Al-Kufa University Journal for Biology / VOL.12 / NO.1 / Year: 2020
Print ISSN: 2073-8854 Online ISSN: 2311-6544

Frequency of ToxA and ExoS genes in *Pseudomonas aeruginosa* isolates obtained from different clinical specimens

Asmaa O. Namuq
M.Sc. Medical Microbiology, Department of Medical Microbiology, College of Medicine, University of Tikrit, Iraq
Email: asmanamuq@yahoo.com

Supervised by:
Dr. Khalid O. Mohammed Ali
Department of Medical Microbiology, College of Medicine, University of Tikrit, Iraq
Email: omar@yahoo.com

Prof. Dr. Ahmed H. Al-Ani
Department of Pediatrics, College of Medicine, University of Tikrit, Iraq
Email: ah70.tucom@gmail.com

Abstract

The present study carried out in Azady teaching general hospital in Kirkuk city during the period from August 2017 to May 2018, to determine the frequency of ToxA gene and ExoS gene in *P. aeruginosa* isolates. *Pseudomonas aeruginosa* (100) isolates had been obtained from (1260) different clinical specimens, High prevalence of *P. aeruginosa* found in isolates obtained from burn swabs. Regarding to genders and ages, *P. aeruginosa* presented in females (58%) more than males (42%), regarding to age groups, *P. aeruginosa* presented more frequently (26%) in age group (20-30) followed by children (1-10) years that formed (22%). Molecular detection of virulence genes had been done by using conventional PCR technique, regarding to prevalence of ToxA and ExoS genes, ToxA gene presented (96%), ExoS gene (92%). Regarding to the distribution of genes in clinical specimens, ToxA gene present (100%) in all clinical specimens except in urine samples (90.9%) and low percentage found in sputum (50%). ExoS gene present (100%) in all isolates, while ExoS gene less frequently presented in ear swab isolates (83.3%) than other specimens, followed by urine specimens (81.8%), in bronchial washes isolates ExoS gene was absent. This study aims to determine the frequency of ToxA gene and ExoS gene in *P. aeruginosa* isolates that obtained from different clinical specimens.

Keyword: *ToxA, ExoS, Pseudomonas aeruginosa, clinical specimens*

Introduction

Pseudomonas aeruginosa is an opportunistic pathogen clinically important involved in many infections, such as nosocomial infection, burns, wound, respiratory tract, urinary tract and eye, etc [(1)]. *Pseudomonas aeruginosa* are commonly occurring in soil and water, they present regularly on the surface of plants and sometimes on the surfaces of animals [(2)]. It can produce number of virulence factors which can cause extensive tissue damage after colonization, bloodstream invasion, and dissemination [(3)]. Pathogenesis of the bacteria depends on multiple virulence factors: endotoxin, exotoxins, and enzymes. *Pseudomonal* endotoxin, like other gram-negative bacteria endotoxin , causes the symptoms of sepsis and septic shock [(4)]. Exotoxin A which is

important factor that cause tissue necrosis since it blocks protein synthesis [5]. Exotoxin A, encoded by the ToxA gene, inhibits protein biosynthesis by transferring an ADP-ribosyl moiety to elongation factor 2 of eukaryotic cells. Exoenzyme S, encoded by the ExoS gene, is also an ADP-ribosyltransferase that is secreted by a type-III secretion system in to the cytosol of epithelial cells [6].

Materials and methods

In the current study 100 isolates of *P.aeruginosa* had been obtain from different clinical specimens that includes: sputum, urine, bronchial wash, swabs (burn, wound, and ear swabs). All those specimens cultured on Blood agar and MacConkey agar and brain heart infusion broth (BHI) incubated over-night at 37 ^{0}C , then the colonies tested for oxidase production and biochemical reactions for exact strains identification, which had been done by VITEK®2 GN ID card.

DNA extraction

Half of our isolates (50 isolates) had been chosen for molecular study, DNA extracted by using Wizard® Genomic DNA extraction Kit (PROMEGA, Germany) by depending on manufacture procedure, bacterial extracted DNA stored at -20°C for polymerase chain reaction technique.

Primers

In the present study primers of virulence genes designed by, depending on NCBI gene sequence information base. The primer prepared by Integrated DNA Technologies Company, Canada; as show table1.

