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DETECTION AND MOLECULAR IDENTIFICATION OF *PSEUDOMONAS AERUGINOSA* VIRULENCE GENE (*TOX A*, *EXO S* AND *EXO U* GENES) BY USING PCR IN BABYLON PROVINCE

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ABSTRACT : In the presentstudy, including collecting and processing 100 clinical samples from different clinical cases. The identification of *Pseudomonas aeruginosa* by conventional technique through using cultural and biochemical features. Only (40) isolates have been recovered from *P. aeruginosa*, where 8 isolates (20%) obtained from otitis media, 10 isolates (25%) from burns samples, 9 isolates (22.5%) from wounds, 6 isolates (15%) from blood samples and 7 (17.5%) isolates recovered from urine. The result from PCR molecular study revealed that the *tox*A gene was present only from 28 (70%) isolates gave positive results for *P. aeruginosa* isolates, since it investigated from only 6 isolate of otitis media (75%) has *tox*A gene, 8 isolate (80%) from burns followed by 7 isolate (77.7%) from wounds infection, 3 isolate (50%) from blood and finally 4 isolate (57%) from urine has this gene. On the other hand, the results from *exoS* gene recovered only from 27 isolates (67.5%), including5 isolates (62.5%) from otitis media, 7 isolates (70%) from burns, 6 isolates (66.6%) from wounds, 3 isolates (50%) from blood and 6 (85.7%) from urine. While the results from *exo*U gene shown that this gene presence only from 32 isolates (80%) offer positive results from *P. aeruginosa* isolates that including 7 isolate (21.8%) from otitis media, 8 isolate (25%) from burns followed by 7 isolate (21.8%) from wounds infection, 3 isolate (9.3%) from blood and finally 7 isolate (21.8%) from urine has this gene.

Key words : *Pseudomonas aeruginosa*, *tox*A, *exo*U, *exo*S.

INTRODUCTION

Pseudomonas aeruginosa is regard as one from significant cases of opportunistic pathogen and associated with nosocomial infections and able of infecting all tissues. The infections in hospitals mainly affect the patients in intensive care units and those having catheterization, burn, andchronic disasese (Panmanee *et al*, 2008).

P. aeruginosa is responsible for a wide range of diseases, this is in addition to its ability to acquire the genes responsible for resistance, which emerged early and became microbes of great importance in hospitals (Beckert *et al*, 2014; Chiu *et al*, 2009). However, the bacteria associated with a great number of virulence agent like exoenzyme S, exotoxin A, sialidase and elastase, which are tightly adjust via cell to cell signalling method.

On the other hand, Exotoxin A, a highly toxic proteins and has many commotion with the event of Necrosis at the location of infection with the bacterium. The risk of this toxin in the inhibition of the process of protein synthesis (Baradaran *et al*, 2013). In addition this toxin role to penetrate the tissue process and protein bio-synthesis is inhibited via exo enzyme S and exotoxin A is secreted through a kind III section method (Nikbin *et al*, 2012 and Xing *et al*, 2010).

P. aeruginosa utilizer a kind III secretion method to insert toxic proteins effector into cytoplasm of cells eukaryotic. To date, four proteins effector has been described in *P. aeruginosa* : exoS, exoT, exoY and exoU (Roux and Ricard, 2012). The genes translated for protein products are associated to kind III excretion method. Also, in studies clinical, the existence of the toxins is related for a Dysfunction clinical result amongst patients with *P. aeruginosa* infection (Firouzi-Dalvand *et al*, 2016). Genes encoding the cytotoxens *exo*S are in attendance as vareable character and commonly restricted in utmost strains (Agnello and Wong-Beringer, 2012). The purpose of this study is to characterize the presence of the *toxA, exo*S and *exo*U genes in clinically isolated of *P.*

aeruginosa by PCR in Babylon, Iraq. An enhanced comprehension of these virulence factors is significant for the future improvement of drug treatments.

MATERIALS AND METHODS

Collection of bacteria

A total of 100 different clinical samples were taken from patient clinically diagnosed as otitis media, burns, wounds, urinary tract infections and blood. These patients attended the three main hospitals in AL- Hilla city (Babylon Hospital for Maternal and Paediatrics, Al-Hilla Surgical Teaching Hospital and Merjan Medical city). The samples were taken from both sexes and all age group from infants to olderly during the peroid from 1/2/2019 to 1/4/2019 the numbers of clinical samples collected during this study are shown in Table 1.

