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Original Article

Detection of Genes Encoding Pyocin Production of *Pseudomonas aeruginosa*

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ABSTRACT

More than 90% of Pseudomonas aeruginosa strains are pyocins producer and each strain may synthesis several types of pyocins. pyocins displayed growth inhibitory activity against some Gram-positive and Gram-negative bacteria .Fifty bacterial isolates of *Pseudomonas aeruginosa* have been collected from many hospitals in Baghdad (Iraq) include: Al-Kindy hospital, Ibn Al-baladi hospital, wound and burn hospital and Baghdad hospital in medical city during the period from Sep. to Dec. (2014). These isolates were collected from different sources, they were diagnosed depending on morphological and microscopic properties as well as by using biochemical tests, and VITEK2 compact system, pyocins production was detected by using cross streaking method. The results showed that (16) isolates out of (50) isolates have the ability to produce pyocins. Polymerase Chain Reaction (PCR) technique used to detect gene responsible for S type pyocins (S1S2 pyocins and S3 pyocin), results appear that there were (24) isolates from all (50) isolates (48%) contained the S1S2 gene, (21) isolates (42%) contained S3 gene. The total number of isolates which contain genes encoding of S-type pyocin production (both S1S2 and S3 genes) were (31) isolates (62%).

Keywords: *Pseudomonas aeruginosa*, Pyocin Production, PCR

INTRODUCTION

P. aeruginosa is considered as an important bacterial species because it is widespread in the environment and causes wide range of infections for humans (1,2) .animals and plants. Also it is considered as causative agent of nosocomial infections (3,4). Many bacterial species are able to produce bacteriocins which are proteineous components; that kill bacterial cells of the same or closely related species (5,6). Pyocins are narrow-spectrum bacteriocins which are synthesized by most isolates of *P. aeruginosa* and presumed to play a role in niche establishment and protection in mixed populations ,three types of pyocins are described (7,8).The soluble S-pyocin, the rod-shaped R-pyocin and the flexible F-pyocin **(**9). The production of pyocins in *P. aeruginosa* is inducible by treatments that cause damage to DNA and they are dependent on the recA gene. The pyocin also can be classified according to their functions or morphology into pore formation pyocin and DNase activity pyocin (10,11,12).

S-type pyocin is more frequently pyocin produced among *P. aeruginosa* strains which are able to kill the non-immune *P. aeruginosa* strains by a specific receptor and are composed of three functional domains, the receptor-binding domain, the translocation domain and the DNase domain. Pyocins S1 and S2 inhibit the lipid synthesis of sensitive cells (13), the majority of S-type pyocins (S1, S2, and S3) causes death to the cell by DNA breaking down due to an endonuclease C-terminal domain (14,15).In this study we detect the ability of local *P. aeruginosa* isolates to produce pyocin and the genes that coding of S-pyocin in these isolates.

MATERIALS AND METHODS

Collection of Bacterial isolates: Fifty bacterial isolate of *P. aeruginosa* were collected from four hospitals in Baghdad include: Al-Kendi hospital, Ibn Al-baladi hospital, wound and burn hospital, Baghdad hospital in medical city, during October to December 2014. These bacterial isolates were collected from different sources. *P. aeruginosa* isolates identified according to morphological, microscopic and biochemical tests. The VITEK® 2 Compact system (BioMe'rieux, French) is dedicated to the identification and susceptibility testing of clinically significant bacteria.

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Detection of Pyocin Production: In this study we used cross-streaking method to detect pyocin production (16); according to this method bacterial isolates were cultured on liquid media and then incubated at 37Cº for 24 hours for refreshment. Drop from the bacterial culture that is prepared above was taken by a loop cultured on trypticase soy agar as straight line with two repeat for each isolate then plates were cultured at 37Cº for 24hours to permit the pyocin to diffuse in the media. After incubation time was over growth of bacterial isolate was observed on straight line, then the growth was removed by sterile slide edge and in order to remove bacteria completely and to maintenance plate sterile it was exposure to chloroform for 15min then leaved open to evaporated chloroform completely.

Indicators isolates which is sensitive to pyocin were cultured on brain heart infusion broth for 2-3 hours. The strains were cultured by loop as a straight line vertical on the first line (producer isolates) then the plates were incubated to the next day at 37Cº, the results were read according to presence or absence growth region.

Amplification of S-pyocin Genes: All fifty *P. aeruginosa* isolates were subjected to molecular screening for detection of pyocin gene by using PCR amplification technique. The primers used in PCR amplification are specific for S1S2, S3 types of pyocin. The oligonucleotide PCR primers specific and molecular size of expected amplification product was listed in table (1) according to (11).