Table 1 Primers used in PCR amplification for detection of virulence genes

Gene name		primers	Product size
ToxA	F	5'-GGT AAC CAG CTC AGC CAC AT - 3'	352
	R	5'-TGA TGT CCA GGT CAT GCT TC- 3’	
ExoS	F	5'-CTT GAA GGG ACT CGA CAA GG - 3'	504
	R	5'-TTC AGG TCC GCG TAG TGA AT - 3’	

PCR method

INtRON's Maxime PCR PreMix Kit used, Amplification of DNA was carried out at final volume of (25µl) containing the following mixture: Taq PCR PreMix 5µl, Forward primer 1 µl, Reverse primer 1 µl, DNA 1.5 µl, Distill water 16.5 µl, PCR amplification carried out by MultiGeneOptiMax Gradient Thermal Cycler under same conditions for both of **(**ToxA gene and ExoS gene); 35 cycle for 3minute at 95°C, 45seconds at 95°C, 45seconds at 52°C, 1minute at 72°C, 7minute at 72°C. The amplified genes were detected by electrophoresis in a (2%) agarose gel, the gel has been tested by a source of the UV (336 nm) after put the gel in pool contain on 30µl Red safe Nucleic acid staining solution

Statistical analysis

Chi-square statistical analysis test used to determine if ToxA and ExoS genes significantly distributed in different clinical specimens or not. P value was set at 0.05.

Results and discussion

Total (100) isolates of *P. aeruginosa* were obtained from 1260 different clinical specimens (44%) from burn swabs, (22%) urine samples, (14%) ear swabs, (12%) wound swabs, (4%) sputum samples, (2%) oropharyngeal swabs, and (2%) bronchial washes as shown in Fig.1. The present study agrees with Al-Tikrity [7] who found (44.4%) isolates found in burn isolates and (16.6%) wound isolates, urine samples, sputum and ear swabs showed different percentage of isolation, and agree with study carried out in Egypt recorded that *P.aeruginosa* isolated from urine (22%), sputum (3%), ear discharge (16%) and wound (11%) [8]. Another study from India reported a different percentage of isolation from wound (55.8%), sputum (20.8%), urine (5%) ear discharge and tracheal wash (8.3%) respectively [9]. High frequency of *P.aeruginosa* in burn patients because of they are more susceptible to have infections if compared with another patients due to their damaged skin barrier and immune system suppression, in addition to prolonged hospital stay expose them to nosocomial infection[10]. The difference in frequency of isolation is due to the difference in the virulence factors, site of infection and source of clinical specimen [11].

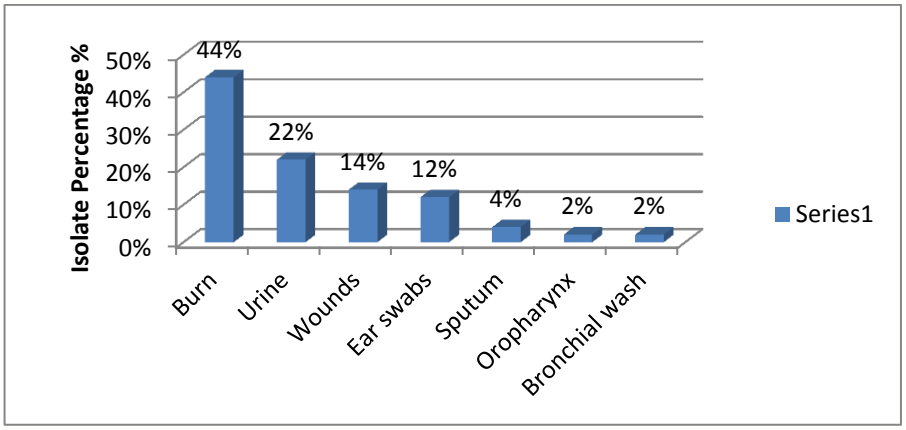


Fig.1 Frequency and percentage of *P. aeruginosa* in current study according to different clinical samples

Regarding to genders and ages, in the present study *P. aeruginosa* presented in females (58%) more than males (42%), regarding to age groups, *P. aeruginosa* presented more frequently (26%) in age group (20-30) followed by (22%) in children in age group (1-10 years). Statistically the distribution of genders were significant for all age groups ($P < 0.05$) as shown in Table 2. The current study results agree with Aljebory [12] in Kirkuk city, reported that high prevalence of *P. aeruginosa* (30%) in age group (23-28 years), and in agreement with Rashid *et al* [13] who reported most of infected case were in (21-40 years) age group, but disagree with Golia *et al* [14] in India, who documented a peak incidence in age group (41-60) years old and patients (>60) years old.

