Isolation of Methicillin Resistant *Staphylococcus aureus* (module 2011) in Taif Area, Saudi Arabia

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium responsible for several difficult-to-treat infections in humans. It may be named multidrug-resistant *S. aureus* or Oxacillin-resistant *S. aureus* (ORSA). MRSA is a strain of *S. aureus* that developed resistance to β-Lactam antibiotics, including Penicillins, which include (Methicillin, Dicloxacillin, Nafcillin, Oxacillin, etc.) and Cephalosporins. The aim of this study was to investigate the new MRSA strain from patient in Taif area who complained from chronic prostatitis and clinically uncured with treatment by Amoxicillin antibiotics for two months; after that the patient was treated by Quinolones antibiotics for three weeks and he was clinically cured. The causative strain isolated was reinvestigated with antibiotics groups to confirm that it is a new module of MRSA that has a different antibiotics sensitivity characteristics in Taif area. The results revealed that the MRSA tested was resistant to antibiotics groups β-Lactam (Penicillin, Oxacillin, Ampicillin and Augmentin) and Glycopeptides (Vancomycin) and of intermediate sensitivity to Macrolides (Erythromycin, Clindamycin and Azithromycin), Aminoglycosides (Gentamycin) and sensitive to Nitrofurantoin from Macrolides, Amikacin from Aminoglycosides and (Ciprofloxacin, Ofloxacin and Norfloxacin) from Quinolones. The study revealed the resistance of new isolated MRSA strain and its sensitivity to little groups of antibiotics that lead to serious health problems if the sensitive antibiotics turned to be resistant or unavailable treated antibiotics in the area. MRSA, widely dangerous for human health as well resistant to antibiotics, is used increasing by time due to fast changes in the genome of *S.aureus* and the misuse of antibiotics by patients. Further studies are required to improve the interaction between human infections by MRSA, prevention, slowing resistant of MRSA and new strong sensitive antibiotics production.

keywords: *S. aureus*, MRSA, ORSA, GISA, VISA, VRSA, CA-MRSA.

Abbreviations:

<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
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<tbody>
<tr>
<td>MRSA</td>
<td>Methicillin Resistant <em>S. aureus</em></td>
</tr>
<tr>
<td>ORSA</td>
<td>Oxacillin Resistant <em>S. aureus</em></td>
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<tr>
<td>GISA</td>
<td>Glycopeptide Intermediate <em>S. aureus</em></td>
</tr>
<tr>
<td>VISA</td>
<td>Vancomycin Intermediate <em>S. aureus</em></td>
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<tr>
<td>VRSA</td>
<td>Vancomycin Resistant <em>S. aureus</em></td>
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<tr>
<td>CA-MRSA</td>
<td>Community Associated MRSA infections</td>
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1. Introduction

*Staphylococcus aureus* is one of the major resistant pathogens, found on the mucous membranes and the human skin of around a third of the population, extremely adaptable to antibiotic pressure. It was one of the earlier bacteria in which Penicillin resistance was found in 1947, just four years after the drug started being mass-produced (Bulent et al., 2003). Methicillin was then the antibiotic of choice, but has since been replaced by Oxacillin antibiotic due to significant kidney toxicity. MRSA strain was first detected in Britain in 1961 and is now in hospitals (Bulent et al., 2003). MRSA was responsible for 37% of fatal cases of sepsis in the UK in 1999, up from 4% in 1991. More than half of *S.aureus* infections in USA are resistant to Penicillin, Methicillin, Tetracycline and Erythromycin (Bulent et al., 2003). Vancomycin is the only effective agent available against MRSA at the time. However, strains with intermediate levels of resistance, termed GISA (Glycopeptides intermediate *S. aureus*) or VISA (Vancomycin intermediate *S. aureus*), began appearing in the late 1990s (Bulent et al., 2003). The first identified case was in Japan in 1996 and strains had since been found in hospitals in England, France and USA. The first documented strain with complete resistance to Vancomycin termed VRSA (Vancomycin-
resistant *S. aureus* appeared in United States in 2002 (Bozdogan et al., 2003).