Bacterial identification

Sample were taken and cultured on blood agar, MacConkey agar and cetrimide agar, the plates were incubated for 72 h at 37°C. In spite of morphology of colonies, all supposed colonies primarily were screened for *P. aeruginosa* by a combination of tests including growth on cetrimide agar medium, growth at 42°C and biochemical tests such as citrate, oxidase, glucose and arginine dihydrolase. The isolates was stored in Luria Bertenibroth contain glycerol 20%, 20°C (Pitt and Simpson, 2006).

Table 1 : Distribution of clinical samples.

Source of isolates	T. No. of samples	No. of *Pseudomonas aeruginosa* isolates	Percentage (%)
Otitis media	20	8	20%
Burn	25	10	25%
Wound	25	9	22.5%
Urine	20	6	15%
Blood	10	7	17.5%
Total	100	40	100%

DNA extraction

The extraction of DNA load out according to the genomic DNA purification kit completed through company manufactured (Geneaid, UK).

Gene Marker Detection of *tox*A, *exo*S and *exoU* genes via PCR technique

The primers and PCR stipulations utilized to amplify gene encoding *toxA, exo*S and *exo*U by PCR are listed in Table 2. 25µl of PCR reaction contain 2.5µl of each downstream and upstream primer, 2.5µl of free nuclease water, 5µl of DNA extraction and 12.5µl of mix master. The PCR amplification produce was visualize via electrophoresis on 1.5% agarose gels for 45 minute at 70 volt. The size of the amplicons was determined through comparison to the 100-2000bp allelic ladder (Promega, USA). The primers utilized in this study were supporting with all conditions needed for carrying out PCR techniques.

RESULTS AND DISCUSSION

A total of 40 isolates *P. aeruginosa* were isolated from 100 samples were taken from patients clinically diagnosed as otitis media, burns, wounds, urinary tract infections and blood. Isolates of *P. aeruginosa* after cultivation on cetrimide agar medium were produced

Table 2 : Primers and PCR stipulations utilized to amplify gene encoding *tox*A, *exo*S and *exo*U.

Genes	Primer sequence (5'-3')	Size of product bp	PCR condition	Reference
Tox A	5' TGA TGT CCA GGT CAT GCT TC 3' 5' GGT AAC CAG CTC AGC CAC AT 3'	352	95ºC 5min 1x 94ºC 1 min 51ºC 5 min 30x 68ºC 8 min 65ºC 10min 1x	Bradbury *et al* (2010)
ExoS	5'CAGGCGTACATCCTGTTCCT 3' 5'ATGTCAGCGGGATATCGAAC3'	230	95ºC 5min 1x 94ºC 1min 51ºC 5 min 30x 68ºC 8 min 65ºC 10 min 1x	Shaver and Hauser (2004)
ExoU	5'AGATCACACCCAGCGGTAAC3' 5' GCTAAGGCTTGGCGGAATA 3'	204	95ºC 5 min 1x 94ºC 1 min 51ºC 5min 30x 68ºC 8 min 65ºC 10 min 1x	Tyson and Hauser (2013)

greenblue pigments. Specific PCR primers were used for the detection of *tox*A, *exo*S and *exo*U genes. The gel electrophoresis showed that the molecular weight of *tox*A gene was 352 bp as shown in Fig. 1, 230 bp of *exoS* gene as in Fig. 2 and 204 bp of *exoU* geneas in Fig. 3.

The results demonstrated that only 28 isolates (70%) of *P. aeruginosa* gave positive amplification for *tox*A gene, since they appear 6 isolates from otitis media strains (21.4%), 8 isolates from burns (28.5%), 7 isolates from wounds (25%), 3 isolates from blood (10.7%) and recovered only 4 isolates from urine (14.2%) as shown in Table 3. The pathogenesis of *P. aeruginosa* in pulmonary damage is mediated by the presence of Exotoxin A encoded by virulence gene *tox*A gene that inhibits biosynthesis of protein among infected individuals so the result investigate the high prevalence of *tox*A gene among *P. aeruginosa* isolates (Morlon-Guyot *et al*, 2009). The result correlated with study conducted by Azhar

