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Prevalence and Antimicrobial Susceptibility Pattern of *Pseudomonas aeruginosa* Isolated from Environmental and Clinical Samples in Upper Egypt

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ABSTRACT

The present study was performed to isolate, characterize and evaluate the prevalence of *Pseudomonas aeruginosa* (*P. aeruginosa*) in 600 samples (environmental and clinical) at Upper Egypt. Antibiotic sensitivity, biotyping and molecular identification of *P. aeruginosa* isolates were determined, as well as hemagram was done on all positive clinical cases. Out of examined 200 environmental samples, 42 *P. aeruginosa* strains (21%) were isolated. Out of examined 400 clinical specimens, 70 and 16 *P. aeruginosa* strains (35 and 8%) were isolated from sputum and urine, respectively. The Multi-Drug Resistant (MDR) *P. aeruginosa* isolates gave resistance to a number of antimicrobial types ranged from 4/14-10/14. The total antimicrobial resistance profile of *P. aeruginosa* isolated from environmental and clinical samples ranged from 3.1% (colistin) to 95.3% (cephalothin, cefuroxime and cefoxitin). The total mean of MIC of tested antimicrobials versus *P. aeruginosa* (n = 128) ranged from $1.13 \pm 0.02 \mu\text{g mL}^{-1}$ (colistin) to $24.7 \pm 2.1 \mu\text{g mL}^{-1}$ (ceftriaxone). The prevalence of *P. aeruginosa* between environmental and clinical samples showed significant correlation. Moreover, significant correlation found in total antimicrobial resistance profile between the tested antimicrobials except imipenem and aztreonam shown non significant ($p > 0.05$). PCR analysis of *P. aeruginosa* indicates that species specific signature sequences were present in 100% of the tested isolates. The hospital environment and healthcare personnel could serve as potential reservoirs of *P. aeruginosa* in the study locality. The obtained results may help in prevention and control strategies of *P. aeruginosa* infection in both the hospital and the community.

Key words: *Pseudomonas aeruginosa*, environmental sample, clinical specimen, antibiogram, PCR

INTRODUCTION

P. aeruginosa has become an important nosocomial pathogen. In addition, MDR *P. aeruginosa* is an emerging nosocomial pathogen worldwide (Mansouri *et al.*, 2011). It is the fifth common pathogen among hospital microorganisms and causes 10% of all hospital infection (Anonymous, 1999) and implicated in urinary tract infections (Harjai *et al.*, 2005; Rello *et al.*, 2006; Manikandan *et al.*, 2011). Moreover, the effectiveness of a multidimensional approach for the prevention of ventilator-associated pneumonia in an adult intensive care unit has been performed

(Guanche-Garcell *et al.*, 2013). Therefore, knowledge of current drug resistance pattern of the *P. aeruginosa* in environmental and clinical samples in Upper Egypt is useful in clinical practice.

Huang *et al.* (2002) showed that leucopenia was found in some sepsis cases and in other cases leukocytosis were seen. Therefore, the isolation of antimicrobial resistant bacteria from hospital environment may represent a public health hazard. *P. aeruginosa* was isolated from swabs of different aqueous environments in hospital (Bradbury *et al.*, 2009).

This study provides an extensive antimicrobial susceptibility report of *P. aeruginosa* isolated from environmental and clinical samples. There is lack of the available literature about the current status of both *P. aeruginosa* occurrence and their antibiotic sensitivity in Upper Egypt.

The present study is aimed at studying the prevalence of *P. aeruginosa* in examined environmental and clinical samples, its antimicrobial sensitivity and molecular typing, as well as clinical hematological profile.

MATERIALS AND METHODS

Study design: A total of 600 samples were collected between December 2009 and September 2010 and examined, of which 200 randomly selected environmental swabs were collected from walls, floors, tables, trays, as well as medical appliances and the surroundings of out-patient clinics at the Al-Azhar University Hospital in Assuit. Four hundred clinical specimens were of origin constituting 200 each of urine (from patients with urinary tract infection) and sputum (from patients with respiratory tract infection). Data such as age and sex of patients were recorded. Ethical approval to undertake the study was obtained from the management board of the hospital.