Infections caused by *S. aureus* with high-level resistance to Vancomycin (VRSA), as only isolated cases have been reported. VRSA developed by the present of the vanA gene, which was transferred from *Enterococcus* with Vancomycin resistance. On the other hand, infections caused with intermediate resistance to glycopeptides (VISA), or heterogeneously expressed intermediate level Glycopeptides resistance (hVISA), are more common. These infections were associated with clinical failure of Glycopeptides therapy. While the biochemical and phenotypic features, including a thickened cell wall of hVISA and VISA, are well known, the genetic basis of these phenotypes remains unknown. Certain genetic regulatory elements such as agr II are associated with reduced susceptibility of *S. aureus* to Glycopeptides. Available data suggested that certain infections might be successfully treated using higher doses of Vancomycin (Ruef, 2004).

CA-MRSA (Community-acquired MRSA) had emerged as an epidemic that is responsible for rapidly progressive (Boyle-Vavra and Daum 2007). The epidemiology of infections caused by MRSA is rapidly changing: in the past 10 years, infections caused by this organism had emerged in the community (Cynthia and Robert, 2007). The two MRSA clones in United States most closely associated with community outbreaks, USA400 (MW2 strain, ST1 lineage) and USA300, often contain Panton-Valentine leukocidin genes and more frequently had been associated with skin and soft tissue infections (Susan and Robert, 2007). Outbreaks of community-associated infections (CA-MRSA) had reported in correctional facilities among athletic teams and in newborn nurseries. CA-MRSA infections appeared to be endemic in many urban regions and caused most infections (Maree et al., 2007).

The Macrolides-resistance due to the effect of *ermC* in MRSA was detected in 67.6% of MRSA and 71.4% of MSSA. These results suggested that SCCmec types IV or V have spread, particularly in MSSA carrying *etb* in the community (Hidemasa et al., 2003).

MRSA is a bacterium responsible for several difficult-to-treat infections in humans. It may also be called multidrug-resistant *S. aureus* or Oxacillin-resistant *S. aureus* (ORSA). MRSA is, by definition, any strain of *S. aureus* that had developed resistance to β-Lactam antibiotics which include the Penicillins and the Cephalosporins (Holten and Onusko, 2000). MRSA is a major cause of hospital-acquired infections that are becoming increasingly difficult to combat because of emerging resistance to all current antibiotic classes. The evolutionary origins of MRSA was poorly understood, no rational nomenclature exists (Mark et al., 2007). A new type of MRSA, designated community-acquired MRSA, became increasingly noticeable in the community, some strains of which caused fatal infections in otherwise healthy individuals. By contrast with hospital-acquired MRSA, community-acquired MRSA was more susceptible to non-β-lactam antibiotics. An investigation of high virulence potential shows certain strains of this bacterium (Christopher, 2002). Species ST8:USA300 was a strain of community-associated MRSA that emerged as a particularly, antibiotic resistant epidemic that was responsible for rapidly progressive infections. The epidemiology of infections caused by MRSA was rapidly changing: in the past 10 years, infections caused by this organism had emerged in the community (Boyle-Vavra and Daum 2007). As two tested MRSA clones in United States that are most closely associated with community outbreaks, USA400 and USA300 often contained Panton-Valentine leukocidin (PVL) genes and, more frequently, CA-MRSA infections that appeared to be endemic in many urban regions, causing most MRSA infections (Diep et al., 2008).

Susceptibility profile of 19 *S. aureus* isolates were acquired from two teaching hospitals and ATCC towards 16 selected antibiotics was analyzed and an antibiogram was generated. Findings also indicated resistance against many of the available antibiotics and thus an urgent need to search for alternative antibiotics (Saiful et al., 2006).

MRSA was determined by mecA gene based PCR., mecA is composed of 50 kb or more of DNA founded, mec-contained mecA, the gene for Penicillin-binding protein 2a (PBP2a); mecI and mecR1, regulatory genes controlling mecA expression and numerous other elements and resistance determinants (Diep et al., 2006). A distinctive feature of Methicillin resistance, its heterogeneous expression, borderline resistance, a low-level type of resistance to Methicillin exhibited by strains lacking mecA, was associated with modifications in native PBPs, β-lactamase hyperproduction, or possibly a mexiticillinase (Diep et al., 2006). The resistance phenotype was influenced by numerous factors, including mecA and β-lactamase regulatory elements, fem factors and yet to be identified chromosomal loci. The heterogeneous nature of Methicillin resistance confounds susceptibility testing. Methodologies based on the detection of mecA are the most accurate (Diep et al., 2006). Vancomycin was the drug of choice for treatment of infections caused by Methicillin-resistant strains. PBP 2a confers cross-resistance to most currently available β-Lactam antibiotics. Investigational agents that bind PBP-2a at low concentrations appear promising but had not been tested in humans. Alternatives to Vancomycin are few due to the multiple drug resistances typical of MRSA (Chambers 1997).

