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Detection of tox A gene in *Pseudomonas* aeruginosa that isolates from different clinical cases by using PCR

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Abstract

Current study obtained (75) isolate of *Pseudomonas aeruginosa* collected from different cases included: 28 isolates from otitis media, 23 isolates from burn infections, 10 isolates from wound infections, 8 isolates from urinary tract infections and 6 isolates from blood, during the period between 1/9/2014 to 1/11/2014

The result revealed that the *tox A gene* was present in 54 isolates (72%) of *Pseudomonas aeruginosa*. The gel electrophoresis showed that the molecular weight of *tox A* gene was 352 bp. The result shows 17 isolates (60.71%) from otitis media has *tox A* gene, 18 isolates (78.26%) from burn followed by 8 isolate (80%) from wound infection and 5 isolates (62.5%) from urinary tract infection, finally 6 isolates (100%) from blood have this gene.

Key words: - Pseudomonas aeruginosa, *tox* A gene.

1. Introduction

Pseudomonas aeruginosa are one of the opportunistic pathogens that infect humans [1]. cause many diseases that lead to the death of a person so it is necessary to follow the accurate and rapid methods in the diagnosis of this type of infection [2]. *Pseudomonas aeruginosa* cause many infections, especially in patients with an immune compromised [3] As well as get infected by respiratory infections, which have a role in the spread of *P. aeruginosa* and transmission from one person to another by tissue and hands of patients and then transmitted directly by skin and the patient's clothing, as well as injuries digestive system and water and food pollution by carriers of the pathogen [4]. *P. aeruginosa* increased resistance to a variety of chemical compounds, including antibiotics, detergents and disinfectants [5]. These bacteria can be stay in disinfectants, liquid medicines, as well as on the floor of the hospitals and this has helped to increase the infection of bacteria in patients which are asleep in hospitals, especially in intensive care unit [6]. This bacterium has many virulence factors including external toxin type A is a highly toxic protein, which is one of the factors which are too severe and cause some deaths when excreted by the bacteria *P. aeruginosa* which cause topical and systemic disease and is linked to the occurrence of necrosis at the site of injury bacterial [7]. Exotoxin A in the inhibition of the process of protein synthesis in the host cells through the formation of the complex 5-Diphosphate Ribosyl with one elongation factors EF2 through the transfer of ADP-ribosyl of NAD to factor EF2 and thus prevents the protein chain elongation on the ribosome therefore exotoxic a mechanism similar to the work of the diphtheria toxin. But the exotoxic are present on the cell surface are different from the diphtheria toxin receptor [8]. And also to the role of this toxin to penetrate tissue, especially in patients with cystic fibrosis it found that strains that are unproductive for external toxin be less virulent strains producing [9]. *tox A* gene encodes Exotoxin A, this toxin when produced by the bacterial cell is linked to specific receptors on the surface of the host cell, which allowed to enter [10].

The aims of study to detection of gene *tox A* in *pseudomonas aeruginosa* that isolated from different clinical cases.

2. Experimental

2.1. Material and method

2.1.1.Collection of bacteria:- Collection the bacteria from different infection cases include (burn and wound infections, otitis media, urinary tract infection and blood samples) during the period between 1/9/2014 to 1/11/2014

2.1.2. Identification of bacteria: - Identification the isolates by culturing on media culture include:- MacConkey agar, Blood agar, Cetrimide agar, Pseudomonas agar and CHROMagar Orientation then identified by using biochemical tests including (oxidase and catalase test) and further identification by using a API20E system [11].

2.1.3. Isolate of DNA: - Use DNA kit to extract DNA of bacteria isolates (Geneaid Biotech kit system, UK) according to the manufacturer's instructions.

2.1.4. Detection of *tox A* gene: -To detection *tox A* gene of *P. aeruginosa* isolates by using the primer in this study:-Primer sequence(5′–3′)F (GGTAACCAGCTCAGCCACAT) R (TGATGTCCAGGTCATGCTTC) size 352bp, Lanotte *et al*. (2004)

Detection of gene *tox A* attended the solution concentration of 10 pekmole / µl (by taking 10 µl from Stock solution and addition of 90 µl of distilled water Alloaona) save the Stock solution under - 20 ° C . Using polymerase chain PCR interactive and conditions described below interaction. Program step 1 (Initial denaturation at 94ºC for 3 min 1 cycle), step 2 (30 cycles) A:- Denaturation DNA template at 94 °C for 30 sec, B:- Annealing at 55 ºC for 1 min, C:- Extension at 72 ºC for 1 min . step 3 (A final extension at 72 ºC for 5 min 1 cycle) [12]

2.1.5. Separation of DNA bands: - PCR products were separated on a 2% agarose with 5µl Ethidium bromide, at 50 volt for 2 hour. Using 100 bp Ladder. The DNA bands were visualized and photographed under UV light [13].

