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Original Article

Serotyping and virulence potential of clinical *Pseudomonas aeruginosa*

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ABSTRACT

Pseudomonas aeruginosa considered as an important pathogen that have the ability to produce virulence factors, like pyocin and biofilm. Objectives of the study are serotyping of clinical *local P. aeruginosa*, determination of the ability to produce biofilm and pyocin and detecting of pyocin genes and their expression in relation to pyocin production. Fifty *P. aeruginosa* isolates were collected from different hospitals in Baghdad during the period from October to December 2014. Serotypes of *P. aeruginosa* were detected by slide agglutination test. The production of pyocin was determined phenotypically and pyocin S genes were detected by Polymerase Chain Reaction (PCR). The ability of bacteria to produce biofilm was tested by two different methods. As conclusion, serotyping of *P. aeruginosa* isolates showed different serotypes. The O7 serotype is appear to be new serotype in Iraq. One third of local *P. aeruginosa* isolates are pyocin producers. Genotypic detection of pyocin is better than phenotypic perhaps due to failure of pyocin gene expression. local *P. aeruginosa* isolates can produce both virulence factors, pyocin and biofilm, increase by that their virulence.

Key Words: *P. aeruginosa*, pyocin, biofilm, serotyping, polymerase chain reaction

INTRODUCTION

P. aeruginosa considered as an important pathogen which considered as one of the causative agents of nosocomial infections as well as, it cause wide range of infections for humans [1] . The ability of *P. aeruginosa* to cause infections returns to its own mechanism of adhesion, capacity to adapt in the environment, development of resistance to antibiotics and production of biofilm [2]. Biofilm formation is a type of virulence factor produce by bacteria which surround itself by exo-polysaccharaide and implanted in matrix of extracellular polymeric constituent [3]. Pyocins are narrow-spectrum bacteriocins which are produced by most isolates of *P. aeruginosa* and are apparent to play a role in niche formation and protection in mixed populations [4]. *Pseudomonas aeruginosa* able to produce three types of pyocin, the rod-shaped R-pyocin the soluble S-pyocin, and the flexible F-pyocin. The production of pyocins in *P. aeruginosa* is inducible by treatments that causing DNA destruction, the pyocin also can classified into pore formation pyocin and DNase activity pyocin according to their functions [5]. S-type pyocin is more frequently produce among *P. aeruginosa* strains which able to kill the non-immune *P. aeruginosa* strains. The majority of S-type pyocins (S1, S2, AP41 and S3) cause death to the cell by DNA destruction due to an endonuclease activity [6] . This study conducted to determine clinical *local P. aeruginosa* serotypes, production of biofilm by two different methods and detecting of pyocin genes and their expression in relation with pyocin production.

MATERIALS AND METHODS

Fifty *P. aeruginosa* isolates were collected from different hospitals in Baghdad during the period from October to December 2014. This isolates diagnosed depending on morphological and microscopic properties as well as VITEK2 compact. Serotypes of *P. aeruginosa* were detected according to a system of Habs (1957)[7], using antisera against somatic antigen (O) by slide agglutination test. The kit was obtained from vaccine and sera institute –Baghdad. The ability of bacteria to produce pyocins was studied by using cross-streaking method, this method applied to all fifty bacterial isolate where followed the method of Essa *et al*(1986)[8] to accomplished this test. The genes responsible for pyocin production were detected by Polymerase Chain Reaction (PCR)

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technique. The primers used in PCR amplification were specific for detection of S1S2 and S3 genes which responsible for pyocin production. The oligonucleotide PCR primer set for detection of S1S2 was S1S2imm-F (CACAAGGGAGGGAAGTGA) and S1S2imm-R (CGGCCTTAAAGCCAGGAA) and the expected product is 287 bp. While the primer set used for amplification of pyocin S3 gene was S3RB- F (CGTATCACGAGACAGGCA) and S3RB-R (TGCCGCTTCTT CCGCTTT) the expected product is 451 bp [5].