Steps for amplification genes include addition of 2µl of bacterial DNA template (prepared by boiling method) on to preloaded of GoTaq®Green master mix,2X,eppendorff tubes 12.5µl followed by the addition of 1.5µl with concentration (10picomol\µl) of each specific primers and then 7.5µl of de-ionized distilled water were added to access the final volume (25µl). PCR was run under the following conditions : primary denaturation step 94°C for 30 sec.; 1 -35 repeated cycles , 53-58 °C annealing temperature (table-1) for 30 sec and 72°C for 1 min for the final extension step at 72 °C for 30 sec. PCR products were electrophoresed in 1% agarose gel and visualized under UV light according to (17).

Table (1) Oligonucleotide primers of S-pyocin genes :

Primers name	primers sequence 5′-------→3′	Product size (bps)	No. of cycles	References
S1S2imm	F - CACAAGGGAGGGAAGTGA R - CGGCCTTAAAGCCAGGAA	287	35	(Al-Shammary *et al*.,2013).
S3 RB	F- CGTATCACGAGACAGGCA R- TGCCGCTTCTTCCGCTTT	451	35	(Al-Shammary *et al*.,2013).

RESULTS AND DISCUSSIONS

Isolation and Identification Methods: In this study, fifty isolates primary diagnosed as *P. aeruginosa* were collected from different clinical samples from different hospitals in Baghdad city including: Al-Kendi hospital, Ibn Al-baladi hospital , wound and burn hospital, Baghdad hospital in medical city, during October to December 2014. These isolates were diagnosed as *Pseudomonas aeruginosa* by viteck-2 compact system. These Gram-negative bacteria considered opportunistic pathogen with most problematic bacterial challenges in the infectious disease community as recognized by the Infectious Disease Society of America (18). Screening of bacteriocins profiles may therefore offer a method for predicting the chronic infections.

Pyocin Production of *P. aeruginosa:* The results of cross-streaking assay have shown that there were (16) isolates were pyocin producer from total (50) isolates, with a percentage (32%). In this study the cross-streaking assay was used and the two different isolate were detected, a producer isolate and indicator isolate. The producer one has the ability to produce pyocin where diffuse on the media while other isolate which is indicator that is sensitive to pyocin which doesn't produce the pyocin or produce other type of pyocins. This can lead to population dynamic cycling; because each strain can be invaded by another strain producers outcompete sensitive, resistant outcompete producers and sensitive outcompete resistant (19).
In cross-streaking assay the inhibition of bacterial growth on the media which is cultured as vertical line on producer bacteria that means the bacteria is produced pyocin which diffuses on the media and made inhibition of bacterial growth Fig 1.

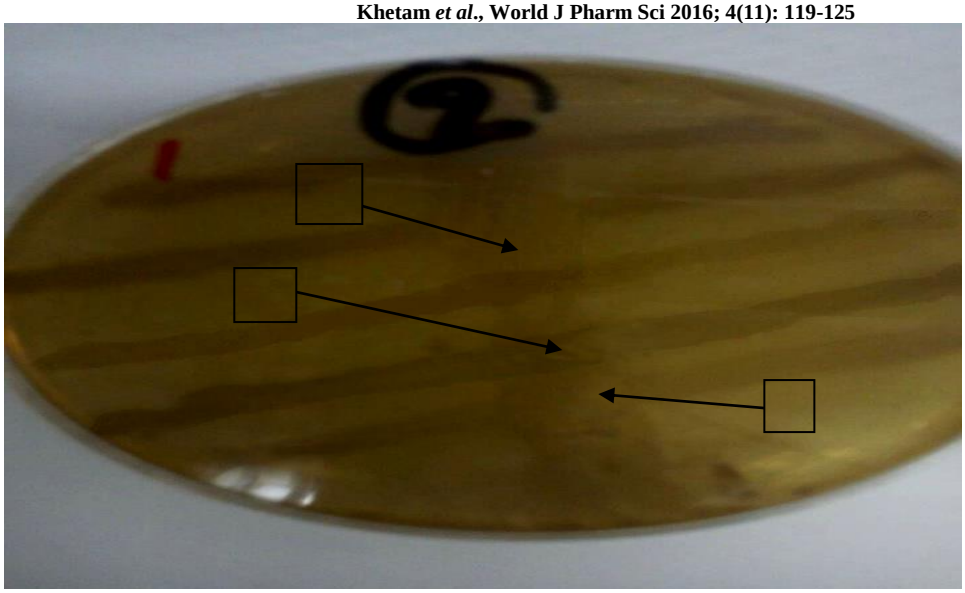

Figure (1) Cross streaking method for detection of pyocin production on trypticase soy agar media.
P= Producer isolate (produce pyocin) , I= Indicator isolate (sensitive to pyocin that produced) , N= Non sensitive to pyocin that

