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Prevalance *Pseudomonas aeruginosa* Among Libyan Patients and its Association with Hospital's Environment in Benghazi

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Abstract

A cross sectional study was carried out which included 300 clinical specimens such as urine, pus, throat swabs, ear swabs, blood, taken from patients admitted to different departments, in addition to 300 samples from the surrounding environment in four hospitals in Benghazi. The aim is to identify the major strains of *P aeruginosa* isolated from various sources in different hospitals from both the patients and surrounding environment, also to determine the level of resistance to the widely used antibiotics in clinical isolates of *P aeruginosa*. *P aeruginosa* were isolated from 91 clinical specimens and 18 from environmental sources. All were Gram negative motile bacilli, oxidase positive and grew at 42ºC. All isolates were confirmed by Analytical Profile Index (API 20 NE) for their biochemical activity, all were positive for arginine dihydrogenase, citrate utilization and gelatin hydrolysis, while 37.4% were positive for aerobic glucose utilization. The antibiotic sensitivity tests were carried out according to the disc diffusion method (modified kirby bauer technique). Out of 91 strains of *P aeruginosa* isolated from clinical specimens and 18 strains from the environment, the most effective antibiotics were respectively Ciprofloxacin (82.2%, 83.3%), Imipenem (80.2%, 72.2%), Amikacin (72.5%, 77.8%) and Tobramycin (49.5%, 50%) Gentamicin showed lowest rate of sensitivity (42.2%, 50%). Other antibiotics tested: ampicillin, chloramphenicol, augmentin, pipemidic acid, colistin sulphate and nalidixic acid. The strains isolated were found to have high or total resistance to them. Pyocin typing was used for the characterization of 109 isolates of *pseudomonas aeruginosa* isolated. The scheme of Gillies and Govan was adopted and the procedure gave 86.8% of clinical isolates were typable, while 50% of environmental isolates were typable, 12 (15%) of 79 typable strains isolates from clinical specimens were classifiable while 67 (85%) were nonclassifiable. More over all typable isolates from environment were nonclassifiable. The study concludes that Pseudomonas infection is high among our patients in Benghazi hospitals, and reflects the hospital environment as source of infection. This study also recommends that there should be a review in the current antibiotics policy.

Keywords: *P aeruginosa*; Bacilli; Dehydrogenase; Ciprofloxacin

Introduction

P aeruginosa is clinically significant and opportunistic pathogen, that often cause nosocomial infections. In addition, these organisms exhibit innate resistance to many antibiotics and can develop new resistance after exposure to antimicrobial agents [1].

P aeruginosa is often encountered in hospital and the main targets are immunocompromised individuals, burn victims, and individuals on respirators or with in dewelling catheters [2]. Infection can occur at many sites and can lead to urinary tract infection, septic pneumonia, pharyngitis, in addition to other problems.

P aeruginosa is rarely found as a cause of infection in healthy individuals, its non-invasive nature limits its pathogenic capabilities [3]. *Pseudomonas aeruginosa* is the most frequently isolated non-fermenter G-ve bacillus in the laboratory.

P aeruginosa has intrinsic resistance to most available antibiotics, and is therefore, a particularly dangerous and dreaded pathogen. The bacterium is naturally resistant to many antibiotics due to the permeability barrier afforded by its outer membrane lipopolysaccharide (LPS) [3].

Only few antibiotics are effective against *Pseudomonas*, including fluoroquinolones, gentamicin and imipenem and even these antibiotics are not effective against all strains [4].

A study done by Revathi et al. (1998), which was undertaken at University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi, to examine the bacterial isolates from the Burns unit and to determine the antibiograms of the isolates to commonly used antimicrobial agents, found that *P aeruginosa* was the most common

(36%), and most susceptible to cefazidime (83%) and cefoperazone (82%), whereas the drugs most effective in other Gram-negative organisms were amikacin, netilmicin and ciprofloxacine [5].

Another study done by Yalcin (1995) [6], of postoperative wound infection was carried out over a two-year period in cumhuriyet medicine faculty hospital in Sivas, Turkey. They examined a total of 4146 surgical wounds and found, the commonest causative organisms were *staphylococci* 21.7%, *Escherichia coli* 19.7%, and *Pseudomonas aeruginosa* 10.7%. Diagnosis of *Pseudomonas* infection depends upon isolation and laboratory identification of the bacterium. Also, local study from Benghazi showed that two hundred isolates of *Pseudomonas aeruginosa* from different clinical specimens and four from other sources like water and soap solution. All were at least sensitive to one antibiotic. The most effective antibiotics were found to be colistin, gentamicin and carbenicillin in descending order. All isolates were typed by the pyocin method except for one strain [7].

Objectives

- To determine the role of *Pseudomonas aeruginosa* in hospitals

acquired infection and its sources in different hospitals from (patients and surrounding environment) in Benghazi.

