

Antimicrobial susceptibility pattern of *Staphylococcus aureus* isolated from clinical specimens in Northern area of Jordan

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ABSTRACT

Background and Objectives: The global spread of methicillin resistant *Staphylococcus aureus* (MRSA) constitutes one of the most serious contemporary challenges to the treatment of hospital-acquired infections. We aimed to screen and assess the antibiotic susceptibility pattern of *Staphylococcus aureus* isolated from clinical specimens in local hospitals of Northern province in Jordan.

Materials and Methods: *Staphylococcus aureus* was isolated and identified using standard methods from various clinical specimens of different infected body sites from 358 patients during the period from January 2008 to November 2012.

Results: Our analysis showed that 31.6% of *S. aureus* infections were MRSA, while 31% were multidrug resistance (MDR) and 42.7% were Oxacillin-resistant (ORSA). Most of these strains were isolated from wound specimens. All isolates were susceptible to vancomycin (100%). They were also susceptible to chloramphenicol, linezolid, nitrofurantoin, rifampicin and teicoplanin (>80%), but showed resistance to erythromycin and penicillin.

Conclusion: Vancomycin was the most effective antimicrobial agent against *S. aureus*. We recommend regular surveillance of hospital associated infections and monitoring antibiotic sensitivity pattern and strict drug policy for antibiotics used within and outside the hospital environments.

Keywords: *Staphylococcus aureus*, MRSA, MDR,

INTRODUCTION

Staphylococcus genus is a heterogeneous group of bacteria consisting of 30 species. *Staphylococcus aureus* has been found to be the most clinically important species, with broad presence in nature. It is part of the normal flora of human body and commonly carried on the skin or in the nose of healthy

individuals, which makes it easy to be transmitted by air or fomites from patients or carriers (1, 2). It been recognized as one of the most common cause of human infections, such as skin infections, wound infections and bacteremia. Nevertheless; the introduction of antibiotics has lowered the mortality rate of *S. aureus* infections. However, the bacteria have rapidly developed resistance mechanisms against many antimicrobial agents (1, 3).

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been isolated and recognized more than 50 year ago. MRSA is a specific strain of the *S. aureus*, which is resistant to methicillin and all β -lactams. Later use of Oxacillin as an alternative to methicillin

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in susceptibility tests resulted in the term 'Oxacillin-resistant *S. aureus*' (ORSA) (2), which is resistant to numerous antibiotics. Before the development of antibiotics, invasive infections caused by *Staphylococcus aureus* have often been fatal (4).

The global spread of MRSA constitutes one of the most serious contemporary challenges to the treatment of hospital-acquired infections (5). MRSA carries a uniquely effective antibiotic resistance mechanism that can protect the microorganisms against all members of β -lactam antibiotics. This makes infections caused by these pathogens very difficult to manage and costly to treat (6, 7).

Centre for Disease Control and Prevention (CDC) published a report in 2004 demonstrating that approximately 60% of all healthcare-associated *Staphylococcus aureus* infections in the United States are caused by MRSA. *Staphylococcus aureus* was the second most common organism causing nosocomial blood stream infections, and the proportion of MRSA isolates increased from 22% in 1995 to 57% in 2001(4, 8). Moreover, data from the National Nosocomial Infections Surveillance system suggest that in intensive care units the proportion of *Staphylococcus aureus* isolates that are resistant to methicillin has increased to approximately 60% (1, 6, 8). Recent reports also suggest that community-associated MRSA infections have become the dominant cause of community-associated *Staphylococcus aureus* skin and soft tissue infections (9). About 15% of reported infections have been considered to be community-associated, which means that the infection occurred in people without documented healthcare risk factors (10, 11).

Currently, vancomycin has been accepted worldwide as the last choice against MRSA infections (11). Rarely, clinical isolates of vancomycin-resistant *S. aureus* (VRSA) have been reported recently (8). The emergence of *S. aureus* isolates resistant to vancomycin and other wide range of structurally unrelated antibiotics have elevated MRSA into a multi-drug-resistant "Superbug", making it more and more dangerous than ever in a hospital environment and also recently, in the healthy community (12, 13).

