ORIGINAL ARTICLE

Detection of Exotoxin a Gene in *Pseudomonas aeruginosa* Strains Isolated from Burn Infections in Tanta University Hospital

[1]Laila T. Sultan*, [2]Hashem M. Ayad, [1]Fawkia M. Mahgoub, [1]Kareman A. Eshra
[1]Department of Medical Microbiology and Immunology, Faculty of Medicine, Tanta University
[2]Department of Surgery and burn Faculty of Medicine, Tanta University, Egypt

Key words:
Burn wound, P.aeruginosa , MDR, EXO-A gene

Corresponding Author:
Laila Taher
Demonstrator of Medical Microbiology & Immunology, Faculty of Medicine, Tanta University, Egypt.
Tel.: +201019909628
lailasultan39@gmail.com

ABSTRACT

Background: *Multi-Drug Resistant (MDR) P. aeruginosa is a major health problem in burn infection. Exotoxin A gene plays an important role in pathogenesis of burn infections* ***Objective:*** *the isolation of MDR p.aeruginosa from infected burns and Detection of Exotoxin-A (Exo-A) gene in MDR P. aeruginosa isolates using conventional Polymerase Chain Reaction (PCR).* ***Methods:*** *The study was carried out on 200 samples of burn patients in a form of burn swabs, these patients admitted to Burn Units at Tanta university Hospitals. PCR was done on isolated MDR P.aeruginosa for detection of exotoxin- A gene.* ***Results:*** *P. aeruginosa was the most common organism isolated from burn infection with a percentage of 32.5% .96.3% of them contained the EXO-A gene while only two cases (3.3%) didn't contain the gene.* ***Conclusion:*** *EXO-A gene is an important virulence factor of P.aeruginosa infected burns. MDR P. aeruginosa incidence increased in hospitals.*

INTRODUCTION

Burned patients are more liable to burn wound infections and other types of health care associated infections due to a state of immunosuppression usually associated with burn[1] .Burn is considered as one of the most common and overwhelming forms of trauma, also patients who suffer from severe burn injury require specialized hospital care to minimize morbidity and mortality. The prognosis of patients with burn has improved in the past few decades due to improvements in modern medical care in specified burn units, mortality rates halved in the past 40 years.[2] *P. aeruginosa* bacteria is one of the most common opportunistic organisms, frequently associated with infections of immunosuppressed patients, and also causes outbreaks of health care associated infections that results in a high mortality.[3]*P. aeruginosa* has high resistance to many antimicrobials and the development of multidrug resistance in healthcare settings is increasing and this complicates anti–*pseudomonal* chemotherapy. [4]Infections which are caused by *P. aeruginosa* are difficult to eradicate because of elevated intrinsic resistance and also the ability to acquire resistance to different antibiotic classes .[5] World Health Organization (WHO) has identified antimicrobial resistance as one of the three most important problems for human health, *P. aeruginosa* represents a phenomenon of resistance since all known mechanisms of antimicrobial resistance can be encountered; nevertheless enzyme production is the major mechanism of acquired resistance in these strains especially with β- lactam antibiotics, which are considered a major line of treatment for *P. aeruginosa*[6]. *P.aeruginosa* has many *virulence* factors which play a major role in the pathogenesis of diseases produced by them, which are described as belonging to adhesins and other secreted toxins, exotoxins are either passively secreted from the cell or actively secreted via type I secretion system (T1SS), type II secretion system (T2SS) or the type III secretion system (T3SS).[7] Exotoxin A causes inhibition of protein synthesis ,so it causes necrosis in burn wounds, exotoxin A is a type II secreted extracellular enzyme encoded by toxA gene , this enzyme by itself alone or synergistically with other hydrolases is causing cell death , severe tissue damage and necrosis in human host, exotoxinA is an ADP ribosyl transferase which transfer an ADP ribosyl moiety to elongation factor 2 resulting in an inhibition of protein synthesis in mammalian cells.[8]

METHODOLOGY

Subjects:

The present study was carried out in The Medical Microbiology and Immunology Department, Faculty of Medicine, Tanta University and Central Research Laboratory of Tanta University, Tanta city on 200 patients admitted to Burn Units of Surgery Department, Tanta university hospitals during the period of research from February 2019 to November 2020.

Ethical approval for this study was provided by Ethics and Research Committee, Faculty of Medicine, Tanta University. A code number was put for each sample for adequate provision to maintain confidentiality of the data.

