Original Article
Molecular detection of virulence genes as markers in *Pseudomonas aeruginosa* isolated from urinary tract infections

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Abstract: Catheter associated urinary tract infections by *P. aeruginosa* are related to variety of complications. Quorum sensing and related circuitry guard its virulence potential. Though *P. aeruginosa* accounts for an appreciable amount of virulence factors, this organism is highly unstable phenotypically. Thus, genotyping of clinical isolates of *P. aeruginosa* is of utmost importance for understanding the epidemiology of infection. This may contribute towards development of immunotherapeutic approaches against this multi drug resistant pathogen. Moreover, no epidemiological study has been reported yet on uroisolates of *P. aeruginosa*. Thus this study was planned to obtain information regarding presence, distribution and rate of occurrence of quorum sensing and some associated virulence genes at genetic level. The profiling of quorum sensing genes *lasI, lasR, rhlI, rhlR* and virulence genes like *toxA, aprA, rhlAB, plcH, lasB* and *fliC* of twelve strains of *P. aeruginosa* isolated from patients with UTIs was done by direct PCR. The results showed variable distribution of quorum sensing genes and virulence genes. Their percentage occurrence may be specifically associated with different levels of intrinsic virulence and pathogenicity in urinary tract. Such information can help in identifying these virulence genes as useful diagnostic markers for clinical *P. aeruginosa* strains isolated from UTIs.

Keywords: Epidemiology, PCR, *Pseudomonas aeruginosa*, urinary tract infections, quorum sensing

Introduction

P. aeruginosa is one of the most important nosocomial pathogen responsible for a variety of infections with limited therapeutic options because of its antibiotic resistance [1]. It produces impressive array of virulence factors, whose coordinated expression is regulated by different regulatory systems. A recent survey showed that *P. aeruginosa* is one of the most frequent pathogen isolated from ICU (Intensive care unit)-acquired infections [2]. Catheter associated urinary tract infections (CAUTIs) are responsible for 40% of nosocomial infections. *P. aeruginosa* within the catheter frequently develops as biofilm by directly attaching to its surface. These surface-associated, matrix-enclosed, microbial communities are responsible for chronicity and recurrence of such infections leading to high morbidity and mortality [3]. Once established, bacteria communicate with each other to coordinate the expression of specific genes in a cell density-dependent fashion. Quorum sensing (QS) via acyl-homoserine lactone (HSL), controls the expression of an array of virulence genes in *P. aeruginosa*. The autoinducer synthase, LasI, synthesises N-(3-oxododecanoyl) homoserine lactone (3OC12-HSL), which regulates the production of elastase, exotoxin A and alkaline protease, while RhlI synthesizes the autoinducer N-butyryl homoserine lactone (C4-HSL), which regulates the production of rhamnolipid, alkaline protease, elastase, cyanide and pyocyanin [4, 5]. Because of the regulatory control of production of virulence factors, QS mechanisms are being proposed as a novel target for development of innovative strategies to control infections. Moreover, importance of QS in the establishment of a successful infection has been shown in different types of animal model studies such as acute pulmonary infection, burn wound infection, microbial keratitis, chronic lung infection and urinary tract infections [6-10].

Table 1. The conditions used for the PCR amplification of QS and virulence genes in *P. aeruginosa* strains

| Gene | The amplification program | | | | | |
	Initial Denaturation	No. of cycles	Denaturation	Annealing	Primer extension	Final extension
lasI	95°C, 2 min.	30	95°C, 40 sec.	50°C/60°C, 1 min.	72°C, 2 min.	72°C, 10 min.
lasR				60°C, 1 min.		
rhlI				60°C, 1 min.		
rhlR				60°C, 1 min.		
toxA	95°C, 2 min.	30	95°C, 40 sec.	50°C, 1 min.	72°C, 2 min.	
aprA				65°C, 1 min.		
rhlAB	95°C, 5 min.	35	95°C, 30 sec.	52°C, 30 sec.	72°C, 30 sec.	
plcH				55°C, 30 sec.		
lasB				55°C, 30 sec.		
fliC	95°C, 2 min.	30	95°C, 40 sec.	55°C, 1 min.	72°C, 2 min.	

Phenotypic typing methods such as biotyping, serotyping, bacteriocin typing and anti-microbial testing are available in literature. However, *P. aeruginosa* is phenotypically very unstable and therefore genotyping tend to be of great value. Infact, an understanding about the distribution of virulence genes in clinical strains can help in understanding the epidemiology of infections. Detection and identification of QS signals provided information about the expression of virulence components of the infecting pathogen [9].

