

Full Length Research Paper

Factors affecting Antimicrobial Sensitivity in Positive *Staphylococcus aureus* clinical isolates from Assir region, Saudi Arabia

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Abstract

This study aimed to assess factors affecting antimicrobial sensitivity in *Staphylococcus aureus* clinical isolates from Asser region, Saudi Arabia. 81 patients presented with *Staphylococcus aureus* infections were involved by collecting nasal swabs at Asser Central Hospital General Laboratory. All age groups and both sex were involved, during the period of Jun 2011- Jan 2012. Laboratory tests performed including; microscopy and culture, antibiotics sensitivity test; minimum inhibitory concentration (MIC) and polymerase chain reactions (PCR) for detection of Mec A gene. Data were collected and analyzed by statistical computer program (SPSS). 50 patients were *Staphylococcus aureus* MecA gene positive that showed: variable resistance to Ciprofloxacin and Fusidin in diabetic and non diabetic patients and 100% resistance to Oxacillin/ Mithicillin. In *Staphylococcus aureus* MecA gene negative (31 cases) showed: variable resistance pattern to Ciprofloxacin and Fusidin and 100% sensitive to Oxacillin/ Mithicillin. Erythromycin in *Staphylococcus aureus* (MecA gene) positive cases (50) showed: 10% resistant in age (0-15 years), 32% in age group (16-50 years) and 24% in (>50 years). Erythromycin in *Staphylococcus aureus* (MecA gene) negative cases (31) showed: 19.3%, 16.1% and 3.9% in age groups respectively. Drugs resistance is a major progressive multi factorial problem facing the treatment of *Staphylococcus aureus* infections.

Keywords: *Staphylococcus aureus*, antimicrobial resistance, oxacillin, mithicillin, ciprofloxacin, fusidin.

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 *Staphylococcus aureus* which is the most common species of *Staphylococcus* causing infections. The reason *Staphylococcus 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 colour of *Staphylococcus 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 *Staphylococcus 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. *Staphylococcus aureus* can also

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present as toxin-mediated disease without bacteraemia or focal infection, such as toxic shock syndrome, scalded-skin syndrome, neonatal toxic shock syndrome-like exanthematous disease and food poisoning. The risk of a secondary or metastatic focus such as endocarditis or other endovascular focus, skeletal and CNS infections and variable abscesses. Usually the presence of secondary infections denote complicated one and it is crucial to successful management of ISA which demand different therapies and follow up these cases.

The *Staphylococcus 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 (Lo et al., 2010). Sometimes there is a clinical suspicion 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 *Staphylococcus aureus* antigens. The antibodies develop during childhood and adult antibody levels are generally reached by age of 15 years. The humeral immune response varies greatly during invasive infections. Hence, the clinical value of diagnosis *Staphylococcus aureus* serology is low (Banchereau R. et al., 2012). 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 *Staphylococcus aureus* infections. Sensitivity of patients with *Staphylococcus aureus* to variable antibiotics varies with the presence of the *MecA* gene (Buckley, 2011, Ling, 2012). 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 (Syed Kashif Nawaz* and Samreen Riaz, 2009) lower levels of antibodies against several antigens in patients with complicated bacteraemia as compared with patients with uncomplicated bacteraemia. Toxic shock syndrome produced by *Staphylococcus 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 (JK Sharp, 2011), Ciprofloxacin (Seif S. and Bazl R., 2011), Fusidic acid (FB McLaws, 2011), Erythromycin (Khan N. W, 2011) and Vancomycin (Antunes and Barth, 2011). 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 (Karakulska, 2011).

In many countries, the number of patients in the hospital either colonized or infected with MRSA has grown dramatically in the last two decades (Bukki and Ostgathe, 2011). 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 (Abdalla, 2011). Zone sizes for *Staphylococcus aureus* for Oxacillin antibiotic; Susceptible (>13 mm), Oxacillin Intermediate (11-12 mm) and Oxacillin Resistant (<10 mm). In this study patients presented with *Staphylococcus 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 Jun 2011- Jan 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).

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:

Microbiological tests

The cultures were carried out on blood agar (all culture media were prepared by bacteriology laboratory, college of medicine, King Khalid University, Abha, Saudi Arabia). 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.

Methods of Antimicrobial Susceptibility Testing

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

Müller-Hinton Agar Medium

Müller-Hinton agar was prepared from a commercially available dehydrated base according to the manufacturer's instructions. Immediately after autoclaving, allowed to cool in a 45 to 50°C water bath.

Poured 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. The agar medium was allowed to cool to room temperature and, unless the plate is used the same day, stored in a refrigerator (2 to 8°C). Plates should be used within seven days after preparation and wrapped in plastic to minimize drying of the agar.

Preparation of dried filter paper discs

What man 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 were made of 20 gauge wire and has a diameter of 2 mm. This delivered 0.005 ml of antibiotics to each disc.

Reading Plates and Interpreting Results

After 16 to 18 hours of incubation, each plate was 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) were 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 plates were 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 2I (Zone Diameter Interpretative Standards and equivalent Minimum Inhibitory Concentration Breakpoints) of the NCCLS M100-S12.

Polymerase chain reaction (PCR)

Nasal swabs were collected and transported to the laboratory using Stuart Medium transport media.. The swabs were placed in lyses buffer (provide with the QIA gen extracting kit). The specimen was concentrated and lysed. An aliquot of the lysates were suspended in absolute alcohol and preserved in Tris EDTA (TE) according to the QIA gen extracting kit protocol. Suspended DNA were added to PCR reagents following QIA gen PCR kit 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 were done automatically by the Cepheid Smart Cycler® software.

PCR steps

Specimen Handling: 24-36 hours at 15-30 C° up to 5 days. *Extracting:* swabs were broken into buffer tube (Blue colour) and vortexed 60 seconds. Concentration / Wash: supernatant were transferred to lysis tube (Yellow colour), centrifuged 5 minutes at 14000Xg and discarded supernatant carefully.

Lysis: 50ul of sample buffer (separate tube) were added to pellet, vortexed 5 minutes, quick spin to bring liquid to bottom of tubes, heated in heating block at 95 C° for 2 minutes and put on ice or cooling block or in freezer. *Reagent Reconstitution:* Added 255ul of diluents to MM tubes, vortexed 5-10 sec, added 225ul of sample buffer to positive control (PC) tube, vortexed 5-10 sec. Aliquot 25ul of MM were added to smart cycler (SC) tubes on SC cooling block. *Addition of Sample (Lysates):* 2.8ul of samples lysates were added to SC sample tubes. *Addition of Controls:* 2.8 ul of PC was added to PC tube and added 2.8ul of Sample Buffer to negative control (NC) tube. All samples and controls were centrifuged 5-10 seconds on the SC centrifuge.

Real – Time PCR Analysis

Using similar protocol of Van Leeuwen (van Leeuwen W, 1996). Approximately 5 ng of DNA was added per PCR 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% gelatine, 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. Species specific set of primers were used. 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 94°C for 4 min, followed by 40 cycles of 1 min at 94°C, 1 min at 258°C, and 2 min at 74°C. 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®.

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.

from 81 patients 50 patients were *Staph aureus* MecA gene positive cases showed: Oxacillin/ Mithicillin, Ciprofloxacin and Fusidin resistant in diabetic patients in this group (13 patients), were 26.0%, 18% and 14% respectively and in non diabetic patients (37 patients), 74.0%, 44% and 40% respectively. All Mec Agene positive patients (50) showed 100% resistance to Oxacillin/ Mithicillin. In *Staph aureus* MecA gene negative (31 cases) showed: Oxacillin/ Mithicillin, sensitivity in diabetic patients (16.1%) and in non diabetic were (83.9%), All Mec Agene negative patients (31) showed 100% sensitivity to Oxacillin/ Mithicillin Table 1. In diabetic patients in this group (5 cases) resistance to Ciprofloxacin and Fusidin were 3.2% and 3.2% respectively Table 2 and in non diabetic patients (26 cases) were 38.7% and 22.6% respectively Table 3.

Erythromycin in *Staph aureus* (MecA gene) positive cases (50) showed: 10% resistant in age (0-15) years, 32% in age group (16-50) years and 24% in (>50 years).

Erythromycin in *Staph aureus* (MecA gene) negative cases (31) showed: 19.3% resistant in age (0-15) years, 16.1% in (16-50 years) and 3.9% in (>50 years) Table 4.

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 high among skin infections specimens specially the diabetic septic foot . A study conducted in Abha, Saudi Arabia in 1996 evaluating nasal carriage and antibiotic resistance of *Staphylococcus aureus* isolates from hospital and non-hospital personnel; showed that all isolate were sensitive to vancomycin. Antibiotic resistance rates, for all other antibiotics tested except cephalothin, were significantly higher for strains from hospital personnel compared to non-hospital patients. Methicillin Resistant *Staph. aureus* (MRSA) was isolated, respectively, from 5.1% and 18.3% of non-hospital and hospital carriers (Bilal NE, 2000). Similarly a study conducted in Al-Noor, King Abdul-Aziz, Hera and King Faisal Hospitals, Makkah, in April 2003 aimed at evaluation of Methicillin resistance among *Staphylococcus aureus* isolates from Saudi hospitals; showed that prevalence of MRSA among *S. aureus* isolates was 38.9% (199/512). From these MRSA isolates, 78.8% showed multidrug resistance to erythromycin, gentamicin and oxytetracycline (El Amin, 2012). A study conducted in King Fahad Hospital of the University Al-Khobar, Saudi Arabia aimed at Emergence of methicillin-resistant *Staphylococcus aureus* as a community pathogen; should that The number of patients with community-acquired MRSA disease increased from a single patient in 1998 to fifteen patients in the year 2000, the percentage of community-acquired MRSA/total number of MRSA increased from 5% to 33% and the community acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infection has become a major pathogen causing significant infection in children in Saudi Arabia. (Bukhari EE, 2009). A study conducted in north Jordan aimed at assessing the nasal carriage of MRSA by hospital staff in north Jordan; showed that the 19.8% were carriers of MRSA. The carriers were four doctors, 23 nurses, three laboratory technicians, one maid and an administrator. It was noted that 78.1% of these carriers were in constant contact with patients in operating theatres, surgical wards or intensive care units (Na'was T, 1991). Another study conducted in Jordan concerned with antibiotic resistance patterns in relation to the Mec A gene *Staphylococcus aureus* isolates from clinical specimens and nasal

Table 1. Resistant and sensitivity to Oxacillin/ Mithicillin among *Staph aureus* isolates

Drug	+ve MecA gene (no. = 50)		-ve MecA gene (no = 30)	
	Diabetic	Nondiabetic	diabetic	Nondiabetic
Sensitivity	0	0	5 (16.1%)	26 (83.9%)
Resistance	13 (26%)	37 (74%)	0	0
Total	13 (26%)	37 (74%)	5 (16.1%)	26 (83.9%)

Isolates composed of positive and negative MecA gene cases in diabetic and non diabetic patients

Table 2. Resistant and sensitivity to Ciprofloxacin among *Staph aureus* isolates

Drug	+ve MecA gene (no. = 50)		-ve MecA gene (no = 31)	
	Diabe	Nondiab	diab	Nondiab
Sensitivity	4 (8%)	15 (30%)	4 (12.9%)	14 (45.2%)
Resistance	9 (18%)	22 (44%)	1 (3.2%)	12 (38.7%)
Total	13 (26%)	37 (74%)	5 (16.1%)	26 (83.9%)

Isolates composed of positive and negative MecA gene cases in diabetic and non diabetic patients

Table 3. Resistant and sensitivity to Fusidin among *Staph aureus* isolates

Drug	+ve MecA gene (no. = 50)		-ve MecA gene (no = 31)	
	Diabe	Nondiab	diab	Nondiab
Sensitivity	6 (12%)	17 (34%)	4 (12.9%)	19 (61.3%)
Resistance	7 (14%)	20 (40%)	1 (3.2%)	7 (22.6%)
Total	13 (26%)	37 (74%)	5 (16.1%)	26 (83.9%)

Isolates composed of positive and negative MecA gene cases in diabetic and non diabetic patients

Table 4. Resistant and sensitivity to Erythromycin in *Staph aureus* isolates among different age groups

Drug	+ve MecA gene (no. = 50)			-ve MecA gene (no = 31)		
	0- 15 yrs	16 -50 yrs	>50 yrs	0 -15 yrs	16 – 50 yrs	>50 yrs
Sensitivity	9 (18%)	7 (14%)	1 (2%)	9 (2.9%)	6 (19.4%)	2 (6.4%)
Resistance	5 (10%)	16 (32%)	12 (24%)	6 (19.3%)	5 (16.1%)	3 (9.7%)
Total	14 (28%)	23 (46%)	13 (26%)	15 (48.4%)	11 (35.5%)	5 (16.1%)

Isolates composed of positive and negative MecA gene cases

carriage; revealed that Mec A gene was detected in all MRSA isolates and they were multi resistant to three antibiotic classes (beta-lactams, amino glycosides, macrolides-lincosamides). This result suggests a serious problem may be encountered in treatment of staphylococcal infections in Jordan (Khalil et al., 2012). Furthermore a study conducted in the laboratory of King Fahad Hospital, Al-Baha, Kingdom of Saudi Arabia among 2001-2004 aimed at identifying of antibiotic susceptibility tests, plasmid profiles and restriction enzyme analysis of plasmid DNA of methicillin susceptible and resistant-Staphylococcus aureus strains

isolated from intensive care units; showed variable MRSA subgroups that emerged from different geographical sites (Tayfour MA, 2005). While a study conducted in National Medical College Teaching Hospital, Birgunj, Nepal aimed at evaluating the rate of MRSA; showed that highest MRSA prevalence rate was among health-care personnel (10.0%), followed by visitors/patient attendants (8.2%) and the patients 3.2%. All MRSA isolates were resistant to Ampicillin and all were sensitive to Erythromycin and Vancomycin (Shakya, 2010). A study conducted in hospital university Sains Malaysia during 2002-2007 aimed at determining

Methicillin-resistant *Staphylococcus aureus* nosocomial infection trends showed; the rate of nosocomial MRSA infection per 1000 admissions was higher than in other studies and the main three attributable factors include; duration of hospitalization, antibiotic use and bedside invasive procedures. The higher MRSA infections were in orthopedic ward (25.3%) followed by surgical ward (18.2%) then intensive care unit (16.4%). Almost all cases were resistance to erythromycin (98%), cotrimoxazole (94%), gentamycin (92%), clindamycin (6%) while all MRSA isolates were sensitive to vancomycin (Hassanain I. Al-Talib, 2010). 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 and 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 (Saxena AK, 2003). Severe community-acquired infections caused by methicillin-resistant *Staphylococcus aureus* in Saudi Arabian 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 (Al-Mendalawi, 2010). Study conducted aimed at evaluating Methicillin-resistant *Staphylococcus aureus* in diabetic septic foot; 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 (Eleftheriadou I, 2010.). 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 (Dumitrescu O, 2010.). Vancomycin-resistant *S. aureus* (VRSA) is a strain of *S. aureus* that has emerged recently in Japan in 1996 (Hiramatsua, 1997) (Chang S 2003) and in the

United States as of 2005 (Menichetti, 2005).

CONCLUSION

The drugs resistance towards Staph. Aureus infections are clearly increased in Saudi Arabia as in worldwide this resistance involved; beta-lactam drugs, vancomycin and amino glycosides. The new trends in treating Staph aureus infections are a combined therapy especially in serious infections such as pneumonia, meningitis and toxic shock syndrome.

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