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Genotypic characterization on antibiotic resistant genes *of Staphylococcus aureus* isolated from conjunctivitis patients

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Abstract:

Bacterial conjunctivitis has a worldwide distribution, affecting persons of all ages and both genders.The study aimed to genotyping determination of antibiotic resistance genes in *S.aureus* .

Thus,40 conjunctival swabs were collected from patients suffering from conjunctivitis were attended to Hospital in Al-Najaf province.The PCR amplification results for the predominance of *S. aureus* antibiotic resistance genes show that :10 (24.39 %) of isolates have Neomycin resistances genes (*neo*) , While 5 (62.5%) and 3 (75 %) of isolates showed Gentamycin resistance gene *(Tn1696 aacC1)* and Chloramphenicol resistance genes *(Tn9 cat)* respectively. *Staphylococcus aureus* is the common Bacterial predominance in conjunctivitis and appear a variable susceptibility for common antibiotics used in the treatment of conjunctivitis .

Keyword: *S.aureus* ,antibiotics resistance genes, *neo* gene.

Introduction

Conjunctivitis a common external eye problem that involves inflammation of the conjunctiva, it is usually associated with symptoms of a red eye , there is a common non-traumatic disease of the eye characterized by pain, conjunctival hyperemia and discharge , watery or pus-like. In addition to itching, stinging, or a scratching sensation are similar to what one would experience if sand was blown into the eyes,

conjunctivitis is most commonly called pink eye when small blood vessels in the conjunctiva become inflamed; they're more visible, both eyes are usually involved; although one may become affected before the other (Randall *et al* ., 2018). Bacterial conjunctivitis is the most common infectious etiology of conjunctivitis in adults and pediatric patients, clinical presentation may be nonspecific and bacterial conjunctivitis can be confused with viral and even with noninfectious sources ,the most common cases of bacterial conjunctivitis are members of *Staphylococcus aureus* , *Streptococcus pneumonia* , *Haemophilus influenzae* and *Moraxella catarrhalis* (Sharma S , 2011) Treatment is often based on clinical suspicion that the conjunctivitis is bacterial, without waiting for the results of microbiological tests, bacterial conjunctivitis in contact lens wearers is of particular concern because of the risk of bacterial keratitis an infection of the cornea accompanying acute or sub-acute corneal trauma. (Keen *et al* ., 2017) .

Mostly, primary eye care providers start the treatment of external ocular infection before the causative microorganisms have been identified, or submitted to antibiotic susceptibility tests, consequently broad-spectrum antibiotics are routinely used in the treatment of bacterial conjunctivitis, of the many antibiotics in use is the group of Fluoroquinolones , particularly Ciprofloxacin, Ofloxacin and Norfloxacin, fluoroquinolones groups were more effective in the treatment of ocular infections than some other broad-spectrum antibiotics e.g. Gentamycin, Chloramphenicol, Tobramycin, Erythromycin and Tetracycline (Sharma *et al* ., 2014) .

Materials and Methods

The study was done at Laboratories of Bacteriology and Molecular in Biology Department, Faculty of Sciences, University of Kufa , Iraq.

Specimens collection and bacterial identification

Eye infection using as a source of 40 samples using in this study with clinical suspicion of Conjunctivitis who attended different hospitals in Al-Najaf provenance according to ethical approval of ministry of Iraqi health . Each specimen was inoculated on culture of selective media namely mannitol salt agar, then inoculated at 37°C for 18-24 hours (Cheesbrough, 2010).

DNA Extraction

Genomic DNA was extracted by using a commercial extraction system (Genomic DNA promega Kit).

Molecular Identification

The PCR assay was performed to detect the *neomycin, chloramphenicol and gentamycin antibiotic* genes for *S. aureus* shown in table (2). These primers were produced by Alpha DNA Company, Canada as in table (1). The amplified PCR products were detected by agarose gel electrophoresis was visualized by staining with ethidium bromide. The electrophoresis result was detected by using gel documentation system. The positive results were distinguished when the DNA band base pairs of sample equal to the target product size (Bartlett and Stirling, 1998). Finally, the gel was photographed using Biometra gel documentation system.

Table (1): Primers used in this study

Primer Type	Target Genes *s . aureus*	Encodes	DNA sequence (5′–3′)	Product Size (bp)	Reference
Tn1696 aacC1	*Tn1696 aacC1*	Resistance to Gentamicin.	F: CGAATCCATGTGGGAGTTTA R: TTAGGTGGCGGTACTTGGGT	616	Verdier *et al.*,(2007)
Tn9 cat	*Tn9 cat*	Resistance to Neomycin.	F: TGAGACGTTGATCGGCACGT R: ATTCAGGCGTAGCACCAGGC	822	Carla *et al.*,(2001)
neo	*neo*	Resistance to Chloramphenicol.	F: ACCTTGCTCCTGCCGAGAAAGTAT R: ATGTTTCGCTTGGTGGTCGAATGG	300	Hou *et al.*, (2012)

