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PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF GENTAMICIN RESISTANCE IN *STAPHYLOCOCCUS AUREUS*

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Abstract

This study was carried out to evaluate Gentamicin susceptibility patterns and the *aacA-aphD* gene in *staphylococcal aureus*. Three hundred various clinical samples of patients obtained from the main Hospital in Maysan Province during a period from October 2018 till March 2019, were subjected to routine laboratory culturing methods and susceptibility patterns were determined according to CLSI guideline, where (103) samples were identified as *S. aureus*. The result of Gentamicin susceptibility patterns revealed that 37(35.9%) of *S. aureus* were resisted. Whilst the result of Polymerase Chain Reaction (PCR) showed that all of *S. aureus* were carried the *aacA-aphD* gene. So the study went to concluded the high rates of prevalence of *aacA-aphD* gene among identified *S. aureus* and distinct differences between Gentamicin susceptibility pattern and molecular detection by PCR which indicated that the PCR may play a role as a golden tool in determine the high resistance which might pass undetectable during the phenotypic test. Which in turn lead to reduce the chance of available treatment especially through the intensive care units.

Keywords : Gentamicin, *S. aureus* and *aacA-aphD* gene

Introduction

Aminoglycosides were introduced in 1944, and by the 1950s aminoglycoside-resistant strains of *S. aureus* had emerged. These drugs enter bacterial cells by energy-dependent binding to the cell wall and energy-dependent transport across the cytoplasmic membrane, finally binding to one or more ribosomal sites, thus inhibiting protein synthesis (Kumar and Singh, 2013). Resistance in staphylococci results from any of three events: a chromosomal mutation leading to altered aminoglycoside binding to ribosomes (Munita and Arias, 2016); ineffective transport of aminoglycosides into the bacterial cell, producing low-level cross-resistance to most aminoglycosides; and, most commonly (Duran *et al*., 2012) enzymic modification of aminoglycosides (Munita and Arias, 2016). In the last case, resistant strains have the aminoglycoside-modifying genes *acc*, *aph* , which code for three classes of enzymes, typically residing on transposable elements in resistant bacteria (Duran *et al*., 2012). These enzymes, the phosphotransferases, acetyltransferases, and adenyltransferases. The *aacA-aphD* aminoglycoside resistance determinant of the *Staphylococcus aureus* transposon Tn*4001*, which specifies resistance to gentamicin, tobramycin, and kanamycin (Malachowa and DeLeo, 2010). The determinant encoded a single protein with an apparent size of 59 kDa which specified both aminoglycoside acetyltransferase [AAC(6')] and aminoglycoside phosphotransferase [APH(2")] activities. Nucleotide sequence analysis of the determinant showed it to be capable of encoding a 479-amino-acid protein of 56·9 kDa (Kadlec *et al*., 2012). Analysis of Tn*/725* insertion mutants of the determinant indicated that resistance to tobramycin and kanamycin is due to the AAC activity specified by, approximately, the first 170 amino acids of the predicted protein sequence and is consistent with the gentamicin resistance, specified by the APH activity, being encoded within the C-terminal region of the protein (Alibayov *et al*., 2014). Gentamicin resistant phenotypically contained at least one of the gentamicin resistance genes [*aac*(*6'*)/*aph*(*2"*), *aph*(*3'*)-*IIIa*, *ant* (*4'*)-Ia]. Aminoglycoside modifying enzymes (AMEs) are major factors responsible for resistance to aminoglycoside in staphylococci (Wendlandt *et al*., 2013).

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Table 1: Primer used in this study.

AacA-aphD	Primer Sequence 5′ ⟶ 3′	Product bp	Reference
F	TAATCCAAGAGCAATAAGGGC	227	Strommenger *et al.,* 2003
R	GCCACACTATCATAACCACTA		

Table 2: Comparison between phenotypic and genotypic patterns of identified *S.aureus.*

Antibiotic	Gene name	Phenotypic resistance pattern No(%)	Genotypic resistance pattern No(%)
Gentamicin	*AacA-aphD*	37(35.9%)	103(100)

The most commonly found AME is *aac*(*6*')/*aph*(*2*"). The bifunctional enzyme *aac*(*6*')/*aph*(*2*") is encoded by the *aac*(*6*')/*aph*(*2*") gene (Partridge *et al*., 2018). Chemically modify the aminoglycosides, which either interferes with drug transport or the binding of the drug at the site of antibacterial action, the 30S ribosomal subunit (Smith and Baker, 2002). The structures of several members of the aminoglycoside-modifying enzyme family are now known, and it is hoped that through a better understanding of these enzymes (Jana and Deb, 2006), both from a structural and mechanistic view-point, could lead to the development of either rationally-designed novel aminoglycosides, or specific structure-based enzyme inhibitors. Such developments could help to bring these compounds back to the forefront of modern antimicrobial chemotherapy (Shakil *et al* ., 2008).

Materials and Methods

To estimation the prevalence of Gentamicin resistance in Misan Provence/Iraq, 103 of *Staphylococcus aureus* which were isolated by using conventional standard bacteriology test (MacFaddin, 2000) and finally confirmed by Vitek 2 system as *S. aureus* were subjected to disk diffusion method (Bauer *et al* ., 1966) as described by CLSI (2017) and the results were interpreted according to CLSI guideline. The molecular detection of *AacA-aphD* gene was performed by using Polymerase Chain Reaction (PCR) technique, the primer used to amplify the target (*AacA-aphD*) gene as described by Strommenger *et al*., (2003) as showing in the table 1.

