Prevalence of multidrug resistance in the Egyptian methicillin-resistant *Staphylococcus aureus* isolates

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**ABSTRACT**

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major health hazards and became of greater public health concern. This work aimed to shed substantial light on prevalence of MRSA in different clinical isolates and their resistance to different antibiotics. Among 258 *Staphylococcus aureus* isolates recovered from different clinical sources (urine, pus, throat swab, blood, seminal fluid, prostatic fluid, sputum swab, ascetic fluid, skin swab, nipple discharge and urinary catheter), 70 isolates were identified as MRSA. The highest percentage of MRSA was recorded from pus samples (57.1%) followed by urine (30%). Antimicrobial susceptibility test using 14 antibiotics showed that all MRSA were resistant to amoxicillin and cefuroxime, while only 50% were sensitive to vancomycin. High minimum inhibitory concentration of oxacillin (256 µg/ml) was detected in 12.9% of MRSA isolates.

**Keywords:** *Staphylococcus aureus*, MRSA, Oxacillin, ORSAB, Clinical.

**INTRODUCTION**

Staphylococci are opportunistic human pathogen capable of causing a wide variety of diseases (Moorem and Lindsay, 2001; Boyd and Brüssow, 2002). Most people are passive carrier of staphylococci (Kluytmans et al., 1997), and in some cases, an infection may arise from self-inoculation of a wound. Staphylococcal infections have been a major problem in hospitals for decades, but the incidence of community-acquired infections has also been increasing (Chambers, 2001). *Staphylococcus aureus* (*S. aureus*) is a bacterium that belongs to the family of staphylococcaceae forming part of the normal flora of skin, intestine, upper respiratory tract and vagina (Lowy, 1998). *S. aureus* can become pathogenic when conditions such as pH, temperature and nutrient availability are altered and become favorable for overgrowth (Mims et al., 2004). Historically, *S. aureus* has been recognized as an important cause of disease around the world and has become a major pathogen associated with both hospital and community-acquired infections (Panlilio et al., 1992; Tong et al., 2015; Lake et al., 2018).

Before the availability of antibiotics, invasive infections caused by *S. aureus* were often fatal. The introduction of penicillin greatly improved the prognosis for patients with severe staphylococcal infections, but after a few years of clinical use resistance appeared in *S. aureus* due to production of beta-lactamases. Methicillin was designed to resist hydrolysis by beta-lactamases, but soon after methicillin was introduced into clinical practice, resistant *S. aureus* strains were identified and designated as Methicillin-resistant *Staphylococcus aureus* (MRSA). The term MRSA has
been retained, although oxacillin has now replaced methicillin for susceptibility testing in laboratories and is the marker for classifying *S. aureus* as MRSA. Until recently, MRSA was predominantly a nosocomial pathogen causing hospital-acquired as well as community-acquired infections (Mera et al., 2011; Tong et al., 2015; Boswihi and Udo, 2018).

Due to the increase of MRSA strains every decade, these bacteria were identified in the early 1980’s as a major cause of nosocomial infections (Boyce et al., 2004). The possibility of transmission of healthcare associated MRSA (HA-MRSA) to the community was obvious. Since 1987, MRSA was increasingly found in the community associated-methicillin-resistant *S. aureus* (CA-MRSA) presented with severe skin and soft tissue infections as well as necrotizing pneumonia (Hayani et al., 2008).

MRSA infections account for one fifth of all hospital-acquired infections, costing the UK National Health Service approximately £1 billion per year (Cepeda et al., 2005). The problem has been aggravated by the rapid spread and high incidence of MRSA in intensive-care units (Cepeda et al., 2005). The continuing rise in MRSA infection rates and its spread worldwide has led to calls for action to control infection and develop novel anti-MRSA agents and vaccines (Cutler and Wilson, 2004; Hancock, 2007).

The aim of this work was to assess the source of MRSA isolates as well as describe the antimicrobial susceptibility patterns of these isolates.

