A Comparative Study for Detection of Methicillin Resistance *Staphylococci* by Polymerase Chain Reaction and Phenotypic Methods

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Abstract: Background: Methicillin-resistant *Staphylococci*; both methicillin resistant *S. aureus* (*MRSA*) and methicillin resistant coagulase negative *Staphylococci* (*MRCNS*) 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 *Staphylococci* in clinical laboratories; cefoxitin disc diffusion (CDD), oxacillin resistance screening agar base (ORSAB) and oxacillin E test, in reference to *mecA* gene based real-time PCR. Also to study the antibiotic resistance pattern of the methicillin resistant *Staphylococci* isolates. **Materials and methods**: A total of 95 clinical isolates of *Staphylococci* were tested for methicillin resistance by CDD test, ORSAB and oxacillin E-test and were compared to *mecA* based real time PCR as reference method. **Results:** *MecA* gene was detected by PCR in 48/95 (51%) of all *Staphylococci* isolates; 28/57 (49%) in *S. aureus* and 20/38 (53%) in *CNS* isolates. CCD test showed 100% sensitivity and 98% specificity for detection of *MRSA* and *MRCNS*. 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 *MRCNS* in minimal resources circumstances. It can also detect other mechanisms of resistance other than *mecA*. Additional confirmatory test is needed with oxacillin screening agar and oxacillin E-test for detection of hetero-resistant strains to methicillin.
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1. Introduction

Infections caused by *Staphylococci* are of great importance for human health. The *Staphylococcus* species are divided into two large groups. The first group is known as coagulase positive *Staphylococci*, which is mainly represented by *Staphylococcus* (*S.*) *aureus,* a pathogen that can cause a variety of infections in immuno-competent patients. The second group, known as coagulase negative *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 *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 *Staphylococci* (*MRCNS*) and were then horizontally transferred among *Staphylococci*. *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 (PBPa) with low affinity for B-lactam antibiotics. Resistance to methicillin in *MRSA* and *MRCNS* is mediated by *mecA* gene, responsible for production of PBP2a. The *mecA* is located on a region of chromosome called *SCCmec (*Vaez *et al., 2011).*

There are many methods for detection of methicillin resistance in *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 *mecA* gene (Medigan and Martinko, 2006 and Kaur *et al*., 2013). The *mecA* gene is highly conserved among the *Staphylococci* species. Therefore the usefulness of polymerase chain reaction (PCR) assay of the *mecA* gene as "gold standard" for the detection of methicillin resistance in *Staphylococci* is well established (Brown, 2001; Adaleti *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 *Staphylococi* 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 -70ºC 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:

a. Cefoxitin disk (30µg) diffusion test (CDD),
b. Oxacillin E- test
c. Oxacillin resistance screen agar base (ORSAB) and
d. 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 McFerland's suspension of the *Staphylococci* isolates and was incubated at 35ºC. 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 (Bioneers, 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 1min at 95ºC, 30s at 55ºC then gradual increase to 95ºC for 30s at a rate of 0.2ºC/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).

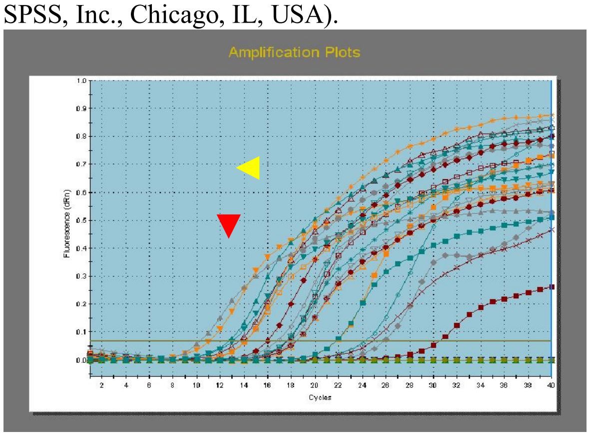

Figure (1): Results of syber Green *mecA* real-time PCR in amplification plot with cycle number on x-axis and florescence on y-axis. (The yellow arrow above = positive cases and positive control, the red arrow below = negative cases and negative control)

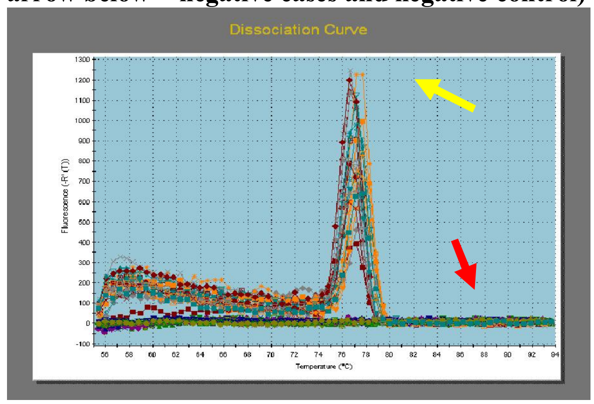

Figure (2): Results of melting curve, average Tm 77.1 ± 0.6 (The yellow arrow above = positive cases and positive control, the red arrow below = negative cases and negative control)

3.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)

Method	CDD	ORSAB	Oxacillin E-test
mecA positive *Staphylococci* (n=48) (28 *MRSA* + 20 *MRCNS*)	**48**	**45**	**43**
Methicillin sensitive *Staphylococci* (n=47) (29 *MSSA* + 18 *MSCNS*)	46	45	46
False positive	1	2	1
False negative	0	3	5
Sensitivity (%)	100	94	90
Specificity (%)	98	96	98
PPV	98	96	98
NPV	100	94	90

CCD: 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

Staphylococci isolates	PCR *mecA*	CDD	ORSAB	E test MIC (µg/ml)
MRSA	Positive	R	R	2 S
MRSA	Positive	R	R	2 S
MRSA	Positive	R	R	1 S
MRSA	Positive	R	R	2 S
MRSA	Positive	R	R	1 S
MRSA	Negative	R	R	2 S
MRSA	Negative	S	R	256 R
MRCNS	Positive	R	S	256 R
MRCNS	Positive	R	S	1 R
MRCNS	Positive	R	S	256 R

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*. Fluit *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, Cherkaoui *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 S*taphylococci* (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 border line 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* (*BORSA*) (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* resisntance 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 (*BORSA*). 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 *mecI* 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*. (20013), 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.

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