PREVALENCE OF MDR STAPHYLOCOCCUS HAEMOLYTICUS IN BLOODSTREAM OF IRAQI INFANTS

Zahra M. Al-Khafaji 1*, Khairiyiah J. Al-Khataua 2

1Institute of Genetic Engineering and Biotechnology for Postgraduate Studies / University of Baghdad, Iraq.
2Ibn- Baladi Hospital / Ministry of Health, Baghdad, Iraq.

ABSTRACT

Antibiotic resistance represent a very big problem especially those microorganisms exhibit multidrug resistance (MDR). The aim of this study was to investigate the prevalence of MDR Staphylococcus haemolyticus isolates among infants which is a striking phenomenon on these days. Among 1560 blood cultures, 67 isolates (4.3%) during 2013 were obtained. Only one isolate was sensitive to all antibiotics (19-21) in use in hospital specialized for infants, the rest (95.7%) were MDR. The highest resistance recorded to Erythromycin as 56 isolates out of 67 (83.6%), which appeared to be due to mechanisms other than methylation. Resistance to other antibiotics recorded for Gentamicin and Tetracycline. However, all the isolates were sensitive to Tigecycline, Nitrofurantion, Vancomycin, large number of isolates were sensitive to many of antibiotics in use, which reduces the effect of the problem and gave a chance for antibiotics use on these days.

KEYWORDS: MDR Staphylococcus haemolyticus, Iraqi infants, mechanisms of antibiotic resistance, antibiogram.

INTRODUCTION

Antibiotic resistance is a form of drug resistance, it is a serious and growing phenomenon in contemporary medicine and has emerged as one of the pre-eminent public health concerns of the 21st century. Any use of antibiotics can increase selective pressure in a population of bacteria to allow the resistant cells to thrive and susceptible cells to die off. So over the
milliseconds, microorganisms have evolved evasion strategies to overcome a myriad of chemical and environmental challenges, including antimicrobial drugs [1]. However, antibiotic resistance is a natural phenomenon and could occur without human action [2], but the current higher – levels of antibiotic resistant bacteria and especially those of nosocomial origin are attributed to the overuse and abuse of antibiotics [2,3]. It has been proven that exchange of antibiotic resistance genes between environmental bacteria and clinical pathogens [4]. So some bacteria are naturally resistant, but also can acquire resistance either by genetic mutations or lateral transfer by conjugation or other methods, accordingly, the resistance can be transferred vertically or horizontally [2]. In addition different types of mechanisms can be used to exhibit the resistance [5].

*Staphylococcus haemolyticus* is a skin flora, coagulase negative colonize the skin of human and is eurythermal organism, lives between 18-45 °C and can grow at 10% NaCl [6], and generally is multidrug resistance [6,7]. It is an opportunistic pathogens and are difficult to eradicate because of its resistance to antibiotics. Whole – genome sequencing of *S. haemolyticus* strain JCSC 1435 uncovers the extreme plasticity of its genome. It has at least three plasmids pSHae A , pSHae B , pSHae C [7]. One of the striking features of the *S. haemolyticus* genome, its richness in repeat sequences including a large number and varieties of insertion sequences (ISs) as well as other types of repeats which make up 5% of the length of the genome and might be responsible for transferring the resistance to other staphylococcal species. The studied strain (*S. haemolyticus* JCSC 1435) chromosome contains 82 ISs elements, 60 of them are intact, while the *S. epidermidis* ATCC 12228 contains 18 intact IS out of 64, and *S. aureus* has 13 intact IS out of 29 [7]. It was accepted that *S. haemolyticus* hardboard a myriad of antiseptic and antibiotic resistance genes that could act synergistically to produce resistant phenotype [8].

The aim of this study was to investigate the *S. haemolyticus* with resistant phenotypes which are prevalent widely in infants in Baghdad city.

**MATERIALS AND METHODS**

During the period 9th Jan, 2013 to 28th Dec 2013, 1560 blood samples from children with ages (one day to one year) were subjected to blood culturing for different reasons and symptoms, in the Ibn-Baladi hospital (at the east of Baghdad city). Diagnosis and antibiogram were carried out using VITEK 2 system (BioMerieux, France), the system offers 64 biochemical tests and antibiotics sensitivity of different antibiotics with different
structural groups. The culturing and determination of antibiogram were done according to manufacture instructions.

RESULTS AND DISCUSSION

During 2013, the hospital specialist for children in Baghdad city (Ibn-Baladi) received 1560 patients with ages of one day to one year for blood culturing. Sixty-seven isolates of *Staphylococcus haemolyticus* (4.3%) were identified. Although *S. haemolyticus* has no particular host location, these isolates were subjected for antibiogram identification. In the hospital different antibiotics (19-21) of different structural classes are in use at different concentrations, some antibiotics are not in use such as Fusidic acid, Fosomycin and Ampicillin. The Vietik system gives the MICs and the results recorded as resistant (R), sensitive (S) and sometimes intermediate (I). The results shown that most of the *S. haemolyticus* isolates are at high resistance rate, among the 67 isolates only one (1.49%) isolate was sensitive to all used antibiotics, while the rest (98.5%) were resistant to different numbers of antibiotics as shown in Fig 1.

