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Research article

Antibiotic Susceptibility of Clinical and Environmental Isolates of *Pseudomonas aeruginosa*

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ABSTRACT

Pseudomonas aeruginosa isolates resistant to several antibiotics. These bacteria are responsible for different disease in patients suffering from immune suppressive diseases. The ability of these isolates to resist several antibiotics is variable according to different factors. In present study, many isolates of *P. aeruginosa* were isolated from sputum of patients suffering from respiratory tract infection (PAC1, PAC2, PAC3, PAC4, and PAC5) and isolated from soil contaminated with oil products (PAE1, PAE2, PAE3, PAE4, PAE5, PAE6, PAE7, and PAE8). Susceptibility of these isolates to several antibiotics (ampicillin, amoxicillin/clavulanic acid, ampicillin/Sulbactam, piperacillin/tazobactam, cefazolin, ceftazidime, ceftriaxone, cefepime, imipenem, gentamicin, tobramycin, ciprofloxacin, levofloxacin, nitrofurantoin, trimethoprim/sulfamethoxazole, tricarcillin, and amikacin). VITEK 2 DensiCheck instrument (bioMe´rieux) was used to check the susceptibility of clinical and environmental isolates. The current study showed that the clinical isolates were resisted to higher number of antibiotics as compared with environmental isolates. The Minimum inhibition concentration (MIC) of antibiotics was high for clinical isolates as compared with environmental isolates. Imipenem was the highest effective antibiotics against all clinical and environmental isolates of *P. aeruginosa*. It can be concluded from current study that the clinical isolates are high resistant to antibiotics as compared to environmental isolates of *P. aeruginosa* and all isolates was sensitive to imipenem.

Keywords: Antibiotics, Clinical Isolates, Environmental isolates, *Pseudomonas aeruginosa*, Resistance, Sensitivity.

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INTRODUCTION

Pseudomonas aeruginosa, a ubiquitous Gram-negative bacterium, is widespread in nature, inhabiting soil, water, plants, and animals [1,2]. It is an opportunistic pathogen with a high incidence of hospital infections that represents a threat to immune compromised patients [2]. Genomic studies have shown that, in contrast to other pathogenic bacteria, clinical and environmental isolates do not show particular genomic differences. In addition, genetic variability of all the *P. aeruginosa* strains whose genomes have been sequenced is extremely low. This low genomic variability might be explained if clinical strains constitute a subpopulation of this bacterial species present in environments that are close to human populations, which preferentially produce virulence associated traits [3].

Resistance to antimicrobial agents is the most important feature of biofilm infections. Infections caused by bacteria that attached to epithelial cells and form biofilm are persistent and very difficult to eradicate [4,5]. Although several mechanisms have been postulated to explain reduced susceptibility to antimicrobials in bacterial biofilms, it is becoming evident that biofilm resistance is multifactorial. The contribution of each of the different mechanisms involved in biofilm resistance is now beginning to emerge [6].

In present study, we evaluate the ability of environmental and clinical isolates of *P. aeruginosa* to form biofilm on abiotic surface and find out the correlation bdtween antibiotic reistance and biofilm formation. Moreover, the present study try to find which antibiotic will be more effective on *P. aeruginosa* isolates.

MATERIALS AND METHODS

Collection of soil samples

Seventy six soil samples were collected from nine different oil industrial areas, Maysan city. In addition, samples were collected from different depth. Collected soils were sealed in sterile polyethylene bags. The details of this method was descried clearly in previous studies [7,8].

Isolation of bacteria

One gram from each soil sample was placed in 9 ml of asparagine broth enrichment medium consisting of 2 g l-1 asparagine L-monohydrate (Fluka, Switzerland), 1 g K2HPO4 (BDH, England) and 0.5 g MgSO4.7H2O (BDH, UK) in order to enhance *Pseudomonas* growth. The samples were incubated for 48 h at 37ºC with vigorous shaking at 200 rpm to provide aeration for the bacteria. A loopful of the resulting bacterial suspension was streaked onto asparagines plates containing 1.5% agar (Oxoid, UK) and incubated at 37ºC until colonies developed.

