ORIGINAL ARTICLE

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Pseudomonas aeruginosa distribution in clinical sample and their antibiogram from Al-Shifa Hospital, Gaza, PNA.

Abstract

Objective:
To assess antibiotic resistance of *Pseudomonas aeruginosa* isolates from four types of clinical specimen at Al-Shifa hospital, and to compare susceptibilities of those isolates according to their source.

Method:
Clinical specimens from Al-Shifa hospital in Gaza were analyzed between January and December 2002. *Pseudomonas aeruginosa* were isolated and identified by conventional methods. The antibiotic resistance rates were measured by modified Kirby-Bauer disk diffusion method. Data were analyzed statistically using SPSS (version 11).

Results:
The number of isolated *P. aeruginosa* was 541, obtained from 4 types of clinical specimens. Pus was the major source of *P. aeruginosa* isolates (64%), followed by urine (24%), sputum (7.0%) and Blood (5.0%). However, considering the number of specimens cultured, sputum showed the highest Pseudomonas isolation rate (49%), followed by Pus (23%), urine (8.0%) and Blood (6.0%). The highest percentage rates of resistance were found against amoxicillin (99% of all isolates), cephalexin (98.5%), cefaclor (97.4%), doxycycline (96.2%), trimethoprim/ sulfamethoxazole (94.7%) and nalidixic acid (93.5 %). Ciprofloxacin was the most effective of all the tested antimicrobials, followed by Gentamicin and Amikacin. Significant statistical ($P \geq 0.05$) difference in isolated strain susceptibility was detected among some of the antimicrobials depending on the specimen source.

Conclusion:
This study showed that antimicrobial resistance of *Pseudomonas aeruginosa* was high and alarming. Significant difference in the resistance pattern of isolates from different specimen type can be useful in clearing the picture of resistance problem and suggests that due care must be taken in hospital settings to adequately diagnose pseudomonal infections and prescribe the antibiotic treatment most effective in preventing the increase in multidrug resistant organisms.
Key words: Antibiotic resistance; *Pseudomonas aeruginosa;* Gaza.

Introduction

Pseudomonas aeruginosa is an aerobic Gram-negative bacilli found in water, soil, plants, animals and humans **[1].** The minimal nutritional requirements of *P. aeruginosa*, its tolerance to a wide variety of environmental conditions, and its relative resistance to antimicrobial agents contribute to its ecological success and to its role as an effective opportunistic pathogen **[1, 2].**

Almost 50 years ago, *Pseudomonas aeruginosa* was rarely considered as a real pathogen. In the 1970s it was recognized as the microorganism associated with bacteraemia in the neutropenic host. Nowadays, it is among the most common pathogens involved in nosocomial infections. Hospital reservoirs of the microorganism include respiratory equipment, antiseptics, soap, sinks, mops, hot tubs, artificial fingernails, and physiotherapy and hydrotherapy pools. **[3].**

Pseudomonas aeruginosa is primarily a nosocomial pathogen, and it rarely affects healthy persons. It is a leading cause of nosocomial infections, ranking first as a cause of nosocomial pneumonia in Brazilian hospitals **[4].** In the United States, *P. aeruginosa* ranked first among all nosocomial pathogens related to pneumonia in intensive care units reported to the National Nosocomial Infection Surveillance System **[5].** The hands of hospital personnel serve as the bridge between the inanimate and animate environments. **[6, 7]**

In the Gaza Strip were antimicrobials are used extensively without prescription, it is expected that *Pseudomonas aeruginosa* has acquired resistance to the most commonly used antimicrobial agent. This study deals with the isolation of *P. aeruginosa* from various types of clinical specimens and testing isolates for their antimicrobial susceptibilities. Data generated by this work is expected to assist physicians in selecting appropriate therapy for pseudomonal infections based on local findings.

Materials and methods

This study was conducted at Al-Shifa Hospital Central laboratory, Gaza Strip, Palestine covering the period of January to December 2002.

Specimen collection

All samples (a total of 9243 samples from both inpatients and outpatients) were collected and delivered within one hour **[8]** by the various hospital departments. Samples that exceeded one hour of collection were discarded as inappropriate for culture.

Specimen processing

Urine

Urine specimen was streaked on a Nutrient agar (NA) plate using 10 µl calibrated loop and plated on MacConkey agar plates and Blood agar plates. Plates were incubated at 37 ^{o}C at the central laboratory. After overnight incubation, culture plates were checked for growth and colonies on NA plates were enumerated and reported as CFU/ml. Urine samples were classified as positive or negative in accordance with WHO recommendations **[9]**.

Sputum

Specimens were streaked on Blood, Chocolate and MacConkey agars,

incubated for 24-48 hours. Pseudomonas isolates were identified according to a test panel consisting of gram stain, color appearance, pigment production and oxidase reaction and growth at 42 ^{O}C.

Blood

Blood sample collected by physicians or nurses were inoculated into hy-lab blood culture broth (Tryptic Soy Broth + SPS), delivered to the laboratory and incubated for one week. A daily check and subculture was conducted. Suspected colonies were identified using appropriate technique depending on the isolate.

