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RESEARCH ARTICLE **OPEN ACCESS**

IL-18 Act as a Costimulus for Production of Interferon Gamma During Stimulation by *Pseudomonas aeruginosa* Infection

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Abstract

***Pseudomonas aeruginosa* (*P. aeruginosa*) is the most important, resistant and dangerous organism in burn wound infections in human. Extracellular and virulence factors lead to tissue damage tissue damage during infection with *P. aeruginosa*. One important virulence factor including exotoxin A(ETA) encoded by the tox A gene. In this study, 45 wound swabs from burned skin patients admitted to Al-Hilla Teaching Hospital/Babylon, 2013 from different localities in Babylon were collected to study the frequency of *P. aeruginosa* and human immune response. Polymerase chain traction technique (PCR) was used for detection ETA gene as a virulent factor producing by *P. aeruginosa* from burned skin wound infections together with interferon gamma and interleukin 18. The results showed that *P. aeruginosa* had a frequency of 51.1% among burned skin patients. The bacteriological culture showed that 19 out of 45 (42.2 %) were positive for *P. aeruginosa* while 23 out of 45 (51.1 %) were positive for *P. aeruginosa.* Only 17 out of 23 (73.91 %) were ETA producing *P. aeruginosa*. Also bacteremia occurred only in 9 out of 17 (52.94 %) in ETA producing *P. aeruginosa. P. aeruginosa* infection was presented to a lesser degree. In addition to that *P. aeruginosa* that expresses the ETA gene were the most common and toxic pathogens in burned patients and lead to bacteremia and septicemia. The using of PCR technique a rapid and accurate technique might be helpful in combating its toxicity. ETA might contributed to the overall virulent factor.**

Keywords: Bacteremia, *Pseudomonas aeruginosa*, burn.

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INTRODUCTION

Pseudomonas aeruginosa was an opportunistic bacteria able to infect humans in addition to animal[1]. In human, it could cause severe infections in nearly many tissues. Infection of respiratory tract with *P. aeruginosa* was a main reason of mortality and morbidity in patients specially those with cystic fibrosis[2]. In hospitals, *P. aeruginosa* mostly affected those who were admitted to units of intensive care, burn wound infections and patients with chronic illnesses[3]. In animals, this pathogen lead to bovine mastitis[4]. Teat wipes contamination is the major source of infection due to various virulence factors causing its pathogenicity[5]. *P. aeruginosa* had ETA, sialidase and exoenzyme S[6]. This bacteria also had secretory system(a type III) which permits toxins delivery directly into host cell cytoplasm[7]. ETA was important cause of tissue necrosis since it blocked protein synthesis[8]. It has been found that interferon gamma is an significant cytokine of protection (innate immunity) of other pathogens like Chlamydia pneumoniae and Toxoplasma gondii but its role in pseudomonas aeruginosa infection is still undiscovered.

In this study, we examined detection of ETA virulence factor gene in *P. aeruginosa* isolates that isolated from burned skin samples by using Polymerase chain reaction technique that based amplification of ETA gene .

MATERIALS AND METHODS

Sample collection

45 wound swabs from burned patients admitted to Al-Hilla Teaching Hospitals /Babylon, 2013 in the sterilized test tubes with aseptic precautions, in agreement with International Dairy Federation standards[9] collected from different fields in Babylon Provinces and the samples transferred into Laboratory for further evaluation.

Bacterial isolation

Collected samples were cultured onto blood , nutrient, brain heart infusion broth and MacConkey's agar[10,11]. All isolates were gram negative bacteria, with positive oxidase test with positive Api-20 system (bio M'rieux)[12].

Extraction of bacterial genomic DNA

Extraction of Genomic DNA of *P. aeruginosa* was done using (PrestoTM) (Geneaid. USA) according to instructions of company. Nano drop spectrophotometer was used to check the results and the extracted materials were stored in refrigerator at -20C until performance of PCR technique.

Polymerase chain reaction

This was done through the use of primers that were specific for ETA gene. These were designed through GenBank (NCBI). Pseudomonas aeruginosa isolate 6 ETA gene, partial cds GenBank: (AF227424.1) through the use of primer3 plus which was online designed. The primers toxA-F primer (CGCAAAGCATCGTCTTCGG) in addition to toxA-R primer (TTCCTGGTCC-TGGGCGTAG), were delivered by (Korea, Bioneer). Then PCR was done according to company instructions.

Interferon gamma and interleukin 18 were assessed using IFN-gamma ELISA kit (BioSource) and Il-18 ELISA kit BioSource, Belgium respectively.

