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ORIGINAL RESEARCH

Molecular characterization of the *pilS2* gene and its association with the frequency of *Pseudomonas aeruginosa* plasmid pKLC102 and PAPI-1 pathogenicity island

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Introduction: *Pseudomonas aeruginosa* is the most common opportunistic pathogen associated with a broad range of infections, including cystic fibrosis, ocular, otitis media, and burn infections. The aim of this study was to show the frequency of the *pilS2* gene, and its association with *P. aeruginosa* plasmid pKLC102 and PAPI-1 pathogenicity island among *P. aeruginosa* strains.
Methods: The samples were collected from patients with cystic fibrosis, ocular, otitis media, and burn infections between January 2016 and November 2017. DNA was extracted using the DNA extraction kit and was used for PCR assay. PCR with 4 primer-pairs including 976 F/PAPI-1R, 4542 F/intF, SojR/4541 F, and intF/sojR was performed to identify PAPI-1. pKLC102 was detected using three other primer-pairs including cp10F/ cp10R, cp44F/cp44R, and cp97F/cp97R.
Results: A total of 112 *P. aeruginosa* isolates were collected from patients with cystic fibrosis (36), burn (20), otitis media (26), and ocular (30) infections. The results of PCR showed that *pilS2* gene was identified in 96 (85%) strains. PAPI-1–attB integration was detected among 38 (33.9%) isolates and the circular form of PAPI-1 detected among 17 (14%) isolates. In addition, 79 (70.5%) strains were found to be positive for pKLC102.
Conclusion: We found that the majority of the isolates may be susceptible to transfer this significant island and the related element pKLC102 into recipient isolates lacking the island owing to high association of the PilS2 pilus with the islands in the studied strains. It is anticipated that strains isolated from burn and eye with the highest rate of *PilS2*, PAPI-1, and pKLC102 association have a high level of antibiotic resistance.

Keywords: *Pseudomonas aeruginosa*, *pilS2*, cystic fibrosis, pKLC102, PAPI-1

Introduction

Pseudomonas aeruginosa (P. aeruginosa) is the most common bacterial pathogen associated with persistent bronchial infections among cystic fibrosis patients.[1] This pathogen is capable of causing several infections including nosocomial pneumonia, nosocomial urinary tract infections, acute diffuse otitis media, conjunctivitis, and keratitis.[2–4] The pathogenesis of *P. aeruginosa* is due to the production of extracellular virulence factors (such as pili, hemolysins, pyocyanin, proteases, alginate, lipopolysaccharide, and the Type III secretion system) and effector proteins (such as ExoU, ExoT, ExoY, and ExoS).[5,6]

P. aeruginosa isolates have shown the ability of adherence to different receptors and production of biofilm by Type IV pili.[7] Type IV pili has two major subfamilies including Type IVa (T4aP) and IVb (T4bP). T4bP are a more heterogeneous group,

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whereas T4aP are a somewhat homogeneous and Tad pili (a monophyletic class of T4bP) were seen in a broad variety of environmental species.[8]

Although T4aP- and Tad-encoding genes are found in all the *P. aeruginosa* isolates, the gene encoding major subunit of the Type IVb pili (*pilS2* gene) is seen in the strains that have related elements such as *P. aeruginosa* plasmid pKLC102 or PAPI-1 pathogenicity island.[8,9] Carter et al showed that PAPI-1 can be transferred to other *P. aeruginosa* strains following excision from the chromosome of the donor. Also, they demonstrated that PAPI-1 is transferred into recipient *P. aeruginosa* by a conjugative mechanism, via a Type IV pilus, encoded in PAPI-1 by a ten-gene cluster (8).