Scarcity of literature exists regarding genotypic analysis of *P. aeruginosa* responsible for causing CAUTIs. Depending upon the site and type of infection, importance and role of a particular virulence factor may differ in a particular strain. However, distribution of virulence genes may be different in UTI strains of *P. aeruginosa*, as no specific virulence genotype has been associated with such strains, rather contradictory reports exist in nature. Role of QS is well established in *P. aeruginosa* induced microbial keratitis, cystic fibrosis [8], pneumonia [6] as well as it has been found to be necessary during UTI [11]. Its role in UTI is controversial as some QS deficient clinical strains have been shown to be capable of causing clinical infections of the humans as well [10, 12]. Thus, the present study was planned to identify specific virulence genotype in *P. aeruginosa* clinical uroisolates. Virulence genes in this study were selected on the basis of their importance. Isolates were screened for QS systems, alkaline protease (*aprA*), rhamnolipid AB (*rhlAB*), phospholipase (*plcH*) and elastase (*lasB*). They were also screened for exotoxin A (*toxA*) which is highly conserved in *P. aeruginosa* and not in other species of this genus. Highly conserved flagellin 'b' and heterologous flagellin 'a' were also screened in the uroisolates. The study provides information about the percentage occurrence and distribution of QS and some associated virulence genes. Information obtained from such studies may provide an insight into identifying virulence genes as useful diagnostic markers which may further contribute towards development of immunotherapeutic approaches for treating UTIs caused by *P. aeruginosa*.

Methods

Clinical strains and phenotypic identification

Twelve clinical strains of *P. aeruginosa* isolated from patients with UTIs obtained from Government Medical College and Hospital, Chandigarh, India, were examined. The isolates were checked for purity, identified by colony morphology, oxidase test and biochemical reactions specific to *Pseudomonas*. In this study, one well defined laboratory strain *Pseudomonas aeruginosa* PAO1 was also used as a positive control. Standard strain PAO1 was generously provided by Prof. Barbara H. Iglewski, University of Rochester, New York, U.S.A. The strains were grown in Luria Bertani (LB) (HiMedia) and maintained in 50% glycerol and stored at -20°C.

Gradient PCR amplification

For the molecular characterization of the genetic support of QS (*las* and *rhl*), extracellular viru-

Table 2. The primer sequences used in PCR assays for QS and virulence genes detection in *P. aeruginosa* strains

Gene	Primer	Nucleotide Sequence	Amplicon size
lasI	forward	5' CGTGCTCAAGTGTTCAAGG 3'	295 bp
	reverse	5' TACAGTCGGAAAAGCCCAG 3'	
lasR	forward	5' AAGTGGAAAATTGGAGTGGAG 3'	130 bp
	reverse	5' GTAGTTGCCGACGACGATGAAG 3'	
rhlI	forward	5' TTCATCCTCCTTTAGTCTTCCC 3'	155 bp
	reverse	5' TTCCAGCGATTCAGAGAGC 3'	
rhlR	forward	5' TGCATTTTATCGATCAGGGC 3'	133 bp
	reverse	5' CACTTCCTTTTCCAGGACG 3'	
toxA	forward	5' GGAGCGCAACTATCCCACT 3'	150 bp
	reverse	5' TGGTAGCCGACGAACACATA 3'	
aprA	forward	5' GTCGACCAGGCGGCGGAGCAGATA 3'	993 bp
	reverse	5' GCCGAGGCCGCCGTAGAGGATGTC 3'	
rhlAB	forward	5' TCATGGAATTGTCACAACCGC 3'	151 bp
	reverse	5' ATACGGCAAAATCATGGCAAC 3'	
plcH	forward	5' GAAGCCATGGGCTACTTCAA 3'	307 bp
	reverse	5' AGAGTGACGAGGAGCGGTAG 3'	
lasB	forward	5' TTCTACCCGAAGGACTGATAC 3'	153 bp
	reverse	5' AACACCCATGATCGCAAC 3'	
fliC	CW45 forward	5' GGCAGCTGGTTNGCCTG 3'	1.02 kb (type a)
	CW46 reverse	5' GGCCTGCAGATCNCCAA 3'	1.25 kb (type b)

