A Comparative Study for Detection of Methicillin Resistance \textit{Staphylococci} by Polymerase Chain Reaction and Phenotypic Methods

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Abstract: Background: Methicillin-resistant \textit{Staphylococci}; both methicillin resistant \textit{S. aureus} (MRSA) and methicillin resistant coagulase negative \textit{Staphylococci} (MR CNS) have a worldwide distribution and are important causes of clinical and epidemiological problems. The aim of this study was to evaluate the usefulness of some phenotypic methods for detection of methicillin resistant \textit{Staphylococci} in clinical laboratories; cefoxitin disc diffusion (CDD), oxacillin resistance screening agar base (ORSAB) and oxacillin E test, in reference to \textit{mecA} gene based real-time PCR. Also to study the antibiotic resistance pattern of the methicillin resistant \textit{Staphylococci} isolates. Materials and methods: A total of 95 clinical isolates of \textit{Staphylococci} were tested for methicillin resistance by CDD test, ORSAB and oxacillin E-test and were compared to \textit{mecA} based real time PCR as reference method. Results: \textit{MecA} gene was detected by PCR in 48/95 (51\%) of all \textit{Staphylococci} isolates; 28/57 (49\%) in \textit{S. aureus} and 20/38 (53\%) in CNS isolates. CDD test showed 100\% sensitivity and 98\% specificity for detection of MRSA and MR CNS. ORSAB and Oxacillin E test had 94\% and 90\% sensitivity and 96\% and 98\% specificity respectively. In our study, MRSA isolates were resistant to ciprofloxacin-78\%, erythromycin-74\%, clindamycin-71\%, Gentamicin-70\%, amikacin-64\%, azithromycin-63\%, doxycyclin-60\%, levofloxacin and linezolid-48\%, trimethoprim-sulfamethoxazole-29\% and vancomycin-0\%. Conclusion: Cefoxitin disc diffusion can be used as a reliable conventional, simple and cheap alternative to PCR for detection of MRSA and MR CNS in minimal resources circumstances. It can also detect other mechanisms of resistance other than \textit{mecA}. Additional confirmatory test is needed with oxacillin screening agar and oxacillin E-test for detection of hetero-resistant strains to methicillin.


Key words: MRSA, MR CNS, Cefoxitin, oxacillin, meca

1. Introduction

Infections caused by \textit{Staphylococci} are of great importance for human health. The \textit{Staphylococcus} species are divided into two large groups. The first group is known as coagulase positive \textit{Staphylococci}, which is mainly represented by \textit{Staphylococcus (S.) aureus}, a pathogen that can cause a variety of infections in immuno-competent patients. The second group, known as coagulase negative \textit{Staphylococci} (CNS) which comprises diverse species that are members of the normal flora of humans, mammals and birds, and they are mostly involved in infectious processes in immuno-compromised patients (Martins and Cunha, 2007).

The first report of methicillin-resistant \textit{S. aureus} (MRSA) was in 1961 after the introduction of methicillin in clinical settings (Brown, 2001). Subsequently, it established a global spread both as hospital acquired infection and in the community population without any apparent risk factor (Pramodhini et al., 2011). MRSA infections constitute a worldwide pandemic (Spellberg et al., 2008).

It is assumed that methicillin-resistance genes had evolved first in coagulase-negative \textit{Staphylococci} (MR CNS) and were then horizontally transferred among \textit{Staphylococci}. \textit{Staphylococci} naturally have a protein in its cell wall, penicillin binding protein (PBP) which play a key role in cell wall synthesis and is the target for B-lactam antibiotics. The methicillin-resistant strains produce modified (PBPs) with low affinity for B-lactam antibiotics. Resistance to methicillin in MRSA and MR CNS is mediated by \textit{mecA} gene, responsible for production of PBP2a. The \textit{mecA} is located on a region of chromosome called \textit{SCCmec} (Naez et al., 2011).

There are many methods for detection of methicillin resistance in \textit{Staphylococcal} species. They include disk diffusion method which is used by most laboratories, the Minimal Inhibitory Concentration (MIC) by broth dilution or E-test, chromogenic screening agars, automated identification and susceptibility and molecular methods for detection of \textit{mecA} gene (Medigan and Martinko, 2006 and Kaur et al., 2013). The \textit{mecA} gene is highly conserved among the \textit{Staphylococci} species. Therefore the usefulness of polymerase chain reaction (PCR) assay of the \textit{mecA} gene as “gold standard” for the detection of methicillin resistance in \textit{Staphylococci} is well
established (Brown, 2001; Adal et al., 2008; Ekrami et al., 2010; Mathews et al., 2010; Shariati et al., 2010).