There are two tRNALys genes located in the *P. aeruginosa* genome, known as PA0976.1 and PA4541.1. pKLC102 is a 103,532 bp integrative and conjugative element that integrates into one of these positions.[10] However, PAPI-1 can integrate into the attB sites of the both PA0976.1 and PA4541.1 positions.[11] In addition to this integrated form of PAPI-1, the island can also exist in some populations of bacteria as an extrachromosomal circular form.[12]

Interestingly, the 10 PAPI-1-encoded pilus proteins are well conserved in several *P. aeruginosa* strains that carry this island, including PA2192, PA7, C3710, PACS2, and PSE9 (PAGI-5), and in *P. aeruginosa* clone C strain that carry a pKLC102-like element.

The PAPI-1 *pilS2* gene encodes for a major pilin subunit, which is a 176-amino-acid protein containing a conserved PilS superfamily domain. Horizontal gene transfer is known as a main evolutionary mechanism and contributes to the virulence properties of many bacterial pathogens (V).

There is no specific study on the prevalence of the *pilS2* gene and its association with *P. aeruginosa* plasmid pKLC102 and PAPI-1 pathogenicity island among *P. aeruginosa* in Iran. This study could be the basis for further research in the future.

So, the aim of this study was to evaluate the prevalence of the *pilS2* gene, and its association with *P. aeruginosa* plasmid pKLC102 and PAPI-1 pathogenicity island among *P. aeruginosa* strains isolated from patients with cystic fibrosis, ocular, otitis media, and burn infections.

Methods

Study design, data, and specimens collection

A prospective cross-sectional study was conducted between January 2016 and November 2017. The research was approved by the ethical committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran with ethics number: IR.SBMU.RETECH.REC.1395.460. As a part of the Shahid Beheshti University of Medical Sciences policy, written informed consent was obtained from all patients. The study was conducted in accordance with the Declaration of Helsinki. The clinical samples were collected from inpatients with otitis media, burn wound infections, keratitis, and cystic fibrosis who were hospitalized in Shahid Motahari Hospital (level I burn care center). The sputum samples of the patients with cystic fibrosis were examined macroscopically for the presence of salivary contamination. Following this, briefly, sputum was incubated with an equal volume of sputolysin for 15 minutes at room temperature and plated onto chocolate agar. The deep oropharyngeal swab samples were collected and cultured on blood agar and MacConkey agar media (Merck, Germany).[13]

For bacterial isolation from the burn patients, surface culture swabs were collected from them and were inoculated onto blood agar, MacConkey agar, and tryptic soy broth media (Merck, Germany).[14] The ocular specimens from the patients with conjunctivitis and keratitis were collected by an ophthalmic surgeon and ophthalmologist. These specimens were transferred into 2 mL of brain-heart infusion broth (Oxoid, Hampshire, UK).[15]

The outer ear of the patients with otitis media was cleaned with normal saline and the pus discharges were collected with sterile cotton swab sticks. Pus swabs were added to stuart transport medium (Merck, Germany) and cultured on blood agar, chocolate agar, and MacConkey agar media (Merck, Germany).[16]

The isolated colonies from the positive cultures were investigated for biochemical characteristics of *P. aeruginosa*. Briefly, the biochemical tests include gram staining, pigment production, colony morphology, growth at 42°C on Mueller–Hinton agar, the ability of oxidase and catalase production, and oxidative-fermentative test.[1]

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method according to Clinical & Laboratory Standards Institute[17] recommendations. The susceptibilities of all isolates to different antibiotics were determined using cefepime (30 µg), amikacin (30 µg), ceftazidime (30 µg), tobramycin (10 µg), ciprofloxacin (5 µg), meropenem (10 µg), cefoperazone (75 µg), piperacillin (100 µg), imipenem (10 µg), gentamicin (10 µg), ceftazidime (30 µg), piperacillin-tazobactam (110/10 µg), colistin (10 µg), cefoperazone-sulbactam (100/10 µg), and cotrimoxazole (10 µg).

