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Isolation of Clinical Strains of *Pseudomonas aeruginosa* Harboring Different Plasmids

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Abstract: Aim of this study was to investigate the presence of plasmids among the strains of *P. aeruginosa* isolated from clinically diagnosed cases in Tehran in 2006. A total of 38 strains of *P. aeruginosa* were isolated. With the exception of one isolate, all *P. aeruginosa* strains harbored at least one plasmid band. The electrophoretic analysis of plasmid DNAs showed different number of plasmid bands among the strains tested. The DNA band of 1.4 kbp was evident in 84.2% of the strains. Approximately 71 and 21% of the isolates harbored concomitantly two and three plasmids, respectively. Isolation of strains with diverse types of plasmids suggests the different cluster of *P. aeruginosa* might be disseminated during the current study period.

Key words: *Pseudomonas aeruginosa*, plasmid determination, Tehran

INTRODUCTION

Pseudomonas aeruginosa is a nonfermentative gram-negative bacterium that has minimal nutritional requirements. This organism rarely cause serious infections in otherwise healthy persons and is infrequently identified as normal microbial flora in healthy individuals (Karlowsky *et al*., 2003). However, *P. aeruginosa* can cause severe and life-threatening infections in immunosuppressed hosts such as patients with burns, patients suffering from respiratory diseases, chemotherapy cancer patients and children and young adults with cystic fibrosis (Rezaee *et al*., 2002). The spectrum of human infections caused by *P. aeruginosa* ranges from superficial skin infections to fulminant sepsis. *P. aeruginosa* is the leading cause of nosocomial respiratory infections and is of particular concern for intubated persons and patients with ventilator-associated pneumonia. *P. aeruginosa* has been documented previously to be responsible for morbidity and mortality in AIDS patients with advanced disease and, as a result of recent improvements in patient management, to be less commonly involved in febrile neutropenia and burn wound sepsis than previously observed (Karlowsky *et al*., 2003).

Many methods have been introduced for the epidemiological investigation of infections caused by *P. aeruginosa*. In different parts of the world, biotyping, serotyping, antibiogram, phage typing, bacteriocin typing, plasmid profile and more recent techniques like pulsed-field gel electrophoresis and random amplified polymorphic DNA analyses have been used in typing the organism (Hernandez *et al*., 1997). In current research we studied the strains of *P. aeruginosa* harboring different plasmids isolated from clinically diagnosed cases admitted in a burn hospital in Tehran.

MATERIALS AND METHODS

Bacterial strains: *P. aeruginosa* strains were isolated between Jan. and May 2006 from patients who hospitalized in a burn hospital in Tehran. All isolates were characterized by gram staining, biochemical reactions, motility, oxidase activity, arginine dihydrolase and urease (MacFaddin, 1980).

Plasmid determination: The High Pure plasmid isolation kit (Roche, Mannheim, Germany) was used to isolate the bacterial plasmids in accordance with the manufacturer's recommendations. Extracted plasmids were then separated on a 0.8% agarose gel in tris-borate-EDTA buffer (TBE×1)(pH 8.2) by electrophoresis.

RESULTS

A total of 38 strains of *P. aeruginosa* were isolated. All except one strain harbored at least one plasmid band. The electrophoretic analysis of plasmid DNAs showed the existence of 1 to 4 DNA bands ranging from 1.4 to larger than of 30 kbp in the strains tested (Fig. 1). The

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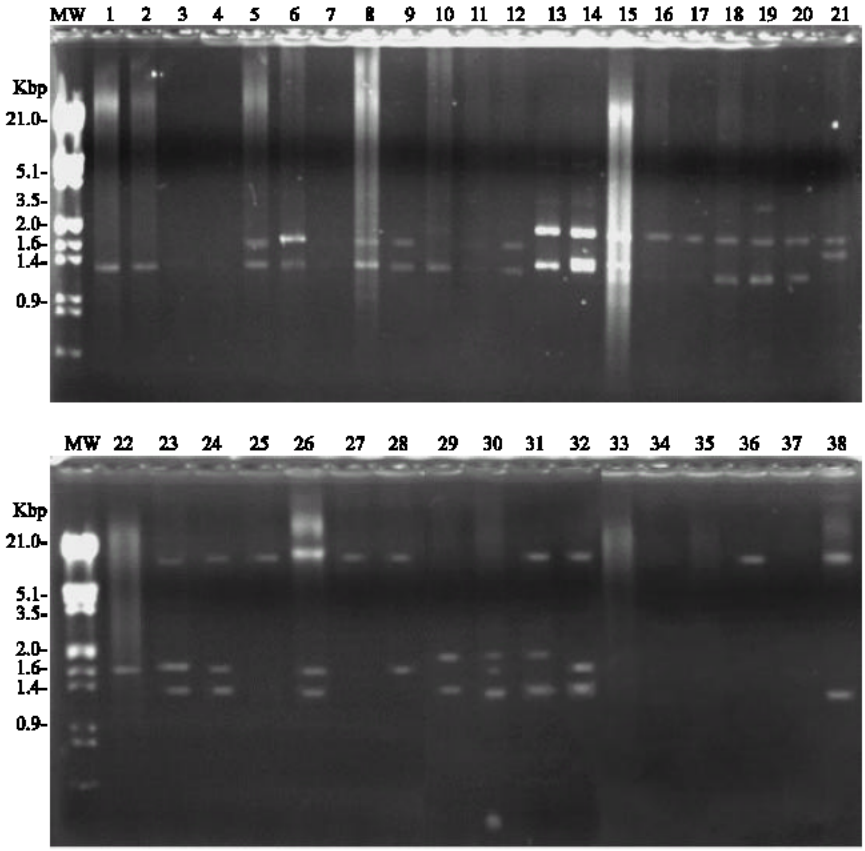

