
Prevalence and Antibiotic Susceptibility Pattern of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolated from Clinical Specimens in Eastern Province, Saudi Arabia

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Summary:

Staphylococcus aureus is environmental organism commonly found and most of them are opportunistic pathogens that colonized in human bodies cause various human disease. To determine the prevalence and antibiotic susceptibility pattern for multidrug and non-multidrug methicillin/oxacillin resistant *S. aureus* (MRSA) 133 *S. aureus* strains, isolated from different clinical specimens submitted to Department of Microbiology, Saad Speciality Hospital Laboratory, Saudi Arabia between November 2008 and September 2010. The percentage of MRSA and MDR were 39.1 % and 54.9 %, respectively. While the remaining strains were considered as Oxacillin sensitive *S. aureus* (OSSA) represented (60.9 %) more than half percentage of total isolated *S. aureus*. From 14 sample source of clinical specimens, the most common source of all isolated species combined was abscesses 62 (46.5%) isolate, followed by nasal swab 19 (14.3%) isolate, respiratory sample 14 (10.5%) isolate, wound 13 (10%) isolate, while the other teen source combined 25 (18.7%) isolate of all isolated *S. aureus*. *S. aureus* strains in general showed highly sensitive percentage to Vancomycin (100%), Linezolid (100%), Rifampin (98.5%), Nitrofurantoin (97.7%) and Trimethoprim-Sulfamethoxazole (93.2). Moreover, the lowest sensitive rate was observed with Penicillin (3%), Ampicillin (4.5%), Ceftriaxone (22%).

Introduction:

Staphylococcus aureus is environmental organism commonly found around us in (soil, water, food and air) and is also found in the mucous membranes and on the skin of healthy individuals.^{1,2} At least 30% of the population may permanently or intermittently carry *S. aureus*. These organisms are easily recognized in the laboratory by their tendency to produce coagulase enzyme that causes citrated plasma to clot and classic golden pigmented colonies with beta- hemolysis on blood agar and the appearance of clustered, grape-like Gram-positive cocci on Gram staining.^{2,3}

Most of *S. aureus* strains are opportunistic pathogens that colonized in human bodies, without symptoms, for short or long period of time, and causing disease when the immune system becomes compromised. Diseases caused by *S. aureus* had high mortality rates.⁴

S. aureus strains have been recognized for a long time as one of most important causative agent of human infection in hospitalized and

nonhospitalized patients all over the world. It is associated with infections in all age groups, including surgical wounds, skin abscess, osteomyelitis, septicemia, food poisoning and toxic shock syndrome, and considered as the second most common cause of hospital-acquired (nosocomial) bloodstream infections.³⁻⁵

More than 50 years ago *S. aureus* developed drug resistance strain, that resistant to beta-lactams including Methicillin. Oxacillin was used as an alternative to Methicillin in susceptibility tests resulted in the term 'Oxacillin-resistant *S. aureus*' (ORSA).⁶ Methicillin-resistant *S. aureus* (MRSA) has become widespread in healthcare settings globally which is resistant to numerous antibiotics and causing severe infections including septicemia, endocarditis and meningitis. It is common cause of community and hospital acquired infections for both patients and the healthcare system. This infection increased hospitalization costs and high mortality/morbidity.^{7,8}

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The resistance of MRSA is commonly mediated by an altered penicillin-binding protein (PBP2a), which is encoded by the *mecA* gene which is chromosomally encoded. *MecA* confers resistance not only to beta-lactams, but also to antibiotics such as Aminoglycosides.^{8,9} MRSA now represent a global problem. Some outbreaks of MRSA have been reported from different parts of the world. Antibiotics abuse, prolonged hospitalization, intravascular instrumentation and hospitalization in an intensive care unit are considered as risk factors that contribute to MRSA. The common sources of these infections are human patients and carriers of MRSA, especially in the nasal cavity, even along medical personnel. There is considerable variation in numbers of clinical infections among units, hospitals and countries.^{10,11}

There is a relationship between colonization rates and the rates of infection. Rapid and accurate identification of MRSA strains and patients infections are important in controlling the spread of infection.^{12,13}

The resistances of MRSA for many types' drugs make therapeutic options for MRSA infection by common antibacterial drugs. They are susceptible only to the glycopeptide antibiotics such as Vancomycin.^{14,15} Vancomycin and few expensive drugs such as Linezolid, Teicoplanin are now the best therapeutic options for MRSA infections.^{6,15} Few MRSA strains resistance to Vancomycin or linezolid were reported around the world.^{16,17}

The aim of the present study was to determine the prevalence and antibiotic susceptibility pattern for multidrug and non-multidrug Methicillin/Oxacillin resistant *S. aureus* (MRSA) isolated from different clinical specimens in Eastern Province, Saudi Arabia using common use antibiotic.

