

LINEZOLID RESISTANCE AMONG *STAPHYLOCOCCUS HAEMOLYTICUS* ISOLATED IN BAGHDAD CITY

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ABSTRACT

Linezolid is an oxazolidinone antibiotic in clinical use for the treatment of serious infections of resistant Gram-positive bacteria. *Staphylococcus haemolyticus* considered as the most antibiotic-resistant CoNS species. The aim of this study was to identify the linezolid susceptibility among *S. haemolyticus* isolates which isolated from Iraqi patients. Among 120 *Staphylococcus* isolates isolated from blood cultures, 46 isolates were identified as *S. haemolyticus* during 2014 -2015. Only two isolates were resistance to linezolid at all Linezolid used concentrations (*i.e.*, 2µg/ml, 4µg/ml, 8µg/ml and 20µg/ml), the rest (95.65%) were sensitive to it at 2µg/ml. The susceptibility of ten representative isolates (8 sensitive to linezolid and

2 resist to it) showed the highest resistance recorded to Erythromycin as 10 isolates from 10 (100%), which appeared to be due to methylation mechanisms in 9 isolates and one isolate due to other mechanism. Resistance to Clindamycin and Chloramphenicol was recorded.

KEYWORDS: Linezolid Resistance, *Staphylococcus haemolyticus*, Baghdad City, Iraq.

INTRODUCTION

Staphylococcus haemolyticus is a gram positive, non-motile, non-spore forming, facultative anaerobic bacteria^[1]. It is coagulase negative colonize the skin of human and is eurythermal organism, lives between 18-45 °C and can grow at 10% NaCl, *S. haemolyticus* is an opportunistic bacterial pathogen found in the normal skin flora, commonly isolated from the axillae, perineum and inguinal areas from humans^[2].

Among the coagulase-negative staphylococci (CoNS), *S. haemolyticus* is now the second most frequently isolated species from human blood cultures, after *S. epidermidis*. *S. haemolyticus* is often resistant to commonly used antimicrobial agents and is ranked as the most antibiotic-resistant CoNS species^[3].

Linezolid is a synthetic drug and a member of the oxazolidinone class of antibiotics. It acts as a protein synthesis inhibitor by binding to the ribosomal peptidyltransferase center (PTC) and stopping the growth of bacteria^[4]. The action of linezolid involves the inhibition of protein synthesis, through binding to the domain V region of the 23S rRNA in the 50S ribosomal subunit^[5]. The most frequent mechanism of linezolid resistance in staphylococci is a G2576T point mutation within domain V of the 23S rRNA; mutations in ribosomal L3 and L4 of the peptidyltransferase center, besides the *cfr* gene codified by a plasmid, contribute to decreased susceptibility to linezolid^[6].

The first clinical isolates of linezolid-resistant staphylococci were reported in 2001^[7]. Recently, linezolid-resistant staphylococci have become an increasing problem, with several outbreaks in European countries and the USA^[8,9].

Macrolides and related compounds are antibiotics that bind to the 23S rRNA, close to the peptidyltransferase centre of the 50S subunit, which catalyzes formation of peptide bonds during elongation. They block extension of the peptide chain and lead to dissociation of peptidyl- tRNA^[10].

Binding of oxazolidinones to the large ribosomal subunit was inhibited by chloramphenicol, lincomycin and clindamycin, drugs known to interact with the ribosomal peptidyltransferase center not due to chromosomal mutations, but by hindrance by methylation^[11].

The present study aimed to investigate the prevalence of linezolid resistance among *S. haemolyticus* in Baghdad city.

MATERIALS AND METHODS

Samples Collection

A total of (120) *Staphylococcus* isolates were isolated from blood cultures, at the Ibn-Baladi hospital (at the east of Baghdad city) and in Baghdad Medical City / Child Welfare Teaching Hospital during the period from November / 2014 to January / 2015.

Identification of Isolates

All collected samples were inoculated on blood agar medium for primary identification. They were incubated aerobically at 37°C for overnight. All staphylococcal isolates were identified to the genus level based on the standard biochemical and microbiological methods such as: morphologic appearance on Gram-stain (Gram positive cocci forming clusters) and catalase test. VITEK2 system was used to confirm identification^[12].

Antimicrobial Susceptibility Test

This was carried out using agar plating method with different concentrates of linezolid (2µg/ml, 4µg/ml, 8µg/ml and 20µg/ml). The antimicrobial susceptibilities of all *S. haemolyticus* isolates to linezolid susceptibility to other antibiotic was carried out using disc diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI). Susceptibility testing results were interpreted based on the CLSI criteria^[13].

Mutation Rate Measurements

This was done according to Cassie *et al.*,^[14]. Briefly, 20-30 full growth cultures in Muller-Hinton broth (10% medium / container volume) incubated aerobically at 37°C for overnight. Drop method was used to estimate the viable count and mutation frequency resist to 2µg/ml linezolid for all these saturated growth cultures on the second day.

