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Antibiotic and Enzymatic Pattern of *Pseudomonas aeruginosa* Isolates Recovered from Environmental and Clinical Samples

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ABSTRACT

Pseudomonas aeruginosa is a Gram negative and motile bacterium with different virulence factors. Nowadays, the reports from worldwide illustrated antibiotic resistance of this opportunistic bacterium. Hence, the present study tried to isolate clinical and environmental samples and compare the antibiotic susceptibility as well as enzyme profiles of the isolates. For this purpose, 100 specimens from environmental sources and clinical sources have been collected. The samples were cultivated on cetrimide agar and blood agar, then the plates were incubated at 35°C for 24-48 hrs. The suspected colonies were purified and preliminarily characterized using Gram reaction, catalase, oxidase and motility test. Then they were identified using Api 20 NE kits. Afterward, antimicrobial susceptibility of the isolates was evaluated using Kirby-Bauer test, and enzyme profile were checked using API ZYM kit. The results obtained from this study indicated that out of 50 specimens, 10 isolates belonged to the environmental samples and 13 belonged to the clinical one. In addition, the highest isolation rate of clinical samples was belonging to the wound specimens. Furthermore, the results indicated that there is no difference between the enzyme profiles of the isolates, but regarding to the antibiotic susceptibility, the environmental samples were more sensitive than the clinical one.

INTRODUCTION

Pseudomonas aeruginosa is a Gram negative, motile bacterium with positive catalase and oxidase test. The organism is commonly found in the environment (soil and freshwater) (Fito-Boncompte et al. 2011, Roy et al. 2013), but the reports illustrated that sometimes the organism is also found within the hospital environments (Kapoor et al. 2011, Festini et al. 2006). Although the organism is not usually pathogenic, in immuno-compromised people, it can cause severe infections, especially within burn units, ventilated patients in ICUs, patients receiving cancer therapy and also patients with cystic fibrosis (Ugur et al. 2012). Indeed, the bacterium could survive on surfaces for several days and this factor may play an important role for cross transmission in a health care setting (Ivanov et al. 2011). In 1998, Bert and his colleagues have reported an outbreak in neurosurgical unit and they thought that the bacterium had been cross transmitted by healthcare worker's hands or by contaminated water (Bert et al. 1998). Afterward, Halabi and his group worked on tap water of one hospital and they have reported that a high number of non touch taps were contaminated with *Pseudomonas aeruginosa* (Halabi et al. 2001). On the other hand, several reports have been illustrated that the bacterium were isolated from different parts of medical instruments including latex gloves, dental chair units, surgical instruments, aseptic solutions, cosmetics and medical products (Franzetti & Scarpellini 2007). Therefore, because of their presence in different places, the present study was conducted to isolate and characterize clinical and environmental samples and then to compare their enzyme and antibiotic susceptibility profile of the isolates.

MATERIALS AND METHODS

Environmental sample collection: Bacteria were studied from freshwater as well as soil samples. For this purpose they were isolated from 10 various locations around Shiraz and Isfahan province, Iran. The samples (50) were aseptically collected from the sites and transported to the laboratory. They were serially diluted and 0.1 mL of each dilution was spread on cetrimide and blood agar (Brown & Lowbury 1965). The plates were kept at 35°C for 24-48 hrs and after purification the suspect isolates were primarily identified.

Clinical sample collection: Totally, 50 specimens were examined including, urine (from patients with urinary tract

infection) and wound swabs (from patients with wound burns). The samples were collected from hospitalized patients and they were cultivated on cetrimide and blood agar for 24-48 hrs at 35°C (Brown & Lowbury 1965). The plates were evaluated for the specific bacterium and after purification they were primarily characterized.

Bacteriological analysis: After purification of the isolates, suspected colonies were presumptively identified using Gram reaction, colony morphology, pigment formation, haemolysis on blood agar, positive oxidase and catalase test, grape-like odour, growth at 42°C on nutrient agar and positive motility test (Cheesbrough 2000). Then the isolates were confirmed using the API 20NE (BioMerieux, France) kit according to the manufacturer's instruction. The results were obtained in duplicate and they were analysed using the Apilab Plus Software (Biomerieux) (Ugur et al. 2012).

Antibiotic susceptibility test: For evaluation of antibiotic susceptibility pattern of the isolates, the disc diffusion (Kirby-Bauer) technique, which was recommended by the National Committee for Clinical Laboratory Standards (2000) was used (Ndip et al. 2005). For this purpose, a small inoculum of each bacterial isolate was emulsified in 3 mL sterile normal saline and the turbidity compared with barium chloride standard (0.5 McFarland). A sterile cotton swab was dipped into the standardized solution of bacterial cultures and used for inoculation of Mueller-Hinton plates. After 5 mins the antibiotic discs with the following drug contents: gentamicin (10 μg), amikacin (30 μg), ciprofloxacin (5 μg), cefotaxime (30 μg), ceftazidime (30 μg) and imipenem (10 μg) were placed on the plates. Subsequently, the plates were incubated at 37°C for 24 hours and evaluated for inhibition zones around the discs for determining the susceptibility or resistance (Pitout et al. 1998, Ndip et al. 2001).

