

Comparison of Different Phenotypic and Molecular Methods for Detection of Methicillin-Resistant *Staphylococcus aureus* in Intensive Care Patients

¹Hebat-Allah G. Rashed, ²Noha A. Afifi, M. ³Samy Abdel-Raheem, ¹Mohamed Z. Abdel-Rahman

Departments of ¹Clinical Pathology, ²Medical Microbiology & Immunology and ³Anesthesiology, Faculty of Medicine, Assiut University, Assiut, Egypt.

Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) has been problematic ever since its discovery. This work was performed to evaluate the effectiveness of different phenotypic methods for MRSA detection in intensive care patients. Three hundred and eighty-nine specimens from 100 patients were inoculated onto mannitol salt agar. All *S. aureus* isolates were examined by four selective culture media [ORSAB, MSO, MSA-Cefox, OAS], two disc diffusion methods [CDD and ODD], and MicroScan panel for MRSA. Polymerase chain reaction (PCR) for *mecA* gene was performed as the gold standard. *S. aureus* isolates were revealed from 56 patients, 41 of them were found to be MRSA by PCR. CDD yielded the best sensitivity (97.6%), followed by ODD and MSA-Cefox (92.7%). CDD, MSA-Cefox and OAS showed the best specificity (100%). In conclusion, MSA-Cefox and CDD showed improved sensitivity and excellent specificity compared to other methods. It is advisable to use the two methods for MRSA detection if PCR is not available.

S *taphylococcus aureus* continues to be a serious health problem worldwide due to its intrinsic nature of virulence, its ability to cause a wide array of infections and its capacity to develop resistance to several antibiotic types (Shittu *et al.*, 2007). Over the last decade, Methicillin-resistant *S. aureus* (MRSA) strains have emerged as serious pathogens causing hospital- and community-acquired infections; often such strains are multiresistant to several antibiotic classes (French, 2006). Currently, MRSA infections are widely recognized as an alarming public health burden (Struelens *et al.*, 2009).

The *mecA* gene confers microbial resistance to methicillin in *S. aureus*. The gene, located on the staphylococcal chromosome cassette *mec* encodes penicillin-binding protein 2a (PBP2a) which is located in the bacterial cell wall and has a low binding affinity for β -lactams (Broekema *et al.*, 2009). MRSA strains possessing the *mecA* gene are either heterogeneous or homogeneous in their

expression of drug resistance. The heterogeneous expression occasionally results in borderline minimal inhibitory concentrations; consequently, the isolates may be interpreted as susceptible (Anand *et al.*, 2009). Identification of the *mecA* gene is the most reliable method of detecting methicillin resistance in staphylococcal isolates, but not all laboratories can include molecular biological techniques in their routine clinical practice. For this reason, phenotypic methods that can detect such MRSA in clinical samples obtained from patients in a rapid and accurate manner may be used to implement the appropriate antibiotic treatment thereby avoiding the spread of such resistant isolates in the hospital environment (Diab *et al.*, 2008). The accurate and early determination of methicillin resistance is of key importance in prognosis of MRSA infection, as it requires treatment with vancomycin, whereas infection caused by methicillin-susceptible strains and *mecA*-negative borderline-resistant strains may be treated with beta-lactam agents

(Chambers, 1997). Misidentification of methicillin-susceptible *S. aureus* will promote unnecessary and inappropriate use of vancomycin; in addition to elaborate procedures to isolate MRSA-infected patients and implement barrier precautions that are not warranted for patients infected with susceptible strains (Ribeiro *et al.*, 1999).

Phenotypic detection of MRSA was reported problematic ever since its discovery in the early 1960s. Accurate detection of oxacillin/methicillin resistance may be difficult due to the presence of two subpopulations; one susceptible and the other resistant that may coexist within a culture of *Staphylococci*. All the cells in a culture may carry the genetic information of antibiotic resistance, however, only a small number of them may express such resistance *in vitro*. Cells expressing such heteroresistance grow more slowly than the oxacillin-susceptible populations and may be missed at temperatures above 35°C (Matouskova & Janout, 2008).