Bacteriological analysis: Collected samples were transported to the laboratory following standard methods (Koneman *et al.*, 1992; Forbes *et al.*, 1998; Cheesbrough, 2000). The samples were plated primarily onto blood agar and incubated at 37°C for 24-48 h. Suspected isolates were presumptively identified by colony morphology, pigment formation, mucoidy, haemolysis on blood agar, positive oxidase test, grape-like odor, growth at 42°C on nutrient agar, positive motility and Gram reaction (Cheesbrough, 2000).

Antimicrobial susceptibility testing: The disc diffusion technique was used as previously described (Bauer *et al.*, 1966). The interpretation of the results was done according to Clinical Laboratory Standard International (CLSI, 2007). Briefly, a sterile cotton swab of bacterial suspension was streaked onto Mueller-Hinton Agar (MHA) plates (Biotec, UK). Thereafter, antimicrobial discs with the following drug contents amikacin (30 µg), gentamicin (30 µg), entrapenem (10 µg), imipenem (10 µg), meropenem (10 µg), cephalothin (30 µg), cefuroxime (30 µg), cefoxitin (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefepime (30 µg), aztreonam (30 µg), ampicillin (25 µg) and colistin (25 µg) were placed on the plates. The plates were incubated at 37°C for 16-18 h. All antimicrobial discs were obtained from Oxoid (England).

Minimum inhibitory concentration: The MICs of the same antimicrobials (Sigma-Aldrich) tested for resistance were determined by the agar dilution method according to Clinical and Laboratory Standards Institute (CLSI, 2006) on MHA. Overnight cultures of tested organisms on Mueller Hinton Broth (MHB) were diluted to the initial cell density of 10^7 CFU mL⁻¹ with fresh MHB. Inocula of 10^5 CFU per spot were applied to the surface of dry MHA plates containing graded

concentrations (from 1-1024 mg L⁻¹) of the respective antimicrobials. Plates were incubated at 37°C for 24 h and MICs were calculated. Spots with the lowest concentrations of antimicrobial showing no growth were defined as the MIC.

Hematological examination: Blood samples were collected from positive *P. aeruginosa* cases by venipuncture with aseptic procedures. Samples were taken for Complete Blood Count (CBC) in vacutainer EDTA tubes and for coagulation screening in vacutainer 3.2% sodium citrate tubes. Samples then sent to laboratory for examination. All blood samples (n = 86) were analyzed according to the standard haematological procedures.

CBC was done by CBC analyzer (Haematology Analyzer Siemens, Germany), giving total WBC, RBC (Red blood cells), Hb (hemoglobin), PCV (Packed cell volume), MCV (Mean corpuscular volume), MCH (Mean corpuscular haemoglobin), MCHC (Mean corpuscular haemoglobin concentration), platelet count and differential leucocytic count. Coagulation screening of Prothrombin Time (PT) and activated Partial Thromboplastin Time (aPTT) was performed in each case by coagulation analyzer (Coagulation Analyzer Condor-TECO, China). Both PT and aPTT were measured (NCCLS, 1996).

PCR detection of *P. aeruginosa*

Preparation of DNA: DNA was prepared from isolates as described previously (Liu *et al.*, 2002). Briefly, a single colony was suspended in 20 µL of lysis buffer containing 0.25% (v/v) Sodium Dodecyl Sulfate and 0.05 N NaOH. After heating for 15 min at 95°C, 180 µL of Milipore water was added and the lysis suspension was stored at -20°C.

Primers: The used primers: PA-SS-F (5' GGGGGATCTTCGGACCTCA 3') and PA-SS-R (5' TCCTTAGAGTGCCACCCG 3') were designed previously by Spilker *et al.* (2004) to amplify only *P. aeruginosa* as it targeted species specific signature sequences.