Table (2): PCR programs of primers that apply in the thermocycler

Gene	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension	Cycles
Tn1696 aacC1	95 ˚C for 5min	94 ˚C for 1 min	55 ˚C for 1min	72˚C for 1min	72 ˚C for 5min	30
Tn9 cat	95 ˚C for 5 min	94 ˚C for 30 sec	58 f ˚C or30 sec	72˚Cfor 1min	72 f or 5 min	29
neo	95 ˚C for 5 min	94 ˚C for 45 sec	59˚C for 45 sec	72˚Cfor 90 sec	72 ˚C for 7 min	35

Results and Discussion

Molecular detection of *neomycin, chloramphenicol and gentamycin antibiotic* genes

The PCR technique was used to investigate the predominance of antibiotics genes among *S. aureus* isolates .The PCR ampliphication results revealed that the *neo* gene (300bp) encoding for Neomycin resistance was found in 24.39% of isolates, as in figure (1).

Momtaz *et al*. (2013) pointed out that the frequency of gene resistance to Neomycin was observed in 60% of the isolates ,there was a diminished vulnerability to Neomycin in the greater part of the detaches, this could be because of the specific weight which happened for the delayed use of Neomycin either as bolster added substances or for the treatment of contamination. In addition , (Gomez- Sanz *et al* ., 2013) Reported in his study that *S. aureus* strain possess two plasmids pUR1902 and pUR2941 that carrying Kanamycin /Neomycin resistance gene .

The amplification results of PCR technique for *Tn1696aacC1* (616bp) (which encoded for Gentamycin resistance) , revealed that 62.5% of *S.aureus* isolates gave positive result for *Tn1696aacC1* figure (3).

Martinez *et al.*,(2004) noted that all separates of *S. aureus*, which are secluded from patients with bacterial conjunctivitis, are touchy to

Ciprofloxacin and Gentamycin, and 94.7%of the segregates are delicate to antibiotic medication.

Silverman and Bessman (2003) have demonstrated that Gentamycin is compelling against *S. aureus* strains, and it is utilized as a salve for the treatment of bacterial conjunctivitis. One examination detailed that the greater part of the bacterial separates was vulnerable to Chloramphenicol , Ciprofloxacin and Gentamicin.

The amplification results of PCR study for *Tn9cat* (822bp) (that encoding Chloramphenicol resistance) revealed that 75% of *S. aureus* isolate gave positive for *Tn9cat gene,* as in figure (3).

One case of non-mutational obstruction is the procurement of the characteristic cfr (Chloramphenicol-Florfenicol opposition) quality, which is a plasmid-conveyed quality encoding a protein which catalyzes the posttranscriptional methylation of the C-8 molecule of a key buildup (A2503) in the 23S rRNA (Grove *et al* ., 2013) .

Chloramphenicol and Thiamphenicol, it indicates action against numerous gram-positive and gram-negative microscopic organisms, the bacterial protection from Chloramphenicol and Thiamphenicol is most generally interceded by mono-and diacetylation by means of Chloramphenicol acetyltransferase (CAT) catalysts. Because of the substitution of the hydroxyl assemble at position C-3 with a fluorine buildup, the acceptor site for acetyl bunches was basically adjusted in Florfenicol, this change rendered Florfenicol impervious to the inactivation by CAT chemicals, and subsequently, Chloramphenicol-safe strains, in which opposition is exclusively in view of CAT movement, are helpless to Florfenicol (Schwarz *et al* ., 2005).

As of late appeared to code for a rRNA methylase which intervenes consolidated protection from Chloramphenicol, Florfenicol, and

Clindamycin by methylation of the 23S rRNA at position A2503 .Antibiotic weakness of 30 *S. aureus* detaches uncovered shifting degrees of powerlessness designs against the antimicrobial specialists, for the most part, Cefoxitin 76.7% (23/30), Chloramphenicol 83.3% (Akanbi *et al* ., 2017) .

Ruiz *et al.*, (1993) have pointed that Chloramphenicol and Erythromycin are the most specialists dynamic against *S. aureus* strains separated from patients with conjunctivitis, aside from Penicillin. One investigation announced that the majority of the bacterial disconnects were vulnerable to Chloramphenicol, Ciprofloxacin and Gentamicin. (Lohr and associates ,1988) discovered that Trimethoprim– Polymyxin , Gentamycin, and Sodium Sulfacetamide are to be similarly powerful in treating bacterial conjunctivitis.

