Assessment of the Multifactorial Effect on Antimicrobial Sensitivity in Positive Staphylococcus Aureus Clinical Isolates from Assir Region, Saudi Arabia

NAZAR M ABDALLA,1 WALEED O HAIMOUR,1 AMANI A OSMAN,1 HASSAN ABDULAZIZ1

Abstract:

General objectives: This study aimed at assessment of factors affecting antimicrobial sensitivity in Staphylococcus aureus clinical isolates from Assir region, Saudi Arabia.

Materials and Methods: In this study, eighty one patients presented with Staph. aureus infections either nosocomial or community acquired infections were involved by collecting nasal swabs from them at Aseer Central Hospital General Lab. These patients were from all age groups and from males and females during the period of Jan 2011- Jun 2011. These samples were undergone variable laboratory procedures mainly; bactech, culture media, antibiotics sensitivity test using diffusion disc test (MIC) and molecular (PCR) for detection of mec A gene. Clinical and laboratory data were recorded in special formats and analyzed by statistical computer program (SPSS).

Results: Showed that; Descriptive and analytical statistical analysis were performed and final results were plotted in tables. In Staph aureus MecA gene positive cases (50) showed: Oxacillin/ Mithicillin, Ciprofloxacin and Fusidin resistant in diabetic patients were 13, 26.0%, 9, 18% and 7, 14% respectively and in non diabetic patients were 37, 74.0%, 22, 44% and 20, 40% respectively. While no sensitivity in diabetic and non diabetic patients using Oxacillin/ Mithicillin. In Staph aureus MecA gene negative cases (31) showed: Oxacillin/ Mithicillin, sensitivity in diabetic patients (5, 16.1%) and in non diabetic were (26, 83.9%). While no resistant in diabetic and non diabetic patients. In Ciprofloxacin and Fusidin resistant in diabetic patients were 1, 3.2% and 1, 3.2% respectively and in non diabetic patients were 12, 38.7% and 7, 22.6% respectively.

Erythromycin in Staph aureus (MecA gene) positive cases (50) showed: resistant in age (0-15) years were (5, 10%), (16-50) years were (16, 32%) and (›50 years) were (12, 24%). Erythromycin in Staph aureus (MecA gene) negative cases (31) showed: resistant in age (0-15) years were (6, 19.3%), (16-50) years were (5, 16.1%) and (›50 years) were (3, 9.7%).

Conclusion: Drugs resistance is a major progressive multifactorial problem facing the treatment of Staph aureus infections.

Keyword: Staphylococcus aureus, antimicrobial, resistance, MecA gene.

Introduction:
Staphylococcus aureus is a facultative anaerobic gram positive coccal bacterium. It is frequently found as part of the normal skin flora on the skin and nasal passages. It is estimated that 20% of the human population are long-term carriers of S. aureus which is the most common species of Staphylococcus causing Staph infections. The reason S. aureus is a successful pathogen is a combination of bacterial immune-evasive strategies. One of these strategies is the production of carotenoid pigment staphyloxanthin, which is responsible for the characteristic golden color of S. aureus colonies. This pigment acts as a virulence factor, primarily by being a bacterial antioxidant which helps the microbe evade the reactive oxygen species which the host immune system uses to kill pathogens. Clinically infections by S. aureus (ISA) has broad clinical presentations from bacteraemia with primary superficial focus such as skin, soft tissue infection and arthritis to deep infections such as abscesses from various organs, respiratory and urinary tract infections. S. aureus can also present as toxin-mediated disease without bacteraemia or focal infection, such as toxic shock syndrome, scalded-skin syndrome, neonatal toxic