PCR: Amplification of targeted DNA was carried out in 25 µL reaction volume as described by Spilker *et al.* (2004). Each containing 2 mM MgCl₂, 50 mM Trizma (pH 8.3; Sigma, St. Luis, Mo.), 250 µM (each) triphosphates (Promega, Madison, Wis.), 0.4 µM of the primer (Invitrogen, Germany), 0.5 U of *taq* polymerase (Qiagen, Germany) and 2 µL of whole-cell bacterial lysate and adjusted to 25 µL PCR-molecular biology grade water. Amplification was carried out in thermal cycler (Biometra, TProfessional Thermocycler, Germany). After an initial denaturation for 2 min at 95°C, 25 cycles were completed, each consisting of 20 sec at 94°C, 20 sec at 58°C and 40 sec at 72°C. A final extension of 1 min at 72°C was applied. The expected amplified band is 956 bp. Amplification products were electrophorased in agarose gel (Biometra, Compact XS/S Horizontal Gel Electrophoresis Apparatus, Germany).

Statistical analysis: The Chi-square (χ^2) test was performed with SPSS 16 statistical software for Windows and a probability value of 0.05 or less was considered to be significant. The mean and standard deviation were done using the same statistical software.

RESULTS

In the present study, out of 600 examined environmental (200) and clinical (400) samples, 128 (12.3%) were positive to *P. aeruginosa*. Concerning the environmental samples, 21% (42/200) were positive for *P. aeruginosa*, while the examined sputum and urine specimens were with a total frequency of 35% (70/200) and 8% (16/200), respectively (Table 1).

Table 1: Prevalence of *P. aeruginosa* in environmental and clinical samples and their multi-resistant isolates/No. of resisted antimicrobial types

Source	Total No. of samples	Positive No. (%)	Multi-resistant isolates No. (%) / No. of resisted antimicrobials							p-value
Environmental	200	42 (21)	1 (2)/4	4 (10)/5	20 (48)/6	5 (12)/7	5 (12)/8	6 (14)/9	1 (2)/10	0.000
Sputum	200	70 (35)	13 (19)/4	21 (30)/5	11 (16)/6	9 (13)/7	8 (11)/8	6 (9)/9	2 (3)/10	0.000
Urine	200	16 (8)	1 (6)/4	1 (6)/5	7 (44)/6	2 (13)/7	1 (6)/8	3 (19)/9	1 (6)/10	0.000
Total	600	128 (12.3)	15 (12)/4	26 (20)/5	38 (30)/6	16 (13)/7	14 (11)/8	15(12)/9	4(3)/10	

Table 2: *In vitro* antimicrobial resistance profile of *P. aeruginosa* isolated from environmental and clinical samples

Antimicrobial resistance profile of <i>P. aeruginosa</i> isolated from environmental and clinical samples									
Antimicrobials	Environmental (n = 42)		Sputum (n = 70)		Urine (n = 16)		Total (n = 128)		p-value
	No.	%	No.	%	No.	%	No.	%	
Amikacin	02	04.8	06	008.6	02	12.5	010	07.8	0.000
Gentamicin	05	11.9	18	025.7	04	25.0	027	21.1	0.000
Entrapenem	34	81.0	70	100.0	14	87.5	118	92.2	0.000
Imipenem	09	21.4	41	058.6	03	18.8	053	41.4	0.110
Meropenem	04	09.5	16	022.9	03	18.8	023	18.0	0.000
Cephalothin	38	90.5	70	100.0	14	87.5	122	95.3	0.000
Cefuroxime	38	90.5	70	100.0	14	87.5	122	95.3	0.000
Cefoxitin	38	90.5	70	100.0	14	87.5	122	95.3	0.000
Ceftazidime	04	09.5	18	025.7	03	18.8	025	19.5	0.000
Ceftriaxone	34	81.0	53	075.7	13	81.3	100	78.1	0.000
Cefepime	02	04.8	18	025.7	00	00.0	020	15.6	0.000
Aztreonam	11	26.2	41	058.6	07	43.8	059	46.1	0.424
Ampicillin	37	88.1	70	100.0	14	87.5	121	94.5	0.000
Colistin	00	00.0	03	004.3	01	06.3	004	03.1	0.000

Moreover, the results of MDR *P. aeruginosa* isolates give resistance to a total number of resisted antimicrobial types (n = 4/14-10/14). The frequency of MDR strains in respect to No. of resisted antimicrobials ranged from 4(3%)-38(30%). The prevalence of *P. aeruginosa* between the environmental and clinical samples shown significant correlation (Table 1).