Pus

Pus received in syringes and swabs were cultured within one hour of collection onto Blood, Chocolate and MacConkey plates and fluid thioglycollate.

Identification of Pseudomonas aeruginosa

All suspected *Psudomonas aeruginosa* isolates were examined for a positive reaction to oxidase and production of pyocyanin on Muller Hinton Agar (Difco). Strains giving positive reactions in both tests were accepted as *P. aeruginosa* and were not identified further. Oxidase-positive but pyocyanin-negative strains were identified by the API 20 E system (BioMérieux, Marcy L'Etoile, France).

Susceptibility testing

All identified *Pseudomonas aeruginosa* species were subjected to antimicrobial susceptibility testing using the disk-diffusion technique, as described by the National Committee for Clinical Laboratory Standards (NCCLS) **[10].** Depending on the sample source, a panel of antimicrobials was tested (Table 1). The antimicrobial susceptibility testing results were interpreted using the NCCLS criteria established for non-Enterobacteriaceae **[11].**

Data analysis

Data were analyzed using Statistical Package for Social Sciences "SPSS" software (version 11). The significance of differences in resistance was evaluated using Chi square test. *P* value less than 0.05 was considered statistically significant.

Table 1. Antimicrobials susceptibility testing of *P. aeruginosa* from various sources

Antimicrobial	Specimen			
	Urine	Blood	Sputum	Pus
Amoxicillin	√	√	√	√
Piperacillin	√	√	√	√
Cephalexin	√	√	√	√
Cefotaxime	√	√	√	√
Ceftazidim	√	√	√	√
Amikacin	√	√	√	√
Gentamicin	√	√	√	√
Doxycycline	√	X	√	√
Trimethoprim/ sulfamethoxazole	√	X	X	√
Nalidixic acid	√	X	X	X
Ciprofloxacin	√		√	√
Cefaclor	√	√	√	√

√ tested , X not tested

3. Results

A total of 9243 specimens (Urine, sputum, blood and pus) were processed among which, 3623 were considered positive constituting a 39% (Figure 1). *P. aeruginosa* constituted about 14.9% of all positive cultures. It was noticed that Pseudomonas ranked as the number 1 pathogen isolated from sputum, 2nd pathogen from pus, 3rd from urine and 4th from blood samples.

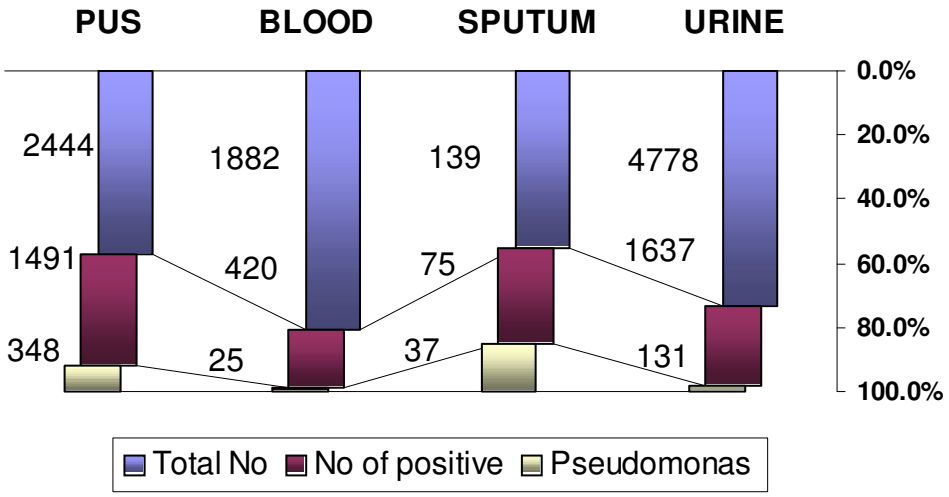

Figure 1. Specimen distribution, number of positive cultures and Pseudomonas isolate

Discussion

The primary aim of this study was to determine the occurrence of *Pseudomonas aeruginosa* in clinical samples and its sensitivity pattern to commonly used antibiotics. The results obtained showed a high incidence of Pseudomonas (14.9%) of all the isolated pathogens. This rate is much lower than those reported in Pakistan **[12].** This is possibly due to the widespread and uncontrolled use of antibiotics in Gaza, where, almost anyone have access to antibiotics.

P. aeruginosa is inherently resistant to many antimicrobial agents mainly due to the synergy between multi-drug efflux systems or a type 1 Amp C β-lactamase and low outer membrane permeability **[13–15]**. Moreover, it is also characterized by its ability to combat effective drugs **[12]**.

