is statically significant ($P< 0.05$), and 40% from endoscope, 25% from respirators, 25% from water tap, 12.5% from air condition outlet and 12.5% from antiseptic solutions. The positive culture of P. aeruginosa from staff hand samples were (13.3%) (Table1).

Table 1. Isolation rate of Pseudomonas aeruginosa from samples obtained from patients, hospital environment and staff hands

Source	No. of samples	No. of isolates	Percentage of positive culture(%)	Chi-square χ^2	*P*-value
Patient samples					
Wounds and burns	30	18	60	17.9	0.000
Sputum	30	8	26.7	0.03	0.86
Urine	27	6	22.2	0.52	0.47
Throat swabs	20	4	20	0.71	0.4
Ear secretions	16	3	18.8	0.74	0.39
CSF	11	2	18.2	0.56	0.46
Blood	6	1	16.6	0.39	0.53
Total	140	42	30	-	-
Environment samples					
Suction apparatus tubing	8	4	50	2.0	0.16
Endoscope	5	2	40	0.37	0.55
Respirators	8	2	25	0.04	0.85
Water tap	8	2	25	0.04	0.85
Air condition outlet	8	1	12.5	0.99	0.32
Antiseptic solutions	8	1	12.5	0.99	0.32
Total	45	12	26.7	-	-
Staff hand samples					
Total	15	2	13.3	1.73	0.19

Significant at p-value ≤ 0.05, No: Number

3.3. Risk Factors for Acquiring P. aeruginosa

The age of the whole sample of 140 patients ranged from months to > 60 years old, the rate of infection among males was (57.1%) whereas females (42.8%). The difference was not significant (P=0.91), and many of those patients were suffering from some chronic diseases (Table2). The risk factors for *P. aeruginosa* infection were in males (57.1%); the age group (> 40-60 years) (42.8%) and surgery disease (47.6%) and ICU (47.5%) (Figure 1).

Table 2. Age, Sex, and diseases risk factors for infection by *Pseudomonas aeruginosa*

Variable	n=140												*P*-value
Age [years]	Infected (n= 42) Sex						Non-infected (n=98) Sex						
	Male	%	Female	%	Total	%	Male	%	Female	%	Total	%	
0-1	2	4.7	-	-	2	4.7	7	7.1	5	5.1	12	12.2	
>1-20	5	11.9	3	7.1	8	19.0	12	12.2	10	10.2	22	22.4	0.51
>20-40	4	9.5	4	9.5	8	19.0	8	8.1	3	3.1	11	11.2	
>40-60	12	28.5	6	14.2	18	42.8	20	20.4	18	18.3	38	38.7	
> 60	1	2.3	5	11.9	6	14.2	8	8.1	7	7.1	15	15.3	
Total	24	57.1	18	42.8	42	-	55	56.1	43	43.9	98	-	0.91
Comorbid conditions													
Diabetes	7	16.6	6	14.2	13	30.9	11	11.2	8	8.2	19	19.4	0.82
Cancer	4	9.5	6	14.2	10	23.8	6	6.1	5	5.1	11	11.2	0.51
Hypertensi-on	8	19.0	5	11.9	13	30.9	10	10.2	9	9.2	19	19.4	0.62
Cardiac disease	5	11.9	4	9.5	9	21.4	6	6.1	3	3.1	9	9.2	0.63
Surgery	8	19.0	12	28.5	20	47.6	21	21.4	18	18.4	39	39.8	0.31