Pyocin producing isolates (p) were inhibited the growth of other *P. aeruginosa* isolates (indicator isolates) (I) these were pyocin sensitive while those were not inhibited were pyocin non sensitive isolates. This test is useful to identify all types of pyocins according to inhibition zones (+) or non-inhibition zones (-) as shown in table (2). Pyocin producers P18 and P17 isolates which inhibit the growth of seven indicator isolates (15, 16, 17, 19, 20, 21, 25), five indicator isolates (16, 18, 25, 29, 39) respectively as shown in table (4), while P16 isolates consider as a most sensitive isolates which are indicator for six different isolates (14, 15, 17, 18, 19, 25).

Table (2) Results of pyocin production by cross streaking method.

p / I	1	4	6	7	10	13	14	23	25	26	39	15	16	19	17	18
5	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
7	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
9	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
1	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	+	-	+	-	-	+	-	+	+	+
27	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
28	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	+	-	+	-	-	-	+	-
17	-	-	-	-	-	-	-	-	+	-	-	+	-	+	-	-
39	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-
15	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
25	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

P= producer, I= indicator, + =No growth of indicator isolates, - =Growth of indicator isolates

one strain of P. aeruginosa usually carries and releases multiple types of bacteriocins known as pyocins, which have a diversity of killing and resistance mechanisms in addition to the toxin–antitoxin systems(20) Theory predicts that structured populations allow all these types to be maintained at appreciable frequencies, whereas sensitive will dominate in unstructured populations (21). We focus on S pyocins because they are composed of a killing and immunity gene.

Detection of Genes Encoding for Pyocin by Using PCR Technique: This technique has been used to detect genes responsible for pyocin production. The DNA of all (50) isolates was extracted by using lysis buffer used as a template for gene amplification. The result of PCR amplification for genes that are responsible for encoding pyocin S1 and S2 indicated that there are (24) isolates of *P. aeruginosa* from all (50) isolates as (48%) appeared that contain this gene (S1andS2, the PCR products were 287bp) while there are (26) isolates corresponding to (52%) of the total (50) isolates were recorded to be negative as shown in figure (2).

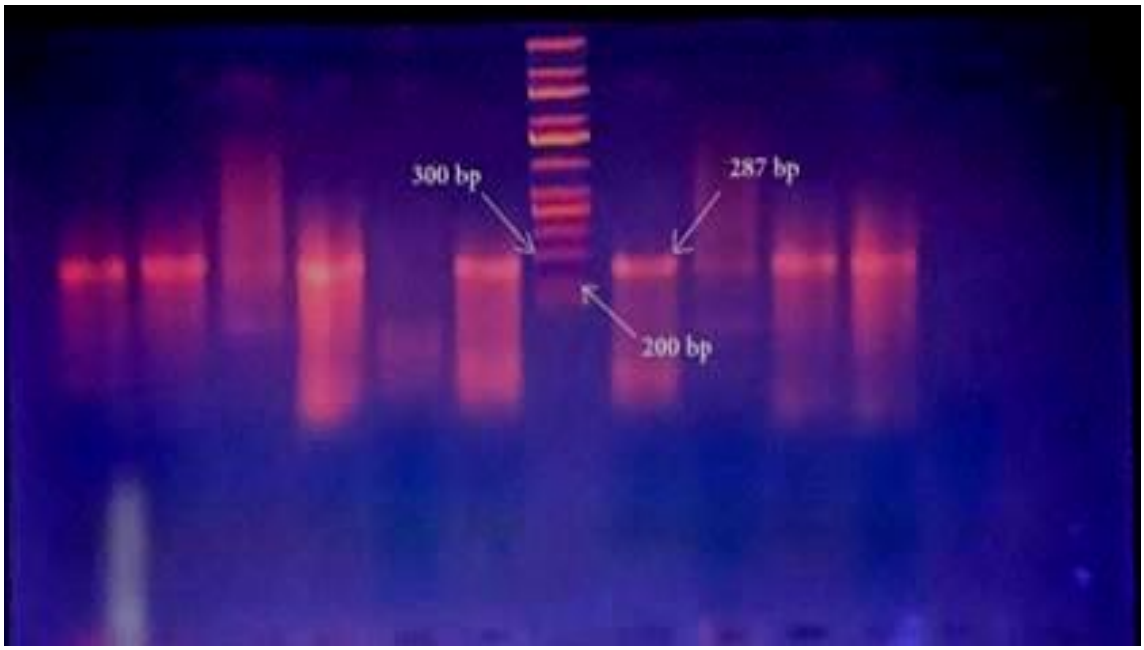

Figure (2) Gel electrophoresis of PCR product for detection of S1S2 gene (287bp) using agarose 1% for 35min at 100 volt.