1. Department of Microbiology, College of Medicine, King Khalid University, Saudi Arabia.

Correspondence: Dr. Abdalla NM, Department of Microbiology, College of Medicine, King Khalid University, Abha, P.O. 641 zip 61421, Saudi Arabia. Tel: 009662417525 Fax: 0096672247800 Email: uofgnazar@hotmail.com
The S. aureus being a gram positive, catalase and coagulase positive furthermore the diagnosis of ISA is based on cultures mostly from normally sterile body sites, often blood. Sometimes there is a clinical suspicious of ISA but cultures are negative or impossible to obtain as in deep abscesses. In patients with bacteraemia it is necessary to have more than one blood samples for culture. Serology against various S. aureus antigens could be useful to differentiate between patients with complicated and uncomplicated infections. Healthy adults have detectable levels against most S. aureus antigens. The antibodies develop during childhood and adult antibody levels are generally reached by age of 15 years. The humoral immune response varies greatly during invasive infections. Hence, the clinical value of diagnosis S. aureus serology is low. This is because of varying sensitivity, specificity and insufficient predictive value of these tests or combination of tests used. It is believed that complicated infections generate a higher antibody response than uncomplicated ones. However, that there is no evidence that any serological assay or combination of assays can distinguish between complicated and uncomplicated S. aureus infections. Sensitivity of patients with S. aureus to variable antibiotics varies with the presence of the MecA gene. However, the reliability of the diagnosis of, for example endocarditis in older studies can be questioned, because of the low use of echocardiogram. The time of sampling is crucial, in some studies samples were collected in the first week after the start of illness showed maximum titer while in other studies sampling during the first week was not accepted. In fact, it has been reported lower levels of antibodies against several antigens in patients with complicated bacteraemia as compared with patients with uncomplicated bacteraemia. Toxic shock syndrome produced by S. aureus can be diagnosed serologically and by determination of specific toxin production from patient’s isolate. This organism has acquired resistance to commonly used antibiotics such as; Oxacillin/ Mithicillin, Ciprofloxacin, Fusidin Erythromycin and Vancomycin. The advent of molecular tool PCR (polymerase chain reactions) has been used to detect different resistance genes that affect the treatment of Staph aureus infections. In many countries, the number of patients in the hospital either colonized or infected with MRSA has grown dramatically in the last two decades. Many factors have been incriminated in this phenomenon, in Saudi Arabia factors such as knowledge, attitude and practice have led to the rising of antimicrobial resistance. Zone sizes for S. aureus for Oxacillin antibiotic; Susceptible (>13 mm), Oxacillin Intermediate (11-12 mm) and Oxacillin Resistant (<10 mm). In this study patients presented with S. aureus infections (ISA) were included to assess the clinical profile and drugs sensitivity tests.

Material and Methods
50 patients with detection of Staphylococcus aureus directly from nasal swab specimens and presented with variable infections; respiratory infection, central nervous system infections, urogenital infection, musculoskeletal (Joints) infections and skin infection were selected from ASEER central hospital, Saudi Arabia during the period from Jan 2011- Jun 2011. These samples were undergone variable laboratory procedures mainly; bactech, culture media, antibiotics sensitivity test using diffusion disc test (MIC) and molecular (PCR) for detection of mecA gene. Clinical and laboratory data were recorded in special formats and analyzed by statistical computer program (SPSS).

1. Collection of samples
The tip of the collection swab was inserted approximately 1 in. (2.56 cm) into the nares and rolled five times in each nostril. Collected specimens were transported and stored at room temperature. Cultures were inoculated and specimens were processed for PCR analysis within 24 hrs of being collected to culture inoculation. Each collection swab was initially inoculated into blood agar. Each sample was examined using the following procedure:

2. Microbiological tests
The cultures were carried out on blood agar. The plates were incubated for 24 to 48 hrs, at 35°C and examined for growth. After incubation each plate was examined to observe the characters of colonial morphology, and the effect of the organism on culture media. The colonies that appeared as medium to large, smooth, entire, slightly raised, translucent, most colonies pigmented creamy yellow, most colonies showed beta-hemolysis. Confirmation of Staphylococcus species were conducted using: microscopic examination of gram stained film, 3% catalase testing , coagulase testing and Staph latex agglutination assay from the colonies grown on the cultured plates.