N: number of samples

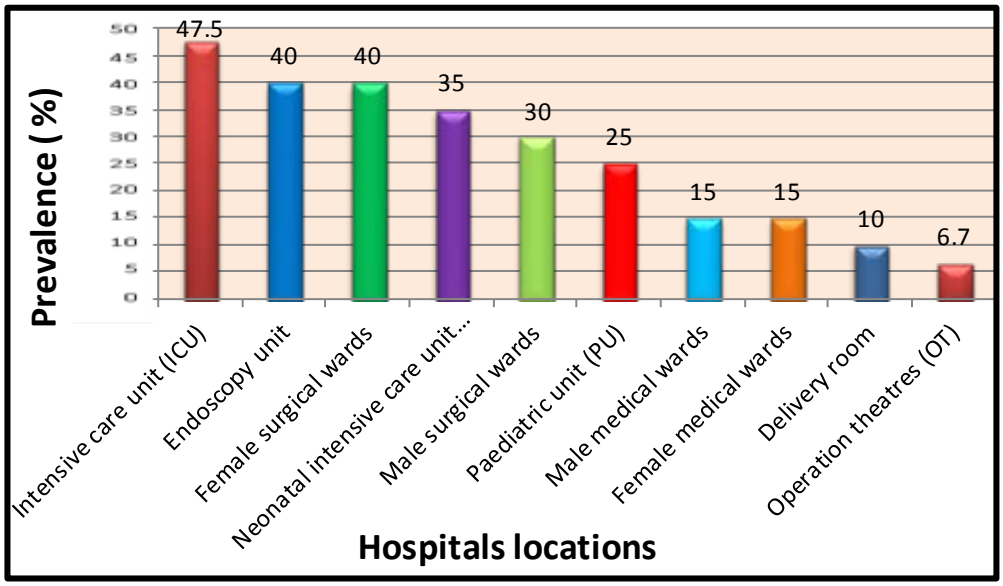

Figure 1. Prevalence of *Pseudomonas aeruginosa* in different locations of participating hospitals

3.4. Antibiotic Susceptibility

Among the 14 used antibiotics, the highest percentage of antibiotic resistance was to Ceftriaxone, Ampicillin, Cefotaxime and Chloramphenicol (100%) and low antibiotic resistance to Imipenem (5.4%) (Table 3).

Table 3. Antibiotic sensitivity tests *Pseudomonas aeruginosa* strains

Antibiotics	No. of esistant Strains	%	No. of ensitive Strains	%	No. of oderate Strains	%
Ceftriaxone	56	100	-	-	-	-
Ampicillin	56	100	-	-	-	-
Cefotaxime	56	100	-	-	-	-
Chloramphenicol	56	100	-	-	-	-
Erythromycin	55	98.2	-	-	1	1.8
Tobramycin	21	37.5	4	7.1	31	55.4
Ceftazidime	19	33.9	34	60.7	3	5.4
Amikacin	19	33.9	17	30.4	20	35.7
Piperacillin	15	26.8	31	55.4	10	17.9
Aztreonam	13	23.2	39	69.6	4	7.1
Ciprofloxacin	10	17.9	43	76.8	3	5.4
Gentamicin	10	17.9	35	60.7	11	19.6
Piperacillin/ tazobactam	6	10.7	45	80.4	5	8.9
Imipenem	3	5.4	53	94.6	-	-

3.5. Antibiogram

Table 4 shows the antibiogram of *P. aeruginosa* strains isolated from clinical, environment and staff hands samples, showing 16 ARP (antimicrobial resistance pattern) including resistance ranged from 5 to 13 antimicrobial. Resistance pattern A1 (48.2%) isolates was the more frequent.

3.6. Molecular Techniques

Out of 51 (91%) isolates *P. aeruginosa* were typeable by ERIC-PCR. The results showed that the size of amplified DNA bands ranged from (150-500) (base pair) with high genetic diversity and yielded 11 ERIC genetic patterns, with 1 to 3 bands (Figure 2).

The majority of strains were from pattern I and pattern II in rate of (24%) for each pattern whereas (18 %) from pattern III, (14%) from pattern IV, (3.9%) from pattern V, VI, VIII and IX and only one isolate (1.9%) from patterns VII, X and XI (Table 5).

Table 4. Antibiogram patterns of *Pseudomonas aeruginosa* strains isolated from Al-Mukalla Hospitals