• To determine the level of resistance to the widely used antibiotics in clinical isolates of *Pseudomonas aeruginosa*.

Materials and Methods

A cross sectional, prospective study was carried out over a 6-months period (from Septemper 2004 to February 2005) in four hospitals in Benghazi City.

The data collection was based on prestructured questionnaire to obtain information on the hospital wards, age, and gender of patients, type of specimens including the possible environmental sources.

The sample included 300 patients taken (Using random table numbers) from Al-Jala hospital (A), Al-Jamahiriya hospital (B), 7th April (C) and 7th October hospital (D), the total number from the four hospitals was estimated according to the bed capacity of each hospital.

The patients

The patients studied were selected from all cases admitted at different departments of surgical wards, medical wards, burn surgical ward, Intensive Care Units (ICU), urology wards, skin ward, gyne and obst. ward, Cardiology Care Unit (CCU), Ear Nose and Throat ward (ENT) and Oncology ward, with variation in the age, sex and final diagnosis.

Specimens were obtained from patients to search mainly the presence of *Pseudomonas aeruginosa* such as urine, wound swabs, pus, blood, throat swabs, ear swabs, sputum, vaginal swabs, bed sore swabs, Nasal swabs, and others

The environment

A total of 300 environmental samples were collected from ventilator equipment, suction apparatus, disinfectants used, beds, air, liquid soaps, walls and floors, tap water and other sources from the various wards of the hospitals studied. Swabs moistened with nutrient broth were sent immediately for culture and sensitivity. Air samples were collected by using the settle plate method.

Isolation procedures

All samples were inoculated on MacConkey agar, blood agar, and CLED agar, and incubated aerobically at 37°C for 18 hours to 24 hours.

P aeruginosa was identified according to colony morphology, pigment production, odor, and gram stain. All typical isolates of *Pseudomonas aeruginosa* were tested for oxidase production by using the wet filter method.

A. Biochemical reaction

All *Pseudomonas aeruginosa* isolates were tested for their ability to utilize citrate by inoculation on simmons citrate agar. One hundred and nine isolates were examined for their biochemical activity by inoculating API 20NE.

B. Growth at 42°C

All *Pseudomonas aeruginosa* isolates tested for growth at 42°C.

C. Antibiotic sensitivity

Sensitivity testing was performed by the disc diffusion method (Kirby Bauer technique).

Results

A total of 600 specimens were obtained from patients and environment (300 specimens for each), *P aeruginosa* was isolated from 109 (18.2%) specimens.

Out of 300 patients admitted over the 6-month period there were 91 isolates from clinical specimens. The isolates were identified according to colony morphology, biochemical and microbiological tests. All the 91 isolates were motile, Gram-negative bacilli, oxidase positive.

Bacteria isolated from clinical specimens

The most frequent bacteria isolated from patients in various hospitals studied was *P aeruginosa*, from 91 clinical specimens (30.3%), followed by *Staph aureus* 23 (7.7%), *E. Coli* spp. 18 (6%), *Klebsella spp.* 10 (3.3%), *Streptococci* 7 (2.3%), *Enterobacter spp.* 4 (1.3%), *Proteus spp.* 2 (0.7%), and Acinetobacter 1 (0.33%), while 144 (48%) of clinical specimens showed no growth.

The number of clinical specimens collected from hospital "A" were 100 clinical specimens, of these 48 isolates (52.7%) were *P aeruginosa*, also the No. of clinical specimens collected from hospital "B" were 100 of these 27 isolates of *P aeruginosa* (29.7%), from hospital "C" the No. of clinical specimens collected were 50 of these 11 isolates were *P aeruginosa* (12.1%), 50 of clinical specimens from hospital "D" of these 5 isolates of *P aeruginosa* (5.5%) (Tables 1 and 2).

In hospital "A" (Al-Jala) 48 were positive for *P aeruginosa*, (30 isolates (62.5%) were from males and 18 isolates (37.5%) were form females). Majority of *P aeruginosa* strains isolated from wound specimen's (Figure 1).

In Al-Jamahyria hospital (hospital B), 27 specimens were positive for *P aeruginosa* (13 isolates (48%) were isolated from males and 14 isolates (52%) were from females) (Figure 2).

A total of 50 clinical specimens were obtained from patients in

Specimen Type	Gender				Total (%)	*Pseudomonas Species*
	Male		Female			
	No	(%)	No	(%)		
Urine	6	54.54	1	9.09	63.6	*P aeruginosa*
Ear swab	1	9.09	2	18.18	27.27	*P aeruginosa*
Absces swab	0	0	1	9.09	9.09	*P aeruginosa*
Total	7	63.64	4	36.36	100	

Table 1: Distribution of specimens collected from hospital "C"(Hospital C)7th April.