Materials And Methods:

A total of 133 *S. aureus* strains, isolated from different clinical specimens included various body sites. Both sexes and all age groups of out or inpatients hospitalized in different wards submitted to Department of Microbiology, Saad Specialty Hospital Laboratory Saudi Arabia, between November 2008 and September 2010.

Clinical specimens were collected using standard collection techniques,^{8,18} and

inoculated on appropriate bacteriological media, including 10% Sheep Blood Agar, Chocolate Agar, Mannitol salt Agar and Oxacillin Blood Agar media. The plates were incubated aerobically at 37°C for 18-24 hours. The blood cultures were performed using an automated blood culture system (BACT / ALERT 3D, BioMerieux), and then inspected for bacterial growth.

The identification of isolates was made according to standard methods (Forbes, 2007) for any potential of being clinically significant growth appear on the culture media on the base quantity, feature of growth, source and site of specimens were recorded.

The primary identification start with basic microbiological methods using colony morphology, gram staining, catalase and coagulase tests,¹⁹ then the final identification and antibiotic susceptibility testing of the bacteria isolated from Clinical specimens were obtained using an autoanalyzer system (MicroScan WalkAway 96 SI, Dade Behring INC., West Sacramento, CA) (20), the results of MicroScan were confirmed manually by using biochemical tests and Kirby – Bauer disk diffusion technique according to NCCLS guidelines on Mueller-Hinton agar where appropriate.²¹

The in-vitro antibiotic susceptibility pattern of all isolated CoNS was determined against 18 antibiotics, the antimicrobial agents used were: Penicillin, Ampicillin, Amoxicillin-Clavulanic Acid, Cefazolin, Trimethoprim-Sulfamethoxazole, Oxacillin, Gentamicin, Erythromycin, Ceftriaxon, Ciprofloxacin, Norfloxacin, Nitrofurantoin, Linezolid, Levofloxacin, Tetracycline, Clindamycin, Rifampicin and Vancomycin all from (Oxoid, UK).

Results:

Based on standard manual and automated methods, a total 133 of *S. aureus* were isolated and identified from various clinical specimens collected from in/out patients in different age and both sex during the study period.

133 *S. aureus* strains were isolated from varying infected body sites. The percentage of MRSA (Resistant to both Oxacillin) and MDR (Resistant to four or more than four antibiotics) were 39.1 % and 54.9 %, respectively (Fig. 1). While the remaining strains were considered as Oxacillin sensitive *S. aureus* (OSSA) represented (60.9%) more than half percentage of total isolated *S. aureus*.

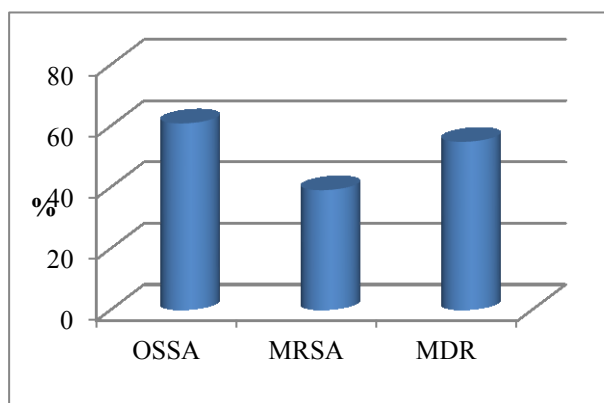


Figure 1: Prevalence of *S. aureus* strains from different clinical specimens.

From 14 sample sources, the most common source of all isolated species combined were abscesses 62 (46.5%) isolate, followed by nasal swab 19 (14.3%) isolate, respiratory sample 14 (10.5%) isolate, wound 13 (10%) isolate, while the other teen source combined 25 (18.7%) isolate of all isolated *S. aureus* (Table 1). The highest percentages of MRSA and MDR strains present in abscesses followed by nasal swabs, wound, respiratory samples and Catheter tips, respectively (Table 1). In all specimens the OSSA strains were present in high percentages except in the nasal swabs the MRSA represent 84.2% from all *S. aureus* strains isolated from this type of specimens (Figure 2).

Table 1: Percentages of *S. aureus* strains isolated from different clinical specimens.

Source of specimens	Total Isolates		OSSA	MRSA	MDR
	NO.	Percentage (%)			
Abscess	62	46.5	53.3	36.6	46.6
Nasal	19	14.3	3.7	30.8	23.3
Respiratory	14	10.5	11.1	9.6	6.8
Wound	13	10	8.7	11.5	8.2
Eye	5	3.7	4.9	1.9	2.7
Blood	4	3	3.7	1.9	1.4
Throat	4	3	3.7	1.9	1.4
Catheter tip	3	2.25	1.2	3.9	4.1
Urine	3	2.25	3.7	0	0
EAR	2	1.5	1.2	1.9	2.7
Cervical	1	0.75	1.2	0	1.4
Semen	1	0.75	1.2	0	0
Tissue	1	0.75	1.2	0	0
Vaginal	1	0.75	1.2	0	1.4

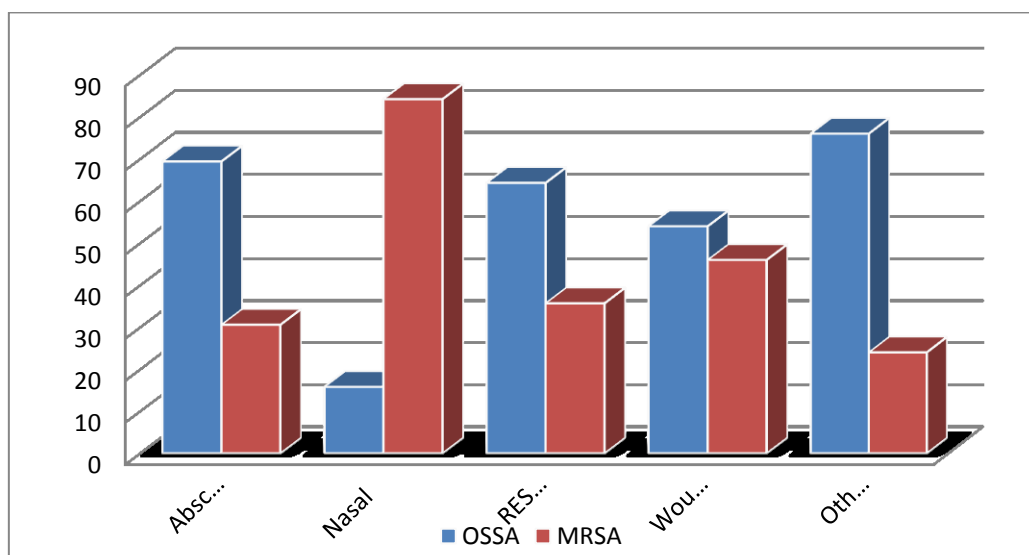


Fig 2: Distribution of OSSA and MRSA among each specimen.

From all identified clinical specimens using for isolated species in present study, 54 (40.6%) *S. aureus* strains isolated from specimens which received to the Microbiology Department Laboratory from outpatient clinics (OPD), 27 (20.3%) from adult Intensive Care Units (ICU), 18 (13.5%) from Emergency department (ER), 15 (11.3%) from Internal Department, 8 (6%) from Nursery Intensive Care Units (NICU), 7 (5.3%) from Pediatrics wards and 4 (3%) from Surgery wards (Table 2). The highest percentages of MRSA and

MDR strains isolated from OPD clinics followed by ICU, Internal department, ER, Pediatrics wards and Surgery wards, respectively (Table 2).

The distribution of all isolated *S. aureus* strains were nearly equal between Inpatients and outpatient (OPD & ER) specimens. The highest percentages of these strains present in outpatient specimens, except the MRSA strains were present in high percentages (53.4%) in inpatient specimens (Figure 3).

Table 2: Percentages of *S.aureus* strains isolated from different clinics of hospital.

	Total Isolates		OSSA	MRSA	MDR
	N0.	Percentage (%)	Percentage (%)		
OPD clinics	54	40.6	38.3	44.3	43.8
ICU	27	20.3	17.3	25	17.8
Emergency	18	13.5	19.8	3.8	9.6
Internal	15	11.3	8.6	15.4	15
NICU	8	6	7.4	3.8	4.3
Pediatrics	7	5.3	4.9	5.8	6.8
Surgery	4	3	3.7	1.9	2.7

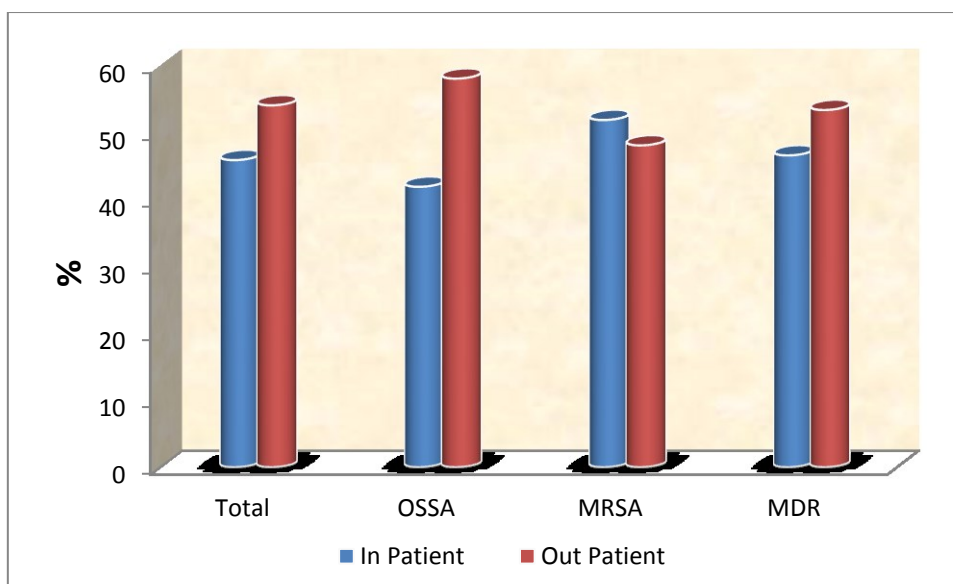


Figure 3: Percentages of *S. aureus* strains among in/ out patient departments.

The susceptibility pattern of *S. aureus* strains were isolated from clinical specimens is presented in Table 3. *S. aureus* strains in general showed highly sensitive percentage to Vancomycin (100%), Linezolid (100%), Rifampin (98.5%), Nitrofurantoin (97.7%) and Trimethoprim-Sulfamethoxazole (93.2%).

Moreover, the lowest sensitive rate was observed with Penicillin (3%), Ampicillin (4.5%) and Ceftriaxon (22%). The OSSR showed highly sensitivity percentage (> 80%) to all used antibiotics except Penicillin and Ampicillin. the highest sensitivity percentages of MRSA and MDR strains showed against

Vancomycin, Linezolid, Rifampin, Nitrofurantoin and Trimethoprim-Sulfamethoxazole. While these two strains

were have high resistance percentages for Amoxicillin-Clavulanic acid, Cefazolin, Ceftriaxon, Penicillin and Ampicillin.

Table 3: Susceptibility pattern of *S.aureus* strains for different antibiotics.

	Total Isolats	OSSA	MRSA	MDR
	Percentage (%)			
Vancomycin	100	100	100	100
Linezolid	100	100	100	100
Rifampin	98.5	98.7	98	97.2
Nitrofurantoin	97.7	100	96.1	95.8
Trimeth./Sulfa	93.2	100	82.6	87.6
Gentamycin	86.5	96.2	71.1	78
Tetracycline	81.2	92.5	63.5	69.8
Norfloxacin	77.4	85.1	65.3	58.9
Clindamycin	76.7	85.1	63.5	58.9
Ciprofloxacin	75.9	85.1	61.5	56.1
Levofloxacin	72.9	83.9	55.7	52
Erythromycin	72	81.4	57.7	50.6
Oxacillin	60.9	100	0	28.7
Amox/clav.	59.4	97.5	0	26
Cefazolin	58.6	96.2	0	24.6
Ceftriaxon	58.6	96.2	0	24.6
Ampicillin	4.5	7.4	0	2.7
Penicillin	3	4.9	0	1.3