ARP no.	ARP (Antibiogram)	No.of strains %	Samples
A1	CRO/CTX/C/E/AMP	27(48.2%)	Urine(3), wound and burn(7), Respirators(2), Throat swabs (4), sputum(4), staff hand, CSF, Endoscope, Suction apparatus tubing (2), Antiseptic solutions, Ear secretions
A2	CRO/CTX/C/E/AMP/CAZ	2 (3.6%)	wound and burn, staff hand
A3	CRO/CTX/C/E/AMP/TOB/AK	4 (7.1%)	CSF, Ear secretions, wound and burn, Air condition outlet
A4	CRO/CTX/C/E/AMP/CAZ/PI	2 (3.6%)	Suction apparatus tubing, Urine
A5	CRO/CTX/C/E/AMP/TOB/AK/CIP/AT/CAZ	2 (3.6%)	wound and burn, Water tap
A6	CRO/CTX/C/E/AMP/TOB/AK/CIP	2 (3.6%)	Water tap, wound and burn
A7	CRO/CTX/C/E/AMP/TOB/AK/CIP/PI	1 (1.8%)	Sputum
A8	CRO/CTX/C/E/AMP/TOB/AK/GEN/PI/CAZ/AT	4 (7.1%)	Suction apparatus tubing, wound and burn(2), Sputum
A9	CRO/CTX/C/E/AMP/TOB/AK/GEN/PI/CAZ/CIP/PIT/IPM	1 (1.8%)	Sputum
A10	CRO/CTX/C/E/AMP/PI/CIP/CAZ	2 (3.6%)	wound and burn, Blood
A11	CRO/CTX/C/E/AMP/TOB/CAZ/PI/GEN	1 (1.8%)	Sputum
A12	CRO/CTX/C/E/AMP/GEN/CAZ/AT	2 (3.6%)	wound and burn, Endoscope
A13	CRO/CTX/C/E/AMP/CIP/AK/TOB/GEN/PI/PIT	1 (1.8%)	wound and burn
A14	CRO/CTX/C/E/AMP/AK/TOB/GEN	1 (1.8%)	Urine
A15	CRO/CTX/C/E/AMP/CIP/AK/TOB/PIT/AT	1 (1.8%)	Ear secretions
A16	CRO/CTX/C/E/AMP/CAZ/PI/PIT/AT/TOB	3 (5.4%)	wound and burn(2), Urine

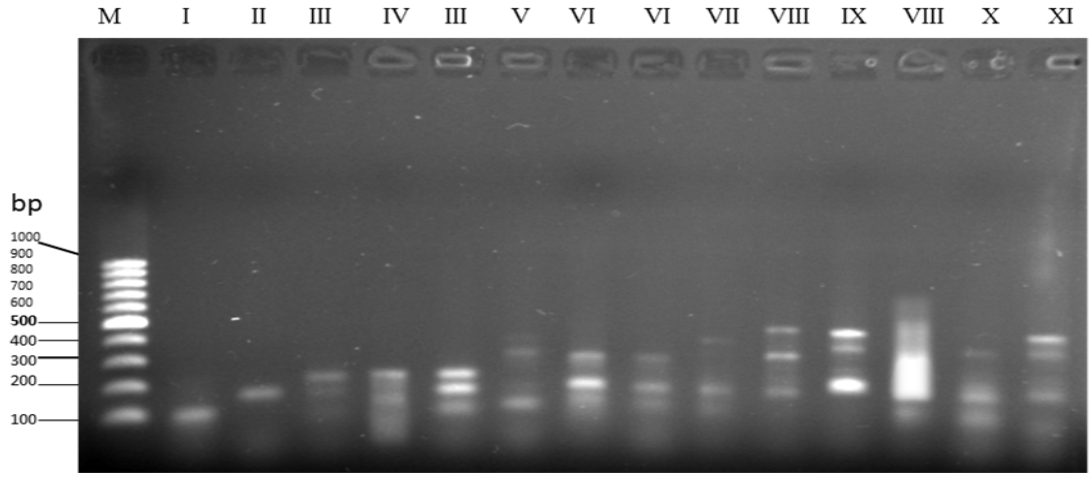

Figure 2. Different eleven patterns enterobacterial repetitive intergenic consensus (ERIC-PCR) of *Pseudomonas aeruginosa* strains

Table 5. (ERIC)-PCR patterns of *Pseudomonas aeruginosa* strains isolated from patients, hospital environment and staff hands

Genetic patterns No.	Bands No.	Size ofamplified DNA bands (bp)	Isolates No.	Isolates %
I	1	100- 150	12	23.5
II	1	150- 200	12	23.5
III	3	100-150, 200, 250-300	9	17.6
IV	2	100-150, 200-250	7	13.7
V	2	100-150, 300-350	2	3.9
VI	3	100-150, 200, 300	2	3.9
VII	2	150-200, 400	1	1.96
VIII	3	150-200, 300, 450-500	2	3.9
IX	3	150-200, 300-350, 400-450	2	3.9
X	2	100-150, 300	1	1.96
XI	3	100-150, 300, 400	1	1.96

3.7. Epidemiological Relationships of Genetic Patterns (ERIC PCR) of Pseudomonas aeruginosa Isolates

From ERIC-PCR typing methods P. aeruginosa strains (Table 6) and (Figure 3) showed horizontal transmission from patient to patient (ERIC-PI, PII, PIII, PIV, PVIII and PIX genetic patterns, respectively), probably from the hands of health care workers or environmental sources. Isolates from patients had ERIC-PI, PII, PIII, PIV and VI pattern linked to all isolates from environmental sources and staff hand samples that indicated that there were direct relationships between them.

