PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF GENTAMICIN RESISTANCE IN STAPHYLOCOCCUS AUREUS

Amany Menam Mtsher¹, Zahid Sadoon Aziz²*

Department of Biology, College of Science University of Misan, Maysan, Iraq

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 Tn4001, 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).

*Author for correspondence: E-mail: dr.aziz_zoomobiology@uomisan.edu.iq
The most commonly found AME is \(\text{aac}(6'/\text{aph}(2'')\). The bifunctional enzyme \(\text{aac}(6'/\text{aph}(2'')\) is encoded by \(\text{aac}(6'/\text{aph}(2'')\) 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

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Gene name</th>
<th>Phenotypic resistance pattern No(%)</th>
<th>Genotypic resistance pattern No(%)</th>
</tr>
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<tbody>
<tr>
<td>Gentamicin</td>
<td>(\text{AacA-aphD})</td>
<td>37(35.9%)</td>
<td>103(100)</td>
</tr>
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</table>

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**Results**

*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 72Cº for 30sec, followed by the Final extension at 72Cº for 4 minutes and Hold at 4Cº. 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).

![Gel Electrophoresis](image)

**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).
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 occurred by (i) reducing the aminoglycoside 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); Argudin 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 Yydýz 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 Argudin et al., (2015) who they reported that the AacA-aphD gene was only in (45), (5%) and (20.9%) respectively.

References