Although several methods were developed to detect MRSA, however, some of them may be too slow, not sufficiently sensitive or specific enough to ensure appropriate treatment of the MRSA-infected patients (Velasco *et al.*, 2005). As the rapid tests were considered more expensive than conventional methods; thereby not all laboratories might use them routinely for financial reasons (Kerttula *et al.*, 2007). The aim of this work was to compare the performance of four culture media, two disc diffusion methods and the MicroScan conventional system in the identification of MRSA isolates in clinical samples obtained from patients admitted at the Intensive Care Unit (ICU), Assiut University Hospital, using the PCR for *mecA* gene as the gold standard.

Patients, Materials and Methods

The present study was conducted over a seventeen-month period (from March 2009 to August 2010) on

100 patients admitted to the trauma and general ICUs, Assiut University Hospital. Criteria for patients enrollment were previous admission at a different ward or hospital, long ICU stay (>15 days), prolonged mechanical ventilation (>7 days), history of major surgery, known to be immunosuppressed secondary to neutropenia (<500/ mm³), diabetes mellitus or malignancy and steroid therapy, chemotherapy or radiotherapy. In addition, patients with cultures positive for Staphylococcal isolates resistant to broad spectrum antimicrobial drugs and/or sensitive to vancomycin were also included in this study. The ethical committee of Faculty of Medicine- Assiut University approved this study and a written consent was obtained before patient enrollment.

In total, studied clinical specimens (n=389) obtained from study patients consisted of sputum samples (n=98), swabs from nostrils (n=90), axilla and groin (n=86), wound discharge (n=35), blood (n=32), vascular catheter tips (n=31) and urine (n=17). The isolation of MRSA from specimens obtained from more than one site for the same patient was only considered as one positive recovery. Infection was considered as nosocomial if it was acquired 48 hours after hospitalization. Otherwise, it was regarded as being a community acquired infection.

Clinical isolates were inoculated onto mannitol salt agar (MSA) plates and incubated at 37°C for 24 h. 74 Staphylococcal isolates were identified by Gram stain and positive catalase test. Both mannitol- fermenting (yellow) and mannitol-nonfermenting (pink) staphylococcal isolates were then differentiated into *S. aureus* (n=56) and Coagulase-Negative *Staphylococci* (CoNS; n=18) by tube coagulase test (Dancer *et al.*, 2009). All *S. aureus* (coagulase positive) isolates were kept frozen at -70°C in Brucella broth supplemented with 20% glycerol (BBL Ltd, USA) until use within six months.

The strains were streaked once from freezer storage and they were grown at 37°C on blood agar plates before use.

We examined the accuracy of different phenotypic methods for the detection of MRSA. These methods were four selective culture media [ORSAB, MSO, MSA-Cefox, OAS], two disc diffusion methods with Mueller-Hinton agar (MHA) [cefexitin (30 µg) and oxacillin (one µg)] and MicroScan conventional system. The polymerase chain reaction (PCR) to detect the *mecA* gene was used as the gold standard test.

- Oxacillin resistance screening agar base (ORSAB) *S. aureus* isolates were subcultured on ORSAB plates (Oxoid Limited, UK) and examined for suspected colonies after 24 and 48 h of incubation at 35°C. The

medium uses aniline blue to demonstrate mannitol fermentation in staphylococci. The dual antibiotic supplement (oxacillin, 2.0 mg/l and polymixin B, 50,000 IU/l) and the presence of 5.5% NaCl have the potential to reduce the growth of nonstaphylococcal organisms and to select for the growth of MRSA (Simor *et al.*, 2001).

- Mannitol salt agar medium supplemented with oxacillin (MSO)

S. aureus isolates were subcultured on MSA agar plates (Oxoid Limited, UK) containing 7.5% sodium chloride