ERIC-PCR typing gave epidemiological relationships among sites: suction apparatus tubing, respirators and staff hand samples that had the same patterns (ERIC-PII genetic patterns), also, it found links to suction apparatus tubing, endoscope, air condition outlet and antiseptic solutions (ERIC-PIII and PIV genetic patterns).

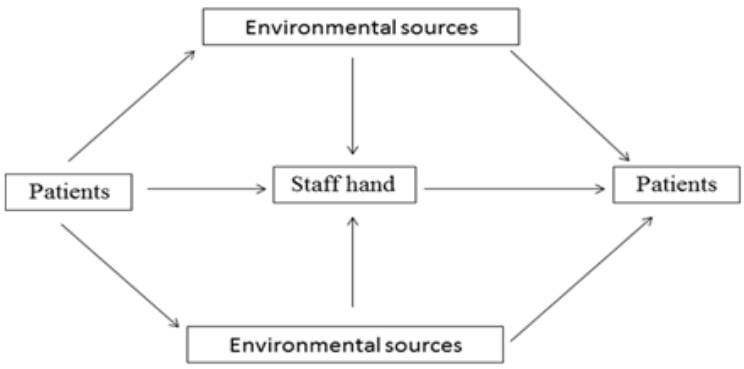

Figure 3. Diagram of epidemiological relationships between strains isolated from patients, environmental sources and staff hand using genotyping method

Table 6. Epidemiological relationships of genetic patterns (ERIC PCR) of *Pseudomonas aeruginosa* strains isolated from samples obtained from patients, the environment and staff hands

Clinical samples	Genetic patterns No.										
	PI	PII	PIII	P IV	P V	P VI	P VII	P VIII	P IX	P X	P XI
Wounds and burns	7	-	3	4	-	-	1	1	1	1	-
Sputum	2	-	2	-	2	-	-	-	-	-	-
Urine	-	4	-	1	-	1	-	-	-	-	-
Throat swabs	-	3	-	-	-	-	-	-	-	-	-
Ear secretions	1	-	-	-	-	-	-	-	-	-	1
CSF	-	-	-	-	-	-	-	1	1	-	-
Blood	1	-	-	-	-	-	-	-	-	-	-
Environment and staff hands samples											
Suction apparatus tubing	-	2	1	1	-	-	-	-	-	-	-
Endoscope	-	-	1	1	-	-	-	-	-	-	-
Respirators	-	1	-	-	-	1	-	-	-	-	-
Water tap	1	-	-	-	-	-	-	-	-	-	-
Air condition outlet	-	-	1	-	-	-	-	-	-	-	-
Antiseptic solutions	-	-	1	-	-	-	-	-	-	-	-
Staff hand samples	-	2	-	-	-	-	-	-	-	-	-
Total	12	12	9	7	2	2	1	2	2	1	1
%	23.5	23.5	17.6	13.7	3.9	3.9	1.9	3.9	3.9	1.9	1.9

P: Pattern

3.8. Distribution of Antibiogram According to the Chromosomal Patterns

The distribution of chromosomal patterns (ERIC-PCR) according to Antimicrobial Resistance Profile (ARP), 51 of P. *aeruginosa* isolated (Table 7) showed 16 ARP including resistance ranged from 5 to 13 antimicrobials.

The strains with ERIC-PCR pattern I 23.5% (the more frequent) were distributed in 10 different ARPs (A1, A3, A5, A6, A8, A9, A10, A11, A15 and A16), there was a statistically significant difference between antibiogram and genetic pattern (ERIC-PCR) in the rate of isolation of *P. aeruginosa* (*P*-value= 0.695) and (Chi-square χ^2 = 1.4).

