Molecular Study on Relatedness between Clinical and Tap Water Isolates of *Pseudomonas aeruginosa* in Two Burn Units

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Abstract: Background: The role of tap water as an environmental source of disease causing *P. aeruginosa* strains was established. **Objective:** to study the relatedness between clinical and tap water *P. aeruginosa* isolates in the burn units of two different hospitals. **Materials and methods:** One hundred and fifty specimens were collected from two burn units of Cairo University Hospital and El Helmeya Military Hospital. These specimens included: 100 pus samples from infected wounds of burned patients, 25 water samples and 25 swabs were taken from hands of medical staff. The samples were cultured and *P. aeruginosa* isolates were identified as according to standards. The selected colonies were subjected to molecular identification as *P. aeruginosa* by PCR tesing using specific PAL1 andPAL2 primers, and tested for relatedness by plasmid profiling and protein electrophoresis. **Results:** All cultures from the hands of the medical personnel were negative for *P. aeruginosa.* The clinical and water samples yielded 52 *P. aeruginosa* isolates and only 19 isolates were confirmed by PCR. These isolates were from 16% of total water samples from 15% of total clinical swabs. Half of these isolates harbored plasmids. The phenotypic characteristics of isolates showed that 30% of isolates (from clinical and water specimens) were related. However, molecular studies did not prove any molecular evidence of relatedness between different clinical isolates or between clinical and environmental isolates. **Conclusions:** *P. aeruginosa* is the cause of 15% of infections in Egyptian burn units, and contaminates 16% of water samples of these units. The simple use of bacterial protein electrophoresis and plasmid profiling ruled out the relatedness between the clinical and the contaminated water samples.
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1. Introduction

The burn wound represents a site susceptible to opportunistic colonization. The situation for patients with *Pseudomonas aeruginosa* infections is particularly problematic since this organism is inherently resistant to many antimicrobial classes and is able to acquire resistance to all effective antimicrobial drugs (1). Although, several authors were able to culture *P aeruginosa* from the hands of hospital personnel, however, in approximately 30% to 60% of the infection , the mode of acquisition of *P aeruginosa* remained unexplained (2).

The role of tap water as an environmental source of disease causing *P. aeruginosa* strains was established (3). The isolate occurrence in water is probably related more to ability to colonize biofilms in plumbing fixtures (i.e., faucets, shower heads, etc.).Taking the measures to reduce the contamination of water taps resulted in a significant decrease of clinical infections (4). However, the complete eradication of contaminants from these sources is nearly impossible (5).

By using phenotypic methods only, environmental *P. aeruginosa* strains, although present in many hospital locations, were not linked to strains causing clinical disease(6). This was explained by environmental factors modifying lipopolysaccharide production such as exposure to antibiotics or availability of nutrients. The advent of molecular typing techniques proved to be more suitable to study relationships between strains. Methods that have been used successfully include pulsed-field gel electrophoresis (PFGE), amplified fragment-length polymorphism (AFLP) analysis, etc.(7) The simplest of these genetic techniques has been the analysis of the plasmid profile of a given organism or group of organisms. Most bacterial species carry plasmids, which are extra-chromosomal pieces of DNA that encode a variety of genes. After isolation, plasmids are separated by electrophoresis and the pattern (number and size) of the plasmids from different organisms are compared (fingerprinting). If an organism has few or no plasmids, this technique provides little assistance.

The aim of this work was to study the relatedness between clinical and tap water *P. aeruginosa* isolates in the burn units of two different hospitals.

2. Material and Methods

Collection of samples

One hundred and fifty specimens were collected from two burn units of Cairo University Hospital and El Helmeya Military Hospital. These specimens included: 100 pus samples from infected wounds of burned patients, 25 water samples and 25 swabs were taken from hands of medical staff.

Water samples were obtained from tap water from patients' bath rooms, as well as from the central hand-washing area of the staff.

Water sampling: A 100 ml water sample was obtained from each patient-related water faucet in the patients ward, as well as from the central hand-washing area of the staff. Taps were opened, and the first flush of water was collected immediately in sterile bottle containing 50 ml of nutrient broth, and then subcultured when turbid(8).

(I) Bacterial isolation and identification

All swabs and turbid broth were cultivated on nutrient agar, blood agar and MacConkey's agar media (Oxoid Basingstoke, UK). Non-lactose fermentative colonies, on Mac Conkey's agar that showed characteristic pigmentation and hemolysis on blood agar, were tested for oxidase activity. These colonies were further studies by growth at 42˚C, oxidation test, nitrate reduction test, gelatin liquefaction test and growth on the following agar media: triple sugar iron agar, lysine iron agar, Christensen's urea, Simmon citrate (Oxoid, Basingstoke, UK) and motility- indole- ornithine. Non- fermenter, indole- negative, urease- negative, motile colonies with positive oxidase test were presumptively identified as P. aeruginosa; these colonies were picked for molecular analysis.