![Diagram showing distribution of resistant isolates depending on number of antibiotics.](image)

**Fig 1**: Distribution of resistant isolates depending on number of antibiotics.

The highest number of resistant isolates were recorded for Erythromycin as 56 isolates were resistant (83.6%), followed by isolates resistant to Tetracycline and Gentamicin (36/67, 53.73%) then followed by the rest as shown in Fig 2.
S. haemolyticus is known for its ability to resists to antibiotics and this ability was clarified when the whole genome was annotated \(^7\). This resistance performed by different mechanisms \(^5\). Efflux of antibiotics could be among the main mechanism as recorded in one study that 95/145 (65.52\%) isolates from hospitalized patients were resistant to 13 antibiotics due to the presence of ABC transporters \(^3\).

Other mechanisms are found in S. haemolyticus which conferred cross-resistance to structurally related antibiotics, and these represented by drug inactivation by enzymes such as esterases, phosphotransferases and others \(^5\). Such multidrug resistant phenotype of S. haemolyticus due to many features, the imbalance into the halves of the bacterial chromosome (i.e., replichore 1) which is longer than replichore 2 and this seems to reflect the accumulation of many exogenous genes in certain chromosomal regions, most notably with in the ori C environ region \(^7,9\). In addition the presence of IS elements which can inactivate a gene by direct integration into the ORF or activate a gene next to its integration site by providing the gene with a potent promoter \(^10\). The abundant IS copies may promote frequent rearrangements in S. haemolyticus genome and confer a potent ability to acquire antibiotic resistance by activating or inactivating these genes that either regulate or mediate antibiotic resistance and accelerate diversification of species for better adaptation towards chemicals including antibiotics \(^7,11\).

In addition some strains of S. haemolyticus carry tow types of antibiotic resistance transposons integrated in the bacterial chromosome, and also have plasmids in an integrated and free forms which contribute to multidrug resistance \(^7\).
The results of this study revealed that the highest resistance was to Erythromycin (83.6%) such resistance recorded early in 1950s and generally in children and differs depending on countries and even in the same country [5], such resistance was found with increase rate as the penicillin resistance increased [12]. The resistance happens when macrolide consumption exceeds a critical threshold of selective pressure [5]. 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 superfamily, MSF), and also using inactivating enzymes like esterases, lyases, phosphotransferases and probably other enzymes [13,14,15]. 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: erm A,B,C,F, classes A and C found in staphylococci; class A found in transposon and erm C 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 Streptogramine B are chemically distinct, but share a similar mode of action [13,16,17], so methylation of target site leads to cross-resistance to Erythromycin and Lincomycin (Lincosamide) i.e., production of MLSB phenotype [5]. This means that resistance to Erythromycin by efflux mechanism remains sensitive to Clindamycin, therefore other mechanisms rather than methylation resulted in resistance to only 1 or 2 classes of antibiotics belonging to MLS group [13] as shown for most isolates of this study, however, isolate # 25 is the only one resistant to one of Streptogramin B members (Quinupristin/dalfopristin) and at the same time was resistant to Erythromycin, but sensitive to Clindamycin, this means that all the Erythromycin resistant isolates is not due to methylation of target.

The second order of resistance was estimated to Gentamicin which can be conferred by efflux systems [8]. Similar rate of resistance was recorded to tetracycline, the latter is broad-spectrum antibiotic, but has side effects and should not be used for children under 8 years and especially during period of tooth development. It has been found that about 45 different acquired tetracycline determinants are working in tetracycline resistance. These determinants of different types, it could be ribosomal protection proteins (RPPs) or efflux systems [13,14,18].
Quinolones such as Ciprofloxacin, these can be outstanding by different mechanisms, among these are efflux pumps\textsuperscript{[13]} or by producing protective proteins which bind to DNA gyrase and protect it from the action of Quinolones. In addition resistance can be due to mutations in the key site of DNA gyrase or topoisomerase IV which decrease their binding affinity to Quinolones and consequently decreasing the drug effectiveness\textsuperscript{[19]}.

The other antibiotics are in use and give a promising application is Teicoplanin, this antibiotic is a glycopeptide mixture of about 10 compounds\textsuperscript{[20]}, inhibits the peptidoglycan polymerization of cell wall. Acquired resistance usually recorded for Teicoplanin and Vancomycin and found in \textit{S. haemolyticus} among staphylococci species\textsuperscript{[11,21]}, some of \textit{S. haemolyticus} strains exhibited the highest Teicoplanin resistance (MIC, 64 mg/lit) ever reported for clinical staphylococcal strains\textsuperscript{[22]}, however, these resistant strains isolated from elderly\textsuperscript{[23]}. In this study only some isolates were found to be resistant to Teicoplanin (MIC, 32 mg/lit) and which exhibited intermediate resistance to Vancomycin (MIC, 8 mg/lit) as in isolate # 26. The others showed Intermediate resistance, isolates #20 showed intermediate resistance to Vancomycin, while the other isolates with intermediate resistance to Teicoplanin #27, #40,#61(MIC, 16 mg/lit), but were sensitive to Vancomycin.

