RESEARCH ARTICLE

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DETECTION OF mecA GENE IN METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS STRAINS RESPONSIBLE FOR WOUND INFECTIONS AND SEPTICEMIA IN MANSOURA UNIVERSITY HOSPITALS.

ABSTRACT:
Methicillin-resistant Staphylococcus aureus (MRSA) is a specific strain of the bacterium Staphylococcus aureus that has developed antibiotic resistance to all penicillins, including methicillin and other narrow-spectrum β-lactamase-resistant penicillin antibiotics. The purpose of this study is to investigate the presence methicillin resistant S. aureus strains in patients suffering from wound infections and septicemia, in Mansoura University Hospitals (MUHs). A total of 29 MRSA strains were isolated from nosocomially infected patients. All isolates were subjected to antibiogram comparison, polymerase chain reaction (PCR) assays for amplifying mecA, oxacillin agar screen test and latex agglutination test. PCR detected 18 out of 29 strains (62.1%), oxacillin agar detected 22 out of 29 strains (75.9%), while latex screen showed 25 out of 29 MRSA strains (86.2%); P<0.001. MRSA strains were completely resistant to Cefuroxime, Cephalexine and Cefotaxime, but appeared to be quite susceptible to vancomycin. This study has demonstrated detection of MRSA strains by different susceptibility procedures and PCR test as a golden standard method for MRSA identification. Our findings call for the prospective potentiality of oxacillin agar screen and latex agglutination tests for detection of MRSA.

KEY WORDS:  
methicillin resistance, mecA gene, PCR, Staphylococcus aureus, nosocomial infection, MRSA.

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INTRODUCTION:  
Staphylococcus aureus is a common commensal of humans and its primary habitat is the moist squamous epithelium of the anterior nares (Peacock et al., 2001). About 20% of the populations are always colonized with S. aureus, 60% are intermittent carriers and 20% never carry the organism. As there is considerable evidence that carriage is an important risk factor for invasive infection (Peacock et al., 2001; von Eiff et al., 2001), it is surprising that so little is known about the bacterial factors that promote colonization of squamous epithelial surfaces and the host factors that determine whether an individual can be colonized or not. S. aureus is the leading cause of postoperative wound infection, and the second-most frequent cause of nosocomial pneumonia (Chastre and Fagon, 2002) and bacteremia (Wisplinghoff et al., 2004).

Despite the major advances in the medical arena, S. aureus remains an important agent of infectious diseases in the human host. Its significance lies in its widespread existence and the broad spectrum of infections it can produce, ranging from inconsequential superficial skin infections to deep-seated life-threatening systemic infections (Lowy, 1998). Indeed, some infections caused by S. aureus, namely bacteremia and endocarditis, are frequently associated with serious complications and high mortality rates (Fowler et al., 2005; Petti and Fowler, 2003). The emergence of antibiotic
resistance has brought renewed attention to staphylococci (Bal and Gould, 1998). Methicillin-resistant S. aureus (MRSA) rates both in hospitalized and ambulatory patients have been escalating, and this resistant phenotype is now considered a major public health problem (Calfee et al., 2003; Enright et al., 2002; Herold et al., 1998).

Methicillin resistance arises by acquisition of a staphylococcal cassette chromosome SCCmec, and is conferred by the mecA gene. Expression of this gene yields PBP2a, a penicillin binding protein with reduced affinity for β-lactam rings. Some strains of S. aureus over-express β-lactamase and appear to be resistant to oxacillin and, rarely, methicillin despite being mecA-negative. They have slightly raised minimum inhibitory concentrations (MICs) and may thus be described as "minimally resistant". Other strains express modified PBPs (not PBP2) and exhibit varying degrees of β-lactam antibiotic resistance. (Guignard et al., 2005)

Wyllie et al., (2006) reported a death rate of 34% within 30 days among patients infected with MRSA, while among Methicillin sensitive S. aureus patients the death rate was 27%. Rapid, precise identification of S. aureus infected with MRSA, while among Methicillin resistant S. aureus, while among Methicillin resistant S. aureus (MRSA) resistant phenotype is now considered a major public health problem (Bal and Gould, 1998). Methicillin-resistant S. aureus (MRSA) rates both in hospitalized and ambulatory patients have been escalating, and this resistant phenotype is now considered a major public health problem (Calfee et al., 2003; Enright et al., 2002; Herold et al., 1998).

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The present study was conducted to detect the mecA gene in MRSA strains implicated in wound infections and septicemia in patients hospitalized Mansoura University Hospitals.

PATIENTS AND METHODS:

A number of 394 isolates suspected to be S. aureus were recovered from 1977 nosocomially infected patients admitted to different wards of Mansoura University Hospitals (MUHs) during a period of 9 months starting from April 2007 till December 2007.

Methicillin disk diffusion susceptibility method:

This method was carried out for suspected MRSA isolates by antibiogram, according to the current Clinical and Laboratory Standards Institute recommended methods. Methicillin susceptibility was determined with 5 μg methicillin disks,
The dried DNA was resuspended in distilled water and used as a template for PCR.