merase (1 U/µl, FIR-Epol). Amplifications were carried out in a Biorad Thermal Cycler for 30 cycles consisting of pre-denaturation, denaturation, annealing, extension and post elongation. The parameters for the amplification cycles used in each PCR experiment are represented in **Table 1**. A set of oligonucleotide primers (Eurofins Genomics) that allowed to amplify whole QS genes (*lasI, lasR, rhlI and rhlR*) [13] were selected. Also, PCR assays were used to detect the extracellular virulence genes encoding alkaline protease (*aprA*) [14], exotoxin A (*toxA*) [15] and rhamnolipid AB (*rhlAB*), phospholipase H (*plcH*), elastase (*lasB*) [7]. A set of conserved oligonucleotide primers CW45, CW46 [16] was also used to analyse flagellin subtypes in the clinical strains. The sequences of specific primers used in PCR reactions and the molecular weight of the obtained amplicons are presented in **Table 2**. After amplification, 10 µl sample was subjected to electrophoresis on a standard 1% agarose gel for 1 h at 100 V, stained with ethidium bromide (Sigma) and detected by UV transillumination.

lence factors (exotoxin A, alkaline protease A, rhamnolipid, phospholipase, elastase) and initial colonization factor flagellin (*fliC*), genomic DNA was extracted from twelve selected *P. aeruginosa* strains and from *P. aeruginosa* reference strain PAO1. One colony of each strain cultured on solid medium was inoculated into 5 ml of LB and grown overnight at 37°C with shaking. From these cultures, DNA extraction was performed by using DNA extraction mini kit (Favorgen) according to the manufacturer's recommendations. At different annealing temperatures (50°C, 55°C, 60°C, 65°C), amplification of above mentioned genes was carried out with PAO1 DNA. Best annealing temperature (intense amplicon band with no primer dimmers) was chosen for the profiling of clinical strains.

PCR protocol

Amplifications were carried out in 25 µl volumes containing template DNA (50 ng), Taq buffer (1X), DMSO (Dimethyl sulfoxide), Magnesium chloride (2 mM), each primer (10 pM/µl), nucleotides (dATP, dCTP, dGTP, dTTP) (200 µM, Thermo Scientific) and Taq poly-

Results

PCR assay for QS genes

The results showed different distribution of *lasI, lasR, rhlI* and *rhlR* genes in *P. aeruginosa* strains. The primers used for detection of QS genes allowed the amplification of the whole genes. Nine (75%) strains namely B1, B2, B3, B4, B5, B6, B7, B9 and B12 were positive for *lasI* gene giving amplification at 60°C at 295 bp (**Figure 1**). Similarly B2, B3, B5, B6, B7, B8, B9, B11 and B12 were positive (75%) for *lasR* gene which gave amplification at 60°C at 130 bp
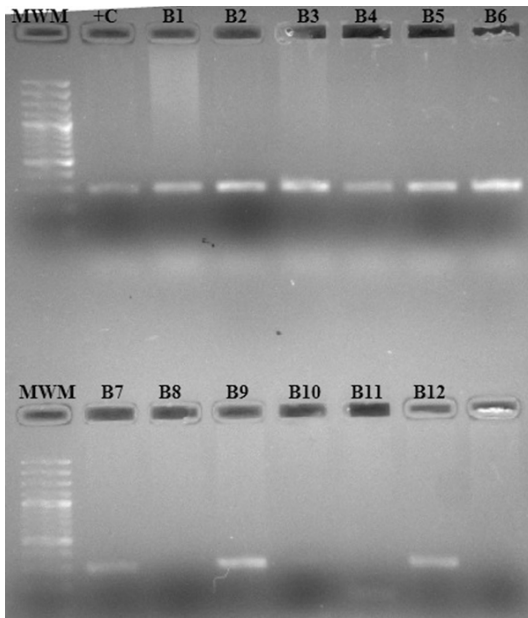
Figure 1. Agarose gel electrophoresis of PCR products after amplification of *lasI* gene. MWM-molecular weight marker (100 bp DNA ladder, # DL004, Geneaid), +C-PAO1 control DNA, B1-B12- different strains of *P. aeruginosa* (*lasI* gene products at 295 bp).
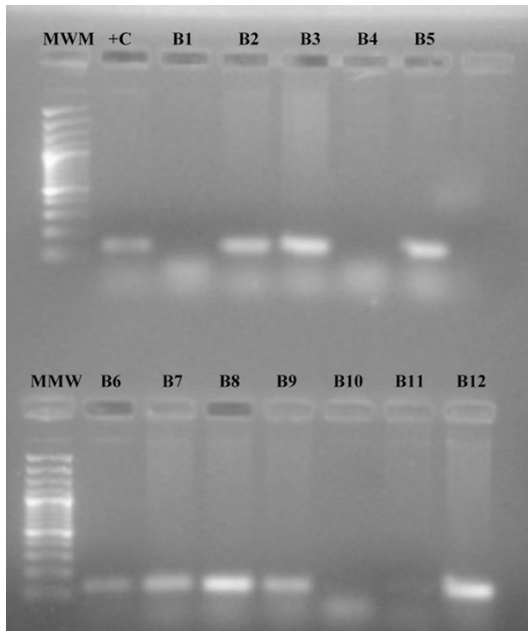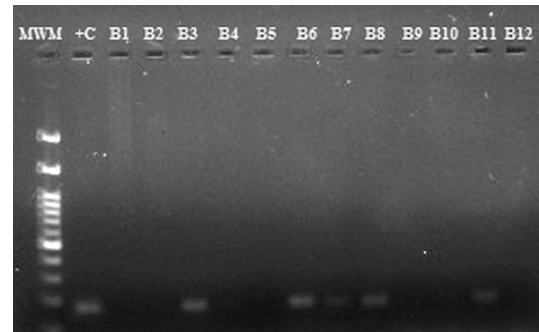