DNA extraction and PCR

The clinical samples were cultured on chocolate agar medium and the isolated single colonies of *P. aeruginosa* were incubated in 5 mL Luria–Bertani medium at 37°C overnight. Then, DNA templates were prepared using the DNA extraction kit (Genet Bio Company, Korea, Cat. No, K-3000) as previously described.[18]

PCR assay with four primer-pairs including 976F/PAPI-1R, 4542F/intF, SojR/4541F, and intF/sojR was performed to identify the *P. aeruginosa* PAPI-1 pathogenicity island.

Likewise, *P. aeruginosa* plasmid pKLC102 was detected using three other primer-pairs including cp10F/ cp10R, cp44F/cp44R, and cp97F/cp97R by PCR method. Primer sequence used in the genetic characterization of *P. aeruginosa* clinical strains and for detecting the *pilS2* gene is shown in Table 1. Amplified products were analyzed by electrophoresis on 1% agarose gel containing safe stain, and finally in the case of DNA replication and seeing the sharp band, it is considered as positive.

Data analysis

The data were analyzed using the SPSS statistical package version 22 (IBM SPSS Statistics, USA) and Microsoft excel 2016 (Microsoft Corporation, USA) statistical software. The results are presented as descriptive statistics in terms of relative frequency.

Results

A total of 112 *P. aeruginosa* isolates were collected from different sources, of which 36 were isolated from patients with cystic fibrosis, 20 from burn wound infections, 26 from otitis media, and 30 from ocular infections.

Antibiotic susceptibility tests using the Kirby–Bauer method revealed that the highest resistance percentage was related to cefepime, gentamicin, and ciprofloxacin 99 (88%), followed by meropenem and imipenem 98 (87%). The results showed that colistin and cefoperazone-sulbactam were the best antibiotics against *P. aeruginosa* isolates. Antibiotic resistance patterns are detailed in Table 2.

Table 1 Primer sequence used in the genetic characterization of *Pseudomonas aeruginosa* clinical strains

Gene/genomic islands	Forward primer sequence (5′–3′)	Reverse primer sequence (5′–3′)	Size of PCR product (bp)	Tm (°C)
pilS2	GCGGTTTCGTTTCCATCGAG	GGTCACTTCTCCGGTGATCG	428	60
pKLC102				
cp10	CGGACCACTAGATAGCCAGG	GGACGCCATTGAGTATGCGC	255	61
cp44	GGGTCCGCAAAACTTTCCGC	GCTTGAGGTTGGGCCAATCG	272	61
cp97	GGATATCTACGTACCCCGGC	CTTTTTACCCGCAGTGGCGG	337	61
PAPI-1				
976F/PAPI-1R	GCCTGACGGTGTCCTGTTAT	GCTGCCTCTCCTACGAACA	2,600	58
4542F/intF	GTGGTGATGACCTCCAACCT	AGCTACATCGAGGCCGACTA	1,600	58
SojR/4541F	CGAGCACAGAAATGTCCTGA	GACAAGACCAGCCACAACCT	1,600	58
intF/sojR	AGCTACATCGAGGCCGACTA	CGAGCACAGAAATGTCCTGA	1,600	58

Table 2 Antibiotic resistance patterns in *Pseudomonas aeruginosa* isolates

Antibiotic	Cystic fibrosis N (%)	Burn wound infections N (%)	Otitis media N (%)	Ocular infections N (%)	Total N (%)
Cotrimoxazole	27 (75)	17 (85)	22 (84)	22 (73)	88 (79)
Cefoperazone-sulbactam	17 (47)	9 (45)	12 (46)	14 (46)	52 (46)
Amikacin	24 (66)	16 (80)	19 (73)	25 (83)	84 (75)
Piperacillin-tazobactam	28 (77)	16 (80)	20 (76)	23 (76)	87 (77)
Piperacillin	32 (88)	14 (70)	21 (80)	24 (80)	91 (79)
Cefepime	31 (86)	17 (85)	22 (84)	29 (96)	99 (88)
Tobramycin	23 (63)	10 (50)	11 (42)	17 (56)	61 (54)
Meropenem	33 (91)	17 (85)	22 (84)	26 (86)	98 (87)
Imipenem	33 (91)	17 (85)	22 (84)	26 (86)	98 (87)
Gentamicin	34 (94)	16 (80)	23 (88)	26 (86)	99 (88)
Ceftazidime	22 (61)	14 (70)	22 (84)	24 (80)	82 (73)
Colistin	0 (0)	0 (0)	2 (7)	3 (10)	5 (4)
Ciprofloxacin	33 (91)	18 (90)	21 (80)	27 (90)	99 (88)
Aztreonam	30 (83)	16 (80)	20 (76)	27 (90)	93 (83)