The bacteria were then transferred to fresh asparagine plates according to the morphological characteristics of colony: color, shape and size. The isolated colonies of Pseudomonas were cultured on nutrient agar [9].

P. aeruginosa clinical isolates

Fifty sputum samples were collected from patients suffering from lower respiratory tract infection. The sputum was subjected in asparagine broth enrichment medium to enhance *Pseudomonas* growth. Similar to procedure of isolation of *P. aeruginosa* from soil samples the *P. aeruginosa* isolates were isolated from sputum. Cultures that grew *P. aeruginosa* were transferred to Luria-Bertani (LB) plates and were stored at 5 ^{o}C.

Identification using the VITEK 2 fluorescent system (ID-GNB card)

The VITEK 2 fluorescent system (ID-GNB card) includes 43 nonenterobacterial gram-negative taxa. Testing was performed according to the instructions of the manufacturer. Briefly, strains were cultured on LB agar for 18 to 24 h at 37°C before the isolate was subjected to analysis. A bacterial suspension was adjusted to a McFarland standard of 0.50 to 0.63 in a solution of 0.45% sodium chloride using the VITEK 2 DensiCheck instrument (bioMe´rieux). The time between preparation of the solution and filling of the card was always less than 1 h. Analysis was done using the identification card for gram-negative bacteria (ID-GNB card) containing 41 fluorescent biochemical tests. Cards are automatically read every 15 min. Data were analyzed using the VITEK 2 software version VT2- R03.1 [10].

Antibiotic susceptibility

The standard method of Mazzariol et al (2008) was followed to test the susceptibility of *P. aeruginosa* to the several antibiotics [Ampicillin (AMP), Amoxicillin/Clavulanic acid (AMC), ampicillin/ Sulbactam (SAM), Piperacillin/Tazobactam (TZP), cefazolin (CFZ), ceftazidime (CAZ), ceftriaxone (CRO), cefepime (FEP), imipenem (IPM), gentamicin (GEN), tobramycin (TOB), ciprofloxacin (CIP), levofloxacin (LVX), nitrofurantoin (NIT), trimethoprim/ sulfamethoxazole (SXT), tricarcillin (TIC), amikacin (AMK)]. VITEK 2 DensiCheck instrument (biome ´rieux) was used to check the supportability of clinical and environmental isolated of *P. aeruginosa* [11].

RESULTS

Isolation of clinical and environmental isolates

Five clinical isolates of *P. aeruginosa* (PAC1, PAC2, PAC3, PAC4 and PAC5) were isolated from 50 sputum samples that collected during time of study. The biochemical and VITEK 2 fluorescent system techniques were used to identify the isolates.

Moreover, from 76 soil sample, 8 environmental *P. aeruginosa* isolates (PAE1, PAE2, PAE3, PAE4, PAE5, PAE6, PAE7 and PAE8) were isolated. Similar method that used in identifying the clinical isolates was used to identify the environmental isolates of *P. aeruginosa*.

Antibiotic Susceptibility

In present study, the susceptibility of the clinical isolates of *P. aeruginosa* to different antibiotics was evaluated. **Table 1** showed that the sensitivity and resistance of clinical isolates differed from isolate to another. But all clinical isolates were resistant to most antibiotics that used in present study. **Table 2** showed the resistance and sensitivity of eight environmental isolates of *P. aeruginosa* to different antibiotics. The result showed that most of environmental isolates was sensitive to several antibiotics and the degree of sensitivity and resistance were different from isolate to another.

PAC1 isolates resistant to the highest number of antibiotics, while PAC3 sensitive to the highest numbers of antibiotics (**Fig.1 a**). **Fig 1b** showed the lowest resistance to antibiotics was found in case of environmental isolates of *P. aeruginosa* (PAE1). The highest sensitivity was found in case of isolate PAE6. **Fig. 1** proved that the clinical isolates of *P. aeruginosa* resist to the most studied antibiotics. However, environmental isolates resist to the few numbers of studied antibiotics.