Figure 2. Agarose gel electrophoresis of PCR products after amplification of *lasR* gene. MWM-molecular weight marker (100 bp DNA ladder, # DL004, Geneaid), +C-PAO1 control DNA, B1-B12- different strains of *P. aeruginosa* (*lasR* gene products at 130 bp).

Figure 3. Agarose gel electrophoresis of PCR products after amplification of *rhlI* gene. MWM-molecular weight marker (100 bp DNA ladder, # DL004, Geneaid), +C-PAO1 control DNA, B1-B12- different strains of *P. aeruginosa* (*rhlI* gene products at 155 bp).
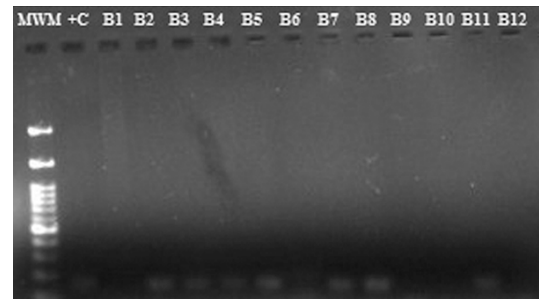

Figure 4. Agarose gel electrophoresis of PCR products after amplification of *rhlR* gene. MWM-molecular weight marker (100 bp DNA ladder, # DL004, Geneaid), +C-PAO1 control DNA, B1-B12- different strains of *P. aeruginosa* (*rhlR* gene products at 133 bp).

(**Figure 2**). Five out of twelve strains (41.6%) B3, B6, B7, B8 and B11 were positive for the presence of *rhlI* gene, giving amplification at 60°C at 155 bp (**Figure 3**). *rhlR* gene was found in seven strains (58.3%) B2, B3, B4, B5, B7, B8 and B11, giving amplification at 60°C at 133 bp (**Figure 4** and **Table 3**).

PCR assay for virulence genes (fliC, toxA, aprA, lasB, plcH, rhlAB)

In the present study, clinical strains were screened for the prevalence of different virulence genes of *P. aeruginosa*. Surprisingly, *aprA* gene had lowest occurrence of 16.6% in only 2 strains, B4 and B8 (**Figure 5**). The prevalence of *lasB and plcH* was found in 75% of strains (**Figures 6** and **7** respectively). *lasB* was found

