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toxA gene as a chromosomal marker for rapid identification of Otitis media *Pseudomonas aeruginosa*

Ibtesam Ghadban AUDA, Israa M.S. AL-KADMY, Alaa Naseer MOHAMMED ALI, Sahira Nsayef MUSLIM & Istabreq Muhammed ALI SALMAN*

Department of Biology, College of Science, Al-Mustansiriya University, Baghdad, Iraq.
*Corresponding author: israaalkadmy@gmail.com; Tel: +9747705988195.

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Abstract

Exotoxin A (ETA) is a powerful chromosomal extracellular virulence factor produced by most of clinical P. *aeruginosa* isolates. Aims of this study is that, identification of aerobic acute and chronic causative agents of otitis media and test whether the chromosomal *toxA* gene can be used as marker for rapid identification of Pseudomonas *aeruginosa*. Ear swabs were taken from 49 patients complaining of symptoms of otitis. Patient's age ranging from 6 months to 85 years, 25 males and 24 females. Ear swabs were collected in period from January to April 2010. Bacterial causative agents were identified. Chromosomal DNA of *Pseudomonas aeruginosa* isolates was extracted and subjected to PCR to amplify *toxA* gene. Staphylococcus *aureus* and Streptococcus *pneumonaie* were the bacterial causative agents of acute otitis media while P. *aeruginosa* and S. *aureus* were predominant in chronic otitis media. *E. coli* and *Proteus* spp. and *Enterobacter* spp were also identified. Amplification of *toxA* showed that 23(100%) of P. *aeruginosa* isolates were positive and were chromosomal encoded. As a conclusion, Staphylococcus aureus and Streptococcus *pneumonaie* were predominant bacterial causative agents of acute otitis media while P. *aeruginosa* and S. *aureus* were predominant ones in chronic otitis media. P. *aeruginosa* Exotoxin A is a chromosomal encoded feature and can be used as a marker in identification of P. *aeruginosa* isolates by molecular methods.

Introduction

Otitis media is inflammation of the lining of the middle ear and one of the most common infections in childhood (Springhouse, 2005). Gram-negative organisms are predominant (Oyeleke, 2009). In acute otitis media, it commonly develops in association with an infection of the upper respiratory tract that extends from the nasopharynx to the middle ear through the eustachian tube (Springhouse, 2005). The principal organisms isolated from patients with chronic otitis media are *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Ghosh *et al.*, 2002; Ghosh, 2006).

Exotoxin A (ETA) is considered one of the most powerful extracellular virulence factors produced by P. *aeruginosa*. The 68 kDa ETA protein, encoded by *toxA*, is an ADP- ribosyl transferase that irreversibly inhibits protein synthesis in eukaryotic cells causing cell death (Todar, 2008). Pseudomonas Exotoxin A is a bacterial toxin that arrests protein synthesis and induced a rapid and dose-dependent induction of apoptosis. Exotoxin A cause mitochondrial permeability, and there is differences in killing were due to steps after the ADP-ribosylation of EF2 (Du *et al.*, 2010).

Patients, Materials and Methods

Ear swabs were taken from 49 patients complaining of symptoms of otitis. Patient's age ranging from 6 months to 85 years, 25 males and 24 females. Ear swabs were collected in period from January to April 2010. Ear swabs were inoculated on blood, chocolate and MacConkey agar plates. All obtained isolates were identified using biochemical tests and according to Forbes *et al.* (2002).

Chromosomal DNA was extracted from all isolates using commercially available DNA extraction kit (Promega-USA). Chromosomal and PCR products DNA were resolved by horizontal agarose gel electrophoresis. Agarose at concentrations of 1.5% and 1% was prepared for PCR products and chromosomal DNA electrophoresis, respectively (Sambrook & Russell, 2001).

The occurrence of *toxA* gene of exotoxin A was detected on the chromosomes of P. *aeruginosa* via PCR procedure using oligonucleotide primers (Palka-Santini *et al*., 2009). Table 1 shows the sequence and molecular weight of PCR products of *toxA* gene.