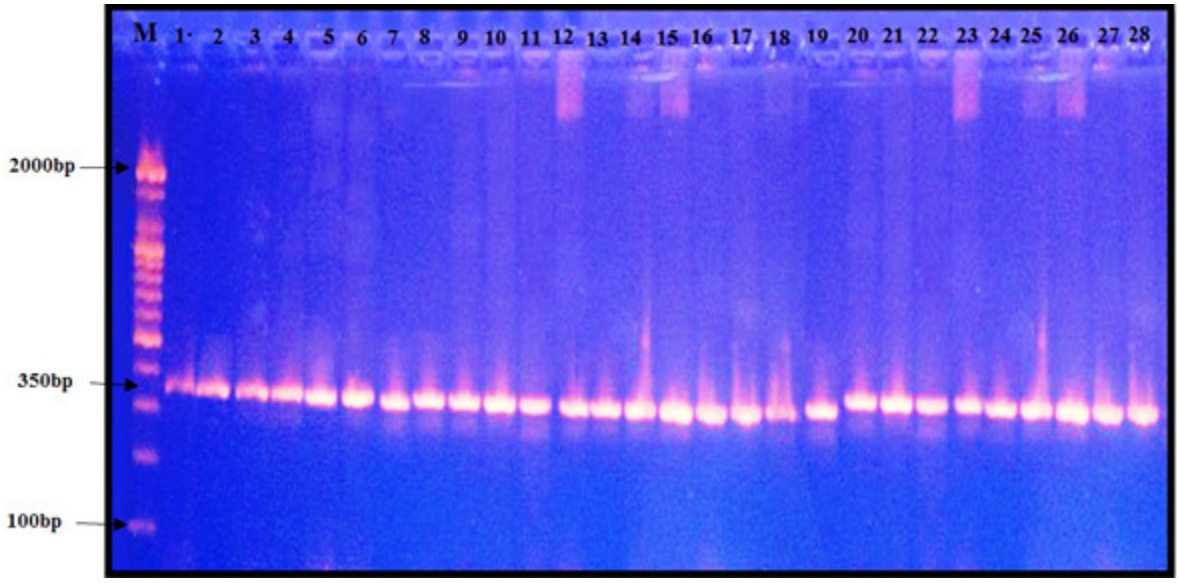

Fig. 1 : Gel electrophoresis of PCR amplified product of *toxA* with 350 bpon 1.5% agarose gel at 70 volt for 45min visualized under UV after staining with ethediumbromid. Lane (M1): DNA marker Ladder 100 -2000 bp Lanes: (1,2,3,4, 5, 6). No. of isolates from otitis media show positive results. Lanes: (7,8,9,10,11,12,13,14) No. of isolates from burns gave positive results. Lanes: (15,16, 17, 18,19, 20): No. of isolates from wounds gave positive results. Lanes: (21,22, 23,24, 25) No. of isolates from UTI have positive results. Lanes: (26,27,28) No. of isolates from blood have positive results.