**B) Amplification of mecA gene:**

Amplification was carried out according to Smyth et al. (2001) as follow: Polymerase chain reaction was carried out in a final volume of 25 μl containing: Taq PCR Master Mix (Metabion, Germany) (12.5 μl), Primers mecA-1, 5’ GCA ATC GCT AAA GAA CTA AG 3’ (positions 693–712) and mecA-2, 5’ GGG ACC AAC ATA ACC TAA TA 3’ (895–914), the expected size of PCR product were 222 bp (1 μl from each), Template DNA: 3 μl, Nuclease free water: 7.5 μl. The samples were gently vortexed and centrifuged briefly to collect all drops to the bottom of the tube. A reagent blank, containing all components of the reaction mixture except template DNA, was included in PCR test as a negative control. The samples were covered with a drop of mineral oil (50 μl). Then the following program was done in the thermalcycler (PTC-200, MJ Research, Inc., Watertown, Mass.): [a]DNA denaturation at 94°C for 3 min prior to amplification, [b]Thirty cycles through-out the following temperature profile: denaturation at 94°C for 10 sec, annealing at 53°C for 20 sec, extension at 72°C for 30 sec and final extension at 72°C for 5 min. [c]After amplification samples were stored at -20°C for longer storage. PCR products were resolved by electrophoresis on a 2% agarose-Tris-borate-EDTA gel (Fermentas, Germany), stained with ethidium bromide and photographed under UV light.

**Disc diffusion method:**

It was done by using the modified Kirby-Bauer sensitivity testing technique (Cheesbrough, 2000). The antibiotic discs were selected according to the protocol of MDICU, as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2000): Neomycin, Cefuroxime, Cephalexine, Cefotaxime, Amoxicillin / K+ Clavulanate, Erythromycin, Tetracycline, Ciprofloxacin, Trimethoprim-sulphamethoxazole, Vancomycin.

![Fig. 1. Antibiotic susceptibility test.](image-url)
The results depicted in Table 3 and Figure 2 revealed that out of 29 S. aureus strains, phenotypically positive methicillin disk diffusion test, 18 strains were positive meca gene by PCR (61%); out of 11 blood cultures 7 were meca positive (63.6%), 10 of 16 wound cultures (62.5%), 0 of 1 urine (0%) and 1 of 1 sputum culture (100%).

Table 3. Distribution of meca gene according to sample type.

| Type of sample (n = 29) | meca gene Positive (n = 18) | meca gene Negative (n = 11) | Total No. | % |
|------------------------|-----------------------------|-----------------------------|-----------|
| Blood                  | 7                           | 4                           | 11        | 37.9 |
| Wound Swab             | 10                          | 6                           | 16        | 55.2 |
| Urine                  | 0                           | 1                           | 1         | 3.4  |
| Sputum                 | 1                           | 0                           | 1         | 3.4  |
| Total                  | 18                          | 11                          | 29        | 100  |

DISCUSSION:

This study aimed to estimate MRSA as a nosocomial pathogen in MUHs and to compare different susceptibility testing methods for diagnosis of MRSA infections. All strains were tested for their susceptibilities to different antibiotics by the disk diffusion method. Identification of methicillin resistance strains was preliminarily done by phenotypic methods (disk diffusion using 5 μg methicillin disks and oxacillin agar screen on Mueller-Hinton agar supplemented with 6 μg oxacillin/ml and 4% NaCl). We selected 29 S. aureus isolates that were phenotypically apparent as methicillin resistant, and were additionally tested by the MRSA Screen latex agglutination test and PCR for meca gene. We performed a head-to-head comparison of previous susceptibility tests, using PCR for meca gene as the ”gold standard” assay. Methicillin resistance was defined as the presence of the meca gene.

Our results revealed that MRSA accounted for 47.2% of all S. aureus nosocomial infections, this is in conformity with Wang et al. (2004), who found that the proportion of MRSA increased from 24.9% in the year 2000 to 45.1% in the year 2004.

In the present study, MRSA accounted 55.1% for wound infections, 50.6% for blood infections and 50% for device related. This is in accordance with Graffunder and Venezia, (2002) who reported that the most common types of infections were bloodstream infections.

Our results revealed that MRSA screen test was the most appropriate to detect MRSA, thus it enabled us to detect 86.2% out of 29 MRSA that were phenotypically resistant by methicillin disk diffusion test. Moreover, the MRSA-Screen test performed better than the oxacillin agar screen did. It is likely practical to process a large number of specimens rapidly. Sakoulas et al., (2001) recommended that molecular susceptibility testing methods could be used to complement conventional susceptibility methods in order to increase the sensitivity and the specificity of MRSA detection, particularly in serious infections in which phenotypically MSSA is isolated from a patient with a prior history of MRSA infection.