Table 1: Oligonucleotide primer sequence and amplicon size of toxA gene of P. aeruginosa .

gene	Sequence of forward Primer	Sequence of reverse primer	Product
toxA	5′-GTGCGCTACA GCTACACG-3′	5′-CTTGCCTTC CCAGGTATC-3′	417bp

Go–Taq green master mix kit (Promega-USA) was used for toxA gene amplification. In an Eppendorf reaction tube, 25 µl master mix was prepared for each test. A master mix contained the following components (according to the manufacturer instruction): 12.5 µl of Go-Taq green master mix, 1.5 µl of each primer (10µM each), 8.5 µl of nuclease free distilled water and 1µl of DNA template (20µg). The cycling was performed using protocol comprising an initial denaturing step at 94°C for 3 minutes, followed by 32 cycles of 94°C for 30 seconds, 57°C for 45 seconds and 72°C for 1 minute (Palka-Santini *et al*., 2009).

Result

Table 2: Aerobic bacterial causative agents of acute otitis media.

Bacteria	No.	Percentage (%)
Staphylococuccus auerus	1	8.33
Streptococcus pneumoniae	1	8.33
No growth	10	83.34
Total	**12**	**100**

Out of 49 patients, 12 patients were diagnosed with acute upper respiratory tract infections and 37 of them were suffering from chronic otitis media. Only 2 ear swabs out of 12 the patients with acute upper respiratory tract infections showed growth of Staphylococcus *aureus* and Streptococcus

pneumonaie. The rest, 10 cases, showed no bacterial growth (Table 2). Culture of ear swabs taken from patients with chronic otitis media showed that 23 isolates were P. *aeruginosa*, 11 were S. *aureus*, two for each *E. coli* and *Proteus* spp. and one *Enterobacter* spp. Some of swabs showed growth of more than one causative agent (Table 3).

Table 3: Aerobic bacterial causative agents of chronic otitis media.

Bacteria	No.	Percentage (%)
Pseudomonas aeruginosa	23	59
Staphylococuccus auerus	11	28.2
E. coli	2	5.12
Proteus spp	2	5.12
Enterobacter spp	1	2.56
Total	**39**	**100**

Chromosomal DNA was extracted from P. *aeruginosa* and electrophoresed, Figure 1 shows agarose gel electrophoreticogram of chromosomal DNA extracted from P. *aeruginosa* isolates. On the left 1000pb molecular marker, other bands represent the chromosomal DNA of P. *aeruginosa* isolates. Electrophoresis was carried out in 1% agarose gel supplied with Ethidium bromide at (5V/cm) for 60 minutes. The number of P. *aeruginosa* isolates that show positive chromosomal *toxA* gene were 23(100%), while negative result was 0 (0%) in Table 4.

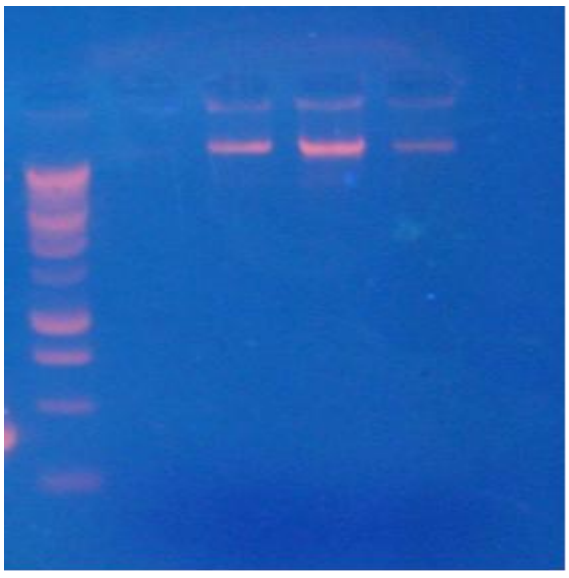

Figure 1: Agarose gel electrophoreticogram of chromosomal DNA

Table 4: Numbers and percentages of occurrence of *toxA* gene on chromosomes of *Pseudomonas aeruginosa* isolates.