Table 3. The distribution of virulence genes among *P. aeruginosa* strains

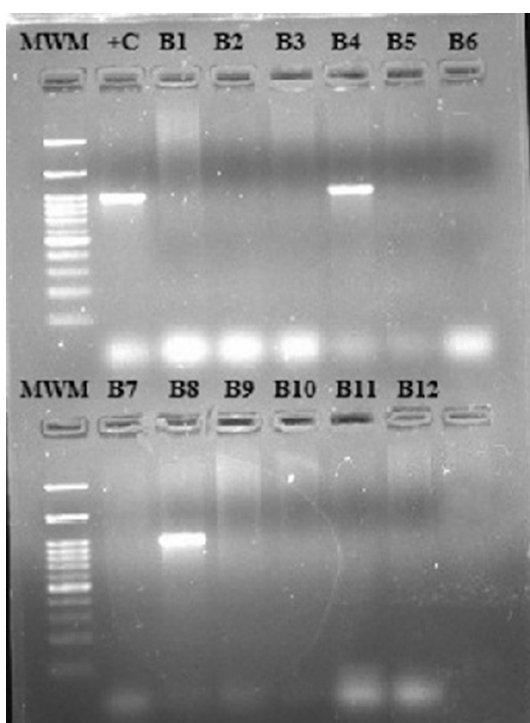| Source of strain isolation	The percent (%) of positive strains for genes encoding different virulence factors										
	lasI	*lasR*	*rhlI*	*rhlR*	*toxA*	*aprA*	*rhlAB*	*plcH*	*lasB*	*fliC*	
Urinary Tract	75%	75%	41.6%	58.3%	100%	16.6%	50%	75%	75%	58.3%	
										a	b
B1	+	-	-	-	+	-	+	+	+	-	+
B2	+	+	-	+	+	-	+	+	+	-	-
B3	+	+	+	+	+	-	+	+	+	-	-
B4	+	-	-	+	+	+	-	+	-	+	-
B5	+	+	-	+	+	-	-	-	+	-	+
B6	+	+	+	-	+	-	+	+	-	-	+
B7	+	+	+	+	+	-	+	-	+	-	-
B8	-	+	+	+	+	+	+	+	+	+	-
B9	+	+	-	-	+	-	-	+	-	-	-
B10	-	-	-	-	+	-	-	+	+	+	-
B11	-	+	+	+	+	-	-	+	+	+	-
B12	+	+	-	-	+	-	-	-	+	-	-

Figure 5. Agarose gel electrophoresis of PCR products after amplification of *aprA* gene. MWM-molecular weight marker (100 bp DNA ladder, # DL004, Geneaid), +C-PAO1 control DNA, B1-B12- different strains of *P. aeruginosa* (*aprA* gene products at 993 bp).

positive for strains B1, B2, B3, B5, B7, B8, B10, B11 and B12. *plcH* was found positive for strains B1, B2, B3, B4, B6, B8, B9, B10 and B11. *rhlAB* was found to be positive in 50% of
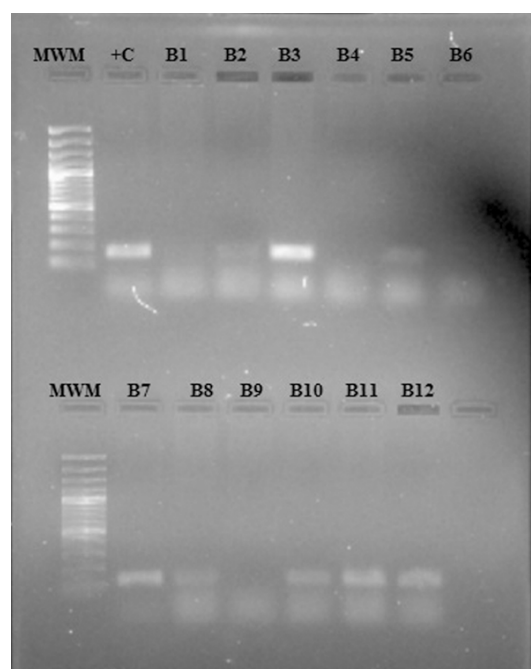

Figure 6. Agarose gel electrophoresis of PCR products after amplification of *lasB* gene. MWM -molecular weight marker (100 bp DNA ladder, # DL004, Geneaid), +C-PAO1 control DNA, B1-B12- different strains of *P. aeruginosa* (*lasB* gene products at 153 bp).