Department (ward)	No.	(%)
Surgical ward	29	31.8
Medical ward	17	18.7
B.S.S.R [1]	12	13.2
I.C.U [2]	9	9.9
Urology	7	7.7
Skin ward	5	5.5
Gyne ward and obstrics	4	4.4
C.C.U [3]	3	3.3
E.N.T [4]	3	3.3
Oncology ward	2	2.2
Total	91	100%

1. Burn surgical shock room
2. Intensive care unit
3. Cardiology care unit
4. Ear, nose and throat ward

Table 2: Number of *P aeruginosa* isolated from various clinical specimens in different departments in hospitals sampled.
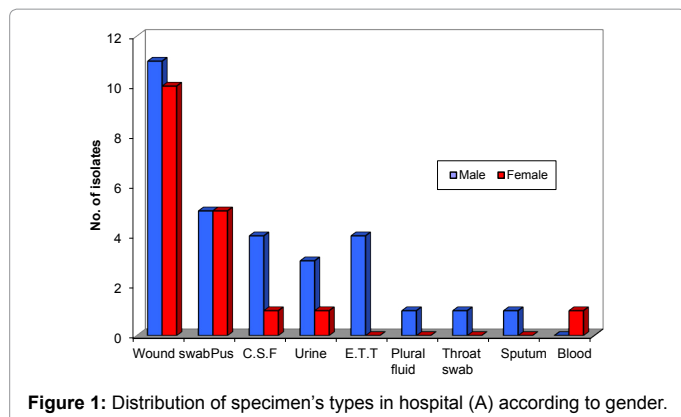
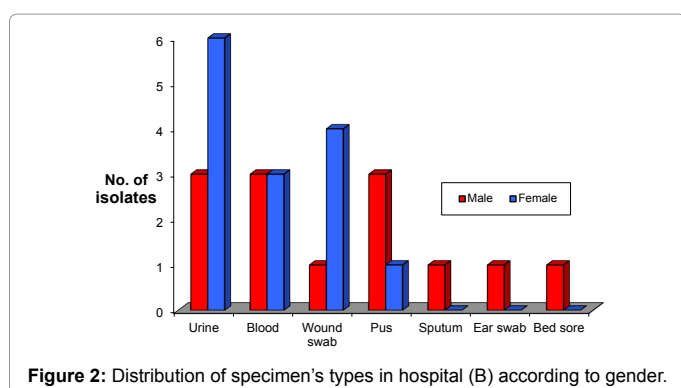**Figure 1:** Distribution of specimen's types in hospital (A) according to gender.

Figure 2: Distribution of specimen's types in hospital (B) according to gender.

Duration of stay in (weeks)	No. of specimens with *P aeruginosa*	No. of specimens not containing *P aeruginosa*	Total of specimens examined
One week	49 (24%)	155 (76%)	204 (100%)
01-Feb	21 (43.8%)	27 (56.2%)	48 (100%)
02-Apr	9 (31%)	20 (69%)	29 (100%)
04-Aug	10 (62.5%)	6 (37.5%)	16 (100%)
08-Dec	2 (66.7%)	1 (33.3%)	3 (100%)
Total	91 (30.3%)	209 (69.7%)	300 (100%)
Chi-Square=17.7, df=4, P=0.001			

Table 3: The relation of duration of stay in hospital (weeks) and the rate of isolation of *P aeruginosa*.

Environmental source	No. of isolates	*Pseudomonas spp.*
Disinfectants	6	*P aeruginosa*
Suction apparatus	3	"
Beds	3	"
Air	1	"
Ventilalator equipment	2	"
Liquid soaps	2	"
Walls and floors	1	"
Total =	18	
* 18 isolates from 300 samples taken from different environmental sources.		

Table 4: Distribution of *P aeruginosa* isolated from environmental sources.

hospital "C" (7th April) 11 of these were positive for *P aeruginosa* (7 isolates (63.6%) were from males and 4 isolates (36.4%) were from females).

In hospital "D" (7th October) a total of 50 clinical specimens 5 of these were positive for *P aeruginosa* (2 isolates (40%) were from males and 3 isolates (60%) were from females), and all *P aeruginosa* isolates were from urine specimens.

The majority *P aeruginosa* isolated from various clinical specimens in different departments in the hospitals sampled were isolated from surgical wards 29 isolates (31.8%), followed by medical wards 17 isolates (18.7%).

Our result showing the distribution of *P aeruginosa* in relation to the age, gender and type of hospital, the age range included ≤ 19 to ≥ 80 years, of 91 isolates of *P aeruginosa* the age group (20-39) in all hospitals are more than other groups (34%).

Table 3 showing the relation of duration of stay in hospitals (weeks) and the rate of isolation of *P aeruginosa*, the duration of stay for all patients in our study was between ≤ 1 and 12 weeks, also shows increase of infection percentage with prolonged stay in the hospital, ≤ 1 week were 49 isolates (24%), stay by (8-12 weeks) were 2 isolates (66.7%), while stay by (2-4 weeks) were 91 isolates (31%), was found statistically significant (P=0.001).