Cycling protocol for the PCR amplification of pyocin S1S2 gene was comprising initial denaturation at 94 ˚C for 30 seconds and 35 cycles of denaturation step at 94 ˚C for 30 seconds, annealing step at 58 ˚C for 30 seconds and extension step at 72 ˚C for 30 seconds. The same protocol was used to amplification of pyocin S3 gene except the annealing temperature was 53 ˚C [5.] The amplified PCR products were analyzed by agarose gel electrophoresis according to Sambrook and Russell (2001)[9] method. The ability of bacteria to produce biofilm was tested by using two different methods, Congo red agar(CRA) method Kala *et al* (2012)[10] and micro titer plate (MTP) method Bose, *et al* (2009)[11] to detect biofilm formation qualitatively and quantitatively.

RESULTS AND DISCUSSION

Results of serotyping of *P. aeruginosa* isolates showed different serotypes, as shown in table 1, the predominant one is O11and this serotype is considered as a more predominant in Iraq as previously reported [8-12]. Serotype classification of *P. aeruginosa* isolates showed the existence of other serotypes. O11 serotype was more common one (48%) whereas O9, O5, O8 and O10 were about 8%, 10% , 12% and 18% respectively. O7 serotype is appear to be new serotypes as compare with local studies [8-12]. Serological classification considered as an indicator of epidemic spread of *P. aeruginosa* in hospitals and responsible for infections after surgery and burn infections [13].

The cross streaking assay revealed that, 16 isolates were able to produce pyocin out of 50 isolates (32%), PCR technique showed that there were 24 isolates of *P. aeruginosa* from 50 isolates (48%) appeared to have genes of S1 and S2 in this study while there are 26 isolates corresponding to 52% of the total (50) isolates were recorded to be negative as shown in figure (1) and table (2). Pyocin S3 gene detection revealed that, 21 isolates (42%) showed the presence of pyocin S3 gene, while 29 isolates (58%) showed the absence of the gene (figure 2 and table 2).

Table 1: serotypes of clinical *P. aeruginosa*

Serotype	No.	Percentage (%)
O11	24	48
O10	9	18
O9	4	8
O8	6	12
O7	2	4
O5	5	10
Total	50	100

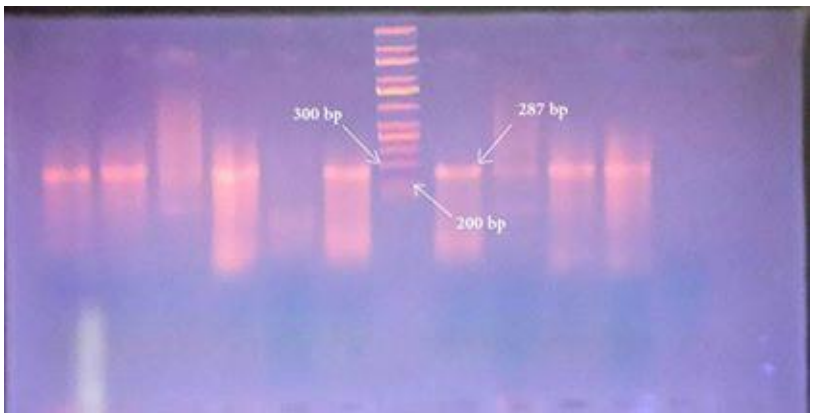

Figure (1): Agarose Gel electrophoresis of PCR product of S1S2 genes (287bp).
Agarose Gel electrophoresis was perform using1% agarose gel and the run last for 45min at 100 volts.

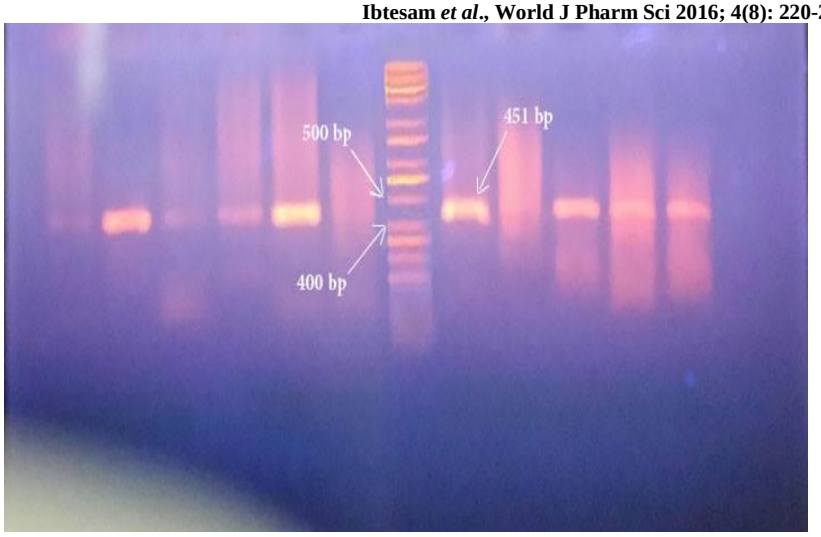

Figure (2): Agarose Gel electrophoresis of PCR product of S3 gene (451 bp).
Agarose Gel electrophoresis was perform using 1% agarose gel and the run last for 45min at 100 volts.

