Incidence of methicillin-resistance and macrolode-lincosamide-streptogramin B resistance in a clinical sample of Staphylococcal isolates: a pharmacodynamic study

Majid A. Lafi* BSc, MPhil, PhD

Summary:
Background: A rapid and accurate identification of Methicillin-Resistant Staphylococci (MRS) is of a particular clinical significance because they have cross-resistance to other antibiotics with high ability to be transmitted among hospitalized patients known as epidemic MRS.

Objectives: The detection of MRS and the susceptibility of isolates to antimicrobial agents, also to determine inducible and constitutive macrolode-lincosamide-streptogramin B (MLSb) resistance mechanisms by pharmacodynamic interpretative reading approaches.

Methods: Standard disk diffusion method was performed for 30 Staphylococcus aureus and 10 Staphylococcus epidermidis isolated from wound, burn patients admitted to Ramadi General Hospital in Ramadi and eczema patients who attended the same hospital, from September 2005 to April 2006, against oxacillin 5µg disk and cefoxitin 30µg disk as indicators to detect the presence of MRS, and against selected antimicrobial agents, double disk tests (D-test) were performed to determine MLSb-inducible resistance mechanism.

Results: Out of 40 isolates, 34 (85%) isolates considered to be MRS according to cefoxitin susceptibility results, but according to interpretative reading of β-lactams susceptibility pattern with oxacillin 24 (60%) of isolates identified to be MRS. High percentage of isolates were non-susceptible to β-lactam antibiotics and 4 (10%) isolates were resistant to imipenem, also 4 (10%) isolates were resistant to vancomycin, the interpretative reading of the susceptibility pattern against erythromycin and clindamycin showed classical 21/40 (52.5%), MLSb-inducible 6/40 (15%), MLSb-constitutive 6/40 (15%), and macrolode efflux mechanism 7/40 (17.5%), -ve D-test.

Conclusion: High percentage (85%) of isolates was MRS, and 15% of them have MLSb-inducible and another 15% have MLSb-constitutive resistance mechanisms inferring the presence of erm gene, 17.5% may have macrolode efflux mechanism encoded by the mrrA gene. The presence of such resistance mechanisms implicates serious problem in hospital regarding control of infection and control of antibiotic use; thus, necessitate an appropriate protocol (to implement) for the control of infection and use of antibiotics.

Keywords: Methicillin-Resistant Staphylococci, MLSb-inducible, MLSb-constitutive, Ramadi

Introduction:
Methicillin-resistant staphylococci (MRS) are important cause of nosocomial and community-acquired infections (1). After methicillin came into clinical use in 1961, methicillin-resistant S. aureus (MRSA) has rapidly emerged and become a major clinical problem, which increased with widely used antimicrobial agents particularly cephalosporins that may be associated with induction, selection and propagation of MRSA (2).

More than 98% of MRSA is mediated by mecA gene which encodes for additional penicillin-binding protein (PBP) called PBP2a with low affinity for all β-lactam antibiotics, and often carry multiple other resistance determinants (3). Strains with intact mec DNA called pre-methicillin resistant Staphylococcus aureus (pre-MRSA) that showed susceptible to methicillin (3).

* Department of Pharmacology, College of Medicine, University of Al-Anbar.

One important fact about MRSA is the frequency of strains exhibiting heteroresistance (i.e., the expression of methicillin resistance occurs in only small subpopulation of bacterial cells (4)). β-lactam antibiotics represented a selective pressure favours the selection and emergence of these mutant strains which express homogeneous resistance from heterogeneous strains (2). Oxacillin tests often fail to detect low level heterogeneous MRSA population, cefoxitin is strong inducer for production of PBP 2a (5), several studies have demonstrated the superiority of cefoxitin for the identification of MRS (4, 5). MRSA organism is of particular clinical significance because it has cross-resistant to other antibiotics with high ability to be transmitted among hospitalized patients so called epidemic MRSA (1). MLSb resistance system in staphylococci encoded by erm gene through target modification, the expression of system may be inducible or constitutive (6), MLSb-inducible strains express resistance to erythromycin which are
good inducer but not to clindamycin, whereas resistance to both drugs expressed by MLSβ-constitutive strains (7). Simple diffusion test has been recommended by the NCCLS (8) detect strains that have the genetic potential (erm genes) to become resistant during therapy from strains that are fully susceptible to clindamycin. In this preliminary study it is aimed to detect the MRS in clinical staphylococcal isolates and show the susceptibility to β-lactam antibiotics and other selected antimicrobial agents, also to determine the MLSβ-inducible and MLSβ-constitutive resistance mechanisms.