Bacteria isolated from environmental sources

Of the 300 samples collected from environmental sources 18 of these were positive for *P aeruginosa* (6%). All 18 isolates from environmental sources were motile, Gram-negative bacilli, oxidase positive, grown at 42ºC, utilized glucose aerobically, and arginine dihydrogenase positive Table 4.

Out of 18 environmental isolates, 6 (33%) *P aeruginosa* isolates were from disinfectants, 3 (17%) isolates from suction apparatus, 3 (17%) isolates from beds, ventilator equipment 2 (11%), liquid soaps 2 (11%), walls and floors 1 (5.5%), finally from air 1 (5.5%) (Figure 3).

Also, our results showing distribution of *P aeruginosa* isolated from environmental sources in various hospital wards, 5 (27.8%) environmental isolates were from Burn Surgical Room, followed by Intensive Care Unit 2 (11.1%), and Surgical wards 2 (11.1%) in hospital "A". In hospital "B" the isolates were from skin ward 2 (11.1%), cardiology care unit 2 (11.1%), in hospital "C" isolates from urology units 2 (11.1%), and hospital "D" all isolates from surgical wards 2 (11.1%) (Table 5).

Antibiotic susceptibility

Table 6 summarizes the resistance pattern of *P aeruginosa* isolated from clinical specimens to antimicrobial agents used. Of 91 isoaltes of *P aeruginosa* from clinical specimens 90 (98.9%) were resistant to Ampicillin, 89 (97.8%) were resistant to Chloramphenicol, 88 (96.7%) resistant to Augmentin, 87 (95.6%) resistant to Pipemidic acid, 77 (84.6%) resistant to Colistin sulphate, 69 (75.8%) resistant to Gentamicin, 46 (50.6%) resistant to Tobramycin, 25 (27.5%) resistant
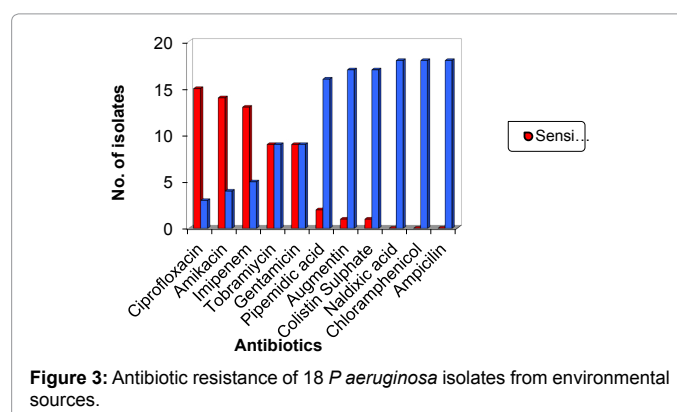

Figure 3: Antibiotic resistance of 18 *P aeruginosa* isolates from environmental sources.

Ward	Hospital "A"	%	Hospital "B"	%	Hospital "C"	%	Hospital "D"	%
B.S.S.R	5	27.8	-	-	-	-	-	-
Urology Units	-	-	-	-	2	11.1	-	-
Medical Ward	-	-	1	5.6	-	-	-	-
Skin Ward	-	-	2	11.1	-	-	-	-
I.C.U	2	11.1	-	-	-	-	-	-
C.C.U	-	-	2	11.1	-	-	-	-
Surgical Ward	2	11.1	-	-	-	-	1	5.6
E.N.T	-	11.1	-	-	-	-	1	5.6
Total=18	9	50	5	27.8	2	11.1	2	11.1

Table 5: Distribution of *P aeruginosa* isolated from environmental sources in various hospital wards.

Antibiotics	Resistant		Sensitive	
	No. of isolates	%	No. of isolates	%
Ampicillin	90	98.9	1	1.1
Chloramphenicol	89	97.8	2	2.2
Augmentin	88	96.7	3	3.3
Pipemidic acid	87	95.6	4	4.4
Colistin Sulphate	77	84.6	14	15.4
Gentamicin	69	75.8	22	42.2
Tobramycin	46	50.6	45	49.5
Amikacin	25	27.5	66	72.5
Imipenem	18	19.8	73	80.2
Ciprofloxacin	16	17.6	75	82.2
Naldixic acid used only for 25 UTI isolates.				

Table 6: Pattern of antibiotics resistance and sensitivity of *P aeruginosa* isolated from clinical specimens.

Hospital	No.	(%)
(A)AL-jala	48	52.7
(B)AL-jamahyria	27	29.7
(C)7th April	11	12.1
(D)7th October	5	5.5
Total =	91	100%

Table 7: Distribution of *P aeruginosa* isolated from hospitals under study.

to Amikacin, 18 (19.8%) resistant to Imipenem and 16 isolates (17.6%) were resistant to Ciprofloxacin. All of the 25 isolates from urinary tract infection shows resistance to Nalidixic acid.