All isolates of this study showed high sensitivity to Linezolid (2 mg/lit). This antibiotic is a synthetic Oxazolidinone approved in 2000 by FDA\textsuperscript{[24]} for treatment of serious infections of G+ve bacteria resistant to other antibiotics such as \textit{S. aureus} (MRSA)\textsuperscript{[25]}, Oxazolidinone bind to P site of the ribosome and inhibit the formation of initiation complex\textsuperscript{[24,25]}. The resistance to these antibiotics, however, had been recorded in strains carrying \textit{cfr} form human and animal origins\textsuperscript{[13]}.

Most of the isolates of this study showed high sensitivity to Steptogramin member antibiotics, especially Quinupristin/Dalfopristin mixture (as mentioned previously). This mixture (30% Quinupristin and 70% Dalfopristin) inhibits protein synthesis in synergistic manner, when they used solely they perform bacteriostatic activity, whereas the combined mixture is bactericidal, they bind to 23S rRNA of 50S subunit and change its conformation and inhibit peptidyl transferase\textsuperscript{[26]}, and therefore sharing the Macrolide action.

The other effective tested antibiotic was Mupirocin (pseudomonic acid A, belongs to carboxylic ester derivatives of fatty acids, isolated from \textit{Pseudomonas fluorescens}), its main use is in topical application, it is a bacteriostatic at low concentrations and bactericidal at
high concentrations. All the tested isolates were sensitive to this antibiotic (MIC, 2 mg/lit), this antibiotic exhibits very selective mechanism of action as it binds to Isoleucyl-tRNA synthetase reversibly and halts the incorporation of isoleucine in bacterial proteins \cite{27}.

All the tested isolates of this study were with high sensitivity to Tigecycline, which is structurally similar to tetracyclines, in that it contains a central four-ring carbocyclic skeleton, it exhibits bacteriostatic activity by inhibiting protein synthesis as it binds to 30S subunit of bacterial ribosome and thereby blocking entry of amino-acyl-tRNA into A site of ribosome during translation process. Results from other studies showed, to date little resistance to Tigecycline has been reported \cite{28}, but with widespread use of drug, resistance will likely to occur.

Among the other effective antibiotics used in this study was Nitrofurantion, this antibiotic could be bacteriostatic or bactericidal depending on concentration and susceptibility of infecting bacteria \cite{29}. Nitrofurantion is highly stable to the development of bacterial resistance, a property thought to be due to its multiplicity of mechanisms of actions. It exhibits effect by inhibiting the synthesis of DNA, RNA, proteins and cell wall after its activation by bacterial flavoproteins (nitrofurantoin reductase) and conversion into active reduced reactive intermediates that modulate and damage the ribosomal proteins especially 30S ribosomal S10, or other macromolecules namely DNA \cite{13,29}.

The last effective antibiotic recorded in this study was Vancomycin, it is one of the glycopeptides effective against G+ve bacteria \cite{30}. Most of the isolates were sensitive to this antibiotic (MIC, 0.5-8 mg/lit). Infectious Disease Society of America recommended to be used in treatment of bloodstream infections and others caused by \textit{S. aureus} (MRSA) \cite{31}. It interferes with cell wall synthesis, resistance could be develop at least by steric hindrance, in addition to other mechanisms \cite{22}.

**CONCLUSION**

Using antibiotics especially those with broad-spectrum for empirical treating of different infections results in selection of more resistant pathogens, which can be easily transmitted to infants, taking into account that \textit{S. haemolyticus} could act as a reservoir of anti-antibiotic determinants and serve as a donor to more virulent staphylococci \cite{32}. So there are some alternatives suggested to preclude the development of the resistance, some people suggest using cytokines instead of antibiotics in animal feed \cite{33}, which represents a big portal for
widespread of antibiotic resistance. The other suggestion is to use old antibiotics against recently developed strains \[^3\] depending on the fact that resistance could be lost with time upon the absence of selective pressure. The other possibility is to design drugs affecting the virulence factors such as biofilms and not kill the bacterial cells, in this case the selective pressure is excluded and eventually reduce the incidence of resistance \[^{34}\].

In Iraq large number of isolates were found to be still sensitive especially these exhibited acceptable ADMET tests results can be continued for using. Antibiotics such as Tigecycline, Nitrofurantion and Vancomycin which are on market can solve the problem at least at the present time.

REFERENCES