However the use of this assay is expensive, need experienced staff and not available in most routine diagnostic laboratories (Medigan and Martinko, 2006; Pramodhini et al., 2011 and Kaur et al., 2013).

The aim of this study was to evaluate the usefulness of some phenotypic methods for detection of MRSA and MRCNS by disk diffusion, minimal inhibitory concentration (MIC) by E-test, oxacillin resistance screening agar (ORSAB) in clinical laboratories, in comparison to the real-time polymerase chain reaction (PCR) for detecting the mecA gene as a reference method. Also to study the antibiotic resistance pattern of the methicillin resistant Staphylococci isolates.

2. Materials and Methods

Bacterial isolates:
A total number of 95 Staphylococci isolates were collected from the microbiology laboratory of Ain Shams University Hospitals which is a referral university hospital in Cairo, Egypt, from August 2011 to September 2011. The collected isolates were stored in tryptic soya broth with glycerol at -70°C till use.

All isolates were identified by their colony morphology, Gram staining, catalase test, coagulase test using both slide and tube methods and deoxyribonuclease test (Brown et al., 2005). All isolates were examined for methicillin resistance by:

- Cefoxitin disk (30µg) diffusion test (CDD),
- Oxacillin E-test
- Oxacillin resistance screen agar base (ORSAB)
- Syber Green real-time PCR confirmed by melting curve analysis for detection of mecA gene (Reference method).

All the Staphylococci isolates were tested for antibiotic susceptibility by the disk diffusion method according to the Clinical Laboratory Standard Institute (CLSI) guidelines (2011). The tested antibiotics were gentamicin (10µg), amikacin (30µg), erythromycin (15µg), azithromycin (15µg), doxycycline (30µg), ciprofloxacin (5µg), levofloxacin (5µg), clindamycin (2µg), trimethoprim-sulfamethoxazole (1.25/23.75µg), linezolid (30µg) and vancomycin (30µg).

Phenotypic detection of methicillin resistance: Cefoxitin disk diffusion test (FOX DD):
The cefoxitin disk (30µg) (Oxoid, UK) was used on Mueller Hinton agar (Oxoid, UK). The inoculum turbidity was adjusted to 0.5 McFarland. Then the agar plates were inverted and incubated at 35°C for 24h. An inhibition zone diameter of ≤21mm and ≤24mm was reported as methicillin resistant and a diameter of ≥22mm and ≥25mm was considered as methicillin sensitive for S. aureus and CNS respectively as recommended by Clinical Laboratory Standard Institute (CLSI) (2011).

E-Test:
Oxacillin E test was used for minimal inhibitory concentration (MIC) testing for both S. aureus and CNS as recommended by CLSI (2011). The oxacillin E-test (BioMerieux, France) was carried out on Mueller Hinton agar (MHA) (Oxoid, UK) supplemented by 2% NaCl. The inoculum turbidity was adjusted to 0.5 McFarland. The agar surface was allowed to dry completely before applying the E-test strips. Then the agar plates were inverted and incubated at 35°C for 24h. The CLSI (2011) MIC break points for defining oxacillin susceptibility categories were used; MIC of ≥4µg/mL and ≥0.5µg/mL was considered as resistant and MIC of ≤2µg/mL and ≤0.25µg/mL was reported as susceptible for S. aureus and CNS respectively.

Oxacillin resistance screen agar (ORSAB):
(Oxoid, UK) ORSAB is intended as a medium for the screening for MRSA directly from routine swab samples. It is based on Mannitol Salt Agar with a reduction in NaCl concentration to 5.5% and 2µg/mL oxacillin. The media was inoculated with swab of a 0.5 McFarland’s suspension of the Staphylococci isolates and was incubated at 35°C. The plates were examined for intense blue colonies of MRSA and yellow colonies of MRCNS. The plates were examined twice after 24h and 48h.