**MATERIALS AND METHODS**

**Clinical isolates**

A total of 379 Gram positive clinical isolates were kindly provided from El-Demerdash hospital in Cairo, Egypt during the period between January 2016 and December 2017. These isolates were recovered from different clinical sources including; urine, pus, throat swab, blood, seminal fluid, prostatic fluid, sputum swab, ascetic fluid, skin swab, nipple discharge and urinary catheter.

**Identification of *Staphylococcus aureus***

All isolates were purified on nutrient agar (Oxoid. UK Code: CM0003) then preliminary identified by conventional microbiological methods including Gram staining (Isenberg, 1992), morphology and growth on selective media including Baird-Parker agar medium (Difco) (Baird-Parker, 1962; Bannerman, 2003), blood agar medium (Oxoid, UK, CM0271) (Murray et al., 1999) and mannitol salt agar medium (Oxoid, UK, CM0085) (Koneman, 1992). All media were incubated at 37°C for 48 h. After incubation period, biochemical testes; coagulase and catalase, were carried out according to Bannerman (2003) and Kloos and Schleifer (1986). Colonies that were Gram positive with black, β-hemolysis and yellow growth on Baird-Parker agar, blood agar and mannitol salt agar media, respectively, with positive coagulase and catalase were identified as *S. aureus* and stored on nutrient agar in 4°C for further investigations.

**Identification of MRSA isolates**

*S. aureus* isolates were streak on oxacillin resistance screening agar base medium (ORSAB) (Oxoid, UK, CM1008) and incubated at 37°C for 48 h (CLSI, 2017 and Kluymans et al., 2002). In addition, they were tested for their susceptibility towards cefoxitin (30 μg) and oxacillin (1 μg) by disk diffusion as per CLSI (2017). Positive MRSA showed growth on ORSAB and were resistance to both cefoxitin and oxacillin.

**Antimicrobial susceptibility test**

Antimicrobial susceptibility of MRSA isolates against 14 antibiotics was carried out using agar disc diffusion method according to CLSI (2017). The antibiotic disks used in this study were purchased from Oxoid, England. The antibiotics used were vancomycin (VA 30
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µg), amikacin (AK 30 µg), tobramycin (Tb 10 µg), erythromycin (E 15 µg), clarithromycin (CLR 15 µg), amoxicillin (AX 25 µg), clindamycin (DA 2 µg), chloramphenicol (C 30 µg), meropenem (MRP 10 µg), imipenem (IPM 10 µg), cefuroxime (CXM 30 µg), ciprofloxacin (CLR 5 µg), ofloxacin (OFX 5 µg), tetracycline (TE 30 µg).

**Determination of minimum inhibitory concentration (MIC) of oxacillin**

The MIC of oxacillin was determined by the well agar diffusion method using plates containing Mueller Hinton agar medium (Oxoid, England) inoculated with MRSA isolates (10⁴ CFU/ml) according to NCCLS (2017) and EUCAST (2000). MIC was defined as the lowest antibiotic concentration that showed no bacterial growth after incubation period.

**RESULTS AND DISCUSSIONS**

**Identification of the *Staphylococcus aureus* isolates**

Among 379 isolates, 258 (68%) were identified as *S. aureus*. The isolates of *S. aureus* were recovered from different clinical sources including: urine (40%), pus (33.3%), throat swab (12.8%), blood (3.1%), seminal fluid (3.1%), prostatic fluid (3.1%), sputum swab (1.2%), ascetic fluid (0.8%), skin swab (1.5%), nipple discharge (0.8%) and urinary catheter (0.3%) (Fig. 1).

![Fig. (1): Percentages of *S. aureus* recovered from different clinical sources.](image)

**Phenotypic identification of MRSA**

*S. aureus* isolates were subcultured on ORSAB plates for 48 h at 37°C. Among the 258 *S. aureus* isolates, only 70 were identified as MRSA which gave blue growth on ORSAB agar medium (Fig. 2). Moreover, 70 of these isolates were resistant to cefoxitin 30 µg (zone of inhibition ≤ 21) and 1 µg oxacillin disc (zone of inhibition ≤ 11).
Distribution of MRSA in clinical samples

Higher percentage of MRSA was recovered from pus (57.1%) followed by urine (30%), skin swab (5.71%), throat swab (2.9%), while 1.43% from blood, urinary catheter, sputum swab collectively. No MRSA isolates were detected in nipple discharge, seminal fluid, prostatic fluid or ascetic fluid (Fig. 3). According to the relation between source of MRSA and gender, higher MRSA isolates were detected in females compared with males except those recovered from pus (male 65%, female 35%) (Fig. 4).

Fig. (2): Methicillin resistant *S. aureus* (MRSA) grown on ORSAB agar medium.

![Blue growth of MRSA on ORSAB medium](image)

Fig. (3): Distribution of MRSA in clinical samples.

![Bar chart showing distribution of MRSA by source](image)

Fig. (4): Percentage of MRSA distribution in clinical sources by gender.
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**Antibiotics susceptibility of MRSA isolates**

All MRSA isolates were resistant to amoxicillin and cefuroxime. Higher resistance for clarithromycin, erythromycin, imipenem, meropenem (78.6%), amikacin, tobramycin (77.1%), chloramphenicol, ciprofloxacin, ofloxacin (74.3%), tetracycline (71.4%) and clindamycin (62.8%). Only 50% of the isolates were sensitive to vancomycin (Fig. 5).

![Antibiotic sensitivity test of MRSA isolates.](image)

**Fig. (5): Antibiotic sensitivity test of MRSA isolates.**

Vancomycin (VA 30µg), amikacin (AK 30µg), tobramycin (Tb 10µg), erythromycin (E 15µg), clarithromycin (CLR 15 µg), amoxicillin (AX 25 µg), clindamycin (DA 2 µg), chloramphenicol (C 30 µg), meropenem (MRP 10 µg), imipenem (IPM 10 µg), cefuroxime (CXM 30 µg), ciprofloxacin (CIP 5 µg), ofloxacin (OFX 5 µg), tetracycline (TE 30 µg).

**Determination of minimum inhibitory concentration (MIC) of oxacillin**

According to MIC, all MRSA isolates were resistant to 4 µg/ml oxacillin. Higher MIC (256 µg/ml) was detected in 12.9% of MRSA isolates, while lower one (8 µg/ml) was observed in 21.4% of the isolates (Fig. 6).

![Minimum inhibitory concentration (MIC) of MRSA against oxacillin.](image)

**Fig. (6). Minimum inhibitory concentration (MIC) of MRSA against oxacillin.**
DISCUSSION

*Staphylococcus aureus* is a common opportunistic bacterium which is an important pathogen due to combination of toxin-mediated virulence, invasiveness, and antibiotic resistance (Qiu et al., 2010). Previously, *S. aureus* infections were primarily treated with β-lactam antibiotics, including oxacillin, a second-generation penicillin. Soon after the emergence of MRSA, these early generation β-lactam antibiotics were eliminated from the treatment options. To date according to Miller et al., (2020), MRSA has become one of the most prevalent multi-drug resistant pathogens, and is responsible for most nosocomial and community-acquired infections worldwide (Tong et al., 2015; Stryjewski and Corey, 2014). In this study, the percentage of identified MRSA isolates among 258 *S. aureus* clinical isolates was 27%. This finding agreed with previous surveillance study carried out to measure the resistance in *S. aureus* and revealed the presence of methicillin resistance in 32% of *S. aureus* isolates (Udo et al., 2008). The present study showed that the percentage of agreement between susceptibility to both Cefoxitin (30 μg) and oxacillin (1 μg) antibiotics and culturing on selective ORSAB medium for detection and identification of MRSA was 100%. These results agreed with Zeeshan et al. (2007) and Stoakes et al. (2006) who used these phenotypic methods for better sensitivity to MRSA identification. However, other studies reported that growing on ORSAB for 48h showed 98% sensitivity (Becker et al., 2002) and 96% sensitivity (Nguyen Van et al., 2006; Nsira et al., 2006).

In this work, higher MRSA isolates were observed in females compared with males in most of the clinical samples. This was congruent with Terry Alli et al. (2012) who found that MRSA was prevalent among female patients.

In the present study, the highest MRSA isolates were recovered from pus (57.1%). This higher frequency of MRSA in pus specimen has been previously reported especially in diabetic foot infections, surgical wounds, and burn patients (Garoy et al., 2019). On the other hand, the higher MRSA isolates recovered from the investigated urine clinical samples agreed with the studies of Akortha et al. (2008) and Aboderin et al. (2009) who reported increasing prevalence of *Staphylococcus aureus* in urinary tract infection. The presence of MRSA in a urine culture has important consequences for patients, both in the community and the hospital setting. MRSA in urine clearly warrants treatment in symptomatic patients, but even in asymptomatic patients, it may require eradication before certain elective procedures such as endourological surgery (Looney et al., 2017). No MRSA isolates were detected in prostatic fluid which was incongruent with Carroll et al. (2017) who reported 26 staphylococcal prostatic abscesses caused by MRSA.

In the present study, the antibiotic susceptibility revealed that all MRSA isolates were resistance to amoxicillin and cefuroxime. In addition, 78.6% of MRSA isolates were resistant to clarithromycin, erythromycin, imipenem, meropenem. Similar results were reported by Prakash et al. (2007). Only 50% of the isolates were sensitive to vancomycin which is regarded as the drug for treatment of infections caused by MRSA. However, emergence of vancomycin resistant *Staphylococcus aureus* has been reported by Kshetry et al. (2016). Higher MICs for MRSA isolates were detected in this study which indicated that the phenomena of multiple drug resistance in MRSA became more and more serious.

Conclusion

MRSA isolates from different clinical sources showed high prevalence in pus followed by urine. All MRSA were resistant to amoxicillin and cefuroxime and 50% to vancomycin. Higher concentration
of oxacillin (256 μg/ml) was needed to inhibit the growth of 12.9% of MRSA isolates.

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انتشار مقاومة الأدوية المتشددة في عزلات المكورات العنقودية الذهبية المقاومة للميثيسيلين

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الملخص

تعتبر المكورات العنقودية الذهبية المقاومة للميثيسيلين (MRSA) واحدة من المخاطر الصحية الرئيسية والتي أصبحت مصدر قلق كبير للصحة العامة. يهدف هذا البحث إلى إلقاء الضوء بشكل كبير على انتشار بكتيريا MRSA في العزلات السربرية المختلفة ومقاومتها للمضادات الحيوية المختلفة. يمثل 258 من المكورات العنقودية الذهبية التي تم أخذها من مصادر سربرية مختلفة (البول، القهش، مسحة الحجرة، الدم، السائل المنووي، السائل البروستاتي، مسحة البلغم) سائل الزهد، مسحة الجلد، إفراز الحلمة والقسطرة البولية) تم تحديد 70 على أنهم MRSA. تم تسجيل أعلى نسبة من عينات الصيدل (57.1%) بليبيا البول (50%). أظهرت الدراسة أن استخدام مضادات الميكروبيات باستخدام 14 مضادًا حيويًا MRSA كانت مقاومة للأموكسيسيلين وسيفاموكسيم، بينما كان 50% فقط حساسة للفانكوميسين. تم الكشف عن أن الحد الأدنى لتركيز الببتيد للأموكسيسيلين (25 ميكرغرام/مل) وجد في 12.9% من عزلات MRSA.