Sample collection and transportation:

All samples were collected under complete aseptic precautions. The samples included infected burn swabs, samples were labeled and delivered as soon as possible to the laboratory in Medical Microbiology and Immunology Department.

Processing of samples:[9]

Isolation and identification of the infecting organism:

All samples were cultured on MacConkey, nutrient agar plates and then Gram stain smears were made after the culture to avoid the contamination of the sample and examined microscopically. All plates were incubated at 37°C for 24 h and then the isolates in the primary plates were identified by: Colony morphology, Gram stained film, different biochemical reactions (catalase test, oxidase test, coagulase test, citrate test, indole test, sugar fermentation reactions, and triple sugar iron agar) and Biochemical identification of Pseudomonas aeruginosa with API 20 NE.

Antibiotic sensitivity testing

Antimicrobial susceptibility of the isolates was determined by modified Kirby Bauer disc diffusion method using antibiotics discs on Mueller Hinton agar plates according to Clinical and Laboratory Standard Institute (CLSI) guideline.[9] The antibiotic discs used were (ceftazidime30 µg,piperacillin-tazobactam100/10 µg, gentamycin 10 µg, obramycin 10 µg,amikacin30 µg,ciprofloxacin5 µg,levofloxacin5 µg, cefepime 30 µg, meropenem10 µg,imipenem10 µg, ofloxacin 5 µg, colistin 10 µg

Detection of *MDR P.aeruginosa*, which is resistant to at least one agent in three or more antimicrobial category.

Genotypic detection of exo A gene (the materials used for DNA extraction kits: (Wizard® Genomic DNA Purification Kit) (Promega)

Nucleic acid Extraction:

Nucleic acid Extraction was done according to manufacturer instructions.

Nucleic acid amplification:

All reagents were thawed and the GoTaq® Long PCR Master Mix was vortexed to ensure proper mixing and prevent formation of magnesium gradients.

Agarose gel electrophoresis of the amplified DNA: The PCR products were visualized and photographed under UV light after electrophoresis for 45 min at 100 V through 1% agarose gel containing ethidium bromide (1 µg/ml).

RESULTS

This study was carried out on 65 clinical isolates *of Pseudomonas aeruginosa (P.aeruginosa)* all were collected from 200 patients admitted to Burn Units of Tanta University Hospitals, and laboratory identification was done in Medical Microbiology and Immunology Department, Central Research Laboratory of Tanta University. Of the 65 infected patients, 45 were males and 20 were females. Age ranged from 1 year to 60 years old with the mean age of (24.26±20.475) .The duration of hospital stay among *P.aeruginosa* infected patients was from 10 to 35 days with a mean of (17.9± 7.38). Table 1 showed the number and percentage of different microorganisms isolated from 200 samples. It showed that the highest percentage of cases (65cases) were *P. aeruginosa* .followed by *Klebsiella species* (52 cases) then *Staph.aureus* (29 cases) then CONS (23 cases) then *E.coli* represented (16 cases) and *Candida species* (8 cases) while no growth was detected in 7 cases.

Table 1: Isolated microorganisms during this study

Causative Bacteria	No.	%
P. aeruginosa	65	32.5%
Klebsiella species	52	26%
Staph.aureus	29	14.5%
Coagulase Negative Staphylococci (CONS)	23	11.5%
Escherichia coli (E.coli)	16	8%
Candida species	8	4%
No growth	7	3.5%
Total	200	100%

Table 2 illustrated antibiotic susceptibility pattern among *P. aeruginosa* isolates during this study, which was as follow: the resistance was more common with tobramycin (90.8%), levofloxacin (87.7%), gentamycin and ofloxacin (86.2%), meropenem (84.6%), imipenem (81.5). The most effective antibiotics were colisitin followed by cefepime and pip-tazobactam.

Table 2: Antimicrobial susceptibility pattern among *P.aeruginosa* isolates:

Antimicrobials	Sensitive isolate		Intermediate		Resistant	
	NO	%	NO	%	No	%
Ceftazidime	9	13.8	2	3.1	54	83.1
Piperacillin-tazobactam	12	18.5	2	3.1	51	78.5
Gentamycin	8	12.3	1	1.5	56	86.2
Tobramycin	3	4.6	3	4.6	59	90.8
Amikacin	10	15.4	1	1.5	54	83.1
Ciprofloxacin	8	12.3	1	1.5	56	86.2
Levofloxacin	6	9.2	2	3.1	57	87.7
Cefepime	12	18.5	3	4.6	50	76.9
Meropenem	8	12.3	2	3.1	55	84.6
Imipenem	10	15.4	2	3.1	53	81.5
Ofloxacin	9	13.8	0	0	56	86.2
Colistin	20	30.7	-	-	45	69.2

