RESEARCH ARTICLE

Genetic Detection and Identification of Some Virulence Factors Genes Among *Pseudomonas aeruginosa* Samples in Kirkuk province-Iraq

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ABSTRACT

The study aim was to identify the prevalence of some virulence factors genes of *Pseudomonas aeruginosa* isolates which carried out at Kirkuk hospitals, in Kirkuk, Iraq. Totally 150 swaps were collected and cultured (110 patients suffered from burns, and 40 patients suffered from wounds) from different ages and both gender for identification of *P. aeruginosa*, the bacterial swaps were detect by biochemical tests, API 20 E and Vitak 2 system. 51 (34%) isolates of *P. aeruginosa* of total samples were identified, distributed as 39 isolates (35%) from burns and 12 isolates (3%) from wounds. Depending on groups of ages and gender, the study indicated that the rate of *P. aeruginosa* in the male was (53%), and in female patients was (47%) and the maximum rate (28%) was between 24-29 years comparison with the elderly. Bacterial chromosomal DNA was extracted from *P. aeruginosa* by QIAamp DNA mini kit. The average DNA concentration of 51 DNA samples were 85 ng/µl and the average purity were 1.9 . Polymerase chain reaction (PCR) has been used to identified the virulence factor genes (*tox A* and *opr L*). The result indicated that 50 (98%) samples were positive for *opr L* and 51 (100%) for *tox A* genes.

Keywords: *Opr L, P. aeruginosa*, *Tox A* genes and Virulence genes.

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INTRODUCTION

P. aeruginosa is an opportunistic pathogen which causes a large variety of individual infections and opportunistic pathogen. It is a frequent hospital-acquired pathogen and accountable for urinary tract infections, bacteremia, dermatitis, soft tissue infections respiratory infections, bone and joint infections, gastrointestinal infections, and systemic infections diversity, mainly in patients with bed ulcers, severe burns, and AIDS or cancer's patients who are immunosuppressed[1-3] and with limited therapeutic options because of its antibiotic resistance.[4] Resistance to antibiotic constitutes is one of the most serious threats to the worldwide public health and impacts all aspects of therapeutics, animal husbandry and agriculture; it is natural, ancient, and hard wired in the microbial pan-genome.[5] In hospitals, *P. aeruginosa* infections mainly have an effect on the patients in the units of intensive care and those having catheterization, burn, and/or chronic illnesses.[6] The mainly essential virulence factors of P. aeruginosa involved outer membrane-associated protein L and I, exotoxin A (ETA) and quorum-sensing determinant system.[7] Exotoxin A be the main hazardous virulence factor formed by P. aeruginosa.[8] The external membrane proteins (*OprI* and *OprL*) of *P. aeruginosa* play an essential role in the relations of the bacterium with the surroundings, as well as *P. aeruginosa* inherent resistance to the antibiotics. Besides, the specific outer membrane proteins have been concerned in the efflux transport systems that have an effect on cell permeability.[9] Because of these proteins are present only in this organism, they could be a dependable factor for rapid detection of *P. aeruginosa* in clinical samples.[10] The virulence of *P. aeruginosa*, fundamentally depends on two kinds of virulence determinants: virulence factors included in acute infection, they seem often secreted and membrane-bound factors. There are a great number of virulence factors for *P. aeruginosa* such as elastase, sialidase, exoenzyme S, and exotoxin A[11] as well as, there are a number of others extracellular products. Exotoxin A encoded by the *toxA* gene which has the capability of protein biosynthesis inhibition just like diphtheria toxin.[12,13]

MATERIALS AND METHOD

Collection of samples:

One hundred and fifty samples were collected (40 wounds patients, 110 burns patients) from Kirkuk hospitalized burns and wounds care units, in Kirkuk, Iraq. During June to August 2018. Samples were cultured on Cetrimide agar, Blood agar, King A and king B medium, and MacConkey agar. The

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biochemical tests were performed for confirmed the detection *P. aeruginosa* isolates by oxidase, catalase, motility, IMVIC tests.[14] The biochemical tests result of final recognition of *p.aeruginosa* was reliant on Api 20 E, and Vitak 2 systems.

DNA extraction and PCR Method

DNA Extraction

DNAs were extracted by QIAamp DNA mini kit (Qiagen, Germany) depending on the manufacturer's protocols and checked via Electrophoresis instrument in a 1% agarose gel which stained by ethidium bromide, then take a look via ultra violet transilluminator (UVT).