All the chromosomal DNA were extracted according to Prsto ™ Mini g DNA Bactria Kit protocol (Geneaid, Taiwan). PCR amplification was performed for the detection of the Gentamicin resistance gene (*AacA-aphD*), as described by Strommenger *et al*., (2003). Included an initial denaturation of DNA at 94 Cº for 5 minutes; followed by 35 cycles each of Denaturation at 94 Cº for 30 seconds; Annealing at 55 Cº for 30 sec and Extension at 72C° for 30sec, followed by the Final extension at 72Cº for 4 minutes and Hold at 4C°. to visualized the amplicon (227 bp), 1.4% Agarose was used which stained with 5µl (0.5µg/ml) of Ethidium Bromide, an electrophoresis was performed at 65 Volts for 1 hour and finally visualized by using gel documentation (Mishra *et al*., 2013).

Results

Staphylococcus aureus is one of the most common human pathogens with the ability to cause a wide range of infections, Out of 300 samples (103) of isolates were identified as *S. aureus* and then examined toward Gentamicin the results revealed that the rate of frequency of resistance was 37(35.9%). The antibiotic resistance occurs when the targeted microorganism no longer remains susceptible to treatment and survives exposure to one or more antibiotics these resistant bacteria continue to grow and multiply even in the presence of therapeutic levels of antibiotic.

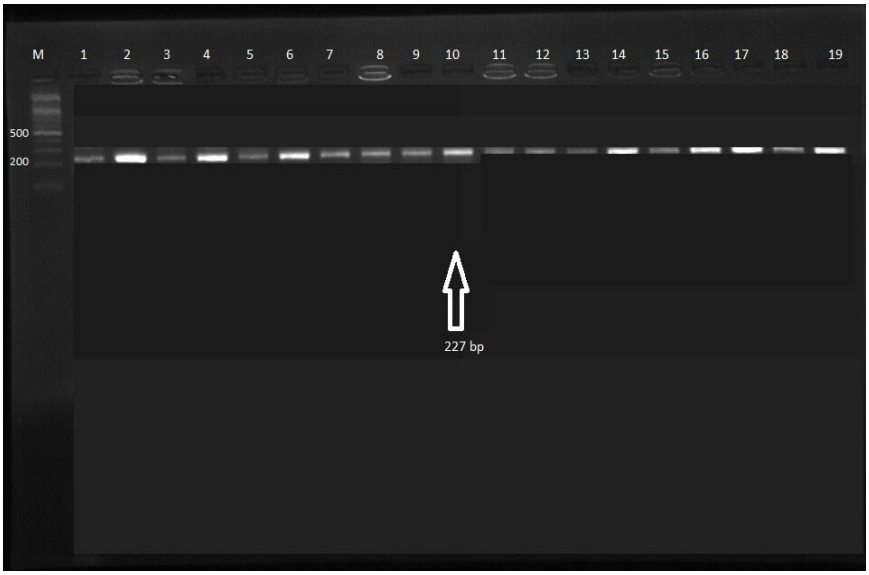

Fig. 1: Ethidium bromide-stained Agarose Gel Electrophoresis of *aacA-aphD* gene of *S.aureus* isolates, Lane M DNA marker (100bp), lane (1-19) refer to the isolates that have *aacA-aphD* gene (227bp).

The *aacA-aphD* gene, coding for a bifunctional enzyme and conferring cross-resistance to clinically used aminoglycosides such as gentamicin, tobramycin, kanamycin, and when over expressed amikacin. In this study, the *AacA-aphD* gene was occurred in all of identified *S. aureus* as showed in the table 2.

Discussion

Gentamicin antibiotic that used in this study, inhibit the protein synthesis by irreversibly binding to 30s subunit of the bacterial ribosome this binding leads to a mistranslation of protein. In general, the resistance mechanisms of aminoglycosides in *S .aureus* can be occured by (i) reducing the aminoglycosid entrance, (ii) ribosomal alteration and (iii) modification of the antibiotic by enzymatic action results from low ribosomal affinity (Kohanski *et al*., 2010). The results of this study appeared that the gentamicin resistance rate was 37(35.9%) this finding was somewhat similitude to Dey *et al*., (2013) who found that the resistance rate was (34%)**.** Whilst the result differ with Al-Dahbi *et al*., (2013); Argudín *et al*., (2015) and Al-Dulimi (2015) they reported resistance rates to gentamicin were (40%), (55.56%) and (29.3%) respectively, while other studies had been conducted to evaluate of resistance rate was 23% (Schmitz *et al*., 1999). In the genotypic study used the most common gentamicin resistance gene (*aac-aphD*) gene the study results revealed that all *S.aureus* carried *aacA-aphD* gene as shown in fig. 1.

This result was closely related to the study of Vakulenko *et al*., (2003) who found that all isolates contains this gene and Yýldýz *et al*., (2014) who found that the rate of Gentamicin resistance gene was in (96%), While the result was disagreement with Kao *et al*., (2000); Ida *et al.,* (2001) and Argudín *et al*., (2015) who they reported that the *AacA-aphD* gene was only in (45), (5%) and (20.9%) respectively.

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