In vitro antimicrobial resistance profiles of *P. aeruginosa* (n = 128) isolated from environmental (n = 42) and clinical (n = 86) sources recorded in Table 2. The highest frequencies of antimicrobial resistance were 95.3% to cephalothin, cefuroxime and cefoxitin followed by 94.5% to ampicillin and 92.2% to entrapenem. The medium resistance rates were 78.1, 46.1 and 41.4% to ceftriaxone, aztreonam and imipenem, respectively. While, the lower resistance rates were 21.1, 19.5, 18.0 and 15.6% to gentamicin, ceftazidime, meropenem and cefepime, respectively, and the lowest resistance rates were 7.8% to amikacin and 3.1% to colistin. Significant correlations were found between the total resistance percentages in the tested antimicrobials except imipenem and aztreonam found non significant (p>0.05) (Table 2).

The results shown in Table 3 reveal the MIC mean of common antimicrobials (n = 14) versus *P. aeruginosa* isolates from environmental and clinical sources. The highest mean of MIC was $24.7 \pm 2.1 \mu\text{g mL}^{-1}$ to ceftriaxone, and the medium MIC mean was $16 \pm 0.0 \mu\text{g mL}^{-1}$ to cephalothin, cefuroxime, cefoxitin and ampicillin. Moreover, low MIC means were 11.36 ± 1.6 and $11 \pm 1.0 \mu\text{g mL}^{-1}$ to amikacin and aztreonam, respectively, while the lowest MIC mean was $1.13 \pm 0.02 \mu\text{g mL}^{-1}$ to colistin.

Table 3: Mean of MIC of common antimicrobials versus *P. aeruginosa* isolated from environmental and clinical samples

Antimicrobials	Mean of MIC ($\mu\text{g mL}^{-1}$)				p-value
	Environmental (n = 42)	Sputum (n = 70)	Urine (n = 16)	Total (n = 128)	
Amikacin	9.9 \pm 6.3	12 \pm 8.5	13 \pm 10.2	11.36 \pm 1.6	0.317
Gentamicin	3.0 \pm 2.3	3.5 \pm 2.5	4.6 \pm 3.1	3.7 \pm 0.8	0.001
Entrapenem	3.7 \pm 1.2	3.7 \pm 1.1	3.8 \pm 0.8	3.73 \pm 0.1	0.001
Imipenem	4.1 \pm 2.9	5.1 \pm 3.1	3.7 \pm 2.8	4.3 \pm 0.7	0.001
Meropenem	2.31 \pm 2.5	3.81 \pm 3.1	2.94 \pm 3.0	3.02 \pm 0.7	0.000
Cephalothin	16 \pm 1.7	16 \pm 1.0	16 \pm 2.0	16 \pm 0.0	0.162
Cefuroxime	16 \pm 0.0	16 \pm 0.0	16 \pm 0.0	16 \pm 0.0	0.162
Cefoxitin	16 \pm 0.0	16 \pm 2.0	16 \pm 0.0	16 \pm 0.0	0.162
Ceftazidime	6.14 \pm 5.5	7.51 \pm 6.2	8.25 \pm 6.4	7.3 \pm 1.1	0.028
Ceftriaxone	23 \pm 10.0	24 \pm 9.5	27 \pm 8.6	24.7 \pm 2.1	0.028
Cefepime	6.2 \pm 6.3	7.7 \pm 6.5	8.9 \pm 6.0	6.7 \pm 1.3	0.549
Aztreonam	10 \pm 5.1	11 \pm 4.8	12 \pm 4.8	11 \pm 1.0	0.000
Ampicillin	16 \pm 0.0	16 \pm 0.0	16 \pm 0.0	16 \pm 0.0	0.162
Colistin	1 \pm 0.0	1 \pm 0.0	1.4 \pm 1.0	1.13 \pm 0.02	0.000

