## RESEARCH NOTE

# PREVALENCE OF GENOMIC ISLAND PAPI-1 IN CLINICAL ISOLATES OF *PSEUDOMONAS AERUGINOSA* IN IRAN

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**Abstract.** *Pseudomonas aeruginosa*, a gram-negative rod-shaped bacterium, is an opportunistic pathogen, which causes various serious diseases in humans and animals. The aims of this study were to evaluate of the presence of genomic island PAPI-1 in *Pseudomonas aeruginosa* isolated from Reference Laboratory of Ilam, Milad Hospital and Emam Khomeini Hospital, Iran and to study the frequency of extended spectrum beta-lactamases (ESBLs) among isolates. Forty-eight clinical isolates of *P. aeruginosa* were obtained during April to September 2010, and were evaluated for ESBLs by screening and confirmatory disk diffusion methods and PAPI-1 by PCR. Fifteen of 48 *P. aeruginosa* isolates were positive for ESBLs and 17 isolates positive for PAPI-1. This was first study of the prevalence of PAPI-1 in clinical isolates of *P. aeruginosa* in Iran, showing that most of PAPI-1 positive strains had high levels of antibiotic resistance and produced ESBLs.

**Keywords:** *P. aeruginosa*, PAPI-1, ESBLs

## INTRODUCTION

It is now well recognized that horizontal gene transfer (HGT) plays a key role in bacterial evolution. The acquisition and retention of cassettes of DNA encoding up to hundreds of gene, represent a rapid mechanism for evolution. HGT can have a more significant and immediate impact on an organism's phenotype when compared with the slower process of accumulation of mutations within individual genes and subsequent selection for the advantageous phenotypes. DNA segments that did not evolve with the bacterial core genome, often referred to as genomic islands (GIs), are acquired by HGT (Hacker and Carniel, 2001). In bacterial genomes, GIs are characterized by several signature features, including

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atypical G+C content, proximity to transfer RNA (tRNA) genes, and presence of genetic determinants responsible for their mobilization and stable maintenance in the recipient genome. It is generally accepted that HGT- mediated gene acquisition involves one of the three mechanisms for DNA uptake; namely, conjugation, transformation and transduction.

One way to classify GIs is based on the functions they encode, such as additional metabolic proteins, antibiotic resistance or properties involved in a particular lifestyle, including symbiosis or pathogenesis (Dobrindt *et al*, 2004). GIs encoding virulence determinants (known as pathogenicity islands, PAIs) have been described in a wide variety of pathogens and, in many instances, have been implicated as the genetic determinants responsible for endowing a nonpathogenic species with virulence traits (Schmidt and Hensel, 2004).

Several GIs, termed PAPI-1, -2, and -3, have been identified in different strains of the opportunistic pathogen *Pseudomonas aeruginosa*, a gram-negative rod shaped bacterium responsible for various serious diseases in humans and animals (Liang *et al*, 2001; Larbig *et al*, 2002). The majority of the proteins encoded within these PAPIs have unknown function, which makes it difficult to assess the selection forces that facilitated their acquisition in the recipient bacterial strains. Recently, PAPI-1 and -2, have been shown to encode several virulence determinants (He *et al*, 2004). PAPI-1 and -2 are located adjacent to tRNALys gene, PA4541.1 and PA0976.1, respectively, according to the annotation of strain PAO1 (Stover *et al*, 2004). These tRNA genes presumably provide an *attB* site for integration of these PAPIs after their acquisition. Mutations in a number of genes in the larger PAPI-1 (108 kb) result in the attenuation of *P. aeruginosa* strain PA14 virulence in several infection models (He *et al*, 2004). Moreover, PAGI-1 carries several regulatory genes, including *pvrR*, which controls biofilm formation of antibiotic resistant variants of *P. aeruginosa* that are associated with chronic infections in individuals with cystic fibrosis (CF) (Drenkard and Ausubel, 2002). The smaller PAPI-2 (11 kb) contains a gene encoding the potent cytotoxin ExoU and is likely a remnant of a larger island (Kulasekara *et al*, 2006). Excision and/or transfer of PAPI-1 require a functional integrase gene (*int*) and an ortholog of the chromosome partitioning gene *soj*, both of which are located in PAPI-1. Mutations in *soj* lead to the deletion of PAPI-1 from *P. aeruginosa* PA14.

Recently, this pathogen has acquired resistance to many therapeutic antibiotics, particularly against extended-spectrum beta-lactams, and this has become a serious problem in laboratories and hospitals (Bradford, 2001; Alipour, 2010). Thus, it is important not only to investigate the occurrence of *P. aeruginosa* infection but also to assess the existence of antibiotic-resistant *P. aeruginosa* strains.

This study evaluated the presence of PAPI-1 in *P. aeruginosa* isolated in Reference Laboratory of Ilam, Milad Hospital and Emam Khomeini Hospital, Iran and determined the frequency of extended spectrum beta-lactamases (ESBLs) among the isolates, both positive and negative for PAPI-1.

## MATERIALS AND METHODS

### Bacterial isolates

Forty-eight clinical isolates of *P. aeruginosa* were obtained during April to September 2010 from Reference Laboratory of Ilam, Milad Hospital, Tehran and

Emam Khomeini Hospital, Ilam, Iran.