3. Methods of Antimicrobial Susceptibility Testing
Antimicrobial susceptibility testing methods are divided into types based on the principle applied in each system.
include: (i) Diffusion: stokes and Kirby-Bauer methods. (ii) Dilution: Minimum Inhibitory Concentration (Broth and Agar dilution). (iii) Diffusion & Dilution: E-Test method. In this study the disk diffusion method have been used. Reagents for the Disk Diffusion Test includes:

3.1. Müeller-Hinton Agar Medium
Müeller-Hinton agar was prepared from a commercially available dehydrated base according to the manufacturer’s instructions. Immediately after autoclaving, allow it to cool in a 45 to 50°C water bath.

Pour the freshly prepared and cooled medium into glass or plastic, flat-bottomed petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. This corresponds to 60 to 70 ml of medium for plates with diameters of 150 mm and 25 to 30 ml for plates with a diameter of 100 mm. The agar medium was allowed to cool to room temperature and, unless the plate is used the same day, stored seven days after preparation and wrapped in plastic to minimize drying of the agar.

3.2. Preparation of dried filter paper discs
Whatman filter paper no. 1 was used to prepare discs approximately 6 mm in diameter, which were placed in a Petri dish and sterilized in a hot air oven. The loop used for delivering the antibiotics is made of 20 gauge wire and has a diameter of 2 mm. This delivered 0.005 ml of antibiotics to each disc.

3.3. Reading Plates and Interpreting Results
After 16 to 18 hours of incubation, each plate is examined. If the plate was satisfactorily streaked, and the inoculum was correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc. Zones are measured to the nearest whole millimeter, using sliding calipers or a ruler, which is held on the back of the inverted petri plate. The petri plate is held a few inches above a black, nonreflecting background and illuminated with reflected light. The sizes of the zones of inhibition are interpreted by referring to the standard table, through 21 (Zone Diameter Interpretative Standards and equivalent Minimum Inhibitory Concentration Breakpoints) of the NCCLS M100-S12.

4. Polymerase chain reaction (PCR):
A nasal specimen is collected and transport to the laboratory using the recommended swab with Liquid Stuart Medium (refer to Materials Required but not provided). For testing, the swab is placed in sample buffer. The specimen is concentrated and lysed. An aliquot of the lysate is added to PCR reagents which contain the MRSA-specific primers used to amplify the genetic target, if present. The assay also includes an internal control (IC) to detect PCR inhibitory specimens and to confirm the integrity of assay reagents. The amplification, detection and interpretation of the signals are done automatically by the Cepheid Smart Cycler® software. PCR steps includes: Specimen Handling: 24-36 hours at 15-30 degrees Celsius up to 5 days. Extracting: Break the swab into buffer tube (Blue color) and vortex 60 seconds. Concentration / Wash: Transfer supernatant to lysis tube (Yellow color), centrifuge 5 minutes at 14000 Xg and discard supernatant carefully. Lysis: Add 50ul of Sample Buffer (separate tube) to pellet, vortex 5 minutes, quick spin to bring liquid to bottom of tube, heating block at 95 degrees celsius for 2 minutes and put on ice or cooling block or in freezer. Reagent Reconstitution: Add 255ul of diluent to MM tube, vortex 5-10 sec, add 225ul of sample buffer to PC tube, vortex 5-10 sec. Aliquot 25ul of MM to SC tubes on SC cooling block. Addition of Sample (Lysate): Add 2.8ul of lysate to SC sample tubes, addition of Controls, add 2.8ul PC to PC tube, add 2.8ul of Sample Buffer to NC tube, centrifuge 5-10 seconds on the SC centrifuge.

5. Real – Time PCR Analysis
Using similar protocol of (Van Leeuwen et al., 1996)17. Approximately 5 ng of DNA was added per PCRRR mixture. The mixture consisted of a buffer system containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 2.5 mM MgCl2, 0.01% gelatin, and 0.1% Triton X-100. Deoxyribonucleotide triphosphates (0.2 mM; Pharmacia Biotech, Uppsala, Sweden) as well as 0.2 U of Taq polymerase (SuperTaq; HT Biotechnology, Cambridge, United Kingdom) were present in the reaction mixture. Five different primers and combinations thereof were used in the assays. The codes and sequences of the primers (50 pmol of primer per reaction) were as follows: ERIC-1R, 59-ATG TAA GCT CCT GGG GAT TCA C-39; ERIC-2, 59-AAG TAA GTG ACT GGG GTG AGC G-39. The PCR mixture was overlaid with 100 ml of mineral oil to prevent evaporation. Amplification of DNA fragments was performed in a Biomed thermocycler (model 60; Biomed, Theres, Germany) with predenaturation at 948C for 4 minutes, followed by 40 cycles of 1 min at 948C, 1 min at 258C, and 2 min at 748C. Amplicons were analyzed by agarose gel electrophoresis containing 1% agarose (Hispanagar; Sphaero Q, Leiden, The Netherlands) in 0.53 Tris-borate-EDTA (TBE) in the presence of ethidium bromide (0.3 mg/ml) at a constant current of 100 mA for 3 h. After photography (high-speed sheet film 57; Polaroid). One positive control and one negative control must be included in each assay run on the Smart Cycler®.
6. Data Analysis:
Clinical and Laboratory data were recorded in special formats and entered in stat computer program (SPSS). Descriptive and analytical statistical analysis were performed and final results were plotted in tables.

Results:
Out of 81 positive cases of Staph aureus confirmed by bacteriological test: 50 cases were positive Mec A gene and 31 cases were negative by PCR.

The sample size includes diabetics and non diabetics patients. Sensitivity of some antibiotics were tested among them which include; Oxacillin/ Mithicillin, Ciprofloxacin, Fusidin and Erythromycin.

Oxacillin/ Mithicillin in Staph aureus Mec A gene positive cases (50) showed: resistant in diabetic patients (13, 26.0%) and resistant in non diabetic in this group were (37, 74.0%). While no sensitivity in diabetic and non diabetic patients. Oxacillin/ Mithicillin in Staph aureus Mec A gene negative cases (31) showed: sensitivity in diabetic patients (5, 16.1%) and in non diabetic in this group were (26, 83.9%). While no resistant in diabetic and non diabetic patients (Table-I).

Ciprofloxacin in Staph aureus (Mec A gene) positive cases (50) showed: resistant in diabetic patients (9, 18%) and resistant in non diabetic in this group were (22, 44%). While the sensitivity in diabetic and non diabetic patients were 4 (8%) and 15 (30%) respectively. Ciprofloxacin in Staph aureus Mec A gene negative cases (31) showed: resistant in diabetic patients (1, 3.2%) and resistant in non diabetic in this group were (12, 38.7%). While the sensitivity in diabetic and non diabetic patients were 4 (12.9%) and 14 (45.2%) respectively (Table-II).

Fusidin in Staph aureus (Mec A gene) positive cases (50) showed: resistant in diabetic patients (7, 14%) and resistant in non diabetic in this group were (20, 40%). While the sensitivity in diabetic and non diabetic patients were 6 (12%) and 17 (34%) respectively. Fusidin in Staph aureus Mec A gene negative cases (31) showed: resistant in diabetic patients (1, 3.2%) and resistant in non diabetic in this group were (7, 22.6%). While the sensitivity in diabetic and non diabetic patients were 4 (12.9%) and 19 (61.3%) respectively (Table-III).

Erythromycin in Staph aureus (Mec A gene) positive cases (50) showed: resistant in age (0-15) years were (5, 10%), (16-50) years were (16, 32%) and (>50 years) were (12, 24%). While sensitivity in same age groups were: 9 (18%), 7 (14%) and 1 (2%) respectively. Erythromycin in Staph aureus (Mec A gene) negative cases (31) showed: resistant in age (0-15) years were (6, 19.3%), (16-50) years were (5, 16.1%) and (>50 years) were (3, 9.7%). While sensitivity in same age groups were: 9 (2.9%), 6 (19.4%) and 2 (6.4%) respectively (Table-IV).

Table-I

Showed resistant and sensitivity to Oxacillin/ Mithicillin among Staph aureus (positive and negative MecA gene) cases in diabetic and non diabetic patients

<table>
<thead>
<tr>
<th>Drug</th>
<th>+ve MecA gene (no. = 50)</th>
<th>-ve MecA gene (no = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxacillin/ Mithicillin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sen</td>
<td>0</td>
<td>5 (16.1%)</td>
</tr>
<tr>
<td>Res</td>
<td>13 (26%)</td>
<td>37 (74%)</td>
</tr>
<tr>
<td>Total</td>
<td>13 (26%)</td>
<td>37 (74%)</td>
</tr>
</tbody>
</table>

Table-II

Showed resistant and sensitivity to Ciprofloxacin among Staph aureus (positive and negative MecA gene) cases in diabetic and non diabetic patients

<table>
<thead>
<tr>
<th>Drug</th>
<th>+ve MecA gene (no. = 50)</th>
<th>-ve MecA gene (no = 31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sen</td>
<td>4 (8%)</td>
<td>4 (12.9%)</td>
</tr>
<tr>
<td>Res</td>
<td>9 (18%)</td>
<td>1 (3.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>13 (26%)</td>
<td>5 (16.1%)</td>
</tr>
</tbody>
</table>
Discussion:
The final results from this study have shown that the presence of the mecA gene in S. aureus isolates will lead to 100% resistance to Oxacillin/ Mithicillin while the absence of this gene in the isolates will lead to 100% sensitivity to Oxacillin/ Mithicillin irrespective of patients being diabetic or non diabetic. Erythromycin resistance is clearly increased in elder patients in both MecA gene positive and negative patients. The effect of diabetes on drugs sensitivity is clear among skin infections specimens specially the diabetic foot infections. A study conducted in Abha, Saudi Arabia in 1996 aimed at Nasal carriage and antibiotic resistance of Staphylococcus aureus isolates from hospital and non-hospital personnel; showed that No isolate was resistant to vancomycin. Antibiotic resistance rates, for all other antibiotics tested except cephalothin, were significantly higher for strains from hospital personnel (P values < 0.001-0.04) compared to non-hospital adults. The antibiograms were also compared with those of 140 clinical isolates. The rates of resistance of the inpatient strains to all the antibiotics tested were significantly higher than those of hospital nasal carrier strains (P < 0.001-0.05). MRSA was isolated, respectively, from 5.1% and 18.3% of non-hospital and hospital carriers; MRSA carriage rates were 1.3% and 4.7%, respectively, for non-hospital and hospital carriers, and 61% of S. aureus isolates from infected patients were MRSA. Only 8% of non-hospital but 44% of hospital carrier strains were multiply resistant (P < 0.001).  

A study conducted in north Jordan aimed at Nasal carriage of methicillin-resistant Staphylococcus aureus by hospital staff in north Jordan; showed that the 109 (19.8%) individuals tested who were nasal carriers of S. aureus, only 32 (5.8%) were found to be carriers of methicillin-resistant Staphylococcus aureus. The carriers were four doctors, 23 nurses, three laboratory technicians, one maid and an...
Almost all cases were resistance to erythromycin (98%), cotrimoxazole (94%), gentamicin (92%), clindamycin (6%) while all MRSA isolates were sensitive to vancomycin.25

A Study conducted from July 1996 to July 1999 aimed at studying the impact of nasal carriage of methicillin-resistant and methicillin-susceptible Staphylococcus aureus (MRSA & MSSA) on vascular access-related septicemia among patients with type-II diabetes on dialysis; showed that The prevalence of type-II diabetes of 28.0% with 72.4% of nasal carriage rate and three folds higher S. aureus related VRS (RR-3.19, p<0.0001) than diabetic non-carriers on HD, was observed. Type-II diabetics also had higher MSSA and MRSA nasal carriage rates (53.4% and 19.0%) than non-diabetic nasal carriers (18.6 and 6.0%) yet, carried a comparable (RR-4.0 vs. 4.5) risk of VRS between MSSA and MRSA nasal carriers. Among diabetic type-II S. aureus nasal carriers, central venous catheters (CVCs) carried 35 and 38 times higher collective risk of developing MSSA and MRSA nasal carriage-related VRS respectively than Arterio-venous fistula (AVF). The AVF recorded the lowest risk of developing MSSA and MRSA nasal carriage-related VRS (0.013 and 0.010 episodes/patient-year) in both diabetic type-II MSSA and MRSA nasal carrier groups.26

In Saudi Arabia a study was conducted from January 2005 to March 2008 aimed at identifying severe community-acquired infection caused by methicillin-resistant Staphylococcus aureus among Saudi children showed that; increased the awareness of clinicians regarding severe CA-MRSA infections and highlight the challenges encountered in the choice of therapy of serious infections caused by this organism.27 Epidemiological study conducted aimed at analysis nasal carriage of methicillin resistant Staphylococcus aureus: the prevalence of patients at risk and the effect of elimination on outcomes among out clinic haemodialysis patients showed that; the prevalence of nasal carriage for staphylococcus aureus was 53 % (41 % MSSA, 12 % MRSA). Compared with patients showing no colonization or with MSSA carriers, the 16 patients with nasal carriage for MRSA were older and more likely to have acquired the bacteria while hospitalized. Genotyping of MRSA isolates revealed different strains in patients and care-providers. Mupirocin eliminated MRSA in all patients, none of these patients experienced an infection caused by staphylococcus aureus, confirming the known value of MRSA elimination from other studies.28

A Study conducted aimed at evaluating Methicillin-resistant Staphylococcus aureus in diabetic foot infections; showed that Infections of mild or moderate severity caused by community-acquired MRSA can be treated with
cotrimoxazole (trimethoprim/sulfamethoxazole), doxycycline or clindamycin when susceptibility results are available, while severe community-acquired or hospital-acquired MRSA infections should be managed with glycopeptides, linezolid or daptomycin. Dalbavancin, tigecycline and ceftobiprole are newer promising antimicrobial agents active against MRSA that may also have a role in the treatment of foot infections if more data on their efficacy and safety become available.  

A study conducted aimed at detecting the Staphylococcus aureus resistance to antibiotics showed that; detection is difficult but necessary because vancomycin MIC creep seems linked to poor outcome in patients. While other study revealed that MRSA remain currently susceptible to several antibiotics in addition to glycopeptides. Linezolid and daptomycin, recently introduced in therapy, but have no indication in children. A Study conducted from October to November 2005 aimed at Screening for MRSA in ICU patients. How does PCR detection capability compared with culture, showed that PCR was less specific and more expensive than CHROM agar MRSA. Another study aimed at Comparison of the BD GeneOhm methicillin-resistant Staphylococcus aureus (MRSA) PCR assay to culture by use of BBL CHROMagar MRSA for detection of MRSA in nasal surveillance cultures from an at-risk community population, showed sensitivity of 89.0% and 91.7%, respectively. Vancomycin-resistant S. aureus (VISA) is a strain of S. aureus that has become resistant to the glycopeptides. The first case of vancomycin-intermediate S. aureus (VRSA) was reported in Japan in 1996, but the first case of S. aureus truly resistant to glycopeptide antibiotics was only reported in 2002. Three cases of VRSA infection had been reported in the United States as of 2005.  

**Conclusion:**  
The drugs resistance towards Staph. aureus infections is clearly increased in Sudi Arabia as in worldwide this resistance involved; beta-lactam drugs, vancomycin and aminoglycosides. The new trends in treating Staph aureus infections is a combined therapy specially in serious infections such as pneumonia, meningitis and toxic shock syndrome.  

**Conflict of Interest:** None.  

**Acknowledgement:** We confer our gratitude to the laboratory of Assir Central Hospital. Our sincere thanks to all the department of microbiology, Ribatt national university, Sudan.  

**References:**  