Table 4: Haemogram of *P. aeruginosa* infected cases in the present study

Gender	Tests/results	RBCs $\times 10^{12} \text{ L}^{-1}$	Hb g dL $^{-1}$	PCV (%)	MCV FL	MCH PG	MCHC (%)	WBCs $\times 10^9 \text{ L}^{-1}$	Platelets $\times 10^9 \text{ L}^{-1}$
Male	Reference range	4-5.4	14-18	42-52	80-94	27-31	33-37	4-11	130-400
	Results	3.81 \pm 0.3	11.05 \pm 2.6	35.45 \pm 2.9	93.04 \pm 0.2	29.00 \pm 4.1	31.17 \pm 4.5	20.49 \pm 8.1	126 \pm 8.5
Female	Reference range	4.2-5.4	12-16	37-47	81-99	27-31	33-37	4-11	130-400
	Results	3.68 \pm 1.1	10.05 \pm 2.2	32.10 \pm 9.1	87.23 \pm 1.7	27.65 \pm 2.1	31.31 \pm 3.4	18.89 \pm 3.6	102 \pm 11.1

*RBCs: Red blood cells, Hb: Haemoglobin, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, WBCs: White blood cells

The statistical analysis evidenced a significant correlation of total MICs between the tested antimicrobials except cephalothin, cefuroxime, ceftazidime, ampicillin ($p = 0.162$), amikacin ($p = 0.317$) and cefepime ($p = 0.549$) found non significant (Table 3).

In the current investigation, CBC for the examined male cases showed that RBCs, Hb, PCV, MCHC and platelets decreased below the reference ranges which were 3.81 \pm 0.3 $\times 10^{12} \text{ L}^{-1}$, 11.05 \pm 2.6 g dL $^{-1}$, 35.45 \pm 2.9 and 31.17 \pm 4.5% and 126 \pm 8.5 $\times 10^9 \text{ L}^{-1}$, respectively. While, both MCV and MCH are within the reference ranges which were 93.04 \pm 0.2 FL and 29.00 \pm 4.1 PG, respectively. Moreover, WBCs count increased to 20.49 \pm 8.1 $\times 10^9 \text{ L}^{-1}$ more than the reference range (Table 4). On the other hand, CBC for the examined female cases showed that RBCs, Hb, PCV, MCHC and platelets decreased below the reference ranges which were 3.68 \pm 1.1 $\times 10^{12} \text{ L}^{-1}$, 10.05 \pm 2.2 g dL $^{-1}$, 32.10 \pm 9.1 and 31.31 \pm 3.4% and 102 \pm 11.1 $\times 10^9 \text{ L}^{-1}$, respectively. While, both MCV and MCH are within the reference ranges which were 87.23 \pm 1.7 FL and 27.65 \pm 2.1 PG respectively. Moreover, WBCs count increased to 18.89 \pm 3.6 $\times 10^9 \text{ L}^{-1}$ more than the reference range (Table 4). Moreover, PT of the blood samples was 27.6 \pm 5.6 sec which was prolonged than being controlled which were 12.8 sec. While, aPTT of the blood samples were 56.5 \pm 15.6 sec which was prolonged than being controlled which were 35.0 sec. (Data not shown). In this study, PCR analysis demonstrated that 100% of the tested *P. aeruginosa* isolates harbored PA-SS gene (Fig. 1).