Table 3 showed that MDR *P. aeruginosa* isolates were (60 isolates) more than not MDR isolates (5 cases)

Table 3: Percentage of MDR P. aeruginosa

	Pseudomonas aeruginosa	
	No	**%**
***Pseudomonas aeruginosa* (not MDR)**	5	7.7%
MDR	60	92.3%
Total	65	100%

Table 4showed that Exo-A gene was detected in most of MDR *P. aeruginosa* isolates about (58 isolates) and it was not detected in (2 isolates). And figures 1, 2, and 3 showed the results of conventional PCR.

Table 4: Results of Exo-A gene detection in *MDR P. aeruginosa by* conventional PCR.

EXO-A gene	NO.	%
Positive	58	96.7
Negative	2	3.3
Total	60	100

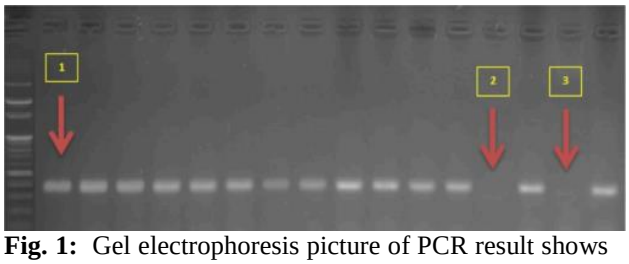

Fig. 1: Gel electrophoresis picture of PCR result shows arrow 1 is a control strain arrow 2 and 3 show negative PCR for Exo-A gene. the remaining islates show positive PCR for EXO_A gene(Molecular weight of positive band was 396 bp).

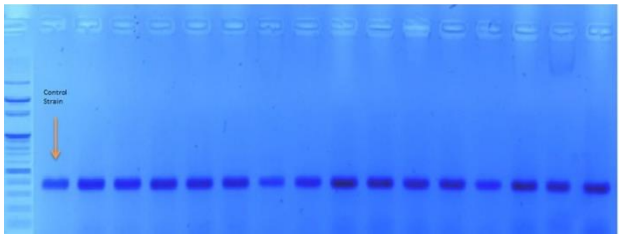

Fig. 2: It shows that all isolates expressed Exo-A gene.

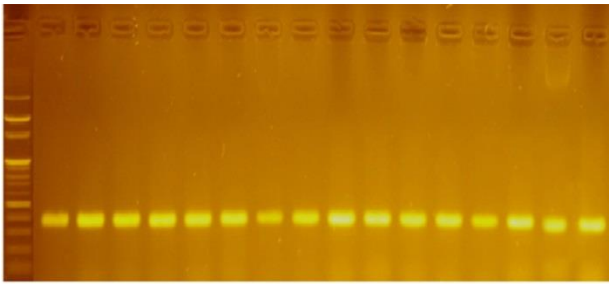

Fig. 3: Shows all isolates in this picture expressed Exo-A gene.

DISCUSSION

Multidrug-resistant *P.aeruginosa* is defined as resistance strain to one anti-microbial agent of three or more anti-*pseudomonal* antimicrobial classes[11] .Hospital acquired infection is identified as one of the most serious complications in health and treatment organizations, factors causing such these infections lead to spread of diseases in different hospital units and to increase the mortality rate[12]**.** Burning is one of the most common and devastating injuries that needs direct attention to avoid its side effects among burn patients[13]. *P.aeruginosa* is one of the most common pathogens that induce infections in burn patients[14]. Exotoxin A (encoded by the tox A gene) is the most introduced exotoxin and it is secreted by type II secretion system. [15], it has the ability to inhibit protein biosynthesis just like diphtheria toxin[16] Exotoxin A has

been demonstrated to be involved in local tissue damage and invasion.[17]. In the present study the relation between risk factors and *P.aeruginosa* infection was as follow; most of cases did not have any risk factors and there was a significant relation between *P.aeruginosa* infection and risk factors as P-value was 0 .036 .An Egyptian study by Nagwa et al.[18] at Cairo University had similar results. In contrary a Japanese study by Puja et al.[19] reported that no significance between risk factors which were DM, hypertension and degree of burn and *P.aeruginosa* infection.

In this study the number and percentage of isolates from 200 burn patients, *P. aeruginosa*(32.5%) was the most common organism isolated from infected burn followed by *klebsiella (26%), staph.aureus (14.5),* the least isolated organism was *candida species (4%)*.This result was in agreement with the Egyptian study of Salah et al. [20] at Ain Shams University Hospitals was reported similar results.

In contrary, a Kuwati study by Mariam et al.[21] was done and reported that the most common isolated organism was *Acinetobacter baumannii* followed by *Klebsiella pneumonia*. Concerning the antimicrobial susceptibility pattern among *P. aeruginosa* isolates during this study, It was found that the most effective drugs were colistin (27.7%) followed by cefepime (18.5%) then amikacin and imipenem (15.4%). The rest of antimicrobials showed highly resistance. Most of *P. aeruginosa* isolates showed antimicrobial resistance as 60 cases of *P. aeruginosa* isolates of 65isolates showed multidrug resistance while only 5 cases were sensitive to antimicrobials. MDR *P.aeruginosa* showed resistance to three or more antibiotic classes. Supportive to the results of this study AMANI et al.[22] Alexandria University, Egypt who found that *P.aeruginosa* had highly antimicrobial resistance as follows azetronam (85.7), ceftazidime (80%), cefepime (79.4%) and meropenem (73.7%)**.** In the present study, we detected Exo-A gene in MDR *P.aeruginosa* isolated from burn infection by conventional PCR and the result was, most of *P. aeruginosa* isolates contained EXO-A gene (96.7%) of the isolated MDR *P.aeruginosa* while only (3.3%) of all isolates did not contain the same gene. The study which was done by Ibraheem[23] at Iraq had higher percentage in comparison to our study as it revealed that the percentage of EXO-A gene was 100% among all isolated MDR *P. aeruginosa*. On the other hand, other studies had more or less similar; an Egyptian study by Yasser et al.[24] at Benha University found that the percentage of EXO-A gene was 72% among *P. aeruginosa* infected cases. Afsoon et al.[25] at Iran found that EXO-A gene presented in 60% of all *P.aeruginosa* isolates [24]. This higher percentage indicate the role of EXO-A gene in the pathogenesis of *P.aeruginosa* infection among burn patients as an important virulent factor because its role in necrosis of tissue and inhibit protein biosynthesis in infected tissues .This difference between studies occurred due to different places, number of cases, infection control measures and time of transport.

CONCLUSIONS

Pseudomonas aeruginosa was the most predominant bacterial isolate in the studied burn infections with higher incidence in male than female. MDR *P. aeruginosa* incidence increased in hospitals. EXO-A gene is an important virulent factor of *P.aeruginosa* infected burn.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

REFERENCES

1. Pruitt Jr BA, Lindberg RB, McManus WF, Mason Jr, AD. Current Approach to Prevention and Treatment of *Pseudomonas aeruginosa* Infections in Burned Patients. Clin Infect Dis; 1983 5: S889-S8.
2. Lionelli GT, Pickus EJ, Beckum OK, Decoursey RL, Korentager R A. A three decade analysis of factors affecting burn mortality in the elderly. Burns; 2005 31:958-963.
3. Wirth FW, Picoli SU, Cantarelli VV et al.: "Metallo-β-lactamase producing Pseudomonas aeruginosa in two hospitals from Southern Brazil," Brazilian Journal of Infectious Diseases; 2009, 13:170–172.
4. Keen EF III, Robinson BJ, Hospenthal DR et al. "Prevalence of multidrug-resistant organisms recovered at a military burn center.Burns; 2010, 36:819–825.
5. Breidenstein EBM, De la Fuente-Nuñez C, Hancock REW. *Pseudomonas aeruginosa*: all roads lead to resistance. Trends Microbiol; 2011; 19:419-426.
6. Hossein HM, Mehdi RM, Masoumeh A, Gholamreza A, Masoud DM. Molecular evaluation of *Pseudomonas aeruginosa* isolated from patients in burn ward, ICU, and ITU , in a number of hospital in Kerman province; 2015, 5: 1428-1431.