Table 1. Minimum inhibition concentrations (MIC) of different antibiotics for clinical isolates of *P. aeruginosa* (PAC1, PAC2, PAC3, PAC4 and PAC5). S, sensitive to antibiotic; R, resistant to antibiotic. The antibiotic MIC was tested by VITEK 2 DensiCheck instrument (bioMe´rieux).

Antibiotic	PAC1	PAC2	PAC3	PAC4	PAC5
AMP	32 (R)	32 (R)	32 (R)	32 (R)	32 (R)
CFZ	64 (R)	64 (R)	64 (R)	64 (R)	64 (R)
CAZ	64 (R)	4 (S)	2 (S)	64 (R)	64 (R)
CRO	64 (R)	16 (R)	16 (R)	64 (R)	64 (R)
FEP	32 (R)	8 (R)	1 (S)	16 (R)	32 (R)
IPM	2 (S)	2 (S)	2 (S)	2 (S)	2 (S)
GEN	16 (R)	1 (S)	1 (S)	16 (R)	16 (R)
TOB	16 (R)	1 (S)	1 (S)	16 (R)	16 (R)
CIP	>4 (R)	1 (S)	0.5 (S)	4 (R)	>4 (R)
LVX	> 8 (R)	4 (S)	4 (S)	8 (R)	>4 (R)
NIT	512 (R)	512 (R)	512 (R)	512 (R)	512 (R)
TIC	64 (R)	32 (R)	2 (S)	-	64 (R)
AMK	64 (R)	4 (S)	2 (S)	-	2 (S)
AMC	32 (R)	32 (R)	32 (R)	32 (R)	32 (R)
SAM	32 (R)	32 (R)	32 (R)	32 (R)	32 (R)
SXT	320 (R)	320 (R)	320 (R)	320 (R)	320 (R)
TZP	128 (R)	4 (S)	4 (S)	128 (R)	128 (R)

The results of present study showed that the clinical isolates showed resistance to high number of antibiotics as compare to environmental isolates.

Table 2. Minimum inhibition concentrations (MIC) of different antibiotics for environmental isolates of *P. aeruginosa* (PAE1, PAE2, PAE3, PAE4, PAE5, PAE6, PAE7, and PAE8). S, sensitive to antibiotic; R, resistance to antibiotic. The antibiotic MIC was tested by VITEK 2 DensiCheck instrument (bioMe´rieux).

Antibiotics	PAE1	PAE2	PAE3	PAE4	PAE5	PAE6	PAE7	PAE8
AMP	-	32 (R)	32 (R)	32 (R)	>32 (R)	>32 (R)	>32 (R)	>32 (R)
CFZ	64 (R)	64 (R)	64 (R)	64 (R)	>64 (R)	>64 (R)	>64 (R)	>64 (R)
CAZ	4 (S)	4 (S)	8 (R)	4 (S)	4 (S)	4 (S)	4 (S)	4 (S)
CRO	16 (R)	32 (R)	16 (R)	32 (R)	32 (R)	2 (S)	2 (S)	16 (R)
FEP	2 (S)	2 (S)	2 (S)	2 (S)	2 (S)	2 (S)	2 (S)	2 (S)
IPM	1 (S)	2 (S)	2 (S)	2 (S)	2 (S)	2 (S)	2 (S)	2 (S)
GEN	1 (S)	2 (S)	2 (S)	2 (S)	2 (S)	2 (S)	2 (S)	2 (S)
TOB	1 (S)	1 (S)	1 (S)	1 (S)	< 1 (S)	< 1 (S)	<1 (S)	<1 (S)
CIP	0.25 (S)	0.25 S)	0.25 (S)	0.25 (S)	<0.25 (S)	<0.25 (S)	<0.25 (S)	<0.25 (S)
LVX	1 (S)	0.5 (S)	0.5 (S)	1 (S)	1 (S)	0.5 (S)	0.5 (S)	0.5 (S)
NIT	-	512 (R)	512 (R)	>512(R)	>512 (R)	>512 (R)	>512 (R)	>512 (R)
TIC	1 (S)	1 (S)	1 (S)	1 (S)	1 (S)	1 (S)	1 (S)	1 (S)
AMK	2 (S)	4 (S)	2 (S)	2 (S)	2 (S)	4 (S)	2 (S)	2 (S)
AMC	32 (R)	32 (R)	32 (R)	>32 (R)	> 32 (R)	4 (S)	> 32 (R)	> 32 (R)
SAM	-	32 (R)	32 (R)	>32 (R)	< 32 (R)	4 (S)	> 32 (R)	> 32 (R)
SXT	160 (R)	80 (R)	80 (R)	160 (R)	80 (R)	80 (R)	80 (R)	80 (R)
TZP	4 (S)	4 (S)	4 (S)	4 (S)	4 (S)	4 (S)	4 (S)	2 (S)