Despite the fact that, comparison between studies from different geographical areas may not be a wise method for presenting data due to variations in clinical application of antimicrobials, interestingly, we found a higher level of resistance to the β-lactams and a lower level of resistance to ciprofloxacin in contrast to other surveys **[16-19].** The incidence of resistance seems to be dependent on the patterns of antibiotic usage. The relationship between the emergence of resistance of group 1 β-lactamase-producing organisms and the prior use of extended-spectrum cephalosporins is clearly proven **[20].**

Table (2): In vitro susceptibility of *Pseudomonas aeruginosa* isolates to commonly used antimicrobial agents

Antimicrobials	Disk potency (µg)	S	R	Total strain tested	% R
Amoxicillin	10	3	298	301	99.0
Piperacillin	100	169	273	442	61.8
Cephalexin	30	8	512	520	98.5
Cefotaxime	30	78	173	251	68.9
Ceftazidim	30	272	152	424	35.8
Amikacin	30	299	160	459	34.9
Gentamicin	10	354	162	516	31.4
Doxycycline	30	18	461	479	96.2
Trimethoprim/ sulfamethoxazole	1.25\23.75	2	36	38	94.7
Nalidixic acid	30	8	116	124	93.5
Ciprofloxacin	5	349	123	472	26.1
Cefaclor	30	14	519	533	97.4

Table 2 includes the number of resistant and susceptible strains of *Pseudomonas aeruginosa* isolated from different samples. High percentage of resistance of the organism against a variety of antibiotics (Amoxycilline 99%, Doxycycline 96.2%, SXT 94.7%, Nalidixic acid 93.5%, Cefaclor 97.4%) is noted. The highest activity against *P. aeruginosa* was exhibited by ciprofloxacin (26.1% resistant), followed by gentamicin (31.4% resistant), Amikacin (34.9% resistant) and finally ceftazidim (35.8% resistant).

Table (3): Distribution and statistical analysis of Pseudomonas antimicrobial resistance according to source.

Antimicrobial	S/R*	Sample type				*P* value
		Urine	Sputum	Blood	Pus	
Amoxycilline	**R**	70	26	18	184	**>0.05**
Piperacillin	**S**	18	3	12	135	**0.001**
	R	41	30	13	189	
Cephalexin	**S**	4	0	1	2	**>0.05**
	R	124	34	19	335	
Cefotaxime	**S**	28	4	3	42	**0.024**
	R	35	24	6	108	
Ceftazidim	**S**	53	15	15	188	**0.049**
	R	29	20	6	97	
Amikacin	**S**	88	15	14	181	**0.001**
	R	24	18	8	110	
Gentamicin	**S**	94	18	13	228	**0.025**
	R	34	19	9	100	
Doxycycline	**S**	4	3	NT**	10	**>0.05**
	R	123	27	NT	311	
Trimethoprim/ Sulfamethoxazol	**S**	0	NT	NT	1	**NC*** **
	R	35	NT	NT	1	
Ciprofloxacin	**S**	83	20	NT	245	**0.000**
	R	46	15	NT	62	
Cefaclor	**S**	6	0	1	6	**>0.05**
	R	125	32	21	341	

*S/R Sensitive/Resistance, **NT = Not tested , *** NC= Not calculated

Chi square test was used to test if significance differences among pseudomonas isolate from different speciemn sources for their susceptibilty against the tested antimicrobials (Table 4). Amoxycilline, Cephalexin, Doxycycline, SXT, Cefaclor did not show any significant differences (***P*** ≤

0.05). While, Piperacillin, Cefotaxime, Ceftazidim, Amikacin, Gentamicin and Ciprofloxacin showed statistically significant differences ($P \geq 0.5$). Ciprofloxacin as the most commonly used Quinolones, showed the best activity against *Pseudomonas aeruginosa* (resistant rate was 26.1%). Resistant bacteria to quinolones had been reported by several investigators. In Bangladesh, high resistance rates against ciprofloxacin were recorded **[21].** In Spain **[22],** it was in a comparative study, the investigators found an increase in ciprofloxacin resistance rate from 3% in 1990 to 20% in 1996. In the USA, a study conducted by Kachroo **[23]**, documented that, the resistance to nalidixic acid was 51%. In China there was an extreme increase resistance to ciprofloxacin that reach about 50% in 1999 **[24].** In contrast, low level resistance rate **(4.8%)** was recorded in "Israel" **[25].**

Aminoglycosides were represented in this study by two antibiotics (Amikacin and Gentamicin). Both agents were superior to all tested antimicrobials with the exception of Ciprofloxacin. However, they are much less effective than reported by Karlowsky, in the United States **[26]**.

Conclusions

Resistance of *P. aeruginosa* to β-lactams appears to be common in the largest hospital in Gaza Strip. Our findings suggest that ciprofloxacin and the aminoglycosides (Gentamicin and Amikacin) may be of significant value for the treatment of severe infections caused by *P. aeruginosa.* However, further studies are recommended to determine the exact reasons for the significance variations in the susceptibilities of clinical isolates to antimicrobials. We also suggest that antimicrobial susceptibility testing results be published periodically in order to assist physicians in selecting the appropriate empirical treatment. Actions should be immediately put in practice to delay, reduce and possibly eliminate antimicrobial resistance. Hospital infection control measures should also be strictly applied to minimize the spread of resistant *P. aeruginosa*

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