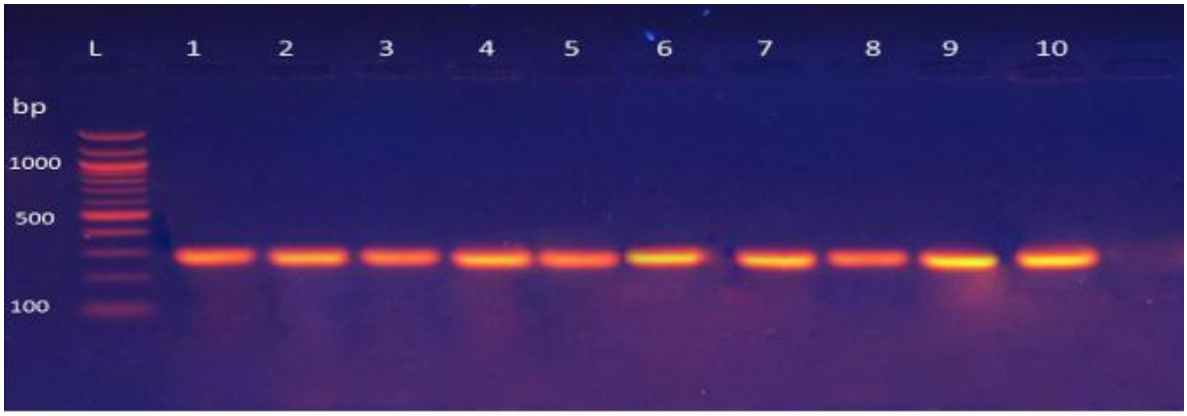

Figure:(1) : Ethidium bromide-stained agarose gel electrophoresis of PCR products from extracted total DNA of *S. aureus* using primer *neo* with product 300 bp. The electrophoresis was performed at 70 volt for 1.5-2hr. lane (L), DNA molecular size marker (100 bp ladder). Lanes (1 to10) show positive results with gene *neo*.

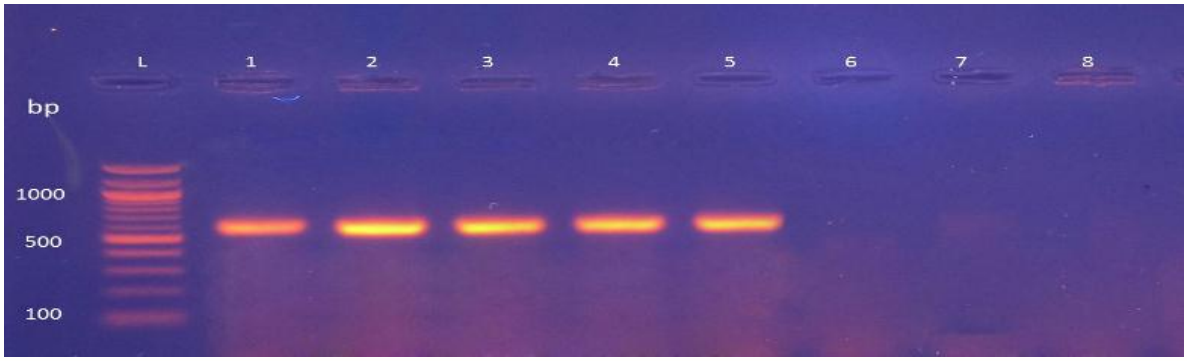

Figure:(2) : Ethidium bromide-stained agarose gel electrophoresis of PCR products from extracted total DNA of *S. aureus* using primer *Tn1696aacC1* with product 616 bp. The electrophoresis was performed at 70 volt for 1.5-2hr. lane (L), DNA molecular size marker (100 bp ladder). Lanes (1 to 5) show positive results with gene *Tn1696aacC1*.

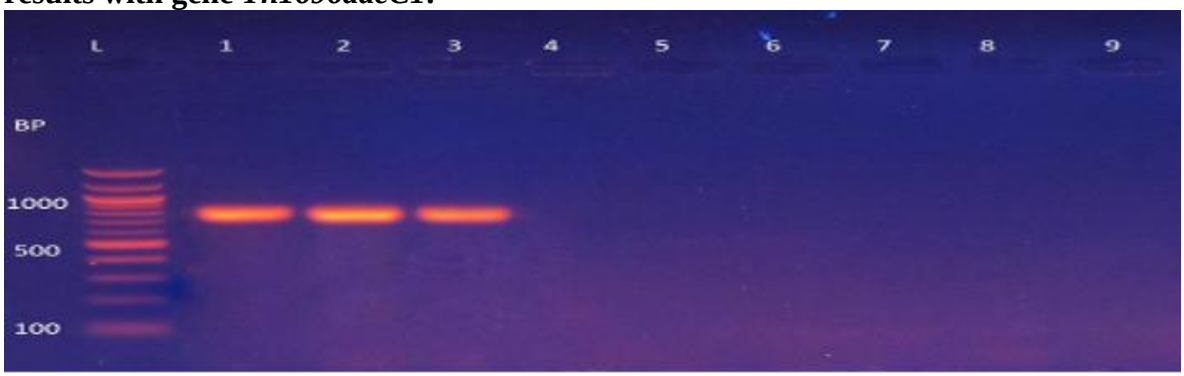

Figure:(3) : Ethidium bromide-stained agarose gel electrophoresis of PCR products from extracted total DNA of *S. aureus* using primer *Tn9cat* with product 822 bp. The electrophoresis was performed at 70 volt for 1.5-2hr. lane (L), DNA molecular size marker (100 bp ladder). Lanes (1 to 3)show positive results with gene *Tn9cat.*

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