In the present study, a PCR reaction was performed using a single set of primers for the amplification of meca gene. Out of 29
MRSA that selected randomly, 18 meca positive strains and 11 meca negative strains were identified; even all 29 strains were confirmed to be methicillin resistant by methicillin disk diffusion susceptibility method. This finding may be explained by false negative PCR reaction due to point mutation or deletion in meca gene or due to presence of inhibitors. This is in harmony with Lee et al. (2004) who demonstrated that out of the 28 MRSA animal isolates, 15 were found to be meca positive and 13 were found to be meca negative by PCR. In addition, Warren et al. (2004) found that three of S. aureus specimens were found to grow on subculture to oxacillin screen agar and therefore were felt to represent phenotypic MRSA. The presence of the meca gene in these three isolates was examined with a meca gene-specific PCR assay. All three isolates were negative for the meca gene, suggesting that methicillin resistance in these isolates was mediated by methods other than PBP2a, such as hyper-production of β-lactamase or modified PBP genes. The role of β-lactamase overproduction in borderline resistance is less clear, although the mechanism is plausible. Because even β-lactamase-stable β-lactam antibiotics may be slowly hydrolyzable by staphylococcal β-lactamase, overproduction of β-lactamase could result in borderline MICs. Culture conditions used to enhance methicillin resistance also favor overproduction of β-lactamase (Chambers, 1997).

In our findings, four MRSA strains, out of those showed meca negative by PCR, were positive oxacillin screen test. This is in concordance with Tai et al. (2006), who found that ten MRSA isolates that did not carry meca were phenotypically resistant to methicillin according to the oxacillin agar screen test.

The present results further demonstrated that seven isolates gave positive result with MRSA-Screen test and was phenotypically resistant by methicillin disk diffusion test, however was negative for meca by PCR. This could be explained on the basis that the initial culture of this isolate was a mixture of MRSA and MSSA. The MRSA-Screen test uses a loopful of bacteria and therefore is much more likely to pick up both MRSA and MSSA isolates from a culture with a mixture of isolates. In the PCR method, a single colony is used, and may give inconsistent results when working with mixed cultures (Sakoulas et al., 2001). In contrary, Felten et al. (2002) found all MRSA were meca positive, and all MSSA were meca negative.

Regarding the antibiogram of MRSA isolates in our study, we selected 10 different antibiotics according to the protocol of MDICU, as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2000) and regarding that each antibiotic represent one class of antibiotic classes, i.e., Neomycin (Aminoglycosides), Cephalexine (1st generation Cephalosporines), Cefuroxime (2nd generation Cephalosporines), Cephotaxime (3rd generation Cephalo-sporines), Amoxicillin / K+ Clavulanate (β-lactamase inhibitor), Erythromycin (Macrolides), Tetracycline (Tetracyclines), Ciprofloxacin (Quinolones), Trimethoprim - sulphamethoxazole (Sulfonamides), Vancomycin (Glycopeptides). All S. aureus isolates showed complete resistance to all cephalosporines (Cephalexine, Cefuroxime, Cephotaxime). This is in accordance with Kernodle et al. (1990) who reported that failure of cephalosporins to prevent S. aureus surgical wound infections is attributed to β-lactamase enzyme that may be involved in degrading the cephalosporin antibiotics, thereby preventing its antibacterial actions.

In the present investigation, 93.1% of S. aureus isolates have been shown to display resistance to amoxicillin-clavulanate, 75.9% to trimethoprim - sulphamethoxazole, 65.5% to erythromycin, and 58.6% to ciprofloxacin. This is in conformity with the results of Saxena et al. (2003) who found 88% of the isolates were resistant to trimethoprim-sulphamethoxazole and amoxicillin-clavulanate followed by 71% to ciprofloxacin, and 53% to erythromycin. Moreover, Tai et al. (2006) reported that 65% of MRSA isolates were resistant to ciprofloxacin, 63.3% were resistant to trimethoprim-sulphamethoxazole. Weber et al. (2003) concluded that exposure to either ciprofloxacin or levofloxacin has been shown to increase the risk for MRSA but not MSSA in hospitalized patients. Furthermore, Blumberg et al. (1991) found that high-level ciprofloxacin resistance was observed within 3 months of ciprofloxacin introduction, and within one year, 79% of all MRSA from hospitalized patients exhibited resistance.

In our study, S. aureus isolates showed lower resistance to tetracycline (48.3%). This was matched with White et al. (2003) who concluded that 40% of isolates were commonly resistant to tetracycline. While none of the isolates showed resistance to vancomycin. This was in accordance with Laplana et al. (2007) and Saxena et al. (2003).

In support of the present findings, it may recall here that Lee et al. (2007) stated that there is a correlation between predisposition to colonization or infection by MRSA and use of broad-spectrum antibiotics, including β-lactams, fluoroquinolones, macrolides,
aminopenicillins, or second-generation cephalosporins suggesting that wide-spread use of these antibiotics could be a major predisposing factor for rapid spread of MRSA in hospital.


REFERENCES:


