DOI:10.24996.ijs.2017.58.1C.2

ISSN: 0067-2904

(Macrolides –Lincosamides - Streptogramins) and Vancomycin Resistance Phenotypes of *Staphylococcus aureus* Isolated From Clinical Samples by Using Vitek 2 Compact System

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Abstract

The study was carried out to investigate MLS and vancomycin resistance phenotypes in *S.aureus* isolated from different clinical samples .A total of 40 of *S.aureus* isolated from Baghdad hospitals from different clinical samples such as blood , urin, sputum ,skin and ear swabs used to identified MLS and vancomycin resistance phenotypes.The susceptibility pattern showed that 3 islolates (7.5) % constitutive resistance to erythromycin ,clindamycin and streptogramins (cMLS) while 9 isolates (22.5)% gave inducible resistance to erythromycin ,clindamycin and streptogramins (iMLS) , 10 isolates (25)% showed resistance to erythromycin and sensitive to clindamycin (M phenotype) and 18 isolates (45)% of *S.aureus* isolates had resistance phenotype to streptogramin A and B (S_{AB}). (5)% of *S.aureus* isolates had resistance to vancomycin (VRSA) (85)% of isolates were vancomycin sensitive (VSSA) and (10)% of *S.aureus* isolates had intermediate resistance to vancomycin (VISA) with heterogeneously VISA phenotype (hetero-VISA or h-VISA).

Keywords: *S.aureus*, iMLS, cMLS, MS, S_{AB} VRSA,VSSA, hVISA.

الانماط المظهرية لمقاومة مضادات (الماكروليدات– اللنكوسامايد– الستربتوغرامين)والفانكومايسين لبكتريا المكورات العنقودية المعزولة من نماذج سريرية باستخدامVitek2copmpact system

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الخلاصة

اجريت هذه الدراسة لمعرفة الانماط المظهرية لمقاومة مضادات الماكروليدات واللنكوسامايد والستربتوغرامين و الفانكومايسين في بكتريا *S.aureus* المعزولة من نماذج سريرية مختلفة . جمعت 40 عزلة من مستشفيات بغداد من الدم والادرار والقشع ومسحات الجلد والاذن لمعرفة الانماط المظهرية المقاومة لهذه المضادات .اظهرت نتائج اختبار الحساسية ان 3 عزلات (7.5)% ذات نمط المقومة الذاتي لمجموعة MLS (cMLS)بينما 9 عزلات (22.5)% اعطت النمط المحفز (iMLS) و 10 عزلات (25)% اظهرت مقاومتها للاريثرومايسين وحساسيتها للكليندامايسين(M phenotype) و 18 عزلة (45)% من العزلات كانت مقاومة لمضادات الستربتوغرامين أ و ب (S_{AB}). (5)% من عزلات *S.aureus* كانت مقاومة لمضاد الفانكومايسين (VRSA) و (85)% من العزلات كانت حساسة له (VSSA) و (10)% من عزلات *S.aureus* لها مقاومة متوسطة للفانكومايسين (VISA or hetero VISA) .

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Introduction

Staphylococcus aureus is a serious problem in the treatment and control as a result of multidrug resistant and ability to cause wide variety of human diseases[1, 2]. *S.aureus* has become a major public health concern as a result of the steadily increasing incidence of antimicrobial resistance , Macrolides –Lincosamides and Streptogramins (MLS) group of antibiotics had the same mode of action which was the inhibition of protein biosynthesis, vancomycin is a glycopeptide antibiotic used for the treatment of Gram-positive bacterial infections , *S.aureus* had resistance to many commonly used groups of antibiotics like beta lactams, aminoglycosides, macrolides, fluoroquinolones, chloramphenicol, and tetracycline [3]. The macrolide lincosamide and streptogramin (MLS) family of antibiotics were first introduced in 1952 served as an alternative therapeutic agent especially with penicillin allergic patients, MLS group of antibiotics had the same target which was the bacterial 50S ribosomal subunit, thereby effectively inhibiting protein synthesis [4]. However, resistance to these antibiotics emerged shortly afterwards in *S.aureus* as resistance genes were already present , use of these antibiotics caused a selective efflux pump of the antibiotics out of the bacterial cell before reached the ribosome [5]. Numerous erythromycin resistance MRSA had been noticed as inducible clindamycin resistance that had been led to clinical failure, because erythromycin can induce cross-resistance among members of the macrolide, lincosamide, streptogramin group , which might be either constitutive or inducible encoded by *msrA* and *msrB* genes through efflux pump mechanism or by enzymic modification mechanism encoded by the *ereA* and *ereB* genes or by ribosomal binding site modification via methylation or mutation in the 23srRNA a nother mechanism which is done by *erm* genes family which have nearly forty types of *erm* genes, expression of only one type can lead to resistance against MLS antibiotics which also had been described in *S.aureus* [6].