Table 7. Distribution of chromosomal patterns (ERIC-PCR) in relation to antimicrobial resistance pattern (ARP) for 51 *Pseudomonas aeruginosa* strains isolated from clinical, environment and staff hands samples from Al-Mukalla Hospitals

ARP no.	ARP (Antibiotypes)	No. of strains (%)	Genotypes (DNA patterns)	Chi-square χ^2	*P*-value
A1	CRO/CTX/C/E/AMP	24(47.1%)	PI, PII(8), PIII(6),PVIII(2), PIV(4), P V (2),PVI		
A2	CRO/CTX/C/E/AMP/CAZ	2 (3.9%)	PII ,P IX		
A3	CRO/CTX/C/E/AMP/TOB/AK	4 (7.8%)	PI, PIII, PIX, P XI		
A4	CRO/CTX/C/E/AMP/CAZ/PI	2 (3.9%)	PII, P VI		
A5	CRO/CTX/C/E/AMP/TOB/AK/CIP/AT/CAZ	1(1.96%)	PI		
A6	CRO/CTX/C/E/AMP/TOB/AK/CIP	2 (3.9%)	PI (2)	1.40	0.695
A7	CRO/CTX/C/E/AMP/TOB/AK/CIP/PI	1(1.96%)	PIII		
A8	CRO/CTX/C/E/AMP/TOB/AK/GEN/PI/CAZ/AT	3 (5.9%)	PI, PIII, P IV		
A9	CRO/CTX/C/E/AMP/TOB/AK/GEN/PI/CAZ/CIP/PIT/IPM	1(1.96%)	PI		
A10	CRO/CTX/C/E/AMP/PI/CIP/CAZ	2 (3.9%)	PI (2)		
A11	CRO/CTX/C/E/AMP/TOB/CAZ/PI/GEN	1(1.96%)	PI		
A12	CRO/CTX/C/E/AMP/GEN/CAZ/AT	2 (3.9%)	PIV, P X		
A13	CRO/CTX/C/E/AMP/CIP/AK/TOB/GEN/PI/PIT	1(1.96%)	P IV		
A14	CRO/CTX/C/E/AMP/AK/TOB/GEN	1(1.96%)	PII		
A15	CRO/CTX/C/E/AMP/CIP/AK/TOB/PIT/AT	1(1.96%)	PI		
A16	CRO/CTX/C/E/AMP/CAZ/PI/PIT/AT/TOB	3 (5.9%)	PI, PII,P VII,		

4. Discussion

Analysis of the results indicated the occurrence of 28% *P. aeruginosa* infections in all hospitals, out of 30% strains were from clinical patient specimens. This is in agreement with rate reported in Egypt and Saudi Arabia by Mansour *et al.* (32.8%) and (30.0%), respectively, and in Saudi Arabia by Al Johani *et al.* (2010) (30.6%). The majority of samples (60%) were isolated from wounds and burns, similar results were obtained in Yemen (Yacin, 2009) and Saudi Arabia (Mansour *et al*., 2013). The high rate of *P. aeruginosa* from wounds and burn this may be due to the ability of *P. aeruginosa* to colonize in damaged skin of burned patients, where the first defense barrier against bacteria is lost (Marquart *et al*., 2005). Also, the high rate in this ward might be due to that *P. aeruginosa* has the ability to survive in different environments and it becomes an opportunistic pathogen for patients especially in the burn ward (Jamasbi and Proudfoot, 2008).

In the present study, the percentage of hospital environment samples were 26.7% strains and 13.3% (*P*-Value= 0.19) of strains belonged staff hand samples. Similar results were obtained in Egypt and Saudi Arabia (Mansour *et al*., 2013) that shown the rate of isolation from environment samples were 25.0% and 23.3%, respectively, and from staff hand samples were 10.0% and 6.7%, respectively. The isolation rate of *P. aeruginosa* from environmental samples was 26.7%, which is slightly higher than a previous study in Egypt (19.5%) (Gad *et al*., 2007). The higher rate of isolation from staff hands could be due to lack of compliance of health care workers to hand-washing practices.

The highest percentage of *P. aeruginosa* isolates was (50%) (*P*-Value= 0.16) from suction apparatus tubing, this may be explained by the failure of sterilization of suction apparatus tubing, this is similarly that found in Egypt and Saudi Arabia (Mansour *et al*., 2013) (57.1%) and (42.9%), respectively. However, these findings are different from those of a previous study in which the number of suction tubing infection was lower than other infection (Pal *et al*., 2010).