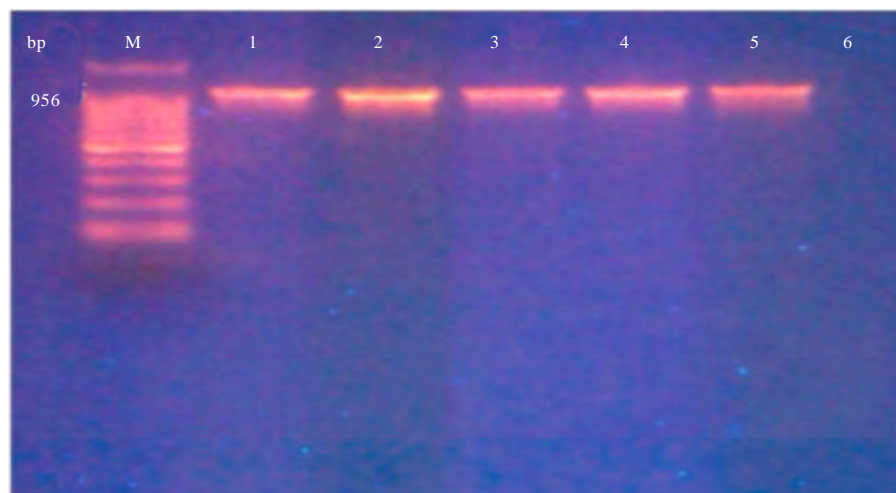


Fig. 1: Agarose gel electrophoresis analysis of PCR amplification using PA-SS pair primers, extracted from *P. aeruginosa* strains isolated from environmental and clinical samples, Lane M: DNA molecular size marker (100 bp ladder) (Qiagen, Germany), lanes 1 and 2: Environmental samples, lanes 3 and 4: Sputum samples, lane 5: Urine sample, lane 6: Negative control

DISCUSSION

In the present study, the antimicrobial sensitivity of *P. aeruginosa* isolates to some commonly used antimicrobials in Egypt was investigated by disc diffusion method (Abukakar *et al.*, 2008; Yesillik *et al.*, 2011; Biswas *et al.*, 2012; Sulaiman *et al.*, 2013).

Concerning the environmental samples, the results indicated that 21% (42/200) were positive for *P. aeruginosa*. This represents a major public health hazard for both hospital and community acquired infection especially for surgical wound contamination. The nosocomial infection of *P. aeruginosa* for surgical wounds was previously reported (Attal *et al.*, 2010; Haleem *et al.*, 2011; Kamel *et al.*, 2011). While the examined specimens were positive for 35% (70/200) and 8% (16/200) in sputum and urine, respectively. Concerning the prevalence of *P. aeruginosa* in sputum and urine specimens, lower frequencies were found previously in Egypt (Kamel *et al.*, 2011) which were 12 (24) and 5 (10%) in sputum and urine, respectively. Moreover, the percentages in Iraq (Haleem *et al.*, 2011) were 7 (4.61) and 15 (9.87%) in sputum and urine, respectively. Accordingly, the recorded findings in India (Attal *et al.*, 2010) were 35 (25) and 32 (22.9%), respectively. Other findings in Iran Tavajjohi and Moniri (2011) were 3 (4) and 17 (22.4%) in sputum and urine, respectively. Nevertheless, similar results in Nigeria (Olayinka *et al.*, 2004) were 38 (41.3%) and 47 (51.1%) in sputum and urine, respectively.

The obtained results may indicate that *P. aeruginosa* in the examined hospital is endemic. Olayinka *et al.* (2004) considered the fact that most patients going in for major surgery tend to get catheterized, so most isolates of *P. aeruginosa* were obtained from urine samples in hospital based cases. Moreover, *P. aeruginosa* is one of the bacteria that commonly contaminates urinary catheters and develops biofilm (Stickler, 1996).

The results of MDR *P. aeruginosa* isolates give resistance to a total number of resisted antimicrobial types (n = 4/14-10/14). The frequency of MDR strains in respect to No. of resisted antimicrobials ranged from 4 (3)-38 (30%). The resistance percentages of environmental and clinical isolates ranged from 3.1-95.3%. Related results have been reported for complete resistance of *P. aeruginosa* against amikacin (Langford *et al.*, 1989) and amoxicillin (Anjum and Mir, 2010; Kamel *et al.*, 2011).

The *in vitro* antibiogram sensitivity test in this work indicate that colistin, amikacin and cefepime are the most effective antimicrobials against the clinical isolates as the recorded overall resistance percentages were 3.1, 7.8 and 15.6, respectively. The present results are near to that found previously (Gales *et al.*, 2002), as they stated that the sensitivity of *P. aeruginosa* against imipenem, meropenem and amikacin were 84, 71.6 and 71.0%, respectively. There is a concord result which showed the sensitivity of *P. aeruginosa* isolates of urinary tract to amikacin, meropenem, gentamycin and cefipime (80.0-93.8%) (Mathai *et al.*, 2001). In addition, prolonged treatment with imipenem in *P. aeruginosa* infected patients allowed the emergence of resistant mutants against it (Okamoto *et al.*, 2001).