The objectives of the present study were to detect the prevalence and identify the multi-drug and non-multidrug Methicillin/Oxacillin resistant *S. aureus* (MRSA, ORSA& MDR) from clinical specimens in Jordan using common used antibiotics.

MATERIALS AND METHODS

Our study included specimens that are collected between January 2008 and November 2012. All clinical specimens were received and collected by laboratory department of assigned hospitals. 358 isolates of *S. aureus* were isolated from both genders and all age groups of out- or inpatients hospitalized in different wards. Clinical specimens were taken from various body sites of infection including blood, wound, sputum, urine and others.

Clinical specimens were collected using standard collection techniques (14, 15), and inoculated on appropriate bacteriological media, including 10% Sheep Blood agar, Chocolate agar, Thioglycollate, MacConkey 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 (BACTEC 9240 and 9050 BD), followed by bacterial growth inspection.

The identification of isolates was made according to standard methods (16) for any potential clinically significant growth appear on the culture media on the base of quantity, feature of growth, source and site of specimens. The primary identification was made with basic microbiological methods using colony morphology, Gram staining, catalase and coagulase tests (14). The final identification and antibiotic susceptibility testing of the bacteria isolated from clinical specimens were obtained using an auto-analyzer system (Biomerieux, VITIC 60, France). The results of VITIC 60 were confirmed manually using biochemical tests and Kirby-Bauer disk diffusion technique according to CLSI guidelines.

The susceptibility of all isolates were determined against penicillin (P, 10ug), azithromycin (AZM, 15ug), chloramphenicol (C, 30ug), gentamicin (GN, 10ug), trimethoprim-sulfamethoxazole (SXT, 1,25/23,75ug), oxacillin (OX, 1ug), erythromycin (E, 15ug), cefoxitin (FOX, 30ug), nitrofurantoin (NIT, 300ug), linezolid (LZD, 30ug), levofloxacin (LEV, 5ug), tetracycline (T, 30ug), teicoplanin (TE, 30ug), clindamycin (CC, 2ug), rifampicin (RA, 5ug) and vancomycin (V, 30ug).

RESULTS

Based on culture, Gram staining, catalase, coagulase and biochemistry tests, 358 *Staphylococci au-*

reus were isolated from different infected body sites. The percentage of MRSA (resistant to both oxacillin and cefoxitin) and MDR (Resistant to four or more than four antibiotics) (17) were 31.6 % and 31 %, respectively (Fig. 1), and the percentage of ORSA was the highest (42.7 %) among the resistant types of *S. aureus*. The remaining strains were considered to be oxacillin susceptible *S. aureus* (OSSA). The OSSA

represented more 57.3 % of total isolates.

In present study, 57% of *S. aureus* were isolated from males and 43 % from females. The percentage of MRSA, ORSA and MDR strains were similar in both genders, while the percentage of *S. aureus* and OSSA were higher in males than females (Fig. 2).

According to age (Table 1), the high percentages of *S. aureus* strains (30.2% - 35.2%) were isolated from

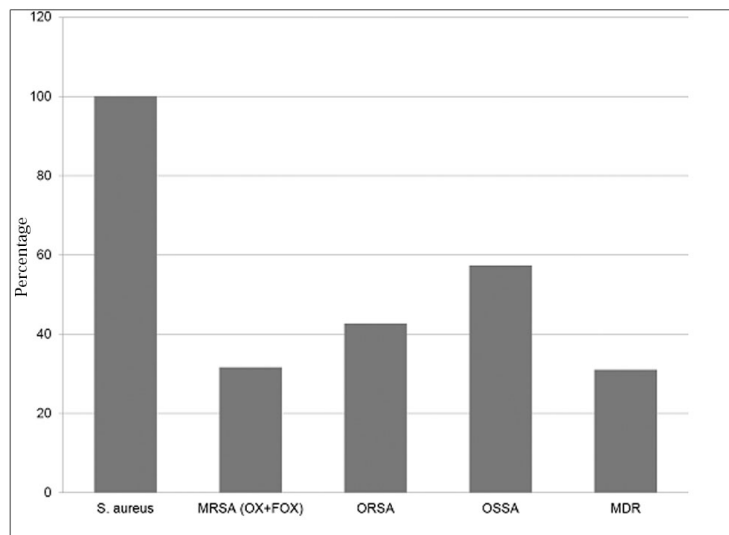


Fig. 1. Percentage of all isolated *S. aureus* strains.