**Detection of ESBLs in *P. aeruginosa***

Standard disk diffusion method (*in vitro* sensitivity testing) using established CLSI (2001) procedure was carried out with cefpodoxime (30 g), cefotaxime(30 g), ceftazidime (30 g), aztreonam (30 g), ceftriaxone (30 g), amikacin (30 g), Cotrimoxazole (30 g), imipenem (30 g), and oxacilin (30 g) (Hi Media, Mumbai, India).

The combined disk method for phenotypic detection also was carried out using ceftazidime (30 g) alone and in combination with clavulanic acid (10 g) (Hi Media, Mumbai, India) (CLSI, 2001).

**Polymerase chain reaction (PCR) for detection of PAPI-1**

PCR amplification was carried out using *soj* forward primer: 5'- CGAGCACAGAAATGTCCTGA-3' and reveres primer: 5'- TAGGAGGTTGTTGGGGTTCT-3'. Thermocycling conditions were as follows: initial denaturation for 5 minutes at 94°C; followed by 35 cycles of 30 seconds at 95°C, 1 minute at 49°C, and 1 minute at 72°C; and a final heating for 10 minutes at 72°C. PCR amplicons were separated by electrophoresis in 1% agarose gel, stained with ethidium bromide (Sigma, St Louis, MO) and photographed using a gel documentation system (Bio Rad Gel Doc 2000 Model Imaging System).

## RESULTS

Out of 48 *P. aeruginosa* isolates 15 (31%) were positive for ESBLs by screening and confirmatory disk diffusion methods (Table 1). Ten isolates from Milad Hospital were resistant to aztreonam and cephalosporin and produced ESBLs, while 4 isolates from Imam Khomeini Hospital

Table 1
Antimicrobial resistance patterns of *P. aeruginosa* isolates.

| Hospital | *P. aeruginosa* *N* (%) | Ceftazidime *N* (%) | Ceftriaxone *N* (%) | Amikacin *N* (%) | Imipenem *N* (%) | Ticarcillin *N* (%) | Aztreonam *N* (%) |
|---|---|---|---|---|---|---|---|
| Milad Hospital | 19 (39) | 7 (37) | 11 (58) | 2 (10) | 2 (10) | 1 (5) | 10 (53) |
| Emam Khomeini Hospital | 16 (33) | 2 (12) | 5 (31) | 1 (6) | 1 (6) | 4 (25) | 4 (25) |
| Reference Laboratory of Ilam | 13 (27) | 4 (31) | 6 (46) | 1 (8) | 1 (8) | 2 (15) | 5 (38) |
| Total | 48 | 17 (35) | 9 (18) | 2 (4) | 4 (8) | 14 (29) | 15 (31) |

and 5 from Reference Laboratory of Ilam 5 were ESBLs positive.

The results of PAPI-1 PCR detection (presence of 680 bp amplicon; data not shown) showed that 7 isolates (35%) were positive for PAPI-1, 42% ($n$ = 8) were found in isolates from Milad Hospital, 29% ($n$ = 5) from References Laboratory of Ilam and 23% ($n$ = 4) from Imam Khomeini Hospital. Interestingly, all the PAPI-1 – positive isolates were ESBLs positive.

## DISCUSSION

Horizontal gene transfer has been recognized as one of the major mechanisms driving the evolution of microorganisms and plays a key role in their ability to adapt to various environments through a rapid, one-step acquisition of several new functions. Sequence analyses of several *P. aeruginosa* genomes revealed that they have a mosaic structure consisting of a core genome of about 5,000 genes and a variable accessory component that can contain anywhere from several hundreds and up to a thousand additional genes (Mathee *et al*, 2008). The majority of the accessory genes are not randomly scattered through out the chromosome but are instead found in clusters (GIs) located at conserved loci. These observations suggest that the majority of the accessory genome was horizontally acquired. This hypothesis is supported by the observations that these GIs are frequently located next to tRNA genes and that, in some instances, their excision and transfer can be demonstrated (Bradford, 2001). In the genomes of certain *P. aeruginosa* strains, phage-related elements can be identified in such GIs, clearly pointing toward their origin via HGT (Winstanley *et al*, 2009).

*P. aeruginosa* strain PA14 possesses PAPI-1 and -2, of which PAPI-1 is the largest GI in pathogenic bacteria and harbors genes for several regulatory functions, including *PVrR* that controls biofilm formation and antibiotic resistance genes, while the smaller GI harbors ExoU cytotoxin gene (Akahane *et al*, 2005).

This is the first study of the prevalence of PAPI-1 in *P. aeruginosa* clinical isolates, which showed that PAPI-1-positive strains play an important role in antimicrobial resistance since these isolates were ESBLs positive. All isolates with PAPI-1 were resistant to imipenem. The majority of PAPI-1 positive strains were from Milad Hospital while the lowest prevalence of PAPI-1 occurred in Emam Khomeini Hospital. As PAPI-1 has an important role in virulence and antibiotic resistance of *P. aeruginosa*, there is a need for having a program for detection of PAPI in *P. aeruginosa* isolates.

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