Methods
Thirty isolates of S. aureus and ten of S. epidermidis isolated from wound, burn admitted to Ramadi General Hospital in Ramadi (from September 2005 to April 2006) and eczema patients attended to the same hospital, and identified depending on the morphology and cultural characteristics on the mannitol salt agar, oxidase and catalase tests (9). Susceptibility testing was performed by disk diffusion on Mueller-Hinton agar (MHA) with 24 h incubation at 37°C. (9). The antibiotic disks from Bioanalyse Company, Ankara-Turkey were used with the following potencies; penicillin G (PG 6μg), amoxicillin (AX 25μg), amoxicillin /clavulanic acid (AMC 20/10μg), oxacillin (OXA 5μg), cephalothin (KF 30μg), tobramycin (TOB 10μg), chloramphenicol (C 30μg), tetracycline (TE 30μg), ciprofloxacin (CIP 5μg), co-trimoxazole (SXT 1.25/23.75μg), erythromycin (ERY 15μg), clindamycin (CIL 2μg), vancomycin (VC 30μg), imipenem (IMP 10μg), the results were interpreted according to the standard zone diameter recommended by Carret et al (10). Screening tests for detection of MRS was performed by interpretative reading of the susceptibility data against β-lactam antibiotics with oxacillin disk (OXA 5μg) used as an indicator for MRS. The susceptibility to oxacillin was made on MHA supplemented with 2% NaCl and high density inoculum (10⁶ cfu/ml used for 18 h at 37°C with critical diameter <20mm (5). The susceptibility of isolates to cefoxitin disk (FOX 30μg) used also as indicator for the presence of MRS, was made on MHA with (10⁶ cfu/ml) inoculum size and critical diameter <27mm (5). S. aureus ATCC 25923 (MSSA β-lactamase-negative strain) used as control strain. In cases of heterogeneous growth, defined as the occurrence of small colonies in the circular growth inhibition area, the diameter of the inner limit of the small colonies' inhibition zone was taken into account. Double disk antagonism test (D-test) was made to demonstrate erythromycin antagonism to clindamycin, a flattening or blunting of the clindamycin zone of inhibition adjacent to the erythromycin disk, giving a D shape to the zone, as described by the NCCLS (8). Screening for VRS in the study isolates was made by brain-heart infusion (BHI) agar containing 6μg/ml vancomycin; S. aureus ATCC 25923 used as negative control (11). The chi-square test was used to determine significant differences between categorical variables. P<0.05 was considered significant.

Results
The susceptibility to antibiotics of 40 isolates is shown in table (1), the interpretative reading of selected β-lactams, and ERY and CIL versus staphylococci isolates showed in table (2) and (3) respectively. In screening test for vancomycin resistant staphylococci four isolates of Staphylococci grew On BHI agar supplemented with vancomycin after 24h.

### Table 1. Susceptible and non-susceptible number and percentage of isolates to the antimicrobial agents used.

<table>
<thead>
<tr>
<th></th>
<th>PG</th>
<th>AX</th>
<th>AMC</th>
<th>OXA</th>
<th>FOX</th>
<th>KF</th>
<th>TOB</th>
<th>C</th>
<th>CIP</th>
<th>TE</th>
<th>ERY</th>
<th>CIL</th>
<th>VN</th>
<th>IMP</th>
<th>SXT</th>
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<tbody>
<tr>
<td>Susceptible (%)</td>
<td>2/40</td>
<td>5%</td>
<td>5/40</td>
<td>25%</td>
<td>10/40</td>
<td>25%</td>
<td>16/40</td>
<td>40%</td>
<td>16/40</td>
<td>40%</td>
<td>15/40</td>
<td>37.5%</td>
<td>22/40</td>
<td>55%</td>
<td>34/40</td>
</tr>
<tr>
<td>Non-susceptible (%)</td>
<td>38/40</td>
<td>95%</td>
<td>35/40</td>
<td>75%</td>
<td>30/40</td>
<td>50%</td>
<td>24/40</td>
<td>60%</td>
<td>33/40</td>
<td>50%</td>
<td>25/40</td>
<td>45%</td>
<td>18/40</td>
<td>45%</td>
<td>14/40</td>
</tr>
</tbody>
</table>

### Table 2. Susceptibility to selected β-lactams, and cefoxitin among all staphylococcal isolates

<table>
<thead>
<tr>
<th></th>
<th>PG</th>
<th>AX</th>
<th>AMC</th>
<th>OXA</th>
<th>FOX</th>
<th>KF</th>
<th>IMP</th>
<th>Interpretation</th>
<th>FOX</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical S. aureus 2/30°</td>
<td>SS</td>
<td>SS</td>
<td>SS</td>
<td>SS</td>
<td>SS</td>
<td>S</td>
<td>Classical S. aureus 2/30°</td>
<td>S</td>
<td>MSSA 6/30 (20%)</td>
<td></td>
</tr>
<tr>
<td>β-lactamase +ve</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>Non-S</td>
<td>MRSA 24/30° (80%)</td>
<td>Non-S</td>
<td></td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Phenotype</th>
<th>MRSA</th>
<th>MRSE</th>
<th>MSSA</th>
<th>MSSE</th>
</tr>
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<tbody>
<tr>
<td>ERY-S, CIL-S</td>
<td>13</td>
<td>3</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>6</td>
<td>34</td>
<td>6</td>
</tr>
<tr>
<td>ERY-R, CIL-R</td>
<td>3</td>
<td>5</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>6</td>
<td>34</td>
<td>6</td>
</tr>
<tr>
<td>ERY-R, CIL-S, D'</td>
<td>4</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>6</td>
<td>34</td>
<td>6</td>
</tr>
</tbody>
</table>