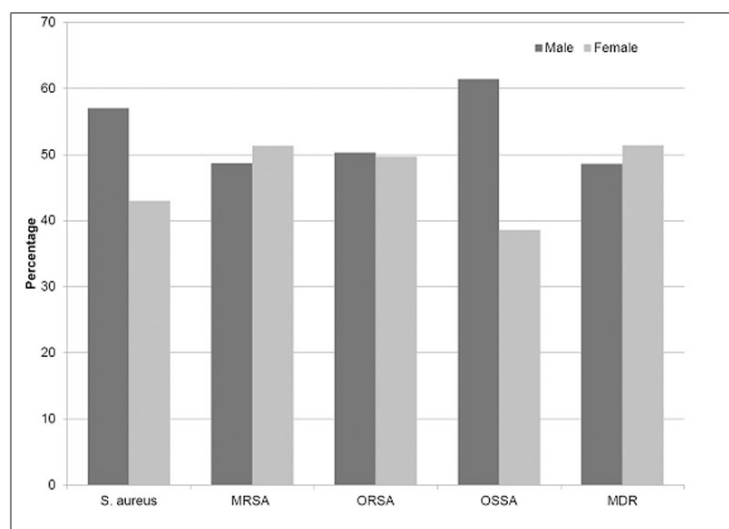


Fig. 2. 2009 Percentage of all of isolated *S. aureus* strains according to gender. (MRSA: Methicillin resistant *S. aureus* (Resistant to both Oxacillin and Cefoxitin). ORSA: Oxacillin resistant *S. aureus*. OSSA: Oxacillin susceptible *S. aureus*. MDR: Multidrug resistant *S. aureus*.)

20 to 39 years age group, and the percentages of these strains (21 % - 30.2 %) were nearly equal for each age group from 40 to 79 years. In the age group less than 19 years the isolated percentage were slightly higher (9.9 % - 16.3 %), while the lowest percentage (2.5 % - 6.3 %) were isolated from >80 years of age group.

Table 2 shows that *S. aureus* was isolated from 10 body sites and were predominant in wound, abscess and blood specimens. The MRSA, ORSA and MDR strains were mainly present in wound specimens.

The susceptibility pattern of *S. aureus* strains is presented in Table 3. The OSSA isolates were susceptible to the majority of tested antibiotics. The MRSA, ORSA and MDR strains showed high rates of susceptibility (>80%) to chloramphenicol, linezolid, nitrofurantoin (for urine samples), rifampicin and teicoplanin, but high resistance to erythromycin and penicillin. All isolate (100 %) were susceptible to

vancomycin.

DISCUSSION

Staphylococcus aureus has been recognized as a major human pathogen. The pathogenicity and virulence of *S. aureus* are associated with its capacity to produce several virulence factors (4). MRSA is a bacterium that has been found to be resistant to antibiotics such as methicillin, oxacillin, penicillin and amoxicillin. This organism had become widespread in healthcare settings globally causing different type of infections. Nosocomial infections represent a burden for both patients and the healthcare system because of their association with increased hospitalization costs and high mortality/morbidity rate (2, 8).

Detection of MRSA by accurate and rapid methods

Table 1. Number of patients reverted to %.

| Age group (year) | % <i>S. aureus</i> | % MRSA | % ORSA | % OSSA | % MDR |
|------------------|--------------------|--------|--------|--------|-------|
| 0-19 | 16.2 | 12.4 | 16.3 | 16.1 | 9.9 |
| 20-39 | 32.1 | 34.5 | 34.6 | 30.2 | 35.2 |
| 40-59 | 21.5 | 25.7 | 22.3 | 21 | 27 |
| 60-79 | 27.2 | 23 | 22.9 | 30.2 | 21.6 |
| up to 80 | 3 | 4.4 | 3.9 | 2.5 | 6.3 |

Table 2. Percentage of isolated *S. aureus* strains according to source of specimens.