7. Strateva T, Yordanov D. *Pseudomonas aeruginosa* is a phenomenon of bacterial resistance. J. Med Microbiol; 2009, 58: 1133–1148.
8. Bradbury RS, Roddam LF, Merritt A, et al. Virulence gene distribution in clinical, nosocomial and environmental isolates of *Pseudomonas aeruginosa*. J Med Microbiol: 2010, 59:881-890.
9. Cheesbrough M: District laboratory practice in tropical countries. 2nd ed. Cambridge, UK: Cambridge University press; 2006.p.65-107
10. Clinical and Laboratory Standards Institute. Methods For dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved guideline M100-S24. Wayne, PA: CLSI; 2020.
11. Magiorakos AP, Srinivasan A, Carey RB, et al.: Multidrugresistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect; 2012, 18:268–81.
12. Azimi, Taher; Maham, Saeid; Fallah, Fatemeh; Azimi, Leila; Gholinejad, Zari :Evaluating the antimicrobial resistance patterns among major bacterial pathogens isolated from clinical specimens taken from patients in Mofid Children's Hospital, Tehran, Iran:. Infection and Drug Resistance, 2019, 12:2089–2102
13. Shariati, Elham Asadian, Fatemeh Fallah, Taher Azimi, Ali Hashemi, Javad Yasbolaghi Sharahi, Majid Taati Moghadam: Evaluation of Nano-curcumin effects on expression levels of virulence 304genes and biofilm production of multidrug-resistant *Pseudomonas aeruginosa* isolated 305 from burn wound infection in Tehran, Iran. Infection and Drug Resistance 2019:12 2223–2235
14. Lan, Yong; Li, Weichang; Jiao, Yanpeng; Guo, Rui; Zhang, Yi; Xue, Wei; Zhang, Yuanming Therapeutic efficacy of antibiotic-loaded gelatin microsphere/silk fibroin scaffolds in infected full-thickness burns. Acta biomaterialia; 2014,10: 3167-3176.
15. Michalska M, Wolf P. *Pseudomonas* exotoxin a: optimized by evolution for effective killing. Front. Microbiol; 2015, 6: 9-63.
16. Hamood AN, Colmer-Hamood JA, Carty NL. Regula on of *Pseudomonas aeruginosa* exotoxin A synthesis. In *Pseudomonas*: Virulence and gene regulation. Academic/plenum publishers, New York: 2004, 38:9–423.
17. Antônio JR, Mario RO, Renan RR, Maria VL, Francisco LL, Soraya LR. *Pseudomonas aeruginosa*: Virulence Factors, Antibiotic Resistance Genes. Brazilian Archives of Biology, and Technology; 2019, 62: e19180-503.
18. Nagwa A. Tharwat, Neveen M. Saleh, Reda E.Hamouda, Rasha H. El Shreif, Sherif. M. Elnagdy1, Ghada Mohamed: combination of ciprofloxacin and silver nanoparticles fortreatment of multi-drug resistant *Pseudomonas aeruginosa* in Egypt.;Az. J. Pharm Sci:2019
19. Puja Neopane, Hari Prasad Nepal, Rajendra Gautam1, Rama Paudel, Shamshul Ansari, Sony Shrestha1,Sangita Thapa:is there correlation of biofilm formation with multidrug resistance and esbl production in *Pseudomonas aeruginosa*; European Journal of Biomedical AND Pharmaceutical sciences;2016,1:366-372.
20. Salah Nasser, Amr Mabrouk, Ashraf Maher. Colonization of burn wounds in Ain Shams University Burn Unit.2003, 29(3): 0–233.
21. Mariam ALfadli1, Eman M. EL-sehsah, Moustapha Ahmed-Maher Ramadan: Risk factors and distribution of MDROs among patients with healthcare associated burn wound infection. GERMS; 2017, 84:199-206.
22. Amani F. Abaza, Soraya a. El shazly, Heba S.A. Selim, Gehan S.A. Aly. Metallo-Beta-Lactamase Producing *Pseudomonas aeruginosa* in a Healthcare Setting in Alexandria, Egypt. Polish Journal of Microbiology; 2017, 66: 297–308.
23. Ibraheem Salih Aljebory: PCR Detection of Some Virulence Genes of *Pseudomonas aeruginosa* in Kirkuk city, Iraq; /J. Pharm. Sci. & Res; 2018, 10: 1068-1071.
24. Yasser M. Ismail, Sahar M. Fayed, Fatma M. Elesawy, Nora Z Abd El-Halim Ola S. El-Shimi: Phenotypic and Molecular Characteristics of *Pseudomonas Aeruginosa* Isolated from Burn Unit; Egyptian Journal of Medical Microbiology: 2021, 30: 19-28.
25. Afsoon Shariat, Sajad Alizadeh, Mehdi Jahangiri Hoseinabadi, Mohammad Movagharnejad. Molecular Detection of Virulence Genes among *Pseudomonas aeruginosa* Strains Isolated from Clinical Specimens by Multiplex PCR. Avicenna Journal of Clinical Medicine; 2018, 87: 2228-3.