The majority of isolates were found resistant to between 5-10 antibiotics used, and 2 (2.3%) isolates were found resistant to all antibiotics.

Of the 18 environmental isolates tested, 3 (16.7%) showed resistant to Ciprofloxacin, 4 (22.2%) were resistant to Amikacin, 5 (27.8%) were resistant to Imipenem, 9 (50%) were resistant to Tobramycin, 9 (50%) were resistant to Gentamicin, 16 (88.9%) were resistant to Pipemidic acid, 17 (94.4%) were resistant to Augmenten, 17 (94.4%) isolates were resistant Colistin sulphate. All 18 environmental isolates showed resistant to naldixic acid, chloramphenicol and ampicillin.

Discussion

Recent studies have shown that infection with *P aeruginosa* is more often the result of hospitalization. Furthermore, the risk of serious infections, particularly septicemia, increases with extended hospitalization. Because of its antibiotic resistance and ubiquitous distribution in the hospitals environment, *P aeruginosa* is one of the most dangerous opportunistic pathogens [8]. As the most frequent bacteria isolated it can be transmitted from person to person or from the environment.

All isolates were oxidase positive, which is one of the most important biochemical characters of *Pseudomonas aeruginosa*, (Anna 1977, Skinner 1979, Allen 1986, Asma 2004, Kenneth 2004) [3,9-12].

P aeruginosa is usually motile and oxidase positive capable of growth in simple minimal media [9]. Also, all isolates from clinical specimens utilized citrate, this is in agreement with a study done by Amal (1988), that reported all isolates except one, utilized citrate [7].

The arginine dihydrolase positive to Pseudomonas, were distinguished by the use of ammonium salt [9]. Thornley in 1960, reported that all strains of *P aeruginosa* hydrolyzed arginine [10], in an early study from Benghazi in 1988, also reported that all strains of *P aeruginosa* were positive to arginine [7].

In the present study, all isolates of *P aeruginosa* showed arginine dihyrolase activity.

However, 34 (37.4%) of isolates utilized glucose aerobically. But 2 (2.2%) utilized Mannitol, and 2 (2.2%) utilized maltose.

In the present study, all isolates from clinical specimens were capable of growth at 42°C. Ajello and Hondley in 1976, reported that no non-aeruginosa strain was capable of growth at 42°C [9]. Our results agree with other reports by Amal in 1988, Kenneth in 2004, Asma in 2004 [3,7,12].

Also In the present study *P aeruginosa* was isolated from 91 (30.3%) of various clinical specimens in different hospitals (Table 7). The largest number of isolates were from hospital (A) Al Jala 48(52.7%) that is due to nature of cases admitted like; burn, surgery, and traffic accidents cases. The largest number of *P aeruginosa* isolates were from wound swabs 26 (28.6%) followed by isolated from urine 25 (27.5%) and pus 14 (15.4%). More over 7 (7.8%) isolates were from blood, 5 (5.5%) from C.S.F, 4 (4.4%) from Ear swabs, 4 (4.4%) from endotrachcheal tube, 2 (2.2%) from sputum, and one isolated from each of throat swabs, plural fluid, bed sores and abscess swabs. Among the isolates as many as 29 (31.8%) were from surgical wards, within hospitals. Surgery and medical services have the highest rates of the hospital acquired infections. Surgery account for 19% of nosocomial infections in USA, about 25% of surgical patients develop post-operative infections [13].

P aeruginosa plays an important role in the post-surgical wound infections, and it is widely spread within hospitals environment, since surgical procedures carry risks, for example, through the use of contaminated surgical instruments, anesthetic apparatus, and ventilators (Figure 4).

Our results are in agreement with the study done by Elyousfi in 2004, who reported that the most frequent bacteria isolated from surgical wards were *P aeruginosa* (5.0%) [13].

While 17 (18.7%) of 91 isolates were collected from medical wards. It is much lower than those reported by a study in Saudi Arabia (27%) [12].

In our study, out of 91 clinical specimens isolated, 12 (13.2%) were obtained from burn patients, as burned patients are very susceptible to microbial infection, partly because of the increased amount of necrotic tissue in the affected areas, the loss of blood supply to the burned field, and loss of neutrophil function. Revathi in 1998, from India reported that *Pseudomonas aeruginosa* was the most common isolate from the Burn unit [6]. In another study from Iraq in 2002, reported the

predominant bacterial pathogen associated with various types of burns was *P aeruginosa* [14] (Figure 5).