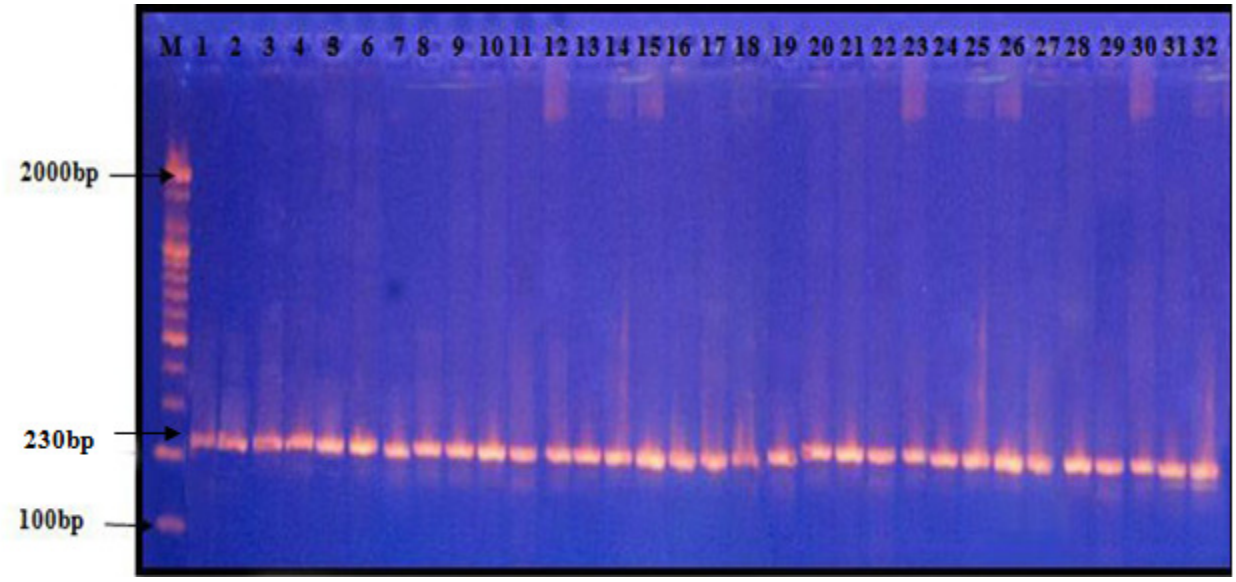

Fig. 2 : Gel electrophoresis of PCR amplified produce of *exoS* with 230bp on 1.5% agarose gel at 70 volt for 45min visualized under UV after staining with ethediumbromid. Lane (M1): DNA marker Ladder 100 -2000 bp ladder. Lanes: (1,2,3,4, 5,6,7) No. of isolates from otitis media show positive results. Lanes: (8,9,10,11,12,13,14,15) No. of isolates from burns gave positive results. Lanes: (16, 17, 18, 19, 20, 21, 22): No. of isolates from wounds gave positive results. Lanes: (23, 24, 25, 26, 27, 28, 29) No. of isolates from UTI have positive results. Lanes: (30, 31, 32) No. of isolates from blood have positive results.

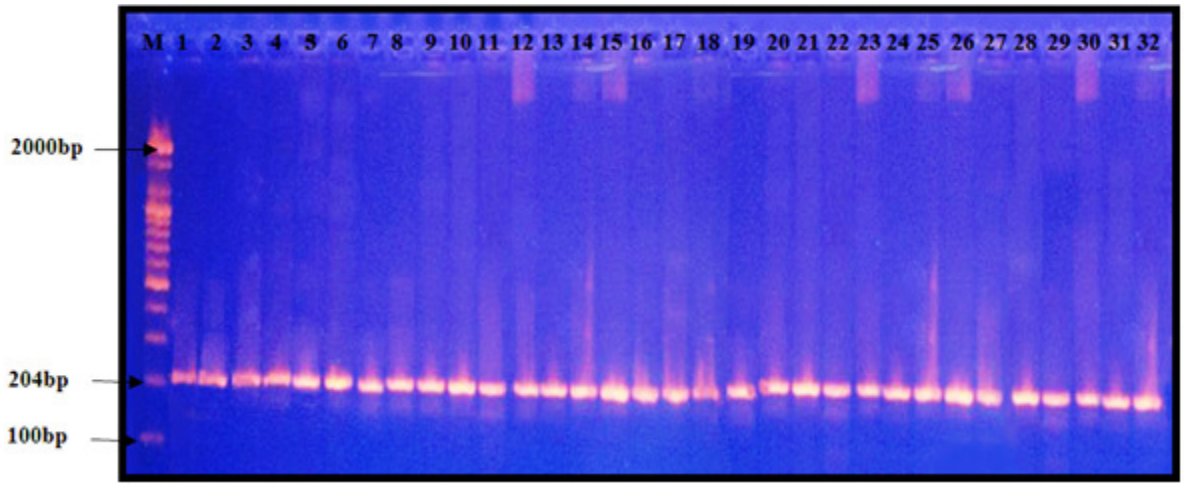

Fig. 3 : Gel electrophoresis of PCR amplified product of *exoU* with 204 bp on 1.5% agarose gel at 70 volt for 45min visualized under UV after staining with ethediumbromid. Lane (M1): DNA marker Ladder 100 -2000 bp ladder. Lanes: (1, 2, 3, 4, 5, 6, 7) No. of isolates from otitis media show positive results. Lanes : (8, 9, 10, 11, 12, 13, 14, 15) No. of isolates from burns gave positive results. Lanes: (16, 17, 18, 19, 20, 21, 22): No. of isolates from wounds gave positive results. Lanes:(23, 24, 25, 26, 27, 28, 29) No. of isolates from UTI have positive results. Lanes:(30,31,32) No. of isolates from blood have positive results.