Enzymatic characterization of *Pseudomonas aeruginosa* using API ZYM profile: In this study the API ZYM system was used to detect presence of 19 enzymes. Indeed, each API ZYM strip contained 20 cupules with dehydrated chromogenic substrates. Hence, bacteria were removed from nutrient agar plates and suspended in 3 mL of sterile saline (0.85% NaCl) to achieve a density approximately equivalent to the McFarland no. 5. Bacterial suspension was added to each cupule and each panel of 20 cupules was incubated within its moist chamber at 37°C for 4 hrs. The kit detects the following enzymes: alkaline and acid phosphatases, butyrate esterase, caprylate esterase lipase, myristate lipase, leucine, valine and cystine aminopeptidases, trypsin, chymotrypsin, phosphoamidase, ot-galactosidase, f-galactosidase, fl-glucuronidase, ax-glucosidase, fl-glucosidase, B-glucosaminidase, x-mannosidase, and cx-fucosidase. For assessment of the enzymes, two reagents were used. Reagent A contained (per 100 mL): Tris, 25 g; 37% HCl, 11 mL; and sodium dodecyl sulphate, 10 g and reagent B contained 0.35% fast blue BB salt in 2-methoxyethanol. After incubation, 1 drop of each reagent was added to the inoculated cupule and the colour was checked after 5 to 15 mins. Then the enzymatic activities were evaluated according to the intensity of colour with the API ZYM colour reaction chart (Poh & Loh 1988, Ugur et al. 2012).

RESULTS AND DISCUSSION

Totally, out of 100 specimens examined, 23 were positive for *Pseudomonas aeruginosa*. Out of that, 10 were positive for environmental samples and 13 for clinical samples. There was no significant difference ($P > 0.05$) between clinical 26% (13/50), and environmental 20% (10/50) specimens, but the highest isolation rate from clinical samples belonged to wound 18% (9/50) and the least were isolated from urine (8%). The isolates were Gram negative, motile, rod with positive oxidase and catalase test. Species identification of the isolates was made by some biochemical tests and API 20 NE test kits and they were confirmed by Apilab Plus Software. Furthermore, the antimicrobial patterns exhibited by the isolates are presented in Table 1. As shown in the table, most of the clinical isolates were resisting to the antibiotics, while the environmental samples were sensitive. Indeed, antibacterial susceptibility pattern between the isolates in-

Table 1: Antibiotic susceptibility results of *Pseudomonas aeruginosa* strain isolated from environmental and clinical samples.

Antibiotics	Isolates Environmental Samples (no. res %)	Clinical samples (no. res%)	Total resistant(%)	Susceptible (%)
Ciproflexacin	1 (4.3)	12 (52.1)	13 (56.4)	10 (43.5)
Ceftazidime	0 (0)	13 (56.4)	13 (56.4)	10 (43.5)
Cefotaxime	2 (8.6)	11 (47.8)	13 (56.4)	10 (43.5)
Gentamicin	1 (4.3)	13 (56.4)	14 (60.7)	9 (39.3)
Amikacin	0 (0)	11 (47.8)	11 (47.8)	12 (52.2)
Imipenem	2 (8.6)	13 (56.4)	15 (65)	8 (35)

Table 2: Enzymatic activities of *P. aeruginosa* strains detected with the API ZYM system
No. of strains testing[a]:

Enzyme	Negative	Weakly Positive	Strongly Positive	Overall reaction
Alkaline phosphatase	0	0	23	+++
Butyrate esterase (C4)	0	0	23	+++
Caprylate esterase (C8)	0	0	23	+++
Myristate lipase (C14)	0	3	20	+++
Leucine arylamidase	0	3	20	+++
Valine arylamidase	20	3	0	±
Cysteine arylamidase	23	0	0	_
Trypsin	23	0	0	_
Chymotrypsin	23	0	0	_
Acid phosphatase	20	3	0	_
Phosphoamidase	23	0	0	_
α-Galactosidase	23	0	0	_
β-Galactosidase	23	0	0	_
β-Glucuronidase	23	0	0	_
α- Glucosidase	23	0	0	_
β-Glucosidase	23	0	0	_
N-acetyl-β Glucosaminidase	23	0	0	_
α-mannosidase	23	0	0	_
α-fucosidase	23	0	0	_

[a] Strains showing different colour intensities ranging from 0 to 5. Strong activity: 3, 4 or 5; weak activity: 1 or 2 and no activity: 0

dicated that there are more differences among clinical and environmental isolates. In fact, antibiotic resistance of this organism is a serious problem in most of the hospitals worldwide (Aka et al. 1987, Kamoun et al. 1992, Rotimi et al. 1994, Poirel et al. 2001) and existence of multi- resistant strains of *P. aeruginosa* should be responsible for nosocomial infections. Therefore, scientists considered that this high level of resistance could be plasmid-mediated (Ndip et al. 2005). Consequently, for remedy and treatment, it needs more attention for prescription of antibiotics and it should be better to evaluate the antibiotic susceptibility test for each specific isolates. On the other hand, scientists supposed that existence of metalo-β-lactamse and extended-spectrum β-lactamse antimicrobial agents seriously complicate the clinical management of patients infected with such multi-drug-resistant strains (Moreira et al. 2002, Pagani et al. 2002). Although, they explained that multi-drug resistance in environmental isolates might be linked to the uncontrolled disposing antibiotics or chemicals into the environment, site of sample collection is an another important factor, because as our results illustrate, the environmental isolates are more sensitive to the antibiotics than the clinical one.

Furthermore, the enzyme activities of the isolates were evaluated by API ZYM enzyme kits (Table 2). The results showed that most of the strains have similar profiles with strong esterase (C4), esterase lipase (C8), and leucine-arylamidase, activities, and none of the examined isolates showed activity on other substrates such as valine arylamidase, cystine arylamidase, α-chymotrypsine, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosidase, α-mannosidase and α-fucosidase. Hence, according to the obtained result it could be concluded that there is no difference between clinical and environmental samples regarding the enzyme profile.

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