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Original Research Article

Molecular Detection of Virulence Genes among *Pseudomonas aeruginosa* Clinical Isolates from Khartoum State Hospitals, Sudan

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Abstract

Pseudomonas aeruginosa possesses a variety of virulence factors that may contribute to its pathogenicity. D*issemination of resistant and highly virulent pathogens is also the main problem worldwide*. Thus this study aimed to determine the frequency of *oprI*, *Las B* and *toxA* virulence genes among the clinical isolates of *P. aeruginosa* species. A total of 100 *P. aeruginosa* clinical isolates were collected from Hospitals and laboratories from different specimens during the period from August 2019 to November 2020. The isolates were re- identified using routine culture techniques, and multiplex PCR was applied to detect the presence of these virulence genes among them. It was found that the frequency of virulence genes were (22%) Las B, (55%) OprI, (15%) Tox A and (8%) mixed of OprI and Tox A. These genes were found with different percentage among the studied variables with the higher frequency among males, wound samples, except OprI were among 26-36 years age group and significantly among inpatients than out patients. The study concluded that all isolates carried either one or two of studied genes in different percentage while OprI represented the most predominant gene. Determination of different virulence genes of *P. aeruginosa* isolates suggests that they are associated with different levels of intrinsic virulence and pathogenicity.

Keywords: Molecular detection, *Pseudomonas aeruginosa*, virulence genes, Khartoum, Sudan.

INTRODUCTION

Pseudomonas aeruginosa (*P. aeruginosa*) is an opportunistic pathogen that it considered as a major health hazard, especially in immunodeficient patients [1]. It is most commonly associated with nosocomial diseases [2]. Its infections in hospitals, mainly affect the patients in intensive care units and those having catheterization, burn, and/or chronic illnesses [3]. It possesses a variety of virulence factors that may contribute to its pathogenicity such as exotoxin A, exoenzyme S, nan 1, and Las genes [4]. The outermembrane proteins of *P. aeruginosa* are OprI and OprLthat play an important roles in the interaction of the bacterium with the environment, as well as the inherent resistance of *P. aeruginosa* to the antibiotics. In addition, the presence of the specific outer membrane proteins has been implicated in the efflux transport systems that affect cell permeability [5, 6].

Exotoxin A, encoded by the toxA gene, which is the most toxic virulence factor detected in this organism that inhibits protein biosynthesis. It has a necrotizing activity on tissues and contributes to the colonization process [7]. Elastase B (lasB) is a multifunctional metalloenzyme, it influences pathogenesis by enhancing adhesion, colonization, and invasion of tissues, causing chronic pulmonary inflammation [8]. Additionally, elastase B is an important protease of *P. aeruginosa*. This enzyme has a tissue-damaging activity, it can degrade a number of plasma proteins and it contributes to the survival of *P. aeruginosa* in infected tissues [9].

LasB is considered to be a potential target for the development of an innovative chemotherapeutic approach, especially against multidrug-resistant strains [6].

So the present study aimed to detect the frequency of some virulence genes (LasB, OprI and Tox A) of clinical isolates of *P.aeruginosa* such few studies were carried out in our country.

METHODS

Design and Setting

This descriptive cross-sectional study encompassed different Khartoum State Hospitals (Soba

Hospital, Bahri Hospital and Military Hospital) during the period from August 2019 to November 2020.

A total of 100 clinical isolates of *P.aeruginosa* that have been collected from different clinical specimens were considered for this study. Data in the present study that included patient's gender, age, and, types of clinical specimens were obtained from Hospitals records.

Sample Processing

Colonies from *P.aeruginosa* clinical isolates were sub-cultured on Nutrient and MacConkey agar media and incubated at 37°C for 24 hours to ensure purity and optimal growth. Then they were re-identified using routine culture techniques

Multiplex PCR for detection virulence gene (oprI,toxAand lasB)

DNA extraction

Bacterial DNA was extracted by using physical method (boiling). Two to three colonies of pure culture of *P. aeruginosa* isolates were suspended in 50µL of distal water (D.W) and vortexed to ensure a homogenous suspension.

The suspension was incubated at 94^{O}C for 10 minutes and then centrifuged. The supernatant was collected in a clean tube and stored at -20°C as a template DNA stock [9].