The mutation rate was calculated according to the following formula^[15].

Mutation rate: $a = h / N$

Where: **a = mutation rate**

H = -ln [No. of cultures with no mutants / No. of cultures with saturated growth].

N = Viable count of saturated growth.

RESULTS AND DISCUSSION

Forty six isolates were identified as *S. haemolyticus*. Results of sensitivity to linezolid are shown in Table (1).

Table (1): Linezolid resistance among *S. haemolyticus* isolates.

No. of <i>S. haemolyticus</i> Strains	%	Linezolid Concentrations			
		(2µg/ml)	(4µg/ml)	(8µg/ml)	(16µg/ml)
44 isolates	95.65%	S	S	S	S
2 isolates (SH9,SH19)	4.35%	R	R	R	R

It is clear the predominant strains are sensitive to the linezolid at 2µg/ml (95.65%), the rest were resistance to all Linezolid used concentrations (*i.e.*, 2µg/ml, 4µg/ml, 8µg/ml and 20µg/ml). The high *S. haemolyticus* sensitive to linezolid was confirmed by very low, mutation rate which ranged from $(0.01 \times 10^{-8} - 0.4 \times 10^{-7})$. This would be expected as the chosen antibiotic linezolid is synthetic, so there is no resistance elements could be transferred from no producers, and it would be expected to be chromosomal resistance.^[16] Ten strains were chosen (8 sensitive: 40, 41, 42, 43, 44, 45, 46, 23 and two resistant isolates: 9 and 19) for further investigations.

Antibiotics act on (PTC) are Macrolides (Erythromycin), Lincosamides (Clindamycin), Amphenicols (Chloramphenicol).^[17] Those were applied for the chosen strains and the results are shown in Table (2).

Table (2): Antibiotic resistance among *Staphylococcus haemolyticus* isolates.

<i>S. haemolyticus</i> Strain	Antibiotics			
	L (≥2µg/ml)	E (≥ 8µg/ml)	C (≤0.25µg/ml)	Ch (3µg/ml)
SH ₉	R	R	R	R
SH ₁₉	R	R	R	R
SH ₂₃	S	R	R	R
SH ₄₀	S	R	R	R
SH ₄₁	S	R	S	S
SH ₄₂	S	R	R	S
SH ₄₃	S	R	R	R
SH ₄₄	S	R	R	S
SH ₄₅	S	R	R	S
SH ₄₆	S	R	R	S

*L=Linezolid, E=Erythromycin, C=Clindamycin, Ch=Chloramphenicol,

(S) Susceptible. (R) Resistant.

Generally resistance to Macrolides (Erythromycin), Lincosamides (Clindamycin), Amphenicols (Chloramphenicol) are carried out by methylation mechanism^[18].

The result of this study revealed that the highest resistance was to Erythromycin (100%) among selected isolates, this result was higher than that recorded by other local study that was done by Al-Khafaji and Al-Khataua(2014)^[19], this would be expected as there is more than one mechanism to resist Erythromycin. The bacteria develop resistance using different mechanisms, including activity of rRNA methylases that modify the ribosomal target sites, the other using ABC transporters, and efflux proteins (of major facilitator super family,

MSF), and also using inactivating enzymes like estrases, lyases, phosphotransferases and probably other enzymes^[20-22], The most important mechanism is the methylation which carried by erm proteins able to methylate the nascent 23S rRNA of 50S subunit, and especially the adenine residue (A2058) that impaired binding of Erythromycin. More than 40 *erm* genes have been reported and grouped in four classes: *ermA*, B, C, F classes A and C found in staphylococci; class A found in transposon and *ermC* on plasmid. However, resistance could be inducible which induced by Erythromycin and confers resistance to Erythromycin only, while the constitutive type confers a characteristic phenotype with high-level cross-resistance to the MLSB (Macrolide-Lincosamide-Streptogramin B) drugs. Macrolides, Lincosamide and Streptogramin B are chemically distinct, but share a similar mode of action^[20,23,24]. so methylation of target site leads to cross-resistance to Erythromycin and Lincomycin (Lincosamide) *i.e.*, production of MLSB phenotype^[15]. This means that resistance to Erythromycin by methylation causes resistance to Clindamycin as shown for most isolates (9/10) of this study and only isolate no. 41 showed sensitivity to Clindamycin, this means that all the Erythromycin resistant isolates are due to methylation of target except one isolate no.41 is not due to methylation mechanism. From mentioned above, nine isolates have constitutive Clindamycin resistant.

The result revealed resistance to Chloramphenicol among selected isolates 5/10 (50%). In conclusion one mechanism cannot be verified and it would be more than one way and this would be expected for *S. haemolyticus* as it considered and having (SCCmec).

In the present study, linezolid resistance was recorded in two isolates. These isolates have resistance to all antibiotics that used in study^[25].

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