| Type of specimens | % <i>S. aureus</i> | % MRSA | % ORSA | % OSSA | % MDR |
|-------------------|--------------------|--------|--------|--------|-------|
| Blood | 25.7 | 22.1 | 22.9 | 27.8 | 23.5 |
| Ear | 1.1 | NA | 0.6 | 1.5 | NA |
| Eye | 2.5 | 2.65 | 2 | 2.9 | 0.9 |
| Nasal Swab | 0.3 | NA | NA | 0.5 | NA |
| Abscess | 25.7 | 21.25 | 24.9 | 26.3 | 22.5 |
| Semen | 0.3 | NA | 0.6 | NA | NA |
| Sputum | 8.1 | 10.6 | 8.5 | 7.8 | 9.9 |
| Catheter tip | 3.4 | 2.65 | 4.5 | 2.4 | 3.6 |
| Urine | 3.6 | 2.65 | 2 | 5 | 3.6 |
| Wound | 29.3 | 38.1 | 34 | 25.8 | 36 |

MRSA: Methicillin resistant *S. aureus* (Resistant to both Oxacillin and Cefoxitin).

ORSA: Oxacillinresistant *S. aureus*.

OSSA: Oxacillin susceptible *S. aureus*.

MDR: Multidrug resistant *S. aureus*.

Table 3. The susceptibility pattern of isolated *S. aureus* strains.

| Types of Antibiotics | <i>S. aureus</i> strains | | | | |
|----------------------|--------------------------|--------|--------|--------|-------|
| | % <i>S. aureus</i> | % OSSA | % MRSA | % ORSA | % MDR |
| Oxacillin | 57.3 | 100 | 0 | 0 | 9 |
| Cefoxitin | 63.7 | 91.2 | 0 | 26.8 | 9.9 |
| Azithromycin | 74.3 | 92.2 | 37.2 | 50.3 | 29.7 |
| Chloramphenicol | 94.4 | 98.5 | 86.7 | 88.9 | 82 |
| Clindamycin | 81.8 | 95.1 | 54.9 | 64.1 | 50.4 |
| Erytromycin | 58.9 | 78.5 | 23 | 32.7 | 7.2 |
| Gentamicin | 79.9 | 96.6 | 47 | 57.5 | 41.4 |
| Levofloxacin | 80.2 | 97 | 42.5 | 57.5 | 39.6 |
| Linezolid | 96.6 | 98.5 | 96.5 | 94.1 | 90.1 |
| Nitrofurantin* | 95.8 | 98.5 | 90.3 | 92.2 | 90 |
| Penicillin G | 37.2 | 52.2 | 5.3 | 17 | 9.9 |
| Rifampin | 92.7 | 95.6 | 85.8 | 88.9 | 82 |
| Tetracycline | 81.8 | 94.1 | 58.4 | 65.4 | 51.4 |
| Trimeth/Sulpha | 83.2 | 97 | 58.4 | 64.7 | 57.7 |
| Teicoplanin | 98 | 98.5 | 97.3 | 97.4 | 96.4 |
| Vancomycin | 100 | 100 | 100 | 100 | 100 |

*Nitrofurantin: Used for urine samples.

is important to choose the best antibiotic for the individual patient and for control of the endemicity of MRSA (18). In present study, we found that the prevalence of MRSA and MDR were equal. The percentage of ORSA was found to be higher than MRSA and MDR. The overall results of MRSA and MDR is lower than the percentage reported by Borg and Al-Zu'bi in 2003 and 2004, and similar to another study in 2005 (19, 20). Moreover, the percentage of OSSA was less than percentage cited in other studies (21-24).

The relationship between the sex and the infection of *S. aureus* types were not clear. The percentage of isolated *S. aureus* types varied from males to females in different studies (21, 25). Some retrospective case-control studies have found that males were one of the risk factors for community-acquired *S. aureus* infections (26, 27). In present study, we did not find any significant difference between both sexes that were infected with resistance types (MRSA, ORSA and MDR), while the percentages of isolated *S. aureus* and OSSA were high in males than females. This percentage may change depending on different factors such as the microbial community in patients bodies, method and the distribution of specimens collection during the period of study.