Fig. 1: Plasmid analysis of 38 *P. aeruginosa* strains. MW: Molecular marker, 1-38 are the number of clinical strains of *P. aeruginosa*

smallest DNA band of 1.4 kbp was evident in 84.2% of the strains. Approximately 44.7 and 34.2% of the strains harbored the plasmid DNA bands of 1.7 and 1.9 kbp, respectively. Seventy-one and 21% of the isolates harbored concomitantly two and three plasmids, respectively.

DISCUSSION

Current study was undertaken to study the presence and types of plasmid bands among the strains of *P. aeruginosa* isolated from clinically diagnosed cases in Tehran in 2006.

Plasmid analysis has been used for demonstrating the similarity of clinical and environmental isolates of bacteria in epidemiological studies. Isolates from the same strain contain the same number of plasmids with the same molecular weights and generally the same phenotype. Strains from different sources have different numbers of plasmids with different molecular weights. (Tompkins, 1985).

The results obtained from electrophoretic analysis of plasmid DNAs showed the presence of plasmid bands in all except one strains tested. Present finding also showed the presence of different number of plasmid bands among the strains harboring plasmid. These findings indicate that the plasmid analysis can be regard as a good method for investigation of the clonal dissemination of *P. aeruginosa* in this area.

Poh *et al.* (1988) found the plasmid profiling was to be a useful adjunct to serotyping for the epidemiological typing of *P. aeruginosa*. Plasmid DNA was present in 15% of 112 clinical isolates of *P. aeruginosa*.

Wu (1993) determined the plasmid profiles of 120 clinical isolates of *P. aeruginosa* from Nanjing City. Only 24.2% of tested strains harbored plasmids.

Plesiat *et al.* (1988) investigated the value of plasmid profile determination as an epidemiological tool in *P. aeruginosa* infections by determining the prevalence of plasmids in 450 *P. aeruginosa* strains and comparing the technique with other epidemiological tools. Since only 13.9% of these strains harbored plasmids and the majority

of these plasmids were antibiotic resistant, the technique appeared to be less appropriate as an epidemiological tool in this organism than other techniques. It was concluded that plasmid profiles provide important epidemiological information on *P. aeruginosa* infections when performed in conjunction with either serotyping or, more importantly, pyocin typing.

In another study, Millesimo *et al*. (1996) applied serotyping, antibiotic resistance pattern and plasmid DNA profile for epidemiological typing of 78 *P. aeruginosa* strains isolated from the respiratory tract of 56 patients, 15 of which were affected by cystic fibrosis (CF). Plasmid DNA analysis showed that 45.2% of strains isolated from patients with and w/o CF harboured 1-3 plasmids ranging in size from 1 to 15 Md. Plasmid prevalence was higher in strains isolated from CF patients in specimens collected after antibiotic therapy.

One of the remarkable results was the presence of DNA band of 1.4 kbp in more than of 84.2% of the strains. This finding raises an assumption that this plasmid is one of the most stable plasmids among the strains tested.

In other aspect, isolation of strains with diverse types of plasmids may suggest the different cluster of *P. aeruginosa* might be disseminated during the current study period.

In conclusion, present results indicate that the plasmid analysis is useful to characterize the circulation of *P. aeruginosa* strains. We hope the results obtained from current study will be useful in epidemiological investigation of *P. aeruginosa* in Iran. Continuous investigation using more powerful techniques are recommended to study the genetic relatedness among *P. aeruginosa* strains in the other parts of this large country.

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