Genotypic detection of mecA gene:
Syber Green real –time PCR:
According to the methodology described by (Paule et al., 2005; Rallapalli et al., 2008). In brief, DNA was extracted from cultured cells using Bacteria DNA Preparation Kit (Jena Bioscience, Germany) according to manufacturer’s instructions. The mecA primers (Bioneer, Korea) were used: Forward: 5’-AAA ATC GAT GGT AAA GGT TGG C and Reverse: 5’-AGT TCT GCA GTA CCG GAT TTG C (Rallapalli et al., 2008). Master mix (Thermo Scientific, EU) components for each 25ul reaction were: Maxima SYBR Green qPCR Master Mix (2X), no ROX (12.5µL), each primer (0.75µL - 0.3µM), ROX solution (0.05µl -100nM), nuclease free water (6µL). The master mix was mixed thoroughly and the appropriate volumes were dispensed into PCR tubes. The template DNA (5µL) was added to the individual PCR tubes. The amplification was then performed by including the reaction mix into a thermo-cycler (Stratagene Mx3000P QPCR Systems, La Jolla, USA). Initial denaturation at 95°C for 10 minutes was followed by 40 cycles of denaturation at 95°C for 15 seconds, followed by annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. The amplification program was followed immediately by a
melt program consisting of 1 min at 95°C, 30 s at 55°C then gradual increase to 95°C for 30 s at a rate of 0.2°C/s with fluorescence acquisition at each temperature transition. Interpretation of results: A positive result for meca gene was considered with a cycle threshold (Ct) of 30 or less, an indeterminate result (requiring a repeated assay) for a Ct of 31 to 35, and a negative result for a Ct of more than 35 (Figure 1). The melting temperature (Tm) of samples which were identical or close to that of positive control were considered the gene of target; average Tm 77.1 ± 0.6 (Figure 2) (Fang and Hedin, 2003).

**Quality Control:**

The reference strain methicillin resistant S. aureus (MRSA) ATCC 43300 was used as positive control, while methicillin sensitive S. aureus (MSSA) strain ATCC 25923 was used as a negative control. They were supported by the Naval American Military Research Unit (NAMRU-3) in Cairo, Egypt.

**Statistical Analysis**

Categorical variables were expressed as number (%). The sensitivity, specificity and the positive and negative predictive values were calculated for determining the diagnostic value of the various phenotypic methods for detecting methicillin resistance. All the analyses were performed with commercially available software (SPSS version 16.0, SPSS, Inc., Chicago, IL, USA).

**Results**

The 95 Staphylococci isolates under study were 57 (60%) S. aureus and 38 (40%) coagulase negative Staphylococci (CNS). The methicillin resistance meca gene was detected by PCR in 48 (51%) of all Staphylococci isolates; 28/57 (49%) of S. aureus and 20/38 (53%) of CNS.

Methicillin resistance was phenotypically detected by cefoxitin disk diffusion (CDD) test, oxacillin resistant screening agar base (ORSAB) and oxacillin (OX) E-test in 49, 44 and 39 isolates respectively. The sensitivity, specificity and the positive and negative predictive values of the various phenotypic methods in comparison to meca PCR reference method, for the detection of MRSA and MRCNS, are summarized in Table 1. Discrepant test results obtained in this study are summarized in table 2.

### Table 1: Comparison of the phenotypic methods for detection of methicillin resistant Staphylococci based on meca-PCR as reference method (n=95)

<table>
<thead>
<tr>
<th>Method</th>
<th>CDD</th>
<th>ORSAB</th>
<th>Oxacillin E-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>meca positive Staphylococci</td>
<td>48</td>
<td>45</td>
<td>43</td>
</tr>
<tr>
<td>Methicillin sensitive Staphylococci</td>
<td>46</td>
<td>45</td>
<td>46</td>
</tr>
<tr>
<td>False positive</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>False negative</td>
<td>0</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>100</td>
<td>94</td>
<td>90</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>98</td>
<td>96</td>
<td>98</td>
</tr>
<tr>
<td>PPV</td>
<td>98</td>
<td>96</td>
<td>98</td>
</tr>
<tr>
<td>NPV</td>
<td>100</td>
<td>94</td>
<td>90</td>
</tr>
</tbody>
</table>

CDD: Cefoxitin disk diffusion test; ORSAB: Oxacillin Resistant Screening Agar Base; PPV: Positive Predictive Value; NPV: Negative Predictive Value
Table 2: Discrepancies between mecA PCR, Cefoxitin disk diffusion, Oxacillin resistant screening agar base and oxacillin E test results