Table 3 : Prevalence of *exoS, exoU* and *toxA* among *P. aerugenosa* attained from several sources.

No	Source	No. of positive isolates	*tox*A (%)	*exo*S (%)	*exo*U(%)
1	Otitis media	8	6(75%)	5(62.5%)	7(87.5%)
2	Burn	10	8(80%)	7(70%)	8(80%)
3	Wound	9	7(77.7%)	6(66.6%)	7(77.7%)
4	UTI	7	4(57%)	6(85.7%)	7(100%)
5	Blood	6	3(50%)	3(50%)	3(50%)
Total		40	28(70%)	27(67.5%)	32(80%)

(2017), who reported that the percentage of *tox*A genes was (92.8%) and (81.8%) in burn and Otitis media respectively of *P. aeruginosa* isolates.

This result also correlated with study done by Rana and Abbas (2017), who revealed that the *tox*A gene was present in 54 isolates (72%) of *P. aeruginosa*. From 75 isolates from different patients. While the study done by Martinez-Solano *et al* (2008) shown that distribution of *toxA* gene is elevated among pulmonary infected patient. The *tox*A gene encodes Exotoxin A, a highly toxic proteins and has many commotion with the event of necrosis at the location of infection with the bacterium. The risk of this toxin in the inhibition of the process of protein synthesis (Baradaran *et al*, 2013) also, for this toxin role to penetrate the tissue process (Xing *et al*, 2010).

Exotoxin A inhibits eukaryotic synthesis of protein through ADP ribosylation of elongation factor 2, which can lead to lysis of cell; stimulates inflammation and hepatotoxicity in animals might aid immune evasion; stimulation of inflammation in the kidney might aid persistence (Chiu *et al*, 2009).

Moreover, the results were detected that only 27 isolate (67.5%) gave positive amplification for (*exoS*) gene, since it observed from only 5 isolates (18.5%) of otitis media, 7 isolates (25.9%) from burns, 6 isolates (22.2%) from wounds, 3 isolates (11.1%) from blood and 6 isolates (22.2%) from urineas shown in Table 3. This result correlated with study conducted by Anmar *et al* (2018), who found that the *exo*S gene was evaluated at (70%) from different clinical specimens of *P.aeruginosa*. On the other hands, the study made by Leila *et al* (2016) investigated that *exoS* gene was present in 62% from *P. aeruginosa* isolated from urinary tract infections patients. The study made by Firouzi and Pooladi (2014) revealed that *exoS* increased action in urinary tract isolates was recently established which, joint with our findings, indicates that this *exoS* enzyme may be significant in the pathogenesis of urinary tract infections and burns caused by *P. aeruginosa*. The *exoS* gene encoding the cytotoxins are present as variable character and are commonly exclusive in most strains of *P. aeruginosa* (El-Solh *et al*, 2012).

In addition the results improved that only 32 isolates (80%) gave positive amplification for *exo*U gene were investigated from 7 isolates (87.5%) from otitis media, 8 isolates (80%) from burns, 7 isolates (77.7%) from wounds, 3 isolates (50%) from blood and 7isolates (100%) from urineas shown in Table 3. This result agree with study conducted by Leila *et al* (2016), who reported that *exo*U gene were found at 73% isolated from patients suffering from urinary tract infections. The *exoU* play an important role in pathogenesis of *P. aerugiinosa* and cause fast death of cell through *in vitro* infections, the

patients with acquired hospital pneumonia caused via *exoU* secreting isolates of *P. aerugiinosa* have worse clinical outcomes than patients infected with isolates, suggesting the protein may contribute to disease severity in humans (Schmalzer *et al*, 2010; Tyson and Hauser, 2013).

This gives the important role of these genes in pathogenesis of *Pseudomonas aeruginosa* among hospitalized patient. This result is correlated with the results done by Bentzmann *et al* (2011), who investigated the high prevalence of *P. aeruginosa* in clinical isolates than commensal isolates.

CONCLUSION

Through the results obtained in this study, it was clearly revealed of virulence genes toxA, exoS and exoU genes was detecting and present in 75%, 66.6% and 80%, respectively and function an significant role in the infections occasion through *P. aeruginosa* was isolated from many cases of infections.

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