Frequency of the *pilS2* gene

The results showed that the *pilS2* gene was identified in 85% (n=96) of the *P. aeruginosa* strains. This major subunit of Type IVb pili was detected among 24% (n=23) of the *P. aeruginosa* strains isolated from cystic fibrosis patients, 21% (n=20) isolated from burn patients, 31% (n=30) isolated from patients with ocular infections, and 24% (n=23) isolated from patients with otitis media. Agarose gel electrophoresis of PCR products after amplification of the *pilS2* gene is shown in Figure 1.

Frequency of the *P. aeruginosa* PAPI-1 pathogenicity island

Overall, the integration of the PAPI-1 to the attB site of positions PA0976.1 and PA4541.1 was identified among 33.9% (n=38) of *P. aeruginosa* isolates. Approximately 18.5% (n=7) of the strains were isolated from cystic fibrosis patients, 39.5% (n=15) isolated from burn patients, 21% (n=8) isolated from patients with ocular infections, and 21% (n=8) isolated from patients with otitis media.

In addition, the PCR assay using primer-pair intF/sojR has detected the circular form of PAPI-1 among 14% (n=17) of *P. aeruginosa* strains isolated from hospitalized patients (including six cystic fibrosis patients, six burn patients, and five patients with ocular infections).

Frequency of the *P. aeruginosa* plasmid pKLC102

Overall, 70.5% (n=79) of the *P. aeruginosa* strains were found to be positive for pKLC102. The highest frequency of this element was detected among patients with ocular (34%) and burn (25%) infections, followed by cystic fibrosis (22%) and otitis media (19%). The results of PCR method for identification of the pKLC102 demonstrated that *CP10*, *CP44*, and *CP97* genes were present in 49%, 51%, and 17% of the *P. aeruginosa* strains, respectively (Table 2). Agarose gel electrophoresis of PCR products after amplification of the *CP44 and CP10* genes is shown in Figures 2 and 3.

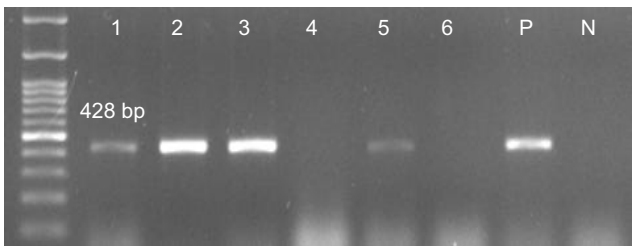

Figure 1 Detection of *pils2* gene (428 bp) in *Pseudomonas aeruginosa* isolates.
Notes: 1–3 and 5 positive samples, 4 and 6 negative samples; molecular pattern: 100 bp.
Abbreviations: N, negative control; P, positive control.

The association of the *pilS2* gene with pKLC102

The *pilS2* gene was simultaneously associated with pKLC102 in 75 (70%) isolates, of which 27 (36%), 19 (25.4%), 15 (20%), and 14 (18.6%) were detected from ocular, burn, otitis media, and cystic fibrosis infections, respectively (Table 3).