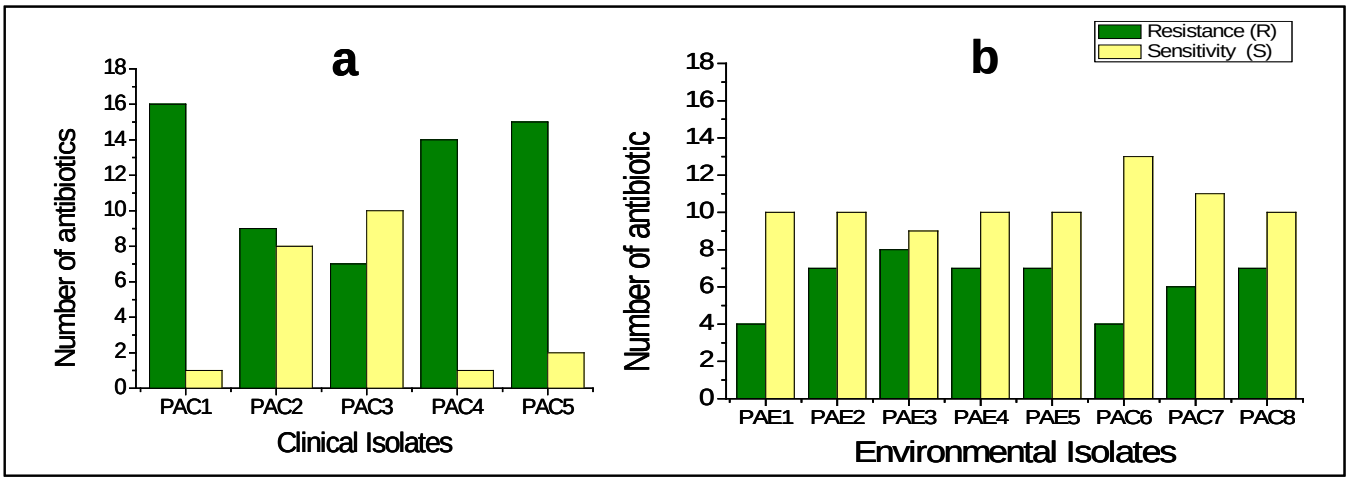

Fig. 1. Number of antibiotics that inhibit (S) and do not inhibit bacterial growth (R) of clinical isolates of *P. aeruginosa* (PAC1, PAC2, PAC3, PAC4, and PAC5) and environmental isolates of *P. aeruginosa* (PAE1, PAE2, PAE3, PAE4, and PAE5, PAE6, PAE7, and PAE8).

Minimum inhibition concentration (MIC) of different antibiotics for different clinical and environmental isolates of *P. aeruginosa* was checked in current study. The results depicted clearly the MIC of antibiotic for clinical isolates was higher than MIC of antibiotics for environmental isolates. The highest difference between clinical and environmental isolates in terms of MIC was in case of Trimethoprim/Sulfamethoxazole (SXT) and Piperacillin/Tazobactam (TZP). The present study showed that Imipenem (IPM) was high effective against clinical and environmental isolates **[Fig. 2**].