In Schleswig-Holstein, a resident was diagnosed with furuncle caused by a Panton-Valentine leukocidin (PVL)-positive (CA-MRSA). As a result of active cased finding, 54% of all residents were screened for MRSA and two further PVL-positive CA-MRSA cases were identified (Dudareva et al., 2011).

A Reported case of empyema caused by MRSA sequence type ST398 was in a 79-year-old Spanish man who had severe chronic obstructive pulmonary disease; he was treated by intravenous Levofloxacin, but he did not clinically improve. The isolates were MRSA from his specimens and nasal swab. Antimicrobial drug therapy was changed to intravenous Linezolid, but the patient's clinical situation rapidly worsened and he died of multorgan failure (Lozano et al., 2011).

MRSA caused high-throughput genomics approach that provides a high-resolution view of the epidemiology and microevolution of a dominant MRSA strain (Simon et al., 2010). This approach revealed the global geographic
structure within the lineage, its intercontinental transmitted through four decades, the potential to trace person-to-person transmission within a hospital environment. The ability to interrogated and resolved bacterial populations was applicable to a range of infectious diseases, as well as microbial ecology (Simon et al., 2010).

Antibiotic resistance was with 40 S. aureus strains isolated from 110 diabetic patients 36% was evaluated to 80% of the isolates showed multidrug-resistance to more than eight antibiotics and 35% isolates were found to be MRSA. All S. aureus strains 100% screened from diabetic clinical specimens were resistant to Penicillin, 63% to Ampicillin, 55% to Streptomycin, 50% to Tetracycline and 50% to Gentamicin. However, low resistance rate was observed to Ciprofloxacin 20% and Rifampicin 8%. In contrast, all 100% S. aureus strains recorded susceptibility to Teicoplanin, which was followed by Vancomycin 95%. Genotypical examination revealed that 80% of the Aminoglycoside resistant S. aureus (ARSA) had Aminoglycoside modifying enzyme (AME) coding genes; however, 20% of ARSA which showed non-AME mediated (adaptive) Aminoglycoside resistance lacked these genes in their genome. In contrast all MRSA isolates possessed mecA, femA genetic determinants in their genome (Raju et al., 2010).

Therefore, the aim of this study was to investigate the new isolated MRSA strain from a clinically uncured patient (from Taif area) with chronic prostatitis and failed treatment with Amoxicillin for two month. He was treated by Quinolones antibiotics for three weeks and thus he clinically improved. The strain was re-investigated with antibiotics for three weeks and thus he clinically cured. The strain was re-investigated with available antibiotics groups as a means of reaching the confirmation that it is a new module of MRSA (resistant to more antibiotics than before) in Taif area.

Case report

A 50-year-old male who had chronic prostatitis was treated for two months by antibiotic Amoxicillin, but no clinical improvement was seen. He was then treated by antibiotic Quinolones group for three weeks and thus he clinically cured. The strain was re-investigated with available antibiotics groups as a means of reaching the confirmation that it is a new module of MRSA (resistant to more antibiotics than before) in Taif area.

2. Materials and Methods

This study was carried out in the Microbiology section of Bioner Lab in Al-Taif area. All clinical specimens were collected in sterile containers, taking care of all the aseptic measures. The specimens were inoculated on sheep blood agar, Mac-Conkey agar and blood chocolate agar. All inoculated media were kept at 37°C in incubator for 24 hours. ATCC strain was used as a control (S. aureus 25923) (CDC 2003). S. aureus isolate, recovered from the patient specimens, was identified by conventional method and confirmed by colony morphology, Grams staining, Catalase, Coagulase, DNase latex agglutination and Mannitol fermentation test (National standard method, 2007).

Pastorex Staph-Plus latex agglutination kit (BIO-RAD) and API-Staph test system (Bio-Merieux, Vitek, Hazelwood, Mo.) were used. Tryptic soy broth and Tryptic soy agar for purify the strain (Difco, Detroit) (CDC 2003) were also used.

The isolate was then tested by the modified Kirby Bauer disc diffusion technique and the results were interpreted as outlined by National Committee for Clinical Laboratory Standards criteria NCCLS. The data were confirmed by Micro-scan and API (Brown et al, 2005). Standardized methods for disc diffusion had been defined by the BSAC5167 and the NCCLS were used for the cultivation and analysis of bacterial cultures, grown at 37°C with vigorous aeration (CDC 2003).

The antibiotics discs tested were differentiated into groups: β-Lactam group (Penicillin 10µg, Oxacillin 1µg, Ampicillin 10µg and Augmentin 20/10µg), Macrolides group (Nitrofurantoin 300µg, Erythromycin 15µg, Clindamycin 2µg and Azithromycin 15µg), Aminoglycosides group (Amikacin 30µg and Gentamycin 10µg), Quinolones group (Ciprofloxacin 5µg, Ofloxacin 5µg and Norfloxacin 10µg) and Glycopeptides group (Vancomycin 30µg). Antibiotics discs were obtained from various manufacturers using a disc diffusion method on Mueller-Hinton agar (Oxoid) according to the CLSI (Clinical and Laboratory Standards Institute) directives (M100-S17M2-A9). MICs of Moxifloxacin and Clindamycin were determined by the E-test method (AB BIODISK) (CDC 2003).

3. Results

The strain of S. aureus (MRSA) was isolated from the infected patient was tested for antimicrobial susceptibility test. The MRSA strain was resistant to antibiotics groups as follow β-Lactam including (Penicillin, Oxacillin, Ampicillin and Augmentin) as the patient was treated by the same group, and Glycopeptides including (Vancomycin) (Table 1). The strain was intermediate sensitive for Macrolides (Erythromycin, Clindamycin and Azithromycin) and Aminoglycosides (Gentamycin). The greatest prevalence of resistance was found to all β-Lactam, Macrolides, Glycopeptides groups and unfortunately resistant to Vancomycin. Fortunately, great Sensitive to Nitrofurantoin from Macrolides group, Amikacin from Aminoglycosides group and (Ciprofloxacin, Ofloxacin and Norfloxacin) from Quinolones group as the patient was cured with the same group.

4. Discussion

The S. aureus strain isolated from the patient was called MRSA because of multi-resistance to antibiotics in-vivo (i.e., during the infection period of patient), and in-vitro (i.e., during the search). The isolated MRSA passed as in infection through the search; it was resistant to all β-Lactam group, tested as in the first period of the infection of the patient, that he was treated by Amoxicillin and thus failed to get clinically cured; it was however sensitive to all Quinolones group, tested as in the second period, which was used to treat the patient with, and thus lead to his cure.

MRSA had become an enormous problem for health care providers, because it hardly treats and it is sometimes called super bug. Multiple studies had been carried out on the growing concern over multidrug resistance.
Table 1 Antibiotics Sensitivity Tests for Isolated MRSA Strain

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Concentration</th>
<th>Sensitivity range</th>
<th>Results mm</th>
<th>Degree</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>β-Lactam:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillin</td>
<td>10 µg</td>
<td>&gt;29 mm</td>
<td>18 mm***</td>
<td>Resistant</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>1 µg</td>
<td>&gt;18 mm</td>
<td>10 mm***</td>
<td>Resistant</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>10 µg</td>
<td>&gt;27 mm</td>
<td>15 mm***</td>
<td>Resistant</td>
</tr>
<tr>
<td>Augmentin</td>
<td>20/10 µg</td>
<td>&gt;28 mm</td>
<td>17 mm***</td>
<td>Resistant</td>
</tr>
<tr>
<td><strong>Macrolides:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>300 µg</td>
<td>&gt;17 mm</td>
<td>18 mm*</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>15 µg</td>
<td>&gt;23 mm</td>
<td>16 mm**</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>2 µg</td>
<td>&gt;30 mm</td>
<td>25 mm**</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>15 µg</td>
<td>&gt;17 mm</td>
<td>12 mm**</td>
<td>Intermediate</td>
</tr>
<tr>
<td><strong>Aminoglycosides:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>30 µg</td>
<td>&gt;17 mm</td>
<td>19 mm*</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>10 µg</td>
<td>&gt;15 mm</td>
<td>13 mm**</td>
<td>Intermediate</td>
</tr>
<tr>
<td><strong>Quinolones:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>5 µg</td>
<td>&gt;21 mm</td>
<td>22 mm*</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>5 µg</td>
<td>&gt;22 mm</td>
<td>24 mm*</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>10 µg</td>
<td>&gt;17 mm</td>
<td>19 mm*</td>
<td>Sensitive</td>
</tr>
<tr>
<td><strong>Glycopeptides:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vancomycin</td>
<td>30 µg</td>
<td>&gt;17 mm</td>
<td>14 mm***</td>
<td>Resistant</td>
</tr>
</tbody>
</table>

* The measure of inhibition zone by mm for sensitive antibiotics.
** The measure of inhibition zone by mm for intermediate antibiotics.
*** The measure of inhibition zone by mm for resistant antibiotics.

MRSA was becoming a problem involving hospital setting and community transferring. The rate of the prevalence of MRSA isolates had increased over the years (Bozdogan et al., 2003; Boyle-Vavra and Daum 2007; Maree et al., 2007). More than half of MRSA was positive for Panton-Valentine Leucocidine PVL which CA-MRSA (Dudareva et al., 2011). The transmission of MRSA as dominant was very easy to trace person-to-person (Simon et al., 2010). The old Spanish man died with MRSA sequence type ST398 which caused empyema (Lozano et al., 2011). The first identified case was in Japan in 1996, and strains have since been found in hospitals in England, France and USA. The first documented strain with complete resistance to Vancomycin appeared in the USA in 2002 (Bozdogan et al., 2003). This study, the pattern of antibiotics sensitivity revealed highly resistant to β-Lactam group (Mark et al., 2007). Thus, more resistance appeared in the tested MRSA strain for β-Lactam group and Vancomycin also (Raju et al., 2010). The MRSA tested acquired strain from community was very resistant to antibiotics including Penicillin, Oxacillin, Ampicillin, Augmentin and Vancomycin (Christopher, 2002). This was due to the changing in the genome of MRSA leading to the increase of the antibiotic resistance contrast; MRSA isolates possessed mecA, femA genetic determinants in their genome (Diep et al., 2008; Raju et al., 2010). The tested MRSA strain was intermediate to Erythromycin, Clindamycin, Azithromycin and Gentamycin due to cross-resistance to most currently available antibiotics related to β-Lactam group (Chambers, 1997). On the other hand, the MRSA strain tested appeared sensitive to some antibiotics Macrolides group (Nitrofurantoin), Aminoglycosides group (Amikacin) and Quinolones group (Ciprofloxacin, Ofloxacin and Norfloxacin) (Raju et al., 2010). So, it showed that the antibiotics currently of choice for the treatment of life-threatening infection caused by MRSA decreased.

5. Conclusion

This study showed high resistance of new MRSA strain to β-Lactam, Glycopeptides groups and some intermediate from other groups (Macrolides and Aminoglycosides). The
study also showed a restricted choice for treatment because the sensitive antibiotics for isolated MRSA strain decreased in number. So the treatment of new MRSA will be difficult, good hygiene practices, infection control and emphasis on hand washing, control misuses of antibiotics, etc. may slow down the process of resistance.

Though the prevalence of MRSA is alarming, the rise of CA-MRSA in recent times has been almost troublesome. Genetic elements allow CA-MRSA to rapidly spread and infection. This has led to poor prevention methods on sporting teams, in prisons and in training centers. The rapid transmission of MRSA infections and the potential for them to progress into life-threatening conditions make these situations quite dangerous. While antibiotic resistance in MRSA increases, the most effective methods of dealing with this threat remain effective preventions of infection.

The next step for confirmation of new MRSA strain will use the molecular methods PCR, including Real-time PCR and Quantitative PCR; they are being increasingly employed in clinical laboratories. This protocol includes detection of nuclease, coagulase, protein A (spa), femA and fmbB, 16S rRNA and surface-associated fibrinogen-binding protein genes, which detects a specific sequence within the internal transcribed spacer (ITS) region of MRSA, have proved to have a crucial and novel molecular approach in the detection of nuclease, coagulase, protein A and negative for the target genes, are essential. 

Acknowledgment

We really appreciate and thank Prof.Dr. Nahla El-Shenawy (Biology Dept., Science Faculty, Taif University) for her aid in this study.

References