strains namely B1, B2, B3, B6, B7 and B8 (**Figure 8**). 100% prevalence of *toxA* gene was found in all analyzed strains (**Figure 9**). *P. aeruginosa* PAO1 was found to have '*b*'-type flagellin, giving PCR amplification at 1.25 kb. Strains B1, B5 and B6 showed '*b*'-type flagellin. Strains
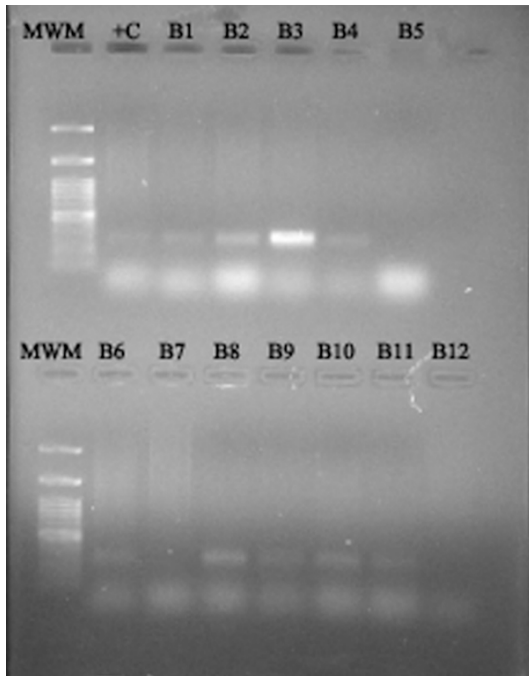
Figure 7. Agarose gel electrophoresis of PCR products after amplification of *plcH* gene. MWM-molecular weight marker (100 bp DNA ladder, # DL004, Geneaid), +C-PAO1 control DNA, B1-B12- different strains of *P. aeruginosa* (*plcH* gene products at 307 bp).
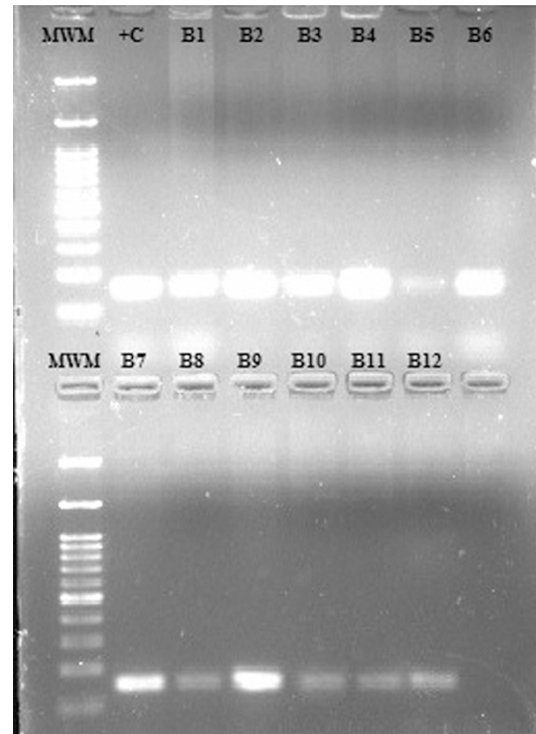

Figure 8. Agarose gel electrophoresis of PCR products after amplification of *rhlAB* gene. MWM-molecular weight marker (100 bp DNA ladder, # DL004, Geneaid), +C-PAO1 control DNA, B1-B12- different strains of *P. aeruginosa* (*rhlAB* gene products at 151 bp).

Figure 9. Agarose gel electrophoresis of PCR products after amplification of *toxA* gene. MWM-molecular weight marker (100 bp DNA ladder, # DL004, Geneaid), +C-PAO1 control DNA, B1-B12- different strains of *P. aeruginosa* (*toxA* gene products at 150 bp).
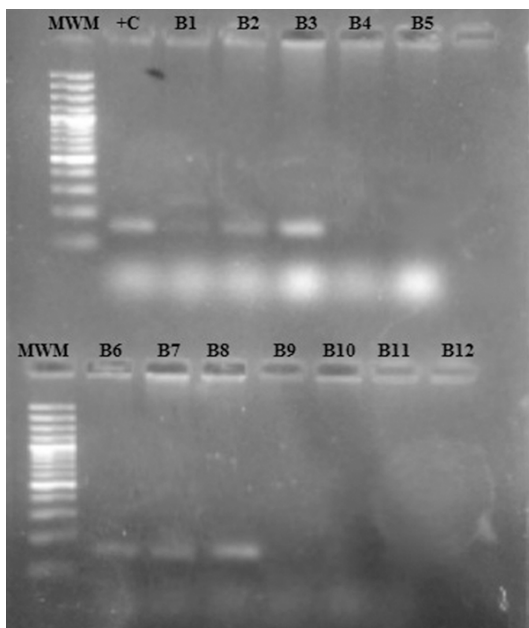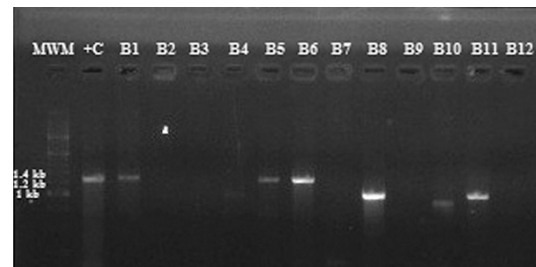

Figure 10. Agarose gel electrophoresis of PCR products after amplification of *fliC* gene. MWM-molecular weight marker (O'Range Ruler 200 bp DNA ladder, # SM0633, Fermentas), +C-PAO1 control DNA, B1-B12- different strains of *P. aeruginosa* (*fliC* gene products at 1.02 kb and 1.25 kb).