Gene	No. of positive isolates	% of positive isolates	No. of negative isolates	% of negative isolates
toxA	23	100	0	0

Figure 2 shows agarose gel electrophoreticogram of *toxA* (417bp) PCR products (amplicons). As shown in this figure, all lanes reveal the band of interest. Lane 1:100bp DNA ladder. Lanes 2-5: toxA gene amplicon (417bp). Electrophoresis was carried out in 1.5% agarose gel supplied with Ethidium bromide at (7V/cm) for 90 minutes.

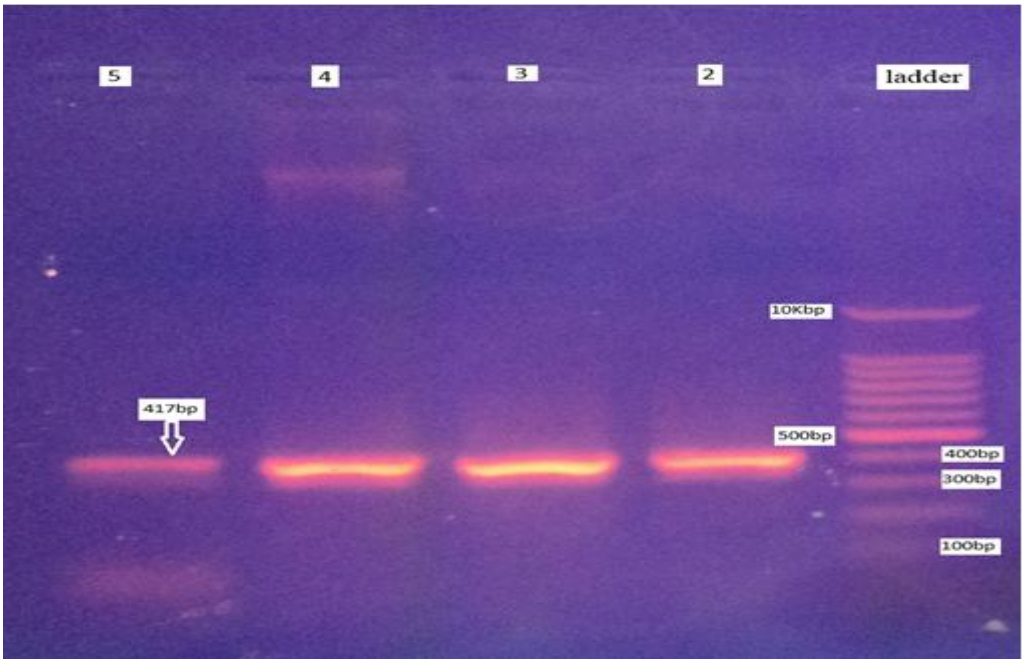

Figure 2: Agarose gel electrophoreticogram of *toxA* PCR products.

Discussion

The results of identification of otitis media causative agents are agree with other researchers' finding, hence they found that the causes of acute otitis media include infection with a cold virus or influenza virus or infection with the bacteria *Streptococcus pneumoniae* (Springhouse, 2005) . These finding explain the negative results in most of the acute otitis media in this study. Other researchers mention the same finding when he separates the causative agents of both acute and chronic otitis media. The researcher found that, the organisms often isolated in cases of acute otitis media as causative

organisms are: *Haemolytic streptococci*, *Staphylococci*, *Heamophilus* or *Pneunococci* while Gram negative bacilli are commonly associated with chronic otitis media particularly *Pseudomonas* and *Proteus* sp (Oyeleke, 2009).