3. Result and discussion

We obtained (75) isolate of *Pseudomonas aeruginosa* collected from different cases included : 28 isolates from otitis media, 23 isolates from burn infections, 10 isolates from wound infections, 8 isolates from urinary tract infections and 6 isolates from blood .

The result revealed that the *tox A gene* was present in 54 isolates (72%) of *Pseudomonas aeruginosa*. The gel electrophoresis showed that the molecular weight of *tox A* gene was 352 bp. The result shows 17 isolates (60.71%) from otitis media has *tox A* gene , 18 isolates (78.26%) from burn followed by 8 isolates (80%) from wound infection and 5 isolates (62.5%) from urinary tract infection , finally 6 isolates (100%) from blood has this gene is shown in table (1) and figure(1). This study was similar with [14] who showed 73% of her isolated was *tox A* and does not have an agreement with 15 who found 81.5% of *P . aeruginosa* that isolated from wound swab and cystic fibrosis patent has *tox A* gene .As well as Nikbin *et al.* [16] found 90% of the isolates of this bacteria own *tox A* gene This high rate is mostly due to the isolates obtained source was one of the swabs burns and this result agrees with the results of the current study that isolate 23 from burns swabs and all of the isolates has *tox A* 100%.

The *tox A* gene encodes Exotoxin A, a highly toxic proteins and has a lot to do with the occurrence of Necrosis at the site of infection with the bacterium The danger of this toxin in the inhibition of the process of protein synthesis [8] Also, for this toxin role to penetrate the tissue process, particularly in patients with cystic [9].

Table (1): Number and percentage of *tox A* gene of *P. aeruginosa*

No.	Sources	No. of *tox A* positive isolates	*tox A* (%)
1	Otitis media	28	17 (60.71)
2	Burn	23	18 (78.26)
3	Wound	10	8 (80)
4	UTI	8	5 (62.5)
5	Blood	6	6 (100)
Total		75	54 (72)

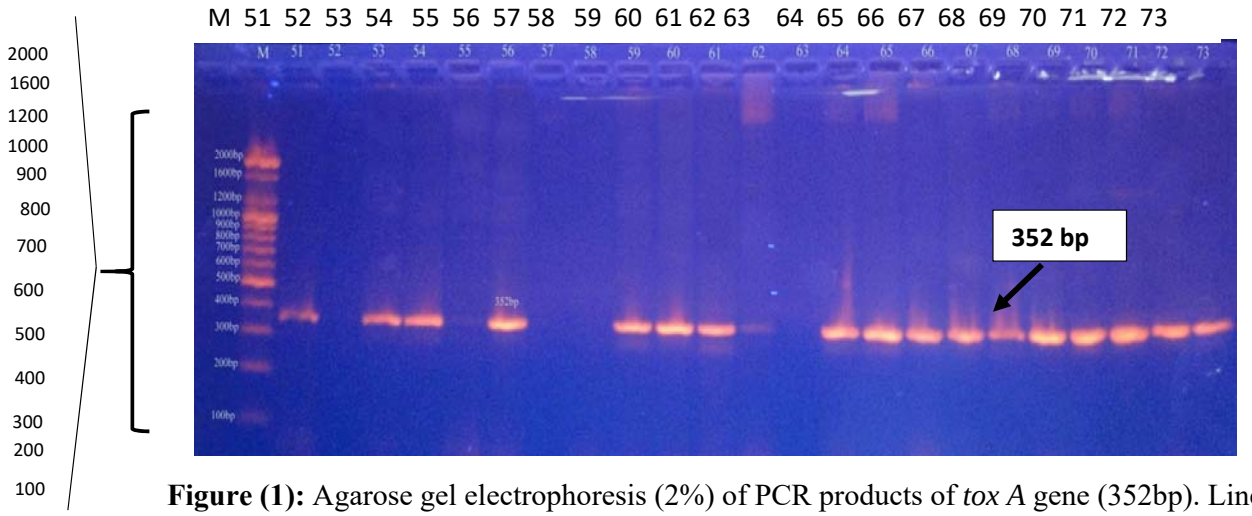

Figure (1): Agarose gel electrophoresis (2%) of PCR products of *tox A* gene (352bp). Line M: DNA marker (100bp -2000 bp ladder, Promega, USA); Lanes 51, 53,54,56,59,60,61, 64, 65,66,67,68,69,70,71,72,73 *Pseudomonas aeruginosa* PCR-positive isolates.

4. Conclusions

The results of detection of virulence genes *tox A* was found in 72 % of *Pseudomonas aeruginosa* isolates. This gene encodes Exotoxin A, a highly toxic proteins and has a lot to do with the occurrence of Necrosis at the site of bacterium infection.

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