Regarding to gender variable, high percentage of *P.aeruginosa* isolates (58%) found in females than males (48%), present study agrees with Al-Salihi *et al* [15] in Kirkuk city, who mentioned (52.98%) were females and (47.02%) were males, also with Ekrem and Rokan [16] in Al-Sulaimania city (Iraq), showed higher occurrence of the bacterium in females. The present study results disagree with Rashid *et al* [17] who documented 294 case of *P.aeruginosa* infection, (60%) were males and (40%) were

females, also with Javiya *et al* [18] in India, reported that maximum numbers of isolate obtained from old aged patients.

Table 2 Distribution of *P. aeruginosa* isolates according to gender and age of patients

Age / Gender	> 1 year	1-10	10-20	20-30	30-40	40-50	50≤years	Total n=100
Male	2/42 (4.7 %)	14/42 (33.3 %)	6/42 (14.3 %)	12/42 (28.6 %)	26/100 (26%)	2/42 (4.8 %)	4/42 (9.5 %)	42/100 (42 %)
Female	0/58 (0 %)	8/58 (13.8 %)	10/58 (17.2 %)	14/58 (24.1 %)	2/58 (3.4 %)	10/58 (17.2 %)	14/58 (24.1 %)	58/100 (58 %)
Total	2/100 (2%)	22/100 (22%)	16/100 (16%)	26/100 (26%)	4/100 (4%)	12/100 (12%)	18/100 (18%)	100/100 (100%)

X_2= 13.464 * p-value = 0.028 (significant)

Molecular study revealed that ToxA gene is present in (96%) of *P. aeruginosa* isolates, the gel electrophoresis showed that molecular weight of ToxA (352bp), as show Fig.2 A and Fig.2 B. the results agree with Sabharwal *et al* [19] who found ToxA (100%) and Khan *et al* [20] that documented ToxA (96%). Regarding to distribution of ToxA gene in clinical specimens ToxA gene present (100%) in all clinical specimens except in urine samples (90.9%) and in sputum (50%), Statistically ToxA gene significantly distributed in all clinical samples (P<0.05), as show Table 3, the result agrees with Neamah *et al* [21] in Diwaniya who documented, ToxA gene (100%) in the wound and (92.8%), (81.8%) in burn and Otitis media respectively, also agree with Al-Kaaby *et al* [22] who reported ToxA gene in burn (94.5%), but he reported low percentage in wound (72.2%). The result disagree with Badamchi *et al* [23] who documented low percentage of ToxA gene (69.4%) , and with Faraji *et al* [24] that showed low percentage of ToxA gene (63.1%) isolated from cystic fibrosis patients, and burn (36.9%). Exotoxin A (ToxA) is highly toxic virulence factor of *P.aeruginosa*, cause protein biosynthesis inhibition, tissues necrosis, Exotoxin A present in each colonization process therefore presented in high level in all clinical specimens [25].