Out of 91 isolates from clinical specimens in this study 9 (9.9 %) were from Intensive Care Units. This is reflected by their longer duration of stay in the unit, the more prolonged and multiple antibiotic therapy, the higher proportion requiring ventilation and the higher mortality of this group. In a study from Saudi Arabia and Kuwait in 1998, the most common bacterial isolate in Jeddah and Kuwait ICUs were *P aeruginosa* (26%) from urine and other clinical specimens [15]. Also, Marchant in 1998 from India, reported in the ICU common isolates were *P aeruginosa* [16].

The prevalence of *P aeruginosa* associated with infected urine indicates that *Pseudomonas aeruginosa* are frequently encountered. Kuzenetsova in 1984, from Russia, reported that the urological and surgical infections were mainly caused by *P aeruginosa* [17]. Our result in agreement with other reports by Ahmed in 1995, Adeyemo in 1994, Lee in 1992 [18,19].

The remainder isolates of *P aeruginosa* were from skin ward, gyne and obstetrics, cardiology care unit, ear nose and throat ward, and oncology ward (Figure 6).

Our results showed no statistically significant correlation between

Figure 4: Photo showing patient infected with *P aeruginosa*.

Figure 5: Photo showing a patient from dermatology ward suffering from *P.aeruginosa* infection.

Figure 6: Photo showing medical waste materials in the intensive care unit which considered source of infection in the hospital.

age of the patient and *P aeruginosa* infection (P>0.05). In the age group the number of patients were 15 (16.5%), while the largest number was in middle age 31 (34.0%) in the age group (20-39) followed by 27 (29.7) age group (40-59) because most the cases studied were in this range of age, but in age group (60-79) 13 (14.3%) were isolated. Out of 91 *P aeruginosa* isolates were 52 (57.1%) patients were males compared with 39 (42.9%) were females, and this difference was found statistically significant (P<0.05). Our results confirmed the results from other studies by Namnyak in 1987, Ihsan in 2002, Asma in 2004 [12,14,20]. All of these studies refer to increase *P aeruginosa* infection among males more than females. Also, some studies showed 5:1 male to female, ratio of *P aeruginosa* infection which could not be explained [21].

The present study also showed an increase of infection among patients hospitalized for longer periods (P=0.001). One week stay were 49 *P aeruginosa isolates* (24%), followed by 21 (43.8%) stay by (1-2 weeks), stay by (2-4) were 9 (31%) and stay by (4-8) weeks were 10 (62.5%), stay by (8-12) were 2 (66.7). Our results agree with studies from Italy, England, United State, Germany which reported the length of hospital stay was positively correlated with wound colonization with *Pseudomonas aeruginosa*, its associated with intensity and duration of exposure to broad spectrum antibiotics [22, 23].

In addition to those *P aeruginosa* isolated from clinical specimens, 18 of 300 samples tested were from environmental sources; like disinfectants, suction apparatus, beds, air, ventilator equipment, liquid floors. This indicates the important role of hospital environment as source of *P aeruginosa* infection, and the capacity of *P aeruginosa* to multiply even in detergents and antiseptics.

These isolates were from environmental sources in various wards in Benghazi hospitals.

The data of present study showed that the isolates from clinical specimens were resistant to ampicillin 90 (98.9%), followed by chloramphenicol 89 (97.8%), augmentin 88 (96.7%), pipemidic acid 87 (95.6%), and colistin sulphate 77 (84.6%). All 25 isolates from U.T.I were resistant to nalidixic acid.

The overall antibiotic sensitivity pattern of *P aeruginosa* isolates from clinical specimens revealed that ciprofloxacin is the most effective antibiotic with 82.4% of all isolates being sensitive. Next in order was imipenem with 80.2% of isolates sensitive, followed by amikacin 72.5% sensitive, and tobramycin with 49.5%. Whereas gentamicin was the least active, gentamicin resistance rate 75.8%.

A total of 18 isolates of *P aeruginosa* from environmental sources showed 83.3% of these strains were sensitive to ciprofloxacin, followed by amikacin with 77.8% of the isolates sensitive, imipenem 72.2% sensitive, while tobramycin and gentamicin with 50% sensitive. Pipemidic acid, augmentin and colistin sulphate are the least effective antibiotic with most isolates resistant to them. All UTI isolates were 100% resistant to nalidixic acid, and all other isolates were resistant to chloramphenicol and ampicillin.

This study showed that all isolates from both clinical specimen and environmental sources were highly sensitive to ciprofloxacin, susceptibility to this agent rate (82.2%, 83.3%), which is in agreement with other reports by Chin in 1986, Makaddas in 1998, Asma in 2004 [12,23-25]. Giachino in 1992, from Italy reported Ciprofloxacin is more effective against all gram-negative bacilli including *P aeruginosa* [26]. In another study from Saudi Arabia and Kuwait centers (1998), reported that about 99% of all *P aeruginosa* isolates were susceptible to ciprofloxacin in both centers [15]. Revathi in 1998 from India,

reported that the drug most effective in *P aeruginosa* organisms was Ciprofloxacin [5]. High sensitivity of Ciprofloxacin reflects the sparing use of this antibiotic in Benghazi hospitals compared to Gentamicin.