The results of current study are differ from those of Al-Shammary *et al* (2013) [5], another local study, hence S1S2 genes were appear to be in 90% of the isolates while S3 gene present in 82% of them. The differences may be due to the variances in methods by which the DNA was extracted or due to differences of sources of the isolates. Sixteen isolates pyocin producers by cross streaking method showed that, there are 6 isolates as 37.5% had S1S2 genes and only 3 (18.75%) had S3 gene. The total number of isolates that had pyocin genes (S1S2and S3) and detected by PCR were 31(62%) were higher than the isolates that produce pyocin by cross streaking method (16, 32%). Nine (29.03%) of *P. aeruginosa* isolates showed positive results by both PCR (genotypic) and cross streaking method (phenotypic). While 22 (70.96%) of them were phenotypically negative in spite of they were positive genotypically, such obtained results may be due to failure of gene expression. It was previously reported that expression of R, F and S pyocins is positively and negatively regulated by PrtN and PrtR proteins [14]. On the other hand, 7 (36.8%) were positive when detected phenotypically but negative with genotypic. This may be due to production of other pyocin types, F or R [15]. while 12 (63.2%) were negative by both phenotypic and genotypic detection (table 3).

Congo red agar (CRA) method for quantitative detection of biofilm formation reveals that, about 33 isolates (66%) were able to produce black to gray color with dry crystalline consistency while numbers of non-biofilm producing bacteria were 17 isolates (34%). The results of MTP method showed that 19 isolates out of 50 isolates (38%) were positive with powerful biofilm production, but non-biofilm former was 18 isolates (36%) while 13 isolates (26%) considered as weak producer isolates (table 4).

On the other hand, there were about 15 isolates (93.75%) and 11(68.75%) from total of 16 pyocin producers are able to produce biofilm by using MTP method and CRA method respectively (table 5). The production of both of these virulence factors, biofilm and pyocin, together increase pathogenicity and virulence of P. *aeruginosa* [16]. Pyocins can stimulate biofilm formation hence the increasing in biofilm occurs due to general response to cellular damage and that occurs due to pyocin that disrupts cellular membranes [17].

As conclusion, serotyping of *P. aeruginosa* isolates showed different serotypes, the predominant one is O11, O7 serotype is appear to be new serotype in Iraq. One third of local *Pseudomonas aeruginosa* isolates are pyocin producers. Genotypic detection of pyocin is better than phenotypic due to failure of pyocin gene expression. local *P. aeruginosa* isolates can produce both virulence factors, pyocin and biofilm, increases by that their virulence.

Table 2: Occurrence of pyocin S genes in *P. aeruginosa* isolates.

Pyocin S genes	Positive (%)	Negative (%)	Total (%)
S1S2	24(48)	26(52)	50(100)
S3	21(42)	29(58)	50(100)

Table 3: Expression of pyocin in relation to pyocin genes occurrence.

Pyocin genes (genotypic)	Pyocin producers (phenotypic) Positive(%)	Negative (%)	Total (%)
Positive(%)	9(29.03)	22(70.97)	31(62)
Negative (%)	7(36.8)	12(63.2)	19(38)
Total (%)	16(32)	34(68)	50(100)

Table (4): Comparison between CRA and MTP method for biofilm formation

Biofilm formation	CRA-method		MTP-method	
	NO	%	NO	%
High producer	33	66.00%	19	38.00%
Moderate	0	0	13	26.00%
Non-producer	17	34.00%	18	36.00%

Table 5: Expression of biofilm along with pyocin.

Method	Positive	Percentage (%)	Negative	Percentage (%)	Total no. of pyocin producers
Congo red agar	11	68.75	5	31.25	16
microtiter plate	15	93.75	1	6.25	16

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