Pseudomonas aeruginosa was predominant among causative agents of chronic otitis media in this study and all of them possess exotoxin A determinants are located on the chromosome. Product of this gene causes extensive tissue damage (Hirakata *et al*., 2002; Gaines *et al*., 2007).

As the identity of the bacteria is very crucial in the treatment, a rapid method to identify the bacteria is desired. The molecular technique for detection of exotoxin A using PCR has been evaluated for identification of this bacteria and shortened the time of identification to less than four hours (Song *et al*., 2000; Xu *et al*., 2004) and because of that toxA gene is a common gene in *P. aeruginosa*, this gene can be used as marker to identify this type of bacteria by molecular techniques as described previously (Gaines *et al*., 2007).

Conclusion

Staphylococcus aureus and *Streptococcus pneumonaie* were predominant bacterial causative agents of acute otitis media while *P. aeruginosa* and *S. aureus* were predominant ones in chronic otitis media. *P.aeruginosa* exotoxin A is a chromosomal encoded feature and can be used as a marker in identification of *P. aeruginosa* isolates by molecular methods.

References

Du, X., Youle, R. J., FitzGerald, D. J. & Pastan, I. 2010. Pseudomonas exotoxin A- mediated apoptosis is Bak dependent and preceded by the degradation of Mcl-1. *Mol. Cell. Biol*. **30**(14): 3444-3452.

Forbes, B. A., Sahm, D. F. & Weissfeld, A.S. 2002. *Diagnostic Microbiology*. (10th edition). Mosby. USA.

Ghosh, S., Panarese, A., Parker, A. J. & Bull, P. D. 2000. Quinolone eardrops for chronic otitis media. *BMJ*. **321**:126-127.

Gaines, J. M., Carty, N. L., Tiburzi, F., Davinic, M., Visca, P., Colmer-Hamood, J. A. & Hamood, A.N. 2007. Regulation of the Pseudomonas aeruginosa toxA, regA and ptxR genes by the iron-starvation sigma factor PvdS under reduced levels of oxygen. *Microbiol.* **153**(12): 4219 - 4233.

Hirakata, Y., Srikumar, R., Poole, K., Gotoh, N., Suematsu, T., Kohno, S., Kamihira, S., Hancock, R. E. W. & Speert, D. P. 2002. Multidrug efflux systems play an important role in the invasiveness of Pseudomonas aeruginosa. *J. Experim. Med*. **196**(1): 109-118.

Oyeleke, S. B. 2009. Screening for bacteria agents responsible for otitis media and their antibiogram. *African J Microbiol Res.* **3**(5): 249-252.

Palka-Santini, M. Cleven, B. E., Eichinger, L., Krönke, M. & Krut,O. 2009. Large scale multiplex PCR improves pathogen detection by DNA microarrays. *BMC Microbiol*. **9**:2180-2189.

Song, K. P., Chan, T. K., Jil Z. L. & Wong, S. W. 2000. Rapid identification of Pseudomonas aeruginosa from ocular isolates by PCR using exotoxin A-specific primers. *Mol. Cell. Prob.* **14**: 199-204

Sambrook, J. & Russell, D. 2001. *Molecular Cloning: Laboratory Manual*. (3rd edition). Cold Spring Harbor, New York, USA.

Springhouse, S. 2005. *Professional Guide to Diseases*. (8th edition). Lippincott Williams and Wilkins.

Todar, K. 2008. *Todar´s Textbook of bacteriology.* (1st edition). Madison and Wisconsin.

Wideman, W. L. 2006. Pseudomonas aeruginosa otitis media and interna in a chinchilla ranch. *Can Vet J.*, **47**(8): 799–800.

Xu, J., Moore, J., Murphy, P. G.; Millar, B. C. & Elborn, J. S. 2004. Early detection of Pseudomonas aeruginosa – comparison of conventional versus molecular (PCR) detection directly from adult patients with cystic fibrosis. *Ann Clin Microbiol Antimicrob*. **3**: 21-25.