PCR amplification procedures

The PCR was carried out by using primers targeting (oprI, toxA and lasB) virulence genes [10]., the PCR assay included 25 µl final reaction mixture which consisted 5 µl of (taq polymerase, reaction buffer., MgCl and DNTPs) (Intron biotechnology, Korea) and 2 µl of each 10 P mol forward and reverse primers specific for these virulence genes, 5 µl from DNA template and 13 µl from (ddH_2O) to complete the volume to 25 µl final reaction mixture .The reaction tubes were cycled in thermal cycler machine (Techne, England). The PCR program was performed with an initial denaturation for 5 min at 94°C, then 35 cycles of denaturation for 1min at 94°C, annealing for 1min at 58°C and 60°C for lasB gene, extension for 1 min at 72°C and final extension for 5 min at 72°C. The amplified products were resolved in %2 agarose gel electrophoresis stained with ethidium bromide and visualized under UV light [10]. Primers used for amplification of virulence genes of *P. aeruginosa* isolates as in Table-1.

Table-1: The amplified primers used in the present study

Amplified gene	Specific Primer	Amplified region (bp)
oprI	PS1, 5'-ATG AAC AAC GTT CTG AAA TTC TCT GCT-3' PS2, 5'-CTT GCG GCT GGC TTT TTC CAG-3'	**250**
toxA	Toxf 5' GGT AAC CAG CTC AGC CAC AT 3' tox r 5' TGA TGT CCA GGT CAT GCT TC 3'	**352**
lasB	lasF 5' GGA ATG AAC GAA GCG TTC TC 3' lasR 5' GGT CCA GTA GTA GCG GTT GG 3'	**300**

Statistical Analysis

Data were analyzed by the statistical package of social science (SPSS) soft program version 22, with reference p-value (0.05), P-value ≤.05 conceder as significant result. Frequencies and percent obtained in frequency tables, chi-square test for goodness of fit used to test these frequencies. The relations between variables tested using cross tables and chi-square (Fisher exact) test for independence

Ethical Statement

Ethical approval for this study was obtained from Al-Neelain University Ethical Committee, and informed consent was obtained from the mentioned hospitals.

RESULTS

Demographical Data

Hundred (100) *P.aeruginosa* clinical isolates were collected from different Hospitals in Khartoum State, Sudan during the period from August 2019 to November 2020. They were obtained from different clinical samples (wound swabs, urine and ear swabs), their identification was confirmed using conventional bacteriological techniques. The isolates were collected from patients from both males (60) and females (40) with age range from 15 to 58 years with a mean age of 43.7 year;

Multiplex PCR result

The amplified PCR results (Figure-1) revealed that all *P. aeruginosa* isolates carried either one or two of studied genes in different percentage, whereas OprI represented the most predominant gene (55%) (Figure-2).

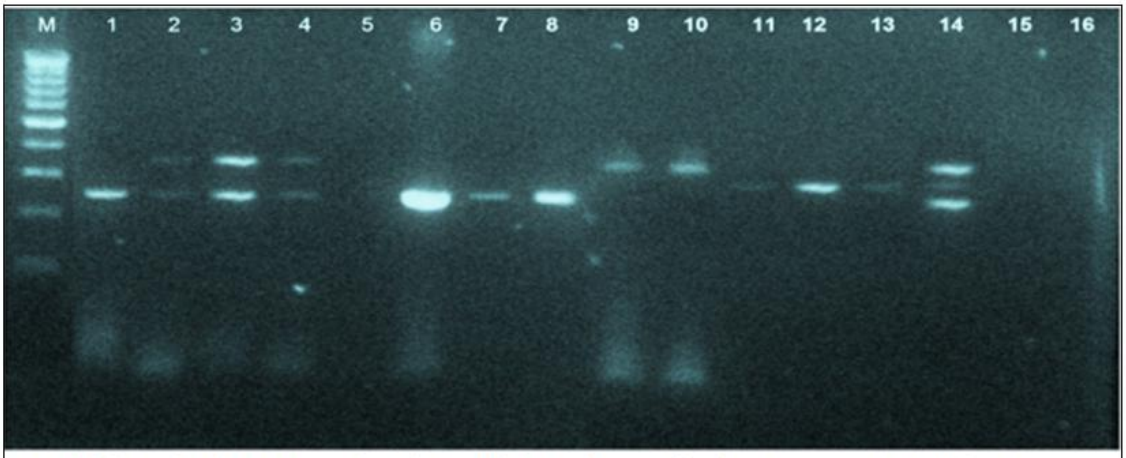

Fig. (1) lane (M) DNA marker (100BP), lane (1,6, 7 & 8) were oprI 250 BP lane (11,12, and 13) were lasB300 BP lane (9,10) were toxA 352 BP lane (2,3, and 4) were oprIand toxA lane (14) mixed positive control lane (15) negative control,

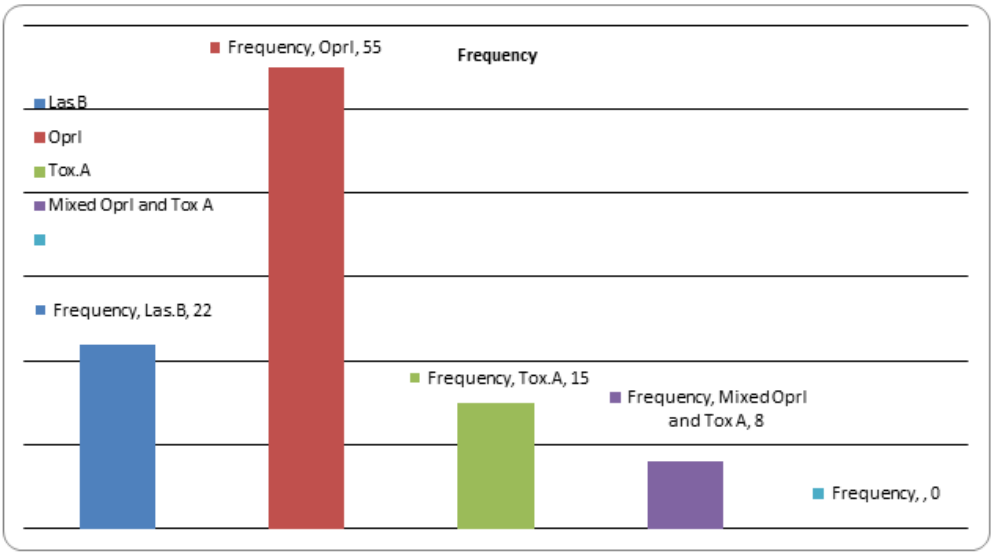

Fig-2: Frequency of virulence genes among *P.aeruginosa* clinical isolates

Frequency of studied virulence genes according to gender, type of samples, age group and hospital stay

Among the 22 (22%) detected Las B, 17**(77.**2%) were detected among males which is the most predominant among them followed by mixed of OprI and ToxA 6/8(75%), Tox.A 10/15(66.7%) and OprI 27/55(49%). In contrast OprI was the most predominant 28(51%) among females followed by ToxA 5(33%), mixed of OprI and ToxA 2(25%) and Las B 5(22%) (Table-2).

Table-2: Frequency of virulence genes among gender

	Males		***Females***		**Total**		**P.value**
Genes	**N**	**%**	**N**	**%**	**N**	**%**	
Las B	17	77.20%	5	22.80%	22	100%	0.09
OprI	27	49%	28	51%	55	100%	
Tox.A	10	66.70%	5	33.30%	15	100%	
Mixed	6	75%	2	25%	8	100%	

The frequencies of the studied genes among the clinical samples were expressed in Table-3. It shows that the most prevalent virulence genes harboring wound swabs, urine and ear swabs were as follow: Las B (72.7%), mixed of OprI and ToxA(50%) and OprI (21.8) respectively. Although OprI was the least common among wound specimens it was the second most common among urine (32.7%) specimens and Las B was not detected among isolates from ear swabs (P value > 0.05).

Table-3: Frequency of *virulence genes according to type of samples*

	Wound		*Urine*		*Ear swab*		*Total*		P.value
Genes	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	0.07
Las B	16	72.70%	6	27.30%	0	0	22	100%	
Oprl	25	45.50%	18	32.70%	12	21.80%	55	100%	
Tox.A	10	66.70%	2	13.30%	3	20%	15	100%	
Mixed	4	50%	4	50%	0	0	8	100%	

The clinical isolates were collected from patients with different age group. They were categorized into four group to detect the prevalence of these virulence genes among them as shown in Table-4, which it reflects that, all of studied virulence genes were detected among the four group, with the commonest genes, mixed of OprI and ToxA (25%) among both the age group (15-25) and (48-58) year, Tox.A (47%), and lasB (40.1%) among the age group (26-36) and (37-47) year respectively (P value > 0.05).

Table-4: Distribution of studied virulence genes according to Age group

	15-25 years		**26-36 years**		**37-47 years**		**48-58 years**		**Total**		**P.value**
Genes	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	0.89
Las B	3	13.6%	9	40.1%	9	40.1%	1	4%	22	100%	
Oprl	13	23.6%	15	27.3%	22	40%	5	9.1%	55	100%	
Tox.A	3	20%	7	47%	4	27%	1	6%	15	100%	
Mixed	2	25%	3	37.5%	2	25%	1	12.5%	8	100%	

Our data targeted the hospital stay as a variable to study the prevalence of these virulence genes among in-patients and out-patients and the result was expressed in Table-5. It shows that there was statistically significance association (P. value = 0.05) between hospital stay and the distribution of studied virulence genes (Las B, OprI and OprI).

Table-5: Frequency of studied virulence genes according to hospital stay

	In patients		*Out patients*		**Total**		**P.value**
Genes	**N**	**%**	**N**	**%**	**N**	**%**	0.05
Las B	17	77.2%	5	22.8%	22	100%	
Oprl	24	43.6%	31	56.4%	55	100%	
Tox.A	9	60%	6	40%	15	100%	
Mixed	4	50%	4	50%	8	100%	

DISCUSSION

Pseudomonas aeruginosa is an opportunistic pathogen capable of infecting virtually all tissues, and possesses a variety of virulence factors that may contribute to its pathogenicity [11]. And it is one of the most common causes of health care- associated infections [12].

In the present study all *P. aeruginosa* clinical isolates represented either one or two of studied genes (Las B, Opr I and ToxA) with the most of isolates (55%) were remarkably positive for *oprI* genes which it relatively similar to Ullah *et al*., [13], who found that *oprI* was (40.74%). Whereas other findings were reported higher percentage (84.61%) and (74.6%) [13, 14]

Khan and Cerniglia [15] also developed a PCR procedure to detect *the prevalence of toxA* gene among *P. aeruginosa* by amplifying the *toxA* gene They reported that of 130 tested *P. aeruginosa* isolates, 125 (96%) contained toxAgene, whereas other species of bacteria did not yield any positive results. Qin *et al*., and Lavenir *et al*., [16, 17] also reported similar results which they differ from our finding that 15 of 100 isolates own *toxA* gene. The present finding is relatively similar to other study that reported (9.9%) prevalence of ToxA among *P. aeruginosa* [18].

Pathogenicity of *P. aeruginosa* is clearly multifactorial. LasB is one of the most important proteases of *P. aeruginosa* [19]. In this study (22%) of examined isolates harbored *lasB* gene. This finding is in agreement with previous reports [13, 18].

The coexistence of more than one virulence factors within the same isolate was found in this study, similar result was reported by Al-Dahmoshi *et al*., [20].

The higher percentage of virulence genes (Las B, ToxA and mixed of OprI and ToxA) were detected among males than females (Table-2). This may be due to strains differences and large sample size of males than females (60% vs. 40%).

The percentage of virulence genes in terms of the site of infection was determined; it was higher in

wounds followed by urine (P value > 0.05). Similar result was reported in previous study [21].

In the present study the frequency of virulence genes were detected with different percentage among different age group (P value > 0.05) and the higher percentage was observed significantly among in-patients than out-patients group. This may be due to the study that reported, the prevalence of *P. aeruginosa* and its virulence genes depends on various causes consisting nature of places, degree of contamination and type, immune status of individual patients and virulence of strains [22].

CONCLUSION

Determination of different virulence genes of *P. aeruginosa* isolates suggests that they are associated with different levels of intrinsic virulence and pathogenicity.

The various distributions of virulence genes among the study variables with significant correlation among in-patients group indicates the potential risk of these isolates especially in nosocomial infections, which it reflect the need for further studies to find out the actual role of these genes in different clinical infections caused by *P. aeruginosa*.

Conflict of interests: The authors declared no conflict of interests.

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