Nanodrop

Chromosomal DNA has measured by the device of nanodrop at 260/280nm, and preserved at (-20˚C) till further uses.

Polymerase chain reaction analysis

The PCR technique has done for factors of virulence genes which were exotoxin A (*toxA*) and outer membrane protein (*oprL*) genes in *P. aeruginosa* by specific primers. PCR amplification was done by thermal cycler instrument (BioRad, USA) with using two specific primers for *oprL* and *toxA* genes. Table 1.

The PCR was done in 50 µL volume reaction mixtures which contain 1µL of primers, 10 µL of DNA template and 25 µl of master mix. The suitable temperature of annealing was performed at 55°C for, *oprL* and *toxA*.[15]

Product analysis

Products of PCR has been analysis via gel electrophoreses instrument in 1.5% agarose gel stained with ethidium bromide then visualized and documented under UVT.

Study Results

In this study, from 150 samples 51 (34%) isolates of *P.aeruginosa,* 39 (35%) from burns, and 12(3%) from wounds as in Table 2.

For 53% (n = 27) and 47% (n = 24) of *P.aeruginosa* samples were isolated from male infected and female infected, respectively as in Table 3.

Also, the ages of patients are ranged between 1-59 years and the greater part between 24–29 years as in below Table 4.

The study results showed that the DNA samples concentration of the fifty-one *Pseudomonas aeruginosa* isolates were 85 ng/uL, and the average purity was 1.9, Figure 1.

Study results showed the distribution of *P. aeruginosa* virulence factors genes were 50 (98%) isolates were positive for *oprL* and 1 (2%) were PCR negative while the *toxA* gene were detected in all of the 51 (100%) *P aeruginosa* isolates as in Table 5.

For identification of virulence factors genes of *P. aeruginosa* (*toxA* and *oprL*), polymerase chain reaction was done and obtained. PCR results of *toxA* gene (352bp) and *oprL* gene (500bp) expression are demonstrated in Figures 2 and 3, respectively.

DISCUSSION

P.aeruginosa is the most important cause of nosocomial infections. Infections caused by it are frequently severe and life-threatening and difficult to treat as the organism is inherently resistant to several drug classes and has the ability to gain resistance to all active antibacterial antibiotics. Over the years, *P. aeruginosa* contributes substantially to morbidity and mortality related to surgical site infection global, the third most frequently reported nosocomial infection.[16] Also, Patients of burn are further liable to have infections in compare

Table 1: primers and their sequence and amplicon.

Amplified gene (Primers)	*Sequence (5®3)*	*Amplicon*
oprL	F, 5'-ATG GAA ATG CTG AAA TTC GGC-3' R, 5'-CTT CTT CAG CTC GAC GCG ACG-3'	500 bp
toxA	F, 5' GGT AAC CAG CTC AGC CAC AT 3' R, 5' TGA TGT CCA GGT CAT GCT TC 3'	352bp

Table 2: Percentage and number of *P. aeruginosa*.

Samples type	*Total samples*	*Positive samples*	*Negative samples*	*Percentage %*
Burns	110	39	71	35%
Wounds	40	12	28	3%

Table 3: Percentage and distribution *P. aeruginosa* depending on gender.

	Male	*Female*
Positive isolates	27	24
Percentage	53%	47%

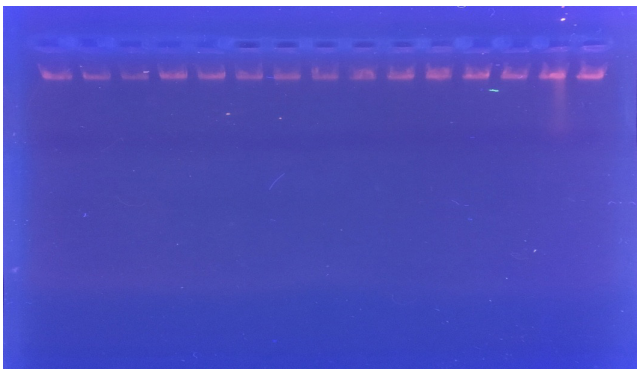

Figure 1: A run of agarose gel electrophoresis of the DNA samples.

Table 5: Number and percentage of genes of positive isolates.

Type of genes	*Positive isolates*	*Percentage*
oprL	50	98 %
toxA	51	100%

Table 4: The wound and Burn frequency of Patients (%) Involved in *P. aeruginosa* Depending on Different Groups of Age.