The association of the *pilS2* gene with PAPI-1 and pKLC102

In this study, 36 (32.1%) *P. aeruginosa* strains encoded all *pKLC102*, *chromosomal PAPI1*, *plasmid PAPI1*, and *pilS2* genes. Moreover, the pKLC102^{+}, chromosomal PAPI1^{-}, plas-

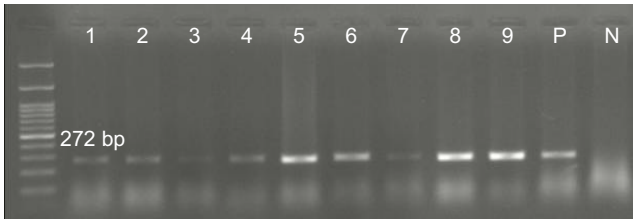

Figure 2 Detection of *CP44* gene (272 bp) in *Pseudomonas aeruginosa* isolates.
Note: Molecular pattern: 100 bp.
Abbreviations: N, negative control; P, positive control.

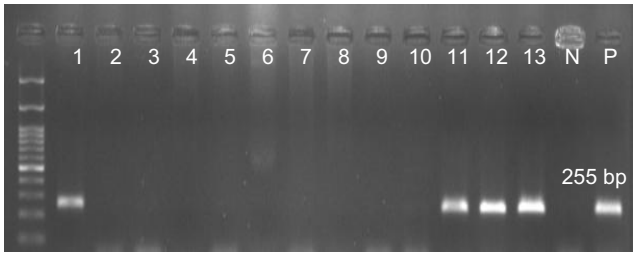

Figure 3 Detection of *CP10* gene (255 bp) in *Pseudomonas aeruginosa* isolates.
Note: Molecular pattern: 100 bp.
Abbreviations: N, negative control; P, positive control.

Table 3 Frequency of the specific genes *CP10*, CP44, and CP97 of pKLC102

Genotype	CF	Eye	Ear	Burn	Total (%)
cp10^{-} cp44^{-} cp97^{-}	18	3	11	1	33 (29.4)
cp10^{+} cp44^{-} cp97^{-}	6	9	2	4	21 (18.7)
cp10^{-} cp44^{+} cp97^{-}	9	0	0	2	11 (9.8)
cp10^{-} cp44^{-} cp97^{+}	0	0	0	0	0
cp10^{+} cp44^{+} cp97^{-}	3	16	0	9	28 (25)
cp10^{+} cp44^{-} cp97^{+}	0	0	0	1	1 (0.9)
cp10^{-} cp44^{+} cp97^{+}	0	1	12	0	13 (11.6)
cp10^{+} cp44^{+} cp97^{+}	0	1	1	3	5 (4.4)

Abbreviation: CF, Cystic fibrosis.

mid PAPI1$^+$, pilS2$^+$ genotype was the most frequent among the isolates (Table 4).

The association of the pKLC102 with PAPI-1

The results showed that all the *P. aeruginosa* strains that are pKLC102 positive encoded also the PAPI-1 element. Although among 37 (33%) strains, pKLC102 was associated with both the chromosomal and plasmid PAPI-1, in 41 (36.6%) strains, pKLC102 was present along with only the plasmid form of the PAPI-1 (Table 5).

Discussion

P. aeruginosa is an opportunistic human pathogen capable of causing a broad range of human life-threatening acute and chronic infections.[19] The bacterium is capable of inhabiting a wide range of environmental niches, in part, owing to have a diverse genomic repertoire that encodes multiple virulence factors such as Type IVb pilus, which is encoded by the island PAPI-1.[6] Also, this pathogen can adhere to different receptors and produce biofilm by its extracellular virulence factor, Type IV pili.[20]

The current study showed the frequency of the gene encoding major subunit of the Type IVb pili, the *pilS2* gene, and its association with *P. aeruginosa* plasmid pKLC102 and PAPI-1 pathogenicity island among *P. aeruginosa* strains isolated from patients with cystic fibrosis, keratitis, otitis media, and burn infection. The results demonstrated that 85% of the *P. aeruginosa* strains encoded the *pilS2* gene. Indeed, 21% (n=20) and 31% (n=30) of the *P. aeruginosa* strains isolated from patients with burn and ocular infections were *pilS2*-positive. Furthermore, patients with otitis media (88.4) and cystic fibrosis (63.8) also had a high prevalence of the *pilS2* gene.