Antimicrobial susceptibility testing: was done according to standard methods by disk diffusion(10). Several antibiotics were tested: ampicillin, ampicillin/sulbactam, cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin, gentamicin, meropenem and imipenem (Oxoid, Basingstoke, UK).

(II) Molecular Biology Studies:

The selected colonies were subjected to molecular identification as *P. aeruginosa* by PCR tesing using specific PAL1 andPAL2 primers (11,12).The confirmed *P. aeruginosa* isolates from clinical and water samples were tested for relatedness by plasmid profiling and protein electrophoresis (13,14).

(A)Polymerase Chain Reaction(PCR)

1.Extraction of genomic DNA(11): The genomic DNA was prepared from non-transformed cells by boiling the cell suspension and by phenol-chloroform extraction. Negative control of non *P. aeruginosa* and positive control of *Pseudomonas aeruginosa* ATCC27853 were used.

2.DNA amplification(12): One type of primer was used to amplify the open reading frame of the OprL gene PAL1, 5 - ATGGAAATGCTGAAATTCGGC- 3 and PAL2, 5 - CTTCTTCAGCTCGACGCGACG- 3`. The PCR reaction was carried out using DNA thermal cycler 480(Perkin Elmer, USA) according to the following profile: 94°C for 2 minutes for1cycle, 94°C for 40 seconds, 56°C for 40 seconds for 30 cycles, 72°C for 50 seconds ,72°C for 10 minutes, finally 4°C for hold. After termination of cycles, the reaction mixture was mixed with DNA loading buffer , electrophoresed on 1.5% agarose gel and visualized using UV trans-illuminator of 312 nm wavelength.

(B)Plasmid profile(13):Plasmid DNA isolation was extracted by Quick and easy Kit for Bacterial Plasmids Isolation. Isolates with identical plasmid profiles were considered to belong to the same plasmid type.

(C) Protein electrophoresis was done according to standard methods (14).

3. Results

(I) Isolation of *P. aeruginosa*

Out of 150 samples, 52 samples yielded *P. aeruginosa* isolates, 41 (41%) from patients samples and 11(44%) from water samples taken from faucets found in patients' rooms. Other water samples were negative.

All cultures from the hands of the medical personnel were negative for *P. aeruginosa.*

The susceptibility testing of these isolates showed sensitivity as follows: ampicilin (0%), ampicillin/sulbactam (0%), cefotaxime (0%), ceftazidime (20%), ceftriaxone (0), ciprofloxacin (22%), gentamicin(22%), meropenem (100%) and imipenem (100%). The antibiogram was identical in 30% of isolates including isolates from clinical and water samples.

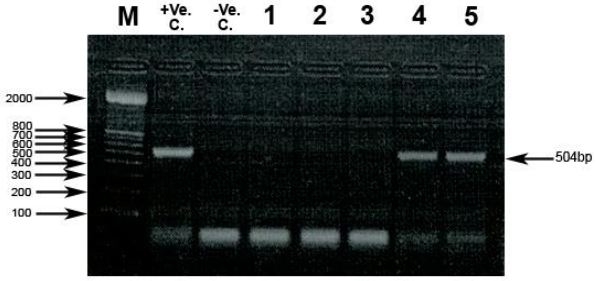


Figure (1): Agarose gel electrophoresis of specific PCR product (504 bp) resulted from amplification of genomic DNA of *Pseudomonas aeruginosa* PAL1 and PAL2 primers. Lane M represents 100 bp ladder markers. Lane 1 represent Positive control, Lane 2 represent Negative control, and Lanes from 3 to 7 represents isolates respectively.

(II) Molecular Biology Studies:

(A)-Polymerase chain reaction(PCR):

The fifty two *Pseudomonas aeruginosa* isolates were identified by PCR and only 19 (36.5% of studied isolates) *Pseudomonas aeruginosa* isolates were confirmed by PCR (Fig.1) . Only 4 isolates were from water samples (16% of total water samples), these were from water faucets of patients' room, and 15 were from clinical samples (15% of total clinical swabs).

(B)- plasmid profile:

From the19 isolates, only 9 (50%) isolates harbored plasmids. No *Pseudomonas aeruginosa* isolates had similar plasmid profiles

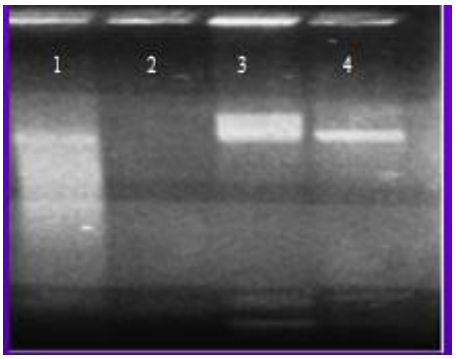


Figure (2): Plasmid profile analysis. Plasmid was detected in isolates number 3 and 4 No. 3 and 4. No plasmids in isolates 1 and 2.