The total mean of MIC of common antimicrobials versus *P. aeruginosa* (n = 128) ranged from $1.13 \pm 0.02 \mu\text{g mL}^{-1}$ (colistin) to $24.7 \pm 2.1 \mu\text{g mL}^{-1}$ (ceftriaxone). Kalantar *et al.* (2012) found that MIC of imipenem exceeded $16 \mu\text{g mL}^{-1}$ against 8 *P. aeruginosa* isolates of clinical specimen in Iran. Moreover, the obtained results are near to those previously reported by Shawar *et al.* (1999), who found the MIC ($\mu\text{g mL}^{-1}$) range 0.5-64, 0.25-512, 2-512 and 1-2048 in amikacin, gentamicin, aztreonam, ceftazidime, respectively. Moreover, Gad *et al.* (2007) reported that MIC of meropenem against *P. aeruginosa* in Egypt ranged from 1-128 $\mu\text{g mL}^{-1}$.

The prevalence of *P. aeruginosa* between environmental and clinical samples showed significant correlation, followed by the same correlation in total antimicrobial resistance profile between the tested antimicrobials except imipenem and aztreonam shown non significant ($p > 0.05$). On the other hand, the statistical analysis evidenced a significant correlation of total MIC between the tested antimicrobials except cephalothin, cefuroxime, ceftazidime, ampicillin ($p = 0.162$), amikacin ($p = 0.317$) and cefepime ($p = 0.549$) found non significant. In relation to these analysis, Upper Respiratory Tract Infection (URTI) Isolates were significantly more susceptible than the *P. aeruginosa* isolates from urine, Lower Respiratory Tract Infection (LRTIs) and wounds and drainages to the following antimicrobials: Amikacin ($p = 0.001$), gentamicin ($p = 0.001$), tobramycin ($p = 0.001$), netilmicin ($p = 0.01$, 0.001 and 0.01 , respectively) and ciprofloxacin ($p = 0.001$) (Strateva *et al.*, 2007).

Hematological examination in this study revealed the low number of RBCs, Hb and PCV that indicated as anaemia. Also, both of MCV and MCH were in the reference range. So, these were cases of normocytic normochromic anaemia. Kaaya and Maxie (1980) and Zirngibl *et al.* (2002) mentioned that *P. aeruginosa* endotoxin had effect on erythroid progenitor cells and consequently to decrease in RBCs production. The obtained findings revealed that leucocytosis was due to sepsis in all examined cases. In the present study, decreased number of platelets and prolongation of both PT and aPTT may be an evidence to sepsis and Dissiminated Intravascular Coagulation (DIC) as cleared previously (Taylor *et al.*, 2001; Rak *et al.*, 2006; Levi, 2007), who studied sepsis and DIC in animal models and found that there is a highly expressed receptors on the surface on hepatocyte. The thrombocytopenia observed in bacteremia and sepsis was not due to increased consumption of coagulation factors anzfird platelets only, but also as a result of this receptor's activity enabling hepatocytes to ingest and rapidly clear platelets from circulation (Grewal *et al.*, 2008).

In spite of a few studies have employed molecular typing methods such as enterobacterial repetitive intergenic consensus polymerase chain reaction and PCR-ribotyping to characterize *P. aeruginosa* in Egypt and abroad (Gad *et al.*, 2007; Agarwal *et al.*, 2002). Moreover, MDR phenomena of *P. aeruginosa* isolated from clinical specimens may complicate the treatment of infections and can adversely affect prognosis and treatment costs impact.

In this investigation, the obtained results from PCR analysis of *P. aeruginosa*, PA-SSgene was present in 100% of the tested isolates. Thus, confirmation of *P. aeruginosa* of clinical samples was previously carried out by PCR (Da Silva Filho *et al.*, 1999; Gad *et al.*, 2007). Thus, PCR assay can be applied as a practical diagnostic method for epidemiological research and the sanitary management of potential environmental contamination with *P. aeruginosa*.

CONCLUSION

It could be concluded that *P. aeruginosa* circulating strains is endemic in hospital ecosystem. Environmental and clinical sources are potential reservoir for *P. aeruginosa* infections. MDR *P. aeruginosa* strain is emerging as a public health hazard. The obtained results from environmental and clinical sources may help in prevention and control strategies of *P. aeruginosa* infection in hospital and community.

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