D = D-test, erm = erythromycin ribosome methylase, msrA = macrolide streptogramin resistance

Discussion
Methicillin resistant staphylococci is an increasing infection control problem and therapeutic challenge. Rapid detection of MRSA with implementation of infection control policies is essential in limiting the nosocomial spread of this organism. The interpretative reading showed that 24(60%) of our isolates with oxacillin inhibition zone diameter 20 mm were identified as methicillin resistant staphylococci, they should be considered as resistant to all β-lactams even if they showed susceptibility in vitro, because the mechanism, PBP2a production, results in cross-resistance for the class (2,6). This may interpret the observation that four isolates (10%) showed to be susceptible to imipenem which has not been used therapeutically in Ramadi General Hospital meeting high expression of resistance in these isolates. Ten out of 24 isolates showed heterogeneous growth around the oxacillin disk. The whole population may be mixed mecA positive plus mecA negative, i.e. MRSA plus MSSA, or heterogeneous mecA positive only with different expressions of resistance (12). Fourteen out of 40 (35%) isolates showed oxacillin inhibition zone diameter 20 mm and were identified as oxacillin susceptible isolates and showed non-susceptible to penicillin G and therefore identified as β-lactamase producing isolates and should be considered as resistant to all penicillins except oxacillin and methicillin (6). But cefoxitin disk diffusion test showed that 34 (85%) isolates gave cefoxitin inhibition zone <27 mm and considered as MRS containing mecA gene (5), 6/40 (15%) isolates showed cefoxitin inhibition zone >27 mm and considered as methicillin susceptible staphylococci (MSS) (5). Among the 34 MRSA isolates ten isolates showed susceptible to oxacillin but not considered as MSS, because cefoxitin does not induce PBP2a production in MSSA strain, unless this strain is pre-MRSA(3). Thus, if only oxacillin results were considered these isolates would have been mis-classified as oxacillin susceptible with direct implication for selection of drug for treatment. Therefore, this result is consistent with those observed by Felten et al (5) who documented that cefoxitin disk had higher sensitivities and specificities than oxacillin disk in detection of oxacillin heteroresistance phenotype among staphylococci. Although the result obtained by Frigatto et al (13) showed that oxacillin more reliable than cefoxitin in detection of methicillin resistant S. epidermidis (MRSE) when cefoxitin critical diameter <25 mm was used, but my ten S. epidermidis gave cefoxitin inhibition zone ranged from 6-20 mm at a time six of them were susceptible to oxacillin. Thus, cefoxitin is better in detecting methicillin resistance than oxacillin (P=0.025) in this sample of S. epidermidis, while no such significant difference was detected with S. aureus (P=0.243). It is suggested that this difference in detection of methicillin resistance may be due to the presence of low-level heterogeneous MRSE population in S. epidermidis isolates in which cefoxitin is stronger inducer than oxacillin for production of PBP2a (4,5). Four isolates showed resistance to vancomycin with zone diameter (6 mm) also showed multiple drug resistance, and gave visible growth on BHI agar supplemented with vancomycin. Therefore, these isolates may be
considered as VRS. Further confirmation by
determining the MIC value for these isolates is
required (11). Although the number of isolates
tested in this study was low, a high number of them
were methicillin-resistant that detected by cefoxitin
disk with different patterns of sensitivity to other
antimicrobial agents were shown. Since MRSA is
considered to be a sensitive indicator of the quality
of hospital hygiene overall (2), the results of this
study infer poor implementation of control of
infection strategies and irrational use of
antimicrobial agents in this hospital. According to
phenotypic interpretative reading of my data as
shown in table 3, 21/40 (52.5%) isolates showed
sensitivity to erythromycin and clindamycin which
represent the classical phenotype and their
frequencies are common. Resistance to both drugs
is expressed in 6/40 (15%) of my isolates inferring
the status of MLSB-constitutive encoded by the
erm gene (8) that mediates resistance to streptogramin-B
(11). Seven out of 40 (17.5%) isolates were resistant
to erythromycin, sensitive to clindamycin and
expressing negative D-test thus may have macrolide
efflux-mechanism encoded by the msrA gene (8).
The most important finding with therapeutic
implication is that 6/40 (15%) of the study isolates
were resistant to erythromycin, sensitive to
clinidamycin and expressing positive D-test
suggesting MLSB-inducible mechanism encoded by
the erm gene (8). Clinically, isolates expressing
inducible MLSB have a high rate of spontaneous
mutation to constitutive resistance, which could
be selected for by use of clindamycin (14). Thus, to
avoid therapeutic failures clindamycin should not be
used in patients with infections caused by MLSB-
inducible resistant Staphylococcus isolates (15).

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