MLS resistance phenotypes are:

- **M Phenotype**: Staphylococcal isolates exhibiting resistance to erythromycin while sensitive to clindamycin and giving circular zone of inhibition around clindamycin [7].
- **Inducible MLS (iMLS)** Phenotype: Staphylococcal isolates show resistance to erythromycin and clindamycin and giving D shape zone of inhibition around clindamycin with flattening towards erythromycin [8].
- **Constitutive MLS (cMLS)** Phenotype : resistant to macrolide, lincosamide and streptogramin antibiotics , this phenotype detects for those Staphylococcal isolates which show resistance to both erythromycin and clindamycin [9].
- **SAB**: resistance to streptogramins A and B antibiotics [10].

Vancomycin Resistance phenotypes:

1. Vancomycin-intermediate *S. aureus* (VISA): These bacterial strains present a thickening of the cell wall, which is believed to reduce the ability of vancomycin to diffuse into the division septum of the cell required for effective vancomycin treatment [11].
2. Vancomycin Resistance *S.aureus* (VRS) : High - level vancomycin resistance in *S. aureus* has been rarely reported, the presence of the vanA gene resulted in resistance to vancomycin [12].
3. Heterogeneous vancomycin-intermediate *S. aureus* (hVISA): is a strain of *S. aureus* that gives resistance to vancomycin at a frequency of 10−6 colonies or even higher [13].

Materials and Methods

Sample collection:

300 clinical specimens including urine, ear, sputum, blood and skin swabs ,were collected from patients attending Baghdad Hospitals, for the period from January to April 2015**.**

Isolation of staphylococci

Isolation of staphylococci from different clinical sample by specific way depending on routine laboratory techniques, all samples were streaked on mannitol salt agar for detecting ability of bacterial isolates to grow on all plates were incubated aerobically for 24 hrs. at 37°C. [14].

Identification of staphylococci

The isolates were identified depending on the morphological features on culture media and biochemical tests: Catalase test, Coagulase test and Oxidase test. according to Bergey's Manual [15]. Confirmed with the use of vitek2 compact system**.**

Antibiotic susceptibility test

Susceptibility test had been carried out through the determination of MIC values for erythromycin , clindamycin , streptogramins and vancomycin by vitek2 compact system to investigate resistance phenotypes in *S.aureus* to these antibiotics.

Results

Identification of *S.aureus*

Analysis of results showed that out of total 300 clinical samples 103 isolates (34.33) % of staphylococci and out of these isolates there were 40 (38.83)% isolates of *S.aureus* which ferment mannitol salt agar,coagulase test positive ,catalase test positive ,oxidase test negative and had B-hemolysis pettern on blood agar.

Antibiotic susceptibility and Resistance Phenotypes

1. MLS resistance phenotypes

S.aureus isolates had (55)% and (32.5)% resistance to erythromycin and clindamycin respectively (MIC>=8 µg/ml for islolates with cMLS and (<=0.25 µg/ml) for isolates which had inducible clindamycin ressistance, (0.4)% of *S.aureus* isolates had intermediate resistance to clindamycin(MIC=2 µg/ml). 3 islolates (7.5) % constitutive resistance to erythromycin ,clindamycin and streptogramins (cMLS) while 9 isolates (22.5)% gave inducible resistance to erythromycin ,clindamycin and streptogramins (iMLS) and 10 isolates (25)% showed resistance to erythromycin and sensitive to clindamycin (M phenotype). resistance to streptogramins determined phenotypically , the results showed that 18 isolates (45)% of *S.aureus* isolates had resistance phenotype to streptogramin A and B (SAB) as shown in Table-1.

Table 1- MLS resistance phenotypes in *S.aureus* isolates.