Nasal and skin carriage has been identified as an

important risk factor for the development of *S. aureus* infections. The carriage rate depends on different factors such as sex and age (28, 29). The patients, whose age over 60 or very young age is susceptible to these infections (30). Hafeez et al. have shown that the distributions of *S. aureus* were similar among different age groups with the exception of newborns and 75 years and older (31). While Jessica et al. reported that the active age group (15 to 60 years old) was more susceptible to *S. aureus* infections to others (32). In this study the highest percentages isolated from (20 to 39 years) age group and the results were nearly equal in two age group between (40 to 79 years) the percentage were declined after 80 years of age (Table 1). Similar findings have been reported by others (16, 31, 33).

It has been reported that 80% of infections with *S. aureus* are endogenous, caused by the colonizing strain when enter the body by any way in both hospitals and the community (34). *S. aureus* has been found to be the most frequent cause of nosocomial pneumonia and surgical-wound infections and the second most common cause of nosocomial bloodstream infections (35). In this study, the majority of *S. aureus* strains of MRSA, ORSA and MDR (34 % to 38.1 %) were isolated from wound specimens. The second higher percentages were isolated from blood and ab-

success specimens, while the bacterial strains in all other specimens were lower (Table 2). These results are compatible with different previous results reported in other parts of world (21, 23, 33, 36, 37).

Hafeez et al. (31) detected higher percentage of MRSA (40 %) in ear swabs. However, in this study MRSA were not detected in the same specimens, but ORSA were isolated in lower percentage. In some studies the MRSA have been isolated in lower percentage from blood specimens (24, 36). These variations may related to many reasons such as the patients population, types of skin normal flora, specimens collection procedures and number of specimens.

S. aureus can cause various types of human infections, the treatment of these infections and those resulted from the drug resistance strains of *S. aureus* is worldwide problem (9, 38). The prevalence of MRSA isolates have increased over the years (11, 39). In our study, isolated *S. aureus* and OSSA showed high rate of susceptibility (more than 80%) for 10/16 and 14/16 of all used antibiotic, respectively (Table 3). Other strains (MRSA, ORSA and MDR) showed higher sensitivity pattern (> 80%) to 6 out of 16 of the used antibiotic. These results may give a potential improvement of therapeutic options to treat the affected people by MRSA infection (1, 12, 28).

Susceptibility testing of MRSA now includes the more stable oxacillin instead of methicillin disk to test the susceptibility of the isolated strains to all β -lactam agents and cefoxitin for cephalosporin (2, 4, 15). The sensitivity pattern showed almost similar differences between the MRSA, ORSA and MDR strains. Consistent with other studies, these three strains were highly susceptible to glycopeptides (90.1 %) and linezolid, 100 % (31, 40). Like previous studies (16, 31, 35), these strains showed higher rates of resistance to macrolides (erythromycin and azithromycin). Lower sensitivity (14.6%) for erythromycin (21) and higher sensitivity (62 %) for azithromycin have also been reported (40).

Vancomycin was the only antibiotic that was effective against all isolates in this study (100%), which makes it a drug of choice for treating multi-drug resistant MRSA. Nitrofurantoin was the second effective antibiotic after vancomycin (90 % to 92.2 %), similar to the report from Iraq (41). It followed by rifampicin and chloramphenicol (80 % to 88.9 %, respectively). Lower sensitivity to rifampicin has been reported by other authors (43% and 43.7%), (38, 39) and chloramphenicol (6.1 %) (34).

Medium sensitivity percentages were found to levofloxacin, gentamicin, clindamycin trimethoprim-sulfamethoxazole and tetracycline (Table 3). Different sensitivity percentages have been reported to these five antibiotics worldwide (21, 31, 41). These differences might be due to prolonged antibiotic treatment, age, type of infection and geographical variation.

In conclusion, the results of this study showed the importance of regular surveillance of hospital associated infections including monitoring antibiotic sensitivity pattern and strict drug policy for antibiotics used within and outside the hospital environments. Moreover, *in-vitro* susceptibility testing of every isolate of MRSA in the clinical laboratories may be helpful for reducing the incidence of these infections.

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