B4, B8, B10 and B11 showed *'a'*-type flagellin giving PCR amplification at 1.02 kb. Non-flagellar strains were: B2, B3, B7, B9 and B12, which surprisingly showed no amplification (**Figure 10**). Percentage *fliC* occurrence was found to be 58.3% (25% 'b'-type flagellin, 33% 'a'-type) (**Table 3**).

Discussion

The large genome and its genetic complexity allow *P. aeruginosa* to thrive in diverse ecologic conditions. Multiple bacterial virulence factors impact the pathogenesis of *P. aeruginosa* infections. The combination of virulence factors expressed by each *P. aeruginosa* strain tend to determine the outcome of an infectious process. However, in the clinical cases, it is often difficult to distinguish between simple colonization and infection, and no diagnostic tool is available to assess the virulence potential of a given isolate [17]. Taking into account the poor availability of information on the patterns of virulence factors possessed by *P. aeruginosa* strains isolated from patients with CAUTIs, the genetic profiling of virulence determinants was carried out.

P. aeruginosa possesses two QS systems, Las and Rhl. Isolates obtained from patients with lower respiratory tract, non-surgical or surgical wound infections and sputa of cystic fibrosis patients showed high percentage of functional QS systems (97.5%) [12]. Similarly, all analysed isolates from respiratory tract, wound secretions and from patients with cardiovascular surgery associated infections possessed QS genes (100%) [7]. In the present study, all the strains were found to have varied distribution of individual QS genes. The primers used for detection of QS genes allowed the amplification of the whole genes. All the four QS genes were not detected in any of the strains tested. Senturk *et al.* revealed that *lasI, lasR, rhlI, rhlR* genes may be differently distributed in clinical isolates. However, presence of all four QS genes may not be necessarily indicative of phenotypic production of C4-HSL and C12-HSL. These deficiencies were linked to combinations of point mutation [10]. The two QS systems do not operate independently. LasR-C12-HSL complex positively regulates transcription of RhlR and RhlI. Expression of *rhlR* is not only dependent on LasR, but on RhlR itself [18]. Since the circuitry is interlinked, the absence of any of the component in clinical strains did not compromise their ability for phenotypic expression of QS system [10, 12].

Another important *P. aeruginosa* virulence determinant is alkaline protease. The proteases promote the development of bacteria within the infected host and interfere with the host immune system. Only 30 - 40% of strains from ear, blood and lungs showed high protease activity [19]. Uroisolates of *P. aeruginosa* in our study showed least presence of alkaline protease (16.6%), indicating that alkaline protease may not be playing a very important role in the pathogenesis of UTI. This was also indicated in another study from our laboratory where 50% of strains possessing alkaline protease were although able to colonize kidney tissue, but were unable to multiply and showed very low bacterial count [20]. Since *aprA* is encoded by both Las and Rhl systems, its percentage occurrence may corroborate with the lower presence of Rhl system. Although less important in establishing UTI, its negligible presence may make these strains different from strains isolated from other infectious sites and hence an important marker in strains causing UTIs. Our results corroborate the finding of Cotar *et al.* who showed that lower presence of a particular virulence factor makes it a more important factor in that particular infection. In this regard, PCR results of an earlier study showed the presence of gene encoding ExoS in 64.87% of strains and ExoU in 56.54% strains. It was suggested that ExoU could be a marker of virulence for strains isolated from respiratory tract and wound secretions [7]. Analysis of *P. aeruginosa* clinical isolates from different sites also highlighted that both the infection site as well as the duration of infection influenced the virulence of the bacteria by altering production of extracellular virulence factors [21]. Thus expression of *aprA* may also be related to an infection site where abundant substrate is available for its growth. The urinary tract may not be providing the substrate for expression of *aprA*, which needs to be exploited.

Elastase (encoded by *lasB*) is a powerful T2SS secreted proteolytic enzyme. This enzyme has a wide range of substrates, including elements of connective tissue such as elastin, collagen, fibronectin and laminen. Phospholipids are hydrolysed by phospholipase C which is encoded by *plcH* gene. Rhamnolipid, being a rhamnose-containing glycolipid biosurfactant, has a detergent-like structure and is considered to solubilize the phospholipids of lung surfactant, making them more accessible to cleavage by phospholipase C [22]. It's encoded by *rhlAB* gene. Production of the QS-dependent virulence factor, rhamnolipid by *P. aeruginosa* isolates is associated with development of VAP (ventilator-associated pneumonia) [6]. In few studies, all isolates from burn, wound and pul-

monary tract infections harbored *lasB*, *plcH* and *rhlAB* gene. Prevalence of a gene in all the environmental and clinical isolates implies the importance of a factor for survival of *P. aeruginosa* in various settings [7, 23, 24]. In the present study the prevalence of *lasB and plcH* was found in 75% of strains. The presence of *lasB* can be corroborated with the percentage occurrence of las genes (*lasI and lasR*) since it is encoded by both Las and Rhl systems. Similarly rhamnolipid is encoded by Rhl system. The presence of *rhlAB* (50%) corroborated with the percentage occurrence of Rhl genes (*rhlI*; 41.6% and *rhlR;* 58.3%).