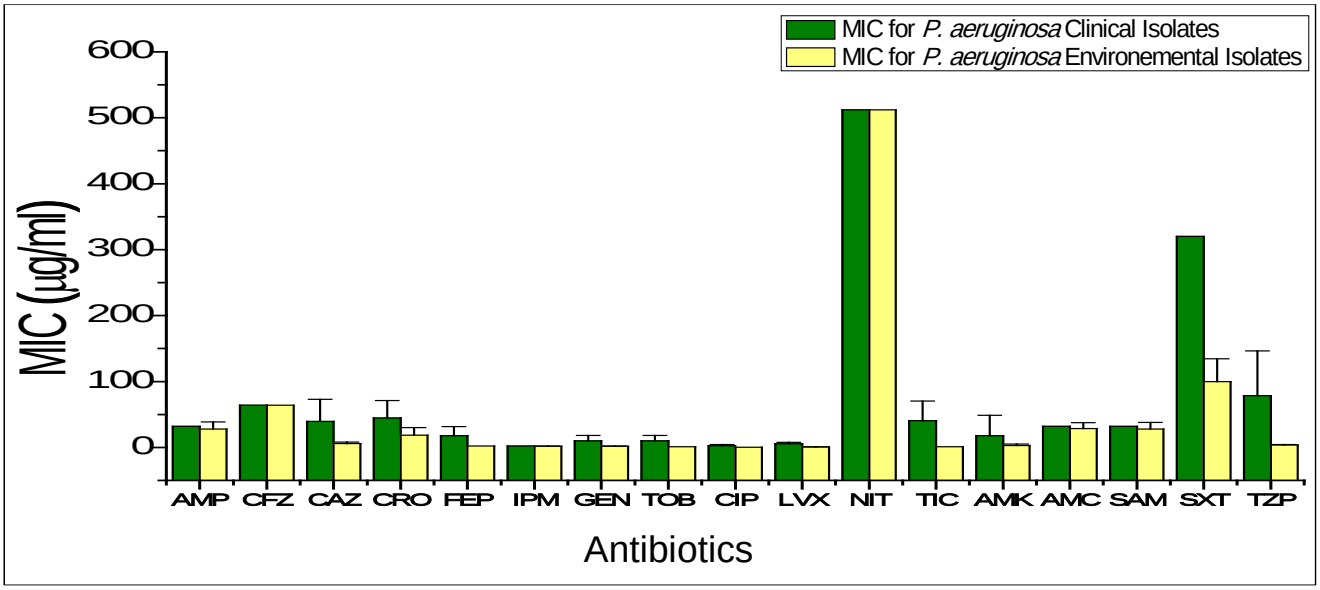

Fig. 2. Minimum inhibition concentration (MIC) of different antibiotics for clinical and environmental isolates of *P. aeruginosa*.

DISCUSSION

P. aeruginosa isolates are resistant to antimicrobials from several different structural classes, either intrinsically or through acquisition of genetic determinants for resistance over time. Most isolates of *P. aeruginosa* are resistant to wide spectrum of antibiotics [12]. The resistance of *P. aeruginosa* to several antibiotics is different from isolate to another. Clinical isolates almost resist to wide spectrum of antibiotics [13]. Thus it can be persisted in the patients' tissues for long time. The studies on the antibiotic sensitivity of environmental isolates of *P. aeruginosa* are very scanty in literature. We did not

find any research paper deals with the compression between clinical and environmental isolates of *P. aeruginosa* in terms of antibiotic sensitivity. That was existed us to cove this area.

In current study, we isolated clinical isolates *P. aeruginosa* from sputum of patients suffering from respiratory tract infection. Environmental isolates of *P. aeruginosa* were isolated from different soil contaminated with oil products. The susceptibility of these isolates to different comment used antibiotics was done. The present study showed clearly that clinical isolates was high resistant to antibiotics as compared with environmental isolates.

Logically the clinical isolates showed by high resistant to antibiotic as this feature helps this kind of isolates to persist in the patients tissues. The high resistant to antibiotic may be yielded because the non scientific using of antibiotic by physicians and because the exposure to high doses of antibiotic these concomitant with other genetic factors produced high resistant isolates of *P. aeruginosa*. In environments especially in industrial area the bacteria generally do not exposed to antibiotic that is why no need to create resistant mechanisms in bacteria against antibiotics. That is why, we did not find that the environmental isolates resist high numbers of antibiotics.

Conflict of interest

The author declares that he has no conflict of interests.

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