Ciprofloxacin can be given by mouth and this may be an advantage if therapy has to be prolonged, Ciprofloxacin exhibits good activity against

P aeruginosa and penetrates well into most tissues. Imipenem is the next most effective antibiotic against *Pseudomonas aeruginosa* with (80.2%) of all isolates from clinical specimens, and (72.2%) of all isolates from environmental sources.

Giachino (1992), reported that imipenem was active against all isolates, including *P aeruginosa* [26]. Zakland in 1997, from Poland reported that Imipenem are highly active *in vitro* against the examined strains of *P. aeruginosa* [27]. Also, Makaddas in 1998, reported that overall Imipenem showed superior activity over the other antibiotics, of the 948 Pseudomonas isolated, (97%) were susceptible to the Imipenem [25]. In another study by Rotimi in 1998, 96% of all isolates were susceptible to Imipenem [15]. The lower number of resistant strains may be a reflection of the low antibiotic consumption of Imipenem in Benghazi hospitals, and both antibiotics (Imipenem and Ciprofloxacin) are very expensive.

Amikacin was the most potent drug tested among aminoglycosides whereas tobramycin and gentamicin were the least active. Of 109 isolates of *P aeruginosa* from patients and the environment respectively, (72.5%), (77.8%) were sensitive to Amikacin.

Varying degree of sensitivity of *P aeruginosa* strain to Amikacin was observed from different parts of the world. Kuznetsova in 1984, from Russia reported that all *P aeruginosa* isolates were resistant to aminglycoside (including Amikacin) antibiotics [17]. Revathi in 1998, from India reported that *P aeruginosa* isolates from the Burn unit in Delhi, was the most susceptible toAmikacin (85%) [5]. More over Mokaddas in 1998, from Italy reported that *P aeruginosa* isolates from wounds, blood, urine etc. from burn patients were susceptible to Amikacin (59%) determined by disk diffusion test. (25) Asma in 2004, reported that the Amikacin susceptibility rate was 85.8%, these *P aeruginosa* were commonly isolated from respiratory tract specimens of patients in intensive care unit in Saudi Arabia [12].

Thus, the antibiotic susceptibility pattern of *P aeruginosa* vary from one geographic area to another as can be seen from the above reports. Hence susceptibility tests should be done in every case of *P aeruginosa* infection as an adjunct to the selection of antimicrobial therapy.

Amikacin is the least used aminoglycoside antibiotic in Benghazyi hospitals in the treatment of *P aeruginosa*.

P aeruginosa isolates from clinical specimens and environmental sources showed increasing resistance to Tobramycin (50.6%, 50%), the dramatic increase in the resistance of *P aeruginosa* isolates to this drug reflect the cross resistance and gradual increasing use of this drug in Benghazi hospitals. These findings agree with other studies which showed the increase resistance to Tobramycin by Shlaes in 1983, from United States they also reported that all *P aeruginosa* isolates plasmids carry tobramycin resistance [28]. Santos in 1985, from England reported 100% of the strains exhibits resistance to Tobramycin [29]. In another study from Russia, by Kuzenetsova, showed high frequency of the strains of gram-negative bacteria, especially *Pseudomonas aeruginosa* due to resistance to aminoglycoside antibiotics such as Tobramycin [17].

Only 22 (42.2%) isolates of *P aeruginosa* from clinical specimens

and 9 (50%) from environmental sources were sensitive to gentamicin in the present study. Despite its toxicity, gentamicin is the most commonly used aminoglycoside antibiotic in the impiric treatment of infections of gram-negative bacilli including *Pseudomonas aeruginosa*. Because of the wide increase in the use of this antibiotic in hospitals, there is a proportionate increase in the resistance by *P aeruginosa*.

Earlier studies at Benghazi by Punjabi in 1978, reported (92.2%) that *P aeruginosa* strains isolated from UTI were sensitive to Gentamicin [30,31].

But these antibiotics, should be used with caution in all persons with anydegree of renal failure [21]. While another local study done by Hussein in 1980, reported that from 84.8% of the swabs taken from burn areas yielded P aeruginosa growth, approximately 27% of these strains were sensitive to gentamicin [3]. Also, Amal in 1988, reported that the gentamicin is the most effective antibiotic against *P aeruginosa* with 70.5% of all isolates being sensitive [7]. Other studies from other parts of the world showed various degrees of sensitivity of *P aeruginosa* strains to gentamicin. Namnyak in 1987, reported in an outbreak in ICU in Dammam central hospital in Saudi Arabia, total resistance of *P aeruginosa* strains to Gentamicin [20]. But from Saudi Arabia, Hassan in 1987, reported that Gentamicin is more effective against *P aeruginosa* isolates from post-operative wound sepsis [32]. Snyder in 1987, from United States reported that *P aeruginosa* isolates showed high resistance to gentamicin [33]. From Holland, Visser reported that Gentamicin may be used in the treatment of infection with gram-negative bacteria including *P aeruginosa*, resistance will only appear in suboptimal or too prolonged courses of treatment and usually is due to multistep mutation [34].