100% prevalence of *toxA* gene in all analyzed strains was found to be related to the presence of *lasI* and *lasR* genes. It plays an important role as a virulence factor of *P. aeruginosa* within catheter associated UTIs [15] and in one of the study, over 80% of isolates from urine were found to possess *toxA* gene [24]. Its presence was also observed in high percentage among *P. aeruginosa* strains isolated from respiratory and burn infections [7]. In case of bacteraemia, 56.7% of *P. aeruginosa* strains produced exotoxin [25]. Khan and Cerniglia developed a PCR to detect *P. aeruginosa* by amplifying the *toxA* gene and reported that 96% of *P. aeruginosa* isolates contained the *toxA* gene, whereas other species of bacteria did not yield any positive results [26]. In another study, 90.7% of *P. aeruginosa* isolates tested from burn, wound and pulmonary tract infections, harbored *toxA* gene [23]. The *ptxR* gene, expression enhancer of *toxA* gene, was only detected in *P. aeruginosa* isolates; whereas other species of *Pseudomonas* did not yield any positive results [27], indicating importance of *toxA* in *P. aeruginosa* and in UTI.

Apart from extracellular factors, the initial attachment mediator (flagella) plays a significant role in initiation of infection. Two types of flagellin proteins have been identified in *P. aeruginosa*, type 'a' and type 'b', which can be distinguished on the basis of molecular size and reactions with type-specific polyclonal and monoclonal antibodies [28]. Type 'a' and 'b' flagellin of *P. aeruginosa* do not exhibit phase variation; a single strain produces single type of flagellin, and no switching between types 'a' and 'b' has been observed. Oligonucleotide primers specific for N-terminal (CW46) and

C-terminal (CW45) conserved regions of flagellin gene were used for PCR amplification of the flagellin gene of *P. aeruginosa* PAO1. In a physical genome analysis of the virulence-associated *fliC* locus in *P. aeruginosa* strains, mapping and sequencing revealed groups of heterologous a-type (1164 bp; 1185 bp) and highly conserved 'b'-type (1467 bp) flagellin genes [29]. Percentage *fliC* occurrence was found to be 58.3% (25% '*b*'-type flagellin, 33% '*a*'-type). Absence of flagellin was observed in almost 40% of the uroisolates obtained from patients with UTIs in our study. The organism must become non-motile to chronically persist. Phagocytic cells respond directly to flagellar motility. This represents a novel mechanism by which the innate immune system facilitates clearance of bacterial pathogens, and provides an explanation for how selective pressure may result in bacteria with down-regulated flagellar gene expression and motility as is evident in isolates causing chronic infections [30]. Thus, variation in the flagellin gene distribution among *P. aeruginosa* isolates from UTI patients may be due to the selective pressure of the disease.

Differences in the distributions of virulence genes in the population strengthens the probability that some *P. aeruginosa* strains are better adapted to the specific conditions found in specific infectious sites. Although genotypic role of extracellular products such as protease and exotoxin A have been shown in corneal infection, respiratory infection and burn wound [7, 14, 24, 25], no genotypic reports are available on *P. aeruginosa* induced UTIs, apart from some phenotypic studies [10, 20]. Determination of different virulence genes of *P. aeruginosa* isolates suggest that they are associated with different levels of intrinsic virulence and pathogenicity. This may have different consequences on the outcome of infection. The present study is first of its kind to show the presence and distribution of four QS genes and six virulence genes viz: *lasI, lasR, rhlI, rhlR, toxA, aprA, rhlAB, plcH, lasB and fliC* across the genome of *P. aeruginosa* uroisolates. These virulence factors could represent a useful diagnostic marker for the investigation of uroisolates of *P. aeruginosa*. Thus, simultaneous detection of genes by PCR provides more confident detection of *P. aeruginosa* from UTIs, gives an idea of percentage and rate of occur-

rence of some virulence genes and source of infection (urinary tract), which further can contribute to the derivation of an immunotherapy against UTI.

Acknowledgements

Disclosure of conflict of interest

None to declare.

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