<table>
<thead>
<tr>
<th>Staphylococci isolates</th>
<th>PCR mecA</th>
<th>CDD</th>
<th>ORSAB</th>
<th>E test MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA</td>
<td>Positive</td>
<td>R</td>
<td>R</td>
<td>2 S</td>
</tr>
<tr>
<td>MRSA</td>
<td>Positive</td>
<td>R</td>
<td>R</td>
<td>2 S</td>
</tr>
<tr>
<td>MRSA</td>
<td>Positive</td>
<td>R</td>
<td>R</td>
<td>1 S</td>
</tr>
<tr>
<td>MRSA</td>
<td>Positive</td>
<td>R</td>
<td>R</td>
<td>2 S</td>
</tr>
<tr>
<td>MRSA</td>
<td>Positive</td>
<td>R</td>
<td>R</td>
<td>1 S</td>
</tr>
<tr>
<td>MRSA</td>
<td>Negative</td>
<td>R</td>
<td>R</td>
<td>2 S</td>
</tr>
<tr>
<td>MRSA</td>
<td>Negative</td>
<td>S</td>
<td>R</td>
<td>256 R</td>
</tr>
<tr>
<td>MRCNS</td>
<td>Positive</td>
<td>R</td>
<td>S</td>
<td>256 R</td>
</tr>
<tr>
<td>MRCNS</td>
<td>Positive</td>
<td>R</td>
<td>S</td>
<td>256 R</td>
</tr>
</tbody>
</table>

CCD: Cefoxitin disk diffusion test; ORSAB: Oxacillin Resistant Screening Agar Base; S: sensitive; R: resistance

The cefoxitin disk diffusion (CDD) test had the best diagnostic performance among the phenotypic methods for detection of the methicillin resistant Staphylococci, MRSA and MRCNS. It has 100% sensitivity and 98% specificity. All mecA gene positive isolates were positive by CDD i.e. no false negative. Only one (1%) of S. aureus was diagnosed as resistant.

The ORSAB test had 94% sensitivity and 96% specificity. Two (2%) of S. aureus were falsely diagnosed as resistant (false positive) and 3 (3%) MRCNS were falsely diagnosed as sensitive (false negative). The results did not change with incubation for 24h and 48h.

The oxacillin E-test had 90% sensitivity and 98% specificity. A complete agreement with mecA PCR for CNS was detected. Furthermore, 57% of MRSA and 60% of MRCNS isolates had MIC values equal or more than 256µg/ml. One (1%) of S. aureus was falsely diagnosed as MRSA (false positive) and 5 (5%) MRSA were falsely diagnosed as MSSA (false negative).

Antibiotics
In our study, MRSA isolates were resistant to ciprofloxacin-78%, erythromycin-74%, clindamycin-71%, Gentamicin-70%, amikacin-64%, azithromycin-63%, doxycyclin-60%, levofloxacin and linezolid-48% and trimethoprim-sulfamethoxazole-29%. All isolates were sensitive to vancomycin.

4. Discussion
The collected Staphylococci isolates were tested by conventional phenotypic susceptibility methods for methicillin resistance; cefoxitin disk diffusion (CDD), ORSAB and oxacillin E-test. The presence of mecA gene was confirmed by SyberGreen real-time PCR as the reference method.

In the present study, among the 95 Staphylococcus isolates tested, 57 (60%) were S. aureus and 38 (40%) were coagulase negative Staphylococci (CNS). Lower results were reported by Ekrami et al. (2010) (Iran) from wound and blood specimens, where the frequencies were 52% S. aureus and 48% CNS respectively.

The overall methicillin resistance by mecA PCR was 48 (51%) among the 95 Staphylococci under study. Within each group, mecA represented 49% of S. aureus and 53% of CNS isolates. In Egypt, according to a multicenter study, the prevalence of MRSA between 2003–2005 was 52% (Borg et al., 2007). In the other northern countries of Africa drenched by the Mediterranean Sea, the prevalence of MRSA varies from 19% in Morocco, 31% in Libya to 45% in Algeria and Tunisia (Falagas et al., 2013). Similarly, Ekrami et al. (2010) (Iran) reported 61% mecA positive methicillin resistant Staphylococci. Comparable results were reported by Felten et al., (2002) (France) and Sasirekha et al. (2012) (India) who detected mecA gene among 55%, and 57.7% of Staphylococcus aureus isolates respectively. Lower prevalence 36%, 35%, 36.4%, 37.57%, 19.2% of MRSA was detected by Karami et al. (2011) (Iran), Datta et al. (2011) (India), Pramodhini et al. (2011) (India), Pillai et al. (2012) (India), and Olowe et al. (2013) (Nigeria) respectively. Perazzi et al. (2006) (Argentina) and Ekrami et al. (2010) (Iran) reported 38% and 60% of MRCNS isolates with mecA gene. Flu et al. (2001) reported that the prevalence of MRSA in Europe varies considerably between different countries and between different hospitals in the same country. The highest prevalence was in Portugal (54%) and Italy (43 to 58%) and the lowest was in Switzerland and The Netherlands (2%). In Spain the prevalence was 34% in Seville, whereas it was 9% in Barcelona hospitals. This difference may be associated with rapid identification of MRSA colonization or infection, strict isolation policies and restricted use of antibiotics.

In the present study, cefoxitin disk diffusion test had the best diagnostic performance among the phenotypic methods used for detection of the methicillin resistant Staphylococci for both MRSA and MRCNS. It detected 100% of all MRSA and MRCNS
isolates with 100% sensitivity and 98% specificity as compared to mecA based PCR. Similar results for detection of MRSA and/or MRCNS were reported by several studies and all researchers used PCR as reference method (Felten et al., 2002; Velasco et al., 2005; Akcam et al. 2007; Mohanasoundaram and Lalitha 2008; Ekrami et al., 2010; Datta et al., 2011; Karami et al., 2011; Pramodhini et al., 2011; Sasirekha et al., 2012; Kaur et al., 2013). While Perazzi et al. (2006) (Argentina) and Martins et al. (2010) (Brazil) reported lower sensitivity of 80% and 91.3% respectively and Olowe et al. (2013) reported lower specificity 78.5%. For detection of MRCNS, comparable results were reported by Perazzi et al. (2006), Swenson et al. (2006). This proposed cefoxitin to be used as a surrogate for detection of mecA-mediated oxacillin resistance of all Staphylococci in routine susceptibility testing including both S. aureus and CNS. It is a more potent inducer of the mecA gene with no special requirements of temperature or medium (CLSI, 2013).

The oxacillin resistance agar (ORSAB) used in our study had 94% sensitivity and 96% specificity for detection of MRSA and MRCNS isolates which did not change with incubation for 24h and 48h. Several researches reported similar results for identifying MRSA (Velasco et al., 2005; Mohanasoundaram and Lalitha, 2008; Ekrami et al., 2010; Pramodhini et al., 2011). However, lower results for detection of MRSA were reported by Pillai et al. (2012) (India) with 87.5% sensitivity and 89.3% specificity. While, Cherkouni et al. (2007) (Switzerland) detected MRSA in clinical specimens with lower sensitivity and specificity of 76% and 67% (after 24h) that increased to 87% and 68% (after 48h) respectively. They proposed for MRSA identification from clinical samples, confirmatory tests should be considered as some coagulase negative Staphylococci (mainly Staphylococcus haemolyticus) appear blue by ORSAB.

In the present study the oxacillin E-test for detection of methicillin resistance had 90% sensitivity and 98% specificity. A complete agreement with mecA PCR for CNS was detected. The oxacillin MIC was more than 256µg/ml in 57% of MRSA and 60% of MRCNS isolates. Similar results were reported by Oberoi et al. (2012) with sensitivity of 90.9%. Ercis et al., (2008) (Turkey), Ekrami et al., (2010), and Martins et al. (2010) reported higher sensitivities for detection of methicillin resistance Staphylococci (100%, 99% and 97.8% respectively). In addition Ekrami et al. (2010) detected MIC >256µg/ml in 93% of MRSA and only 15% of MRCNS isolates. All used mecA PCR as a reference method.

Two isolates lacking the mecA gene were detected as MRSA (false positive). One was detected by both CDD and ORSAB and the other by ORSAB and oxacillin E test. The first one had a borderline MIC (2µg/ml). Similar results were reported by Oberoi et al. (2012). They compared different methods for detection of MRSA and found seven strains of S. aureus resistant to oxacillin but sensitive to cefoxitin, having MIC values of <2 µg/ml. This could also be attributed to the heterogeneous expression of methicillin resistance in this isolate with the borderline of MIC. Under some test conditions, low level resistance may also be seen in isolates which produce large amounts of penicillinase (penicillinase hyper producers), and are referred as borderline oxacillin-resistant S. aureus (BORS A) (Louie et al., 2000; Fluit et al., 2001; Mohanasoundaram and Lalitha, 2008). It may be difficult to distinguish them from true resistant strains that carry the mecA gene, by routine tests. The clinical problem is that during chemotherapy with Beta-lactam antibiotics, production of PBP-2a may be induced, converting them into oxacillin-resistant strains. Hence the use of cefoxitin as a more potent inducer of mecA gene or the detection of mecA gene is useful in clinical laboratories (Pillai et al., 2012). Finally, according to the explanation of the CLSI (2013) the two resistant strains by ORSAB may be due to presence of other mechanism rather than mecA. So resistant isolates with oxacillin MIC, cefoxitin MIC or cefoxitin disc test should also be reported as oxacillin resistant. Mechanisms of oxacillin resistance other than mecA are rare and include a novel mecA homologue, mecC. MICs of strains of mecC which are typically in the resistant range for cefoxitin and/or oxacillin; mecC resistance cannot be detected by tests directed at mecA.

Five mecA positive MRSA isolates and three mecA positive MRCNS isolates were falsely diagnosed as MSSA and MSCNS (false negative) by oxacillin E test and ORSAB respectively. The MICs of the five MRSA and one MRCNS isolates were 1-2 µg/ml (BORS A). The lower sensitivity oxacillin E test and ORSAB may be explained by the absence of, or reduced expression of, the mecA-encoded protein, PBP2 in the hetero-resistant strains. Also, cefoxitin is a better inducer for the expression of the mecA gene than oxacillin (Velasco et al., 2005; Anand et al., 2009; Oberoi et al., 2012). This can be attributed to the fact that accurate determination of methicillin resistance by conventional tests is subject to variations in inoculum size, incubation time, medium pH, medium salt concentration, etc. (Menon and Nagendra, 2001). Another important reason for these methicillin resistant isolates being detected phenotypically as methicillin sensitive is the over-expression of mecR and mecl genes which are co-repressors of mecA gene (Lewis et al., 2000 and Khan
et al., 2007). Misdiagnosis of methicillin resistant *Staphylococci* changes the treatment pattern of the patient to a methicillin sensitive one. So patients are not cured. By time MRSA would spread to other patients or health personnel in the hospital as well as in the community (Pillai et al., 2012).

The characteristics and antimicrobial resistance profiles of *Staphylococci* differs according to geographical regions and in relation to antibiotic usage (Olowe et al., 2013). In the current study, MRSA isolates were resistant to ciprofloxacin-78%, erythromycin-74%, clindamycin-71%, Gentamicin-70%, amikacin- 64%, azithromycin-63%, doxycyclin-60%, levofloxacin and linezolid-48%, trimethoprim-sulfamethoxazole-29% and vancomycin-0%. Methicillin resistant *Staphylococci* had higher pattern of resistance than the methicillin sensitive isolates. In addition most of the methicillin resistant isolates were multi-drug resistant (MDR) to many groups of antibiotics at the same time. Our pattern of resistance was lower than that reported for MRSA by Karami et al. (2011) (Iran) as tetracycline (99.01%), erythromycin (97.16%) clindamycin (97.16%), chloramphenicol (97.16%), ciprofloxacin (96%) and gentamicin (95.3%), rifampicin (70.76%). But our results were similar to several studies in different countries that also show variation in the same country (Mohanasoundaram and Lalitha 2008; Ekrami et al., 2010; Abd El-Moez et al., 2011; Pramodhini S. et al., 2011; Sasirekha et al.; 2012; Kaur et al., 2013; Olowe et al., 2013). According to Falagas et al. (2001), our results were within the variable susceptibility pattern of MRSA isolates in Africa to various antibiotics as: rifampicin 22%–100%, gentamicin 0–100%, vancomycin 82–100%, ofloxacin 40–100%, ciprofloxacin 25–100%, chloramphenicol 0–100%, cotrimoxazole 0–100%, erythromycin 0–100%, fusidic acid 33–100%, tetracycline 0–100%, clindamycin 18–100%, teicoplanin 93–100%, fosfomycin 84–99% and linezolid 85–100%.

**Conclusion**

The cefoxitin disc diffusion method was matching with the results of the mecA gene PCR for detection of both MRSA and CNS. Cefoxitin can also detect other mechanisms of resistance other than mecA. Oxacillin screening agar and oxacillin E-test had lower sensitivity, thus another confirmatory test is needed for detection of hetero-resistant strains to methicillin. Cefoxitin disc diffusion and ORSAB methods are reliable, simple, do not require special technique, and cost effective alternatives to PCR for detection of methicillin resistant *Staphylococci; MRSA* and CNS In countries with restricted resources.

**References**


30. Olowe OA, Kukoyi OO, Taiwo SS, Ojurongbe O, Opaleye OO, Bolaji OS, Adegoke AA, Makanjuola OB, Ogbolu DO, and Alli OT.