The same technique is applied to recognize the genes responsible for S3pyocin, where in this study the results of PCR amplification for the gene encoding pyocin S3applied to all (50) isolates of *P. aeruginosa* bacteria ,the results showed that there were (21) isolates (42%) show the presence of the gene encoding for pyocin S3(the PCR product were 451bp), while there were (29) isolates corresponding to (58%) of a total (50) isolates were showed absence the gene encoding for S3 pyocin table (3). Figure (3) shows the result of PCR amplification of S3 gene.

Figure (3)Gel electrophoresis of PCR product for detection of S3 gene (451bp) using agarose 1% for 35min at 100 volt.

According to the previous results, the prevalent rate of S1S2gene was (48%) while this percentage decreased to (42%) for S3 gene as shown in table (3). The results in this study are different from the result of (11) considered as a local study where the study illustrated that prevalent rate of appearance S1S2 gene is (90%) while prevalent rate of appearance S3gene is (82%).

In table (4), there is a comparison between two methods for pyocin formation a PCR method and Cross streaking method where the first one depends on genetic properties to detect pyocin formation while second method depends on morphological properties to detect pyocin production.

In this study all isolates which were able to produce pyocin by cross streaking methods (16 isolates) were tested by PCR method to detect of S-type pyocin, where results show that there were (6) isolates as a (37.5%) produce S1S2 pyocin and only (3) isolates as (18.75%) able to produce S3 pyocin table (4).

Total numbers of isolates that have pyocin genes (S1S2 and S3) and detected by PCR method (31 isolates,62%) were higher than the total isolates that produce pyocin by cross-streaking method (16 isolates 32%). Nine (29.03%) of *P. aeruginosa* isolates were showed positive results by both PCR (genotypic) and cross streaking method (phenotypic), while 22 (70.96%) of the isolates were negative by phenotypic detection in spite of they were positive by genotypic table (5).

These results can explain as follows, the gene of pyocin failed to express. It was previously showed that expression of R, F and S pyocins is positively and negatively regulated by PrtN and PrtR proteins (22). On the other hand, 8(42.1%) were positive by phenotypic detection and were negative by genotypic detection, this may be due to that, the pyocin that produced by these 8 isolates may have other types of pyocin genes (like F or R) (7,23) while 11(57.9%) isolates were negative by both phenotypic and genotypic detection . The abundance of bacteriocins genes in bacteria like *Pseudomonas*, *Klebsiella* and *Photorhabdus* sugges ts that these systems play an important role in the competition between rival bacteria. The nature of these proteins implies that extensive domain swapping has contributed to the diversification of bacteriocins in γ-proteobacteria.(24).

Table (3) Occurrence of pyocin S gene 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 (4) Comparison between cross-streaking method and PCR technique to detect of pyocin production.

PCR Method		Isolates producing pyocin by Streaking Method
S1S2 gene	S3gene	
-ve	-ve	P_1
-ve	-ve	P_4
-ve	-ve	P_6
-ve	-ve	P_7
-ve	-ve	P_{10}
-ve	-ve	P_{13}
-ve	-ve	P_{14}
-ve	+ve	P_{15}
-ve	-ve	P_{16}
+ve	-ve	P_{17}
+ve	-ve	P_{18}
+ve	-ve	P_{19}
+ve	-ve	P_{23}
+ve	-ve	P_{25}
+ve	+ve	P_{26}
-ve	+ve	P_{39}
37.5%	18.75%	

Table (5): Expression of pyocin according to pyocin genes occurrence

Pyocin genes (genotypic)	Pyocin producers (phenotypic)		Total (%)
	Positive (%)	Negative (%)	
Positive (%)	9(29.03%)	22(70.96%)	31(62%)
Negative (%)	8(42.1%)	11(57.9%)	19(38%)
Total (%)	17(34%)	33(66%)	50(100%)

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