Age group	1–5	6–11	12–17	18–23	24–29	30–35	36–41	42–47	48–53	54–59
Rate %	10	12	16	20	28	4.3	5	3.7	0.5	0.5

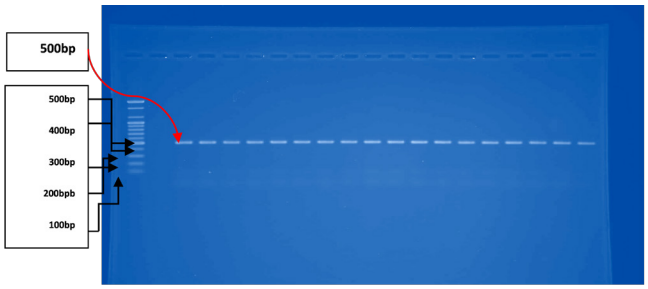

Figure 2: PCR product at Agarose Gel electrophoresis for the identification of *oprL* gene (500bp) by using 1%agarose, stained with ethidium bromide, M: 100bp DNA Ladder, Lanes 1 negative control. Lanes (2-18):Positive for *oprL* gene (500bp).

Figure 3: PCR product at agarose Gel electrophoresis for the identification of *toxA* gene (352bp) by using 1% agarose, stained with ethidium bromide, M: 100bp DNA Ladder, Lanes 1 negative control. Lanes (2–18): Positive for *toxA* gene (352bp).

with other patients due to their damaged of barriers skin and suppressed immune system, in order to extended hospital stay diagnostic procedures and invasive therapeutic.[17] Findings come with same results more or less with others in Karbala city, Iraq,[18] which showed that the highest percentage of bacterial isolate in burn patients had the bacteria *p.aeruginosa* , (45%) and (49%) with Alhamdy.[19] Most of burn patients die because of infections through their hospital courses. The infection rate in burn cases is too high in developing countries.[20,21] This may be as a result of the prevalence of poor socioeconomic groups of patients in whom low-level hygienic conditions prevail.[22] Also varying in common rate among several studies perhaps imputed to varieties in geographical position and hygienic practices. Depending on gender groups and age, the results of study indicates that *P. aeruginosa* rate is 28% for young patients (ages 24 to 29 years) and in the male (53%), comparison with the elderly, agree with Al-Zaidi, et[23] shows males in this group of age are more effective which include different clinical hygiene practices, for hospital environment.. This study is compared with the results of Okon *et al.* in Nigeria ,who registered that the male patients showed a record of 52.8% and the highest frequency of this bacterium was (20.7%) that establish for an age of 29 years old and below.[24] In contrast, these results differ with results in Karbala city, Iraq[18], results of Ekrem and R okan in Al- Sulaimania city, Iraq[25] and Shewatatek et al.[26] in Ethiopia, study results indicated a higher incidence of the bacterium in female and elderly patients. Many of the virulence factors formed by *P.aeruginosa* are ordered with diverse systems.[27] Farther last studies show *P. aeruginosa* is mainly common pathogen which produced several virulence factors gens such as (*ToxA, exoA* , *oprL,* and *oprI*) genes.[28] The results of PCR illustrated that, 50 of 51 *P. aeruginosa* isolates were positive for the *oprL* gene with amplified size (500 bp) in a percentage (98%), similar to this study, the total isolates of *P. aeruginosa* (100%) were positive for both *oprL* and *oprI* genes.[29] *P. aeruginosa* has a diversity of virulence factors that may take part to its pathogenicity. Our results showed that *toxA* gene (352bp) were detected in all 51(100%) tested strains of *P. aeruginosa* , The distributions of virulence factor genes are varieties in the populations that empower the probability of some *P. aeruginosa* strains are best adapted to the specific conditions which found in specific infectious locations[30] that may returned to the different environmental and geographical sources. *P. areugenosa* percentage and frequency of virulence factors genes depending on the numerous reasons such as sites nature, kinds and virulence of strain, immune status of patients, and extent of contamination.[31]

CONCLUSION

Conclusions of the outcome study show that a high rate of infected wounds and burns of *P. aeruginosa* may occur as a result of common apply and antibiotics mistreatment. So the results of the study may be as a recommendation to the accurate antibiotics using for treatment of patients. PCR seems that concurrent use of specific primers different virulence factors genes as (*oprL* and *toxA*) of *P. aeruginosa* provides more confident detection of *P. aeruginosa*. Also, its varieties in the virulence factor genes distributions in the isolated strains need further studies for existing out the real role of these *P.aeruginosa* genes from different sources. PCR showed that all *P. aeruginosa* strains do not necessarily have similar virulence genes.

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