Table 1. Frequency of P. aeruginosa among burned skin infection

	Positive *P. aeruginosa*	Total	%
Human burned skin infection	23	45	51.1

Table 2. Sensitivity, Specificity, Positive predictive value and Negative predictive value of bacterial culture

Test	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Bacterial culture	80.95	84.61	89.47	73.3

Table 3. Frequency of ETA among human *P. aeruginosa* isolated from patients with burned skin

	ETA	Total	%
Human *P. aeruginosa*	17	23	73.91

Table 4. Showed the frequency of human *P. aeruginosa* bacteremia in relation to ETA

ETA	Bacteremia	%
Positive(17)	9	52.94
Negative(6)	0	0

Table 5. Mean levels of interferon gamma and IL-18 among burned patients in relation to pseudomonas aeruginosa infection

	Burned patients with pseudomonas aeruginosa infection	Burned patients without pseudomonas aeruginosa infection	P-value
interferon – γ level (pg/ml)	4.91±2.13	11.58±3.11	<0.05
IL-18(pg/ml)	33.72±11.66	73.94±17.49	<0.05

RESULTS AND DISCUSSION

Table (1) showed that the frequency of *P. aeruginosa* infection was 51.1 % among human burned skin infections. This high occurrence infection rate might be due to the opportunistic nature of this pathogen that lead to its colonization on burned skin when the mechanical or the immunological defect either alone or both were breached[14]. This was consistent with[15] and inconsistent with[16] and[17] who showed that the frequency of *P. aeruginosa* was 36%. This might be due to geographical variation.

Because *P. aeruginosa* was important medically, numerous techniques had been established for quickly recognition of species of *P.aeruginosa*. Though conventional procedures to recognize *P. aeruginosa* were dependable, they need numerous days. PCR had the probable chance for microbial species identification quickly through gene sequences amplification specific to *P. aeruginosa*[18].

Table (2) showed that the sensitivity, specificity, PPV and NPV were (80.95 %, 84.61%, 89.47% and 73.3 % respectively). This might be due to true infection by *P aeruginosa* detected only by PCR(4 cases) with false negative culture results. This might be due to overgrowth of other bacteria or presence of non-cultivable organisms or mutations[18,19]. Therefore we could conclude that the PCR screening is mandatory technique to detect low number of this organisms at their initial colonization, that is a very important clinically in detection of early infection by this dangerous pathogen when there is no or intermittent colonization with this bacteria.

Table (3) showed that 17 out of 23 (73.91%) of human *P. aeroginosa* produce ETA. As the ETA was very important in *P. aeruginosa* disease process, so its high frequency in these isolates reflected that it was a prominent virulent factor for this pathogen and our finding was consistent with[20,21].

Table (4) showed that 9 out of 17 (52.94%) ETA positive *P. aeruginosa* had bacteremia while none of the ETA negative P. aeruginosa had bacteremia. This might be due to the major role of ETA inhibition of elongation factor 2 such as what happened with the diphtheria toxin that lead to death of cell[22,23] with resultant bacterial dissemination to blood. In addition to that ETA had a cytotoxic effect that extend to varied range of mammalian cells. In addition to that, ETA had the ability of inhibition of macrophage and granulocytes[24,25], inhibit the production of IL-1 and altered TNF-alpha production[26]. All of the above might indicate a major role for ETA in the *P. aeruginosa* bacteremia and death amongst patients with burned skin. This finding support

ETA	Bacteremia	%
Positive(17)	9	52.94
Negative(6)	0	0

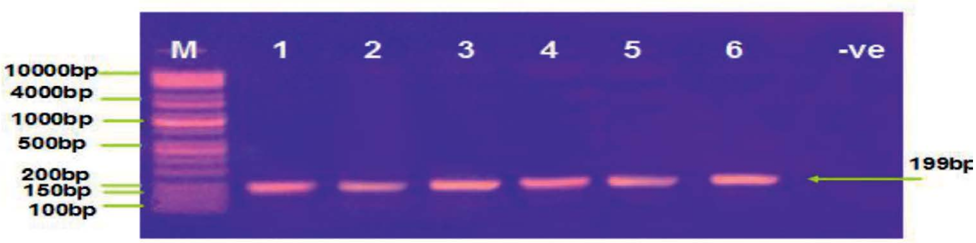

Fig. 1. Electrophoresis agar gel of PCR of *P. aeruginosa* ETA gene . Where, Lane (M) marker of DNA (100bp), Lane ((1-3)) samples that were positive in burn wound infections patients Lane (4-6) positive samples in mastitis milk samples at 199bp and Lane negative ETA gene in *P. aeruginosa* isolate.

what was mentioned by other researchers[27,28].

Table (5) showed that both interferon gamma and IL-18 was significantly decreased in burned patients with pseudomonas aeruginosa infection in comparison to their value in burned patients without pseudomonas aeruginosa infection (P value < 0.05). IL-18 might act as a stimulus for production of interferon gamma during stimulation by bacterial infection of macrophage to drive Th1 T cell development (33&34). So IL-18 might lead to host resistance through the production of interferon gamma. This was consistent with Xi Huang *et al.* 2018[35].

CONCLUSION

1. PCR screening is mandatory technique to detect low number of P. aeruginosa infection at their initial colonization, that is a very important clinically in detection of early infection by this dangerous pathogen when there is no or intermittent colonization with this bacteria.

2. As the ETA was very important in P. aeruginosa disease process, so its high frequency in these isolates reflected that it was a prominent virulent factor for this pathogen .

3. There was a major role for ETA in the P. aeruginosa bacteremia amongst patients with burned skin.

4. IL-18 might act as a stimulus for production of interferon gamma during stimulation by bacterial infection of macrophage to drive Th1 T cell development. So IL-18 might lead to host resistance through the production of interferon gamma.

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None.

CONFLICTS OF INTEREST

The authors declare that there is no conflicts of interest.

AUTHORS' CONTRIBUTION

All authors have made substantial, direct and intellectual contribution to the work and approved it for publication.

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None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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