MLS Resistance phenotypes	Percentages
iMLS (erythromycin resistance, clindamycin inducible resistance)	22.5%
cMLS (both erythromycin and clindamycin resistance)	7.5%
M (resistance to erythromycin)	25%
S_{AB} (resistance to streptogramin A and B	45%

iMLS=Inducible resistance phenotype, cMLS=Constitutive resistance M=Macrolides resistance,S_{AB}=Streptogramins A and B .

The results showed that (37.5)% of *S.aureus* isolates were sensitive to both antibiotic erythromycin and clindamycin (ERY-S,CL-S), while (5)% isolates were sensitive to erythromycin and resist to clindamycin (ERY-S,CL-R) , (2.5)% of isolates were sensitive to erythromycin and had an intermediate resistance to clindamycin (ERY-S,CL-I). resistance.

2. Vancomycin Resistance Phenotypes

Results showed that (5)% of *S.aureus* isolates had resistance to vancomycin (VRSA) (MIC ≥ 32 µg/ml) ,(85)% of isolates were vancomycin sensitive (VSSA) (MIC≤0.5,1 and 2 µg/ml) and (10)% of *S.aureus* isolates had intermediate resistance(MIC4 µg/ml) to vancomycin (VISA) with heterogeneously VISA phenotype (hetero-VISA or h-VISA) as shown in Table-2.

Table 2- Vancomycin resiostance phenotypes in *S.aureus* isolates.

Susceptibility pattern	No. of isolates	Percentage (%)
Resistance (VRSA)	2	5
Sensitive (VSSA)	34	85
Intermediate(VISA) or (hVISA)	4	10
Total	40	100

VRSA=Vancomycin resistance *S.aureus* , VSSA= Vancomycin sensitive *S.aureus*, VISA= Vancomycin intermediate resistance *S.aureus*, hVISA=heterointermediate resistance *S.aureus*

Discussion

High percentage of resistance to erythromycin and clindamycin among *S.aureus* isolates appear as iMLS and cMLS, although the high rate of iMLS in *S.aureus* isolates, erythromycin and clindamycin are recommended as second-line drugs especially for patients with a beta lactam allergy in the treatment of MRSA (CA-MRSA) infections which caused skin and superficial infection as alternative drug [16]. Numerous erythromycin resistance MRSA had been noticed as inducible clindamycin resistance that had been led to clinical failure, because erythromycin can induce cross-resistance among members of the macrolide, lincosamide, streptogramin group , which might be either constitutive or inducible encoded by *msrA* and *msrB* genes through efflux pump mechanism or by enzymic modification mechanism encoded by the *ereA* and *ereB* genes or by ribosomal binging site modification via methylation or mutation in the 23srRNA a nother mechanism which is done by erm genes family which have nearly forty types of *erm* genes, expression of only one type can lead to resistance against MLS antibiotics which also had been described in *S.aureus* [17].

Resistance to streptogramin antibiotics in *S.aureus* resulted from enzymatic inactivation, streptogramin such as pristinamycin and virginiamycin consist of two components, streptogramins A and B, which are synergistic in action. Streptogramin A can be inactivated by an 0- acetyltransferase (*SgA*) and streptogramin B can be inactivated by a hydrolase (*SgB*) [18]. Other genes responsible for streptogramins resistance by efflux pump mechanism like *msrA,vgb* , *vat* and *vga* causing active efflux in *S.aureus* [19].

Negative result for inducible clindamycin resistance in the case of clindamycin susceptibility provided a very good therapeutic option in the treatment of skin , soft tissue infections (SSTIs) and serious infections because of it's efficacy against MRSA and MSSA , clindamycin could be administered orally, good tissue penetration and is tolerable, therefore it is usually used to treat skin and bone infections [20]. There for it was very important to find out the resistance phenotypes to this group of antibiotics in MRSA before the treatmens because the arbitrary use of MLS antibiotics had led to an increase in number of *S.aureus* isolats acquiring resistance to this group of antibiotics as a result of the same target site of action of these antibiotics which is protein biosynthesis [21].

Vancomycin (glycopeptide antibiotic) had bactericidal activity against aerobic Gram positive bacteria, especially staphylococci (including beta lactamase producing and MRSA), it was associated with slower clinical response and longer duration of MSSA bacteremia, and it has been associasted with more frequent complications in patients with endocarditis [22]. Vancomycin has been regarded as the first-line drug for treatment of MRSA [23].

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