These findings are comparable with the study performed by Dabiri et al,[14] in Iran. They evaluate the Type IV pilin subgroups in *P. aeruginosa* strains and showed that *pilS2* gene was found among 67.5% of cystic fibrosis and 73.3% of burn

Table 4 The association of pilS2 with pKLC102, chromosomal PAPI-1, and plasmid PAPI-1

Genotype	Cystic fibrosis N (%)	Eye N (%)	Ear N (%)	Burn N (%)	Total N (%)
pKLC102$^-$ chromosomal PAPI1$^-$ plasmid PAPI1$^-$ pilS2$^-$	6 (16.6)	0	3 (11.5)	0	9 (8)
pKLC102+ chromosomal PAPI1$^-$ plasmid PAPI1$^-$ pilS2$^-$	0	0	0	0	0
pKLC102$^-$ chromosomal PAPI1$^+$ plasmid PAPI1$^-$ pilS2$^-$	3 (8.3)	0	0	0	3 (2.6)
pKLC102$^-$ chromosomal PAPI1$^-$ plasmid PAPI1$^+$ pilS2$^-$	0	0	0	0	0
pKLC102$^-$ chromosomal PAPI1$^-$ plasmid PAPI1$^-$ pilS2$^+$	3 (8.3)	3 (10)	4 (15.3)	0	10 (8.9)
pKLC102$^+$ chromosomal PAPI1$^+$ plasmid PAPI1$^-$ pilS2$^-$	0	0	0	0	0
pKLC102$^+$ chromosomal PAPI1$^-$ plasmid PAPI1$^+$ pilS2$^-$	3 (8.3)	0	0	0	3 (2.6)
pKLC102$^+$ chromosomal PAPI1$^-$ plasmid PAPI1$^-$ pilS2$^+$	0	0	0	0	0
pKLC102$^-$ chromosomal PAPI1$^+$ plasmid PAPI1$^+$ pilS2$^-$	0	0	0	0	0
pKLC102$^-$ chromosomal PAPI1$^+$ plasmid PAPI1$^-$ pilS2$^+$	6 (16.6)	0	4 (15.3)	1 (5)	11 (9.8)
pKLC102$^-$ chromosomal PAPI1$^-$ plasmid PAPI1$^+$ pilS2$^+$	0	0	0	0	0
pKLC102$^+$ chromosomal PAPI1$^+$ plasmid PAPI1$^+$ pilS2$^-$	1 (2.7)	0	0	0	1 (0.8)
pKLC102$^+$ chromosomal PAPI1$^+$ plasmid PAPI1$^-$ pilS2$^+$	0	0	0	0	0
pKLC102$^+$ chromosomal PAPI1$^-$ plasmid PAPI1$^+$ pilS2$^+$	11 (30.5)	17 (56.6)	6 (23)	5 (25)	39 (38.8)
pKLC102$^-$ chromosomal PAPI1$^+$ plasmid PAPI1$^+$ pilS2$^+$	0	0	0	0	0
pKLC102$^+$ chromosomal PAPI1$^+$ plasmid PAPI1$^+$ pilS2$^+$	3 (8.3)	10 (33.3)	9 (34.6)	14 (70)	36 (32)