The analysis showed that the risk factors for *P. aeruginosa* infection were in male (57.1%) (*P*-Value = 0.91), and the age group of (> 40-60) years (42.8%) (*P*-value= 0.51), this is in agreement with rates in previous studies (Zavascki *et al*., 2005; Jamasbi and Proudfoot, 2008; Pal et al., 2010), and surgery disease (47.6%) (*P*-values= 0.31) (Zavascki *et al*., 2005), the rate of *P. aeruginosa* in surgery was higher than that reported from U.S.A (8%) during 1990-1996 (Qarah, 2008).

In the present work, the highest incidence of *P. aeruginosa* (47.5%) (*P*-value = 0.002) was found in ICU, which was followed by endoscopy unit and female surgical wards (40%), NICU (35%), male surgical wards (30%), PU (25%), male and female medical wards (15%), delivery room (10%) and OT (6.7 %); this is in agreement with Pall *et al.* (2010) and Bashir *et al*. (2011). The Gram-negative bacterium *P. aeruginosa* is frequently associated with hospital-acquired infections in ICUs (Driscoll *et al*., 2007). In this connection, intensive care patients are more prone to infection because of the debilitating effect of a prolonged hospitalization and instrumentation (Jarlier *et al*., 1996).

The most resistance antibiotics used were Ceftriaxone, Ampicillin, Cefotaxime and Chloramphenicol (100%), and the most effective antibiotic used was Imipenem (94.6%) (Fazeli *et al*., 2012; Sedighi *et al*., 2015). However, these findings are different from those of previous studies (Kamel *et al*., 2011; Adeyemi *et al*., 2015). The differences in antibiotic susceptibility in different regions could be attributed to the differences in patient population, the duration of hospitalization, cross-infection, and the dose and types of antibiotics (Rossello *et al*., 1992).

The 16 different ARPs observed in the strains isolated in the present study presented resistance to antimicrobials characterizing the analyzed strains as multi-drug resistant, similarly to Loureiro *et al.* (2002). Multiple factors may contribute to *P. aeruginosa* antibiotic resistance. The low outer membrane permeability and active efflux systems have been implicated in this process (Westbrock-Wadman *et al*., 1999). The mechanisms of acquisition of resistance include mutation, transmission of resistant plasmids, and production of inducible b-lactamase enzymes (Pagani *et al*., 2004).

In the present study from ERIC-PCR typing methods *P. aeruginosa* strains showed horizontal transmission from patient to patient (ERIC-PI, PII, PIII, PIV, PVIII and PIX genetic patterns, respectively), probably from the hands of health care workers or environmental sources (Mansour *et al*., 2013). Isolates from patients linked to all isolates from environmental sources and staff hand samples which had the same genetic patterns that indicated there was direct relationships between them. However, these findings indicate transmission of these stains by contamination during the use of medical instruments. A similar observation was described by Yorioka *et al.* (2010).

The strains with ERIC-PCR pattern I were distributed in 10 different ARPs (A1, A3, A5, A6, A8, A9, A10, A11, A15 and A16), the statistically significant was (*P*-value= 0.695) and (Chi-square χ^2 = 1.4).

Antibiogram typing methods explained the link between strains isolated from patients and environmental sources, but failed to show a direct relationship between patients and hands of health staff workers.

A significant decline in susceptibility of *P. aeruginosa* to β-lactams, aminoglycosides, and quinolones has been observed in many countries, including the United States (Livermore, 2002). Nosocomial outbreaks and the spread of MDR in different hospitals have also been documented (Bukholm *et al*., 2002). It appears that the frequency

and rate of resistance to individual antibiotics are different in different regions (Gales *et al*., 2001).

Our ERIC-PCR genotyping indicated the high diversity of *P. aeruginosa*, it was found that some isolates possessed a unique genotype and the most stains with similar genotypic characteristics (Jamasbi and Proudfoot, 2008).

The frequent phenotypic and genotypic changes could complicate the epidemiological studies of *P. aeruginosa* (Jamasbi and Proudfoot, 2008). Therefore, until a more precise molecular technique becomes available, each health care facility should establish a procedure for prompt identification, and perform phenotypic and genotypic analysis frequently, along with an effective infection-control measurement to prevent or reduce the spread of *P. aeruginosa* infections.

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