(C)-protein electrophoresis:

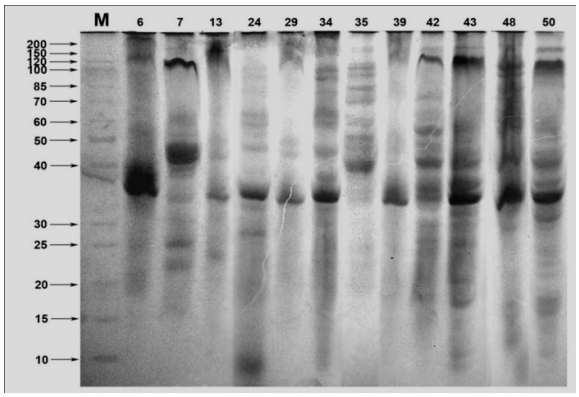
Electrophoretic separation of general protein patterns Revealed a total of 27 protein bands. Protein band of 172 KDa was present in all isolates, the common region could be related to species -specific proteins. Protein band of 45 KDa was present in 11 isolates, protein bands of 71 KDa and 31 KDa were present in 9 isolates, protein bands of 125 KDa, 81 KDa, 61 KDa, 36 KDa and 22 KDa were present in 8 isolates and protein bands of 137 KDa, 97 KDa, 75 KDa and 57 KDa were present in 7 isolates. The densitometric scanning proved diversity of the isolates.

Interpretation of results

The phenotypic characteristics of isolates (biochemical reactions and antibiogram) showed that 30% of isolates (from clinical and water specimens) were related. However, molecular studies ruled out any molecular evidence of relatedness between different clinical isolates or between clinical and environmental isolates.

4. Discussions

The ability of the laboratory to isolate and identify microorganisms is crucial to infection control studies. In this study, when *P. aeruginosa* isolates were identified by culture characteristics and conventional biochemical reactions, 41(41%) of clinical samples and 11(44%) of water samples were mis- identified as *P. aeruginosa* isolates. This falsely represented higher rates than other published studies, where *P. aeruginosa* was isolated from (4.5-26.2%) of water samples and from (21.3%) of pus samples (16,17). But using PCR- based amplification of the specific *P. aeruginosa* primers proved that *P. aeruginosa* contributed only to 4 (16%) of water samples and 15 (15%) of clinical samples, which is comparable to results of other studies.



Fig(3): Electrophoretic separation of protein patterns of *Pseudomonas aeruginosa* isolates. Isolates No. 6,29,34,35,39,42,48,50 shared in 5 bands only.

As cultures from the hands of the medical personnel were negative for *P. aeruginosa*, and *P. aeruginosa* was isolated from both patients' samples and water, we hypothesized that *P. aeruginosa* infections were related to tap water contamination. However, by using simple cheap molecular profiling methods, there was no molecular evidence of relatedness between clinical and water isolates, negating that water was the source of infection.

Other studies using advanced molecular typing techniques; RAPD and PFGE, have shown that up to 50% of nosocomial *P. aeruginosa* acquisitions may result from transmission through tap water. Carriage of *P. aeruginosa* by patients was both the source and the consequence of tap water colonization (17-19). On the other hand, some authors were unable to demonstrate a similarity by PFGE between *P. aeruginosa*, isolates recovered from faucets and the corresponding species from clinical isolates (20). Another study proved that some patients harbored strains not previously isolated from tap water. Thus; in addition to tap water, other environmental or unknown reservoirs appeared to

play a role for the epidemiology of *P. aeruginosa* infection (21).

In this study, only 50% of the isolates harbored plasmids and that have hampered making use of the results of profiling. Some authors assumed that plasmid profiling cannot be used as an epidemiological marker of *P. aeruginosa* strains in the hospital environment and that the study of the more stable and constitutive chromosomal DNA may be more predictive for clonality (22). However, in a setting with low resources, like ours, where the expensive instruments and reagents are not available, plasmid profiling can be a simple tool for epidemiologic studies.

This study has some limitations. First, the small number of water samples, as we obtained cultures from each tap water faucet once. Second, the plasmids were undetected in 50% of studied isolates, so profiling actually compared 10 isolates only.

In conclusion, the study proved that *P. aeruginosa* is the cause of 15% of infections in 2 Egyptian burn units, and contaminates 16% of water samples from the 2 burn units. These isolates were multidrug resistant. Half of the isolates harbored plasmids and the simple use of bacterial protein electrophoresis and plasmid profiling ruled out the relatedness between the clinical and the contaminated water samples. This study emphasizes the need of sterile water in burn units, and the importance of including the water surveillance in epidemiologic investigations.

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