A Study by Asma in 2004, from Saudi Arabia showed agreement with our results, they reported that gentamicin was the least active (22%) antibiotic against *P aeruginosa* [12].

The other antibiotics tested against *P aeruginosa* in the present study revealed a very low sensitivity or total resistance, with (15.4%, 5.6%) sensitivity to colistin sulphate, (4.4%, 11.1%) to pipemidic acid, (3.3%, 5.6%) to augmentin, (2.2%) to chloramphenicol, 1 (1.1%) to ampicillin and none to nalidixic acid. Amal in 1988), also reported total resistance to ampicillin, nalidixic acid, and 1.5% to chloramphenicol, by *P aeruginosa* strains isolated in Benghazi [7]. Also, Punjabi in 1978, reported that chloramphenicol and ampicillin were ascertained to be drugs of poor choice for the treatment of urinary infection with *P aeruginosa* in Benghazi [30]. Giachino in 1992, from Italy reported very high resistance of *P aeruginosa* isolates to these antibiotics mentioned above [26].

Therefore, these antibiotics appear to have no place at this time in the treatment of *P aeruginosa* infections in this area. Our results show only a few antibiotics are effective against *P aeruginosa*; including fluoroquinolnes, imipenem and aminoglycosides, and even these antibiotics are not effective against all strains. either our results are in agreement with the fact that *P aeruginosa* strains isolated from hospitals are often natural more resistant to antibiotics and in direct proportion to their use, and suggests that such strains were exposed to multiple antibiotics over time, which threatens to limit the ability to treat several hospital infections. However, *P aeruginosa* strains vary greatly in antibiotic sensitivity. Therefore, susceptibility testing should be done on all *P aeruginosa* isolates to help in selection of the best antibiotic for therapy.

All isolates (109) of *P aeruginosa* from clinical specimens and environmental sources exhibited multiple antibiotic resistance.

From 91 *P aeruginosa* isolates from clinical specimens, 27 (29.7%) exhibited resistance to 7 antibiotics, 24 (26.4%) exhibited resistance to 8 antibiotics, 16 (17.6%) exhibited to 6 antibiotics, while only 2 (2.3%) showed total resistance to all 11 antibiotics tested.

The remaining isolates were 11 (12.2%) resistant to 9 antibiotics, and 8 (8.8%) were resistance to 10 antibiotics and 3 (3.3%) resistance to 5 antibiotics.

As regards antibiotic sensitivity, the results obtained were more or less similar to other studies carried abroad and any differences reflect the geographical variations in resistance pattern. This fact indicates that different strains are endemic in different countries. The incidence of nosocomial infections with *P aeruginosa* had markedly increased in Benghazi hospitals as seen from published data and hospital records Punjabi in 1978 and Amal in 1988 [7,30].

That indicate the hospital environment has become an important source of this organism, but the resistance of Pseudomonas to most antibiotics has made the treatment of infections a real problem.

On the other hand, our finding showed similarity between *P aeruginosa* isolates from clinical specimens and the environmental sources in the antibiotic sensitivity pattern.

As it is shown from our data that the common antibiotics used in Benghazi hospitals among infected patient with *P aeruginosa* was cephtriaxone the most common used antibiotic used in treatment of 35 (38.4%) infected patient, followed by Augmentin used to treat 14 (15.4%) infected patient, ampicillin used to treat 11 (12.1%) and gentamicin used to treat 6 (6.6%) infected patient. The remaining antibiotics were of low use (tobramycin, ciprofloxacin, amikacin and imipenem).

These data show that, the wide spread use encouraged the development of resistant *P aeruginosa* strains.

Conclusion

All Pseudomonas isolates from clinical specimens and environment, were identified as *P aeruginosa*.

The highest number of isolates were from wound swabs followed by urine and pus, in clinical specimens which taken from different wards in hospitals studied. There was a significant relation between prolonged duration of stay in the hospital and infection with *Pseudomonas aeruginosa*.

Almost one third of environmental isolates were from disinfectants in general use. Most *P aeruginosa* strains were sensitive to ciprofloxacin imipenem, amikacin and tobramycin, and considered the most suitable antibiotics for empiric treatment of *P aeruginosa* infections.

No strains of *P aeruginosa* under study showed sensitivity to naldixic acid.

The typability of *P aeruginosa* strains from clinical specimens was very high but strains from environmental sources showed only half being typable. Pyocin typing of *P aeruginosa* highlighted the association of various pyocin types with infections.

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