Table 5 The association of pKLC102 with chromosomal PAPI-1 and plasmid PAPI-1

Genotype	Cystic fibrosis N (%)	Eye N (%)	Ear N (%)	Burn N (%)	Total N (%)
pKLC102$^-$ chromosomal PAPI1$^-$ plasmid PAPI1$^-$	9 (25)	3 (10)	7 (26.9)	0	19 (16.9)
pKLC102$^+$ chromosomal PAPI1$^-$ plasmid PAPI1$^-$	0	0	0	0	0
pKLC102$^-$ chromosomal PAPI1$^+$ plasmid PAPI1$^-$	9 (25)	0	4 (15.3)	1 (5)	14 (12.5)
pKLC102$^-$ chromosomal PAPI1$^-$ plasmid PAPI1$^+$	0	0	0	0	0
pKLC102$^+$ chromosomal PAPI1$^+$ plasmid PAPI1$^-$	0	0	0	0	0
pKLC102$^+$ chromosomal PAPI1$^-$ plasmid PAPI1$^+$	14 (38.8)	17 (56.6)	6 (23)	5 (25)	42 (37.5)
pKLC102$^-$ chromosomal PAPI1$^+$ plasmid PAPI1$^+$	0	0	0	0	0
pKLC102$^+$ chromosomal PAPI1$^+$ plasmid PAPI1$^+$	4 (11.1)	10 (33.3)	9 (34.6)	14 (70)	37 (33)

patients. In this manner, regarding the importance of Type IV pili in virulence and pathogenesis of *P. aeruginosa*, the results highlight the need for a main program for identification of this pilin and urgently interventions to prevent the development of *P. aeruginosa* strains isolated from hospitalized patients.

In a study by Sadeghifard et al[21] in 2012, the existence of PAPI-1 in clinical *P. aeruginosa* isolates was evaluated; in that study, 35% of isolates were positive for this genomic island, so that most of the PAPI-1-positive isolates showed high levels of antibiotic resistance. In another study done by Morales-Espinosa et al in Mexico, the genetic and phenotypic features of 100 *P. aeruginosa* isolates were studied. They have detected PAPI-1 among 81% of the isolates. This island was reported to be integrated into the chromosome at locus PA0976.1 and PA4541.1 among 65% and 16% of the strains, respectively. However, the circular form of PAPI-1 was not found in any of the strains.[22]

In addition, in Morales-Espinosa et al's work, 87% of isolates carried pKLC102, so that CP10, CP44, and CP97 were positive in 87, 88, and 70 strains, respectively, and more than half of the isolates carried all three genes. Also, Klockgether et al found 50 isolates of *P. aeruginosa* with pKLC102-like islands in their study.

Similarly, we reported that the prevalence of integrated form of PAPI-1 was 33.9%. This rate was the highest among patients with burn infection (39.5%), followed by ocular (21%), otitis media (21%), and cystic fibrosis (18.5%) infections. Interestingly, all isolates positive for pKLC102 in the current study were also shown to carry PAPI-1 (in 70.5% of the isolates) and this association was more frequently detected among eye and burn isolates (Table 4).

Furthermore, as our results revealed, the association of *pilS2* gene with PAPI-1 and pKLC102 was found predominantly in *P. aeruginosa* isolates from burn (*pKLC102*$^+$, *chromosomal PAPI1*$^+$, *plasmid PAPI1*$^+$, and *pilS2*$^+$ genotype) and eye infection (*pKLC102*$^+$, *chromosomal PAPI1*, *plasmid PAPI1*$^+$, and *pilS2*$^+$ genotype) (Table 4); taken together, in 70.8% of the isolates, the association was established and in 9.8% of the isolates *pilS2* gene was only associated with PAPI-1 (*chromosomal PAPI-1*).

Conclusion

In this study, we found that the majority of the isolates may be susceptible to transfer this significant island and the related element pKLC102 into recipient isolates lacking the island owing to high association of the PilS2 pilus with the islands in the studied strains, which may affect the pathogenesis of these isolates.

Moreover, based on this data, it is anticipated that strains isolated from burn and eye with the highest rate of PilS2, PAPI-1, and pKLC102 association have a high level of antibiotic resistance and a higher potential of biofilm formation. Regarding this, more investigations are needed to evaluate the frequency of PilS2, PAPI-1, and pKLC102 and its association with antibiotic-resistance properties and biofilm synthesis in *P. aeruginosa* isolates from Iran.

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Disclosure

The authors report no conflicts of interest in this work.

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