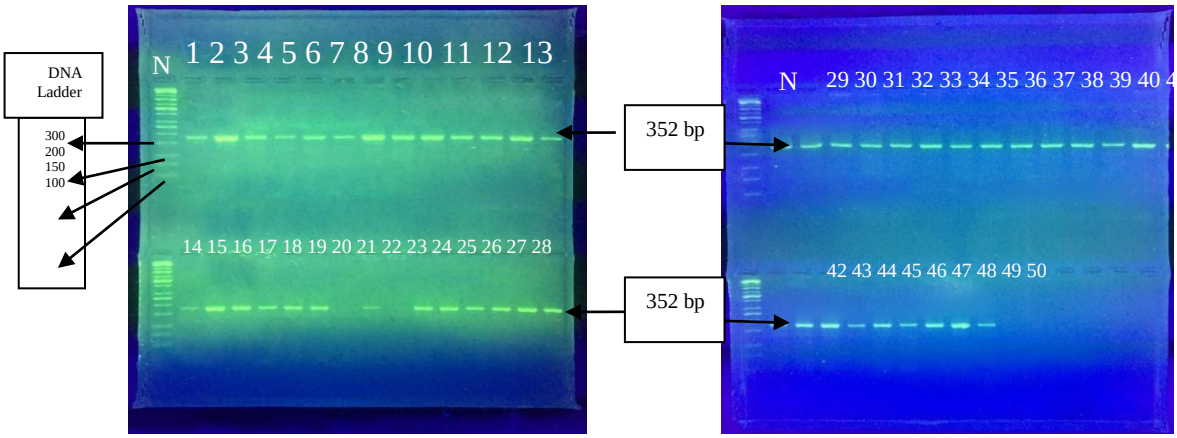
ExoS gene is present in (92%) of *P. aeruginosa* isolates, the gel electrophoresis showed that molecular weight of ExoS (504bp), as show Fig.3A and Fig.3A. Regarding to distribution of genes in clinical specimens ExoS gene present (100%) in all isolates, while less prevalence of ExoS gene found in ear swab isolates (83.3%) followed by urine specimens (81.8%), except in bronchial washes isolates ExoS gene was absent, Statistically ExoS gene significantly distributed in all clinical samples (P<0.05), as show Table 3. The results agree with Nafee *et al* [26] who reported ExoS gene (86.8%) in burn and wound isolates, but disagree with Al-Kaaby *et al* [22] showed that low percentage of ExoS gene (55%) in total isolates and low percentage in burn isolates (67.5%), pulmonary tract isolates (41.6%), and wound isolates (38.3%). ExoS gene play important role in host invasion by *P. aeruginosa*, it is able to transfer ADP-ribosyl portion of NAD to proteins, which often lead to kill eukaryotic cells because its ability in inhibition of DNA synthesis in the cell and cytotoxic as

well as it is responsible for anti phagocytosis [27]. It has been suggested that exoenzyme S may cause impair function of phagocytic cells in the bloodstream and internal organs to facilitate the invasion *P. aeruginosa* [28].

Table 3 Distributions of virulence genes (ToxA and ExoS) according to source of clinical specimens

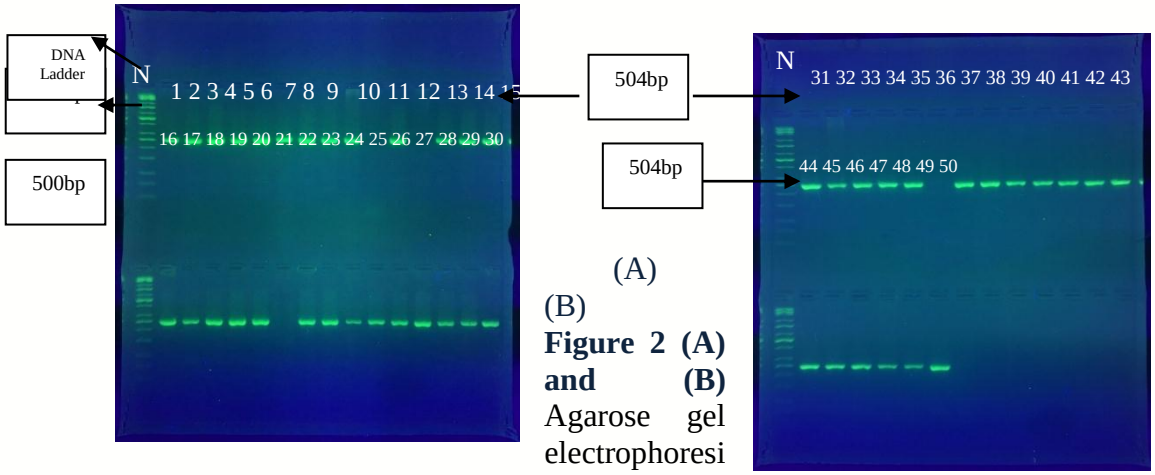
Specimen type	Burn	Urine	wound	Ear swab	sputum	Bronchial wash	Oropharyngeal wash
ToxA gene	100 %	90.9 %	100%	100%	50%	100%	100%
ExoS gene	100 %	81.8	100%	83.3	100%	0%	100%

ToxA gene (*chi-Square=13.305, P-Value = 0.031)
ExoS gene (***** chi-Square=16.444, P-Value = 0.024)



(A) (B)

Figure 2 (A) and (B) Agarose gel electrophoresis for PCR product ToxA gene (the band 352bp). Electrophoresis had run on (2% agarose at 5 volt/cm^2, 1x TBE buffer for 1:30 hours). N: DNA ladder (100) Bp, lanes (1-50) PCR product positive Tox A gene except lanes (20, 22) were negative for Tox A gene



(A)
(B)

Figure 2 (A) and (B) Agarose gel electrophoresis for PCR product ExoS gene (the band 504 bp). Electrophoresis had run on (2% agarose at 5 volt/cm^2, 1x TBE buffer for 1:30 hours). N: DNA ladder (100) Bp, lanes (1-30) PCR product positive ExoS gene, except lanes (10, 11, 21, 36) were negative for ExoS gene.

Conclusions:

Molecular detection of *P.aeruginosa* ToxA and ExoS genes by PCR technique revealed that, ToxA gene presented (96%), ExoS gene (92%) in total clinical specimens. High frequency of both genes found in all clinical specimens except low percentage of ToxA gene found in sputum isolates (50%), and ExoS gene was absent in bronchial washes isolates.

Reference:

1. Abidi SH, Sherwani SK, Siddiqui TR, Bashir A, and Kazmi SU. Drug resistance profile and biofilm forming potential of *Pseudomonas aeruginosa* isolated from contact lenses in Karachi-Pakistan. BMC Ophthalmol. 2013; 13: 57.
2. Todar K. (2004). *Pseudomonas* and related bacteria. Todar's Online Textbook of Bacteriology. Available online at:
http://textbookofbacteriology.net/pseudomonas.html. Accessed 5th July 2006
3. Storey GD, Ujack EE, Rabin HR. Population transcript accumulation of *Pseudomonas aeruginosa* exotoxin A and elastase in sputa from patients with cystic fibrosis. Infect. Immun.1992; 60(11):4687-4694.
4. Latif IA. Bacteriological and Genetical study of *Pseudomonas aeruginosa* Isolated from different human infection. M.Sc. Thesis. University of Tikrit, 2009.
5. Aljarah AK. Molecular Detection of *Pseudomonas aeruginosa* secreting Exotoxin type A in Postsurgical operation wound inflammations. J. Pharm. Sci. & Res. 2018; 10(4): 736-738.
6. Al-Kaaby WA. Molecular Detection of Virulence Factors Genes in *Pseudomonas aeruginosa* Isolated from Different Infections Cases in Al-Diwaniya Hospital. AL-Qadisiyha Journal For Science. 2015; 20(2):1-2.
7. Al-Tikrity IA. Bacteriological and Genetical study of *Pseudomonas aeruginosa* Isolated from different human infections. Msc. Thesis, University of Tikrit, 2009.
8. **Gad GF**, **El-Domany RA**, **Zaki S**, **Ashour HM**. Characterization of Pseudomonas aeruginosa isolated from clinical and environmental samples in Minia, Egypt: prevalence, antibiogram and resistance mechanisms. **J Antimicrob Chemother.** 2007; 60(5):1010-7.

9. Golia S, Manasa SS and Jyoti. Isolation of *Pseudomonas aeruginosa* from various Clinical Isolates and it Antimicrobial Resistance Pattern in a Tertiary Care Hospital. Int.J.Curr.Microbiol.App.Sci. 2016; 5(3): 247-253.
10. Weinstein RA, Mayhall CG. The epidemiology of burn wound infections: then and now. Clinical Infectious Diseases. 2003; 37(4):543-50.
11. Kayser FH, Bienz AK, Eckert J, Zinkernagel MR. Medical Microbiology. New York, Thieme Stuttgart; 2005.
12. Aljebory IS. PCR Detection of Some Virulence Genes of *Pseudomonas aeruginosa* in Kirkuk city, Iraq. J. Pharm. Sci. & Res. 2018; 10(5): 1068-1071.
13. Rashid A, Chowdhury A, Rahman SH, Begum SA, Muazzam N. Infections by *Pseudomonas aeruginosa* and Antibiotic Resistance Pattern of the Isolates from Dhaka Medical College Hospital. Bangladesh J Med Microbiol. 2007; 01 (02): 48-51.

14. Golia S, Manasa SS and Jyoti. Isolation of *Pseudomonas aeruginosa* from various Clinical Isolates and it Antimicrobial Resistance Pattern in a Tertiary Care Hospital. Int.J.Curr.Microbiol.App.Sci. 2016; 5(3): 247-253.

15. AL-Salihi S Sh , Hameed BH , Hameed BH. Antibiosis resistant of *Pseudomonas aeruginosa* isolated from different clinical specimens. Kirkuk University Journal/Scientific Studies (KUJSS). 2014; 9(2): 15-28.
16. Ekrem K, Rokan DK. Antibiotic susceptibility patterns of *Pseudomonas aeruginosa* strains isolated from various clinical specimens. Sky J. Microbiol. Res. 2014; 2(2):13-17.

17. Rashid A, Chowdhury A, Rahman SH, Begum SA, Muazzam N. Infections by *Pseudomonas aeruginosa* and Antibiotic Resistance Pattern of the Isolates from Dhaka Medical College Hospital. Bangladesh J Med Microbiol. 2007; 01 (02): 48-51

18. Javiya VA, Ghatak SB, Patel KR, and Patel JA. Antibiotic susceptibility patterns of *Pseudomonas aeruginosa* at a tertiary care hospital in Gujarat, India. Indian J Pharmacol. 2008; 40(5): 230–234.

19. Sabharwal N, Dhall, S, Chhibber S. and Harjai K. Molecular detection of virulence genes as markers in *Pseudomonas aeruginosa* isolated from urinary tract infections. Int. J. Mol. Epidemiol. Gen. 2014; 5(3): 125-134.

20. Khan AA. and Cerniglia CE. Detection of *Pseudomonas aeruginosa* from clinical an environmental samples by amplification of the exotoxin A gene using PCR. Appl. Environ. Microbiol.1994; 60: 3739-3745.

21. Neamah AA. Molecular Detection of virulence factor genes in *Pseudomonas aeruginosa* isolated from human and animals in Diwaniya province. Kufa Journal for Veterinary Medical Sciences. 2017; 8(1):218-230.

22. l-Kaaby WA. Molecular Detection of Virulence Factors Genes in *Pseudomonas aeruginosa* Isolated from Different Infections Cases in Al-Diwaniya Hospital. AL-Qadisiyha Journal For Science. 2015; 20(2):1-2.
23. Badamchi A, Masoumi H, Javadinia S, Asgarian R, Tabatabaee A. Molecular detection of six virulence genes in *Pseudomonas aeruginosa* isolates detected in children with urinary tract infection. Microbial Pathogenesis. 2017; 107: 44-47.
24. Faraji F, Mahzounieh M, Ebrahimi AO, *et al*. Molecular detection of virulence genes in *Pseudomonas aeruginosa* isolated from children with Cystic Fibrosis and burn wounds in Iran. Microbial Pathogenesis. 2016; 99:1-4.
25. Michalska M, Wolf P. *Pseudomonas* exotoxin A: optimized by evolution for effective killing. Front Microbiol. 2015; 6: 963.
26. Nafee, SK. Isolation and identification of clinical *Pseudomonas aeruginosa* producing exotoxin A and studying its toxic effect in mice. Msc. Thesis, College of Science, Baghdad University, 2012.

27. Fraylick JE, La Rocque JR, Vincent TS and Olson JC. Independent and coordinate effects of ADP-ribosyltransferase and GTPase-activating activities of exoenzyme S on HT-29 epithelial cell function. J. Infect Immun. 2001; 69(9): 5318-5323.
28. Jia J, Wang Y, Zhou L, Jin S. Expression of *Pseudomonas aeruginosa* Toxin ExoS effectively induces apoptosis in host cells. Infection and Immunology. 2006; 74(12):6557-6570.