Discussion:

Staphylococcus aureus is a significant cause of human disease and is one of the most common causes of healthcare acquired infections. MRSA is the most important pathogen among Gram-positive cocci and they are a part of a larger problem of antibiotic resistance. MRSA now more common in medical facilities, community and other institutions.^{5,7,9}

The percentages of isolated MRSA (39.1%) in this study (Figure 1) were nearly comparable to that of reported in India, Iran, Pakistan and in Jeddah city/ Saudi Arabia.²²⁻²⁶ Lower percentages (15%) were reported in Al-jouf province of Saudi Arabia.²⁷ Other researchers were report higher incidence percentages in many area of the world.²⁸⁻³⁰ Geographic variation, personal hygiene, using of antibiotics, length of hospital stay, nutritional status and types of body normal flora may be the most reasons of the variation in MRSA strains prevalence around the world.

S. aureus carriage by nasal and skin has been identified as an important risk factor for the development of *S. aureus* infections. The carriage rate is depending on different factors such as sex and age.^{31,32} Most isolates of *S. aureus* strains were observed in this study were related to skin and respiratory samples. On the other hand, the highest percentages of MRSA and MDR strains are closely related to these two specimens source (Table 1). The population of normal flora and the type of bacteria in the human body environment may be consisting major sources of microbes that cause a serious infection (Fig 2).

Many investigators have reported that higher percentages of MRSA mostly of which originated from skin specimens (abscess, wounds, pus), and from respiratory specimens (nasal swab, sputum and washing) some of them were similar to our results and others high or low with some variation.^{14,23,25,29,33-40} In some studies the MRSA were isolated in

higher percentages from other specimens type such as body fluids, urine and blood and tissue.^{11,23,28,39-40} These differences might be due to prolonged antibiotic treatment of severely sick patients, who generally have longer hospital stays, resulting in enhanced selection pressure. This reflects the fact that critically ill patients have a greater chance of becoming colonized or infected. The results of our study were similar to those seen in other studies.^{23,29,36}

However, an interesting finding was that the highest percentages of *S. aureus* strains in general were isolated (OSSA & MDR) from the outpatient clinics (OPD & ER) except the MRSA percentages were isolated from all inpatient departments higher than outpatient clinics (figure 3 & table 2). This higher number can possibly be explained by looking further into the population of patients being seen in the outpatient Departments. Urinary tract infections and wound infections, if present in higher amounts in that population, could potentially clarify the increased use of microbiology specimens to manage these outpatient conditions. Another possible reason for the OPD specimens having such a high percentage of *S. aureus* strains isolates may be related to poor sample collection techniques and/or multi-sample sources and clinic.^{2,13,41} These results were similar to many previous research results.^{11,14,28,38,42} In addition to that, the length of hospital stay, nutritional status of

the patient, prolonged antibiotic treatment, presence of wounds and/or invasive devices and ICU environment may be explain the highest percentage of MRSA in ICU.^{11,34,38}

Different antibiotics were used to evaluate the susceptibility pattern of isolated *S. aureus* strains according to recommendations of CLSI 2009 fore Gram Positive Bacteria (CLSI).²¹ *S. aureus* strains isolates showed higher sensitivity value to Vancomyc, Linzolid, Rifampin, Nitrofurantin and Trimeth./Sulfa. These five antibiotics may play an important role in treatment and prevent nosocomial infection of *S. aureus*. However, *S. aureus* strains showed remarkable resistance to Ampicillin, Penicillin and other type of antibiotics listed in Table 3. These results are constant with different studied.^{1,4,11,14,24,25,29,33,35,38,39}

It is thus concluded that carefully evaluation of *S. aureus* and performance on susceptibility tests on all *S. aureus* isolates, considered being a cause of infection especially that isolated from blood cultures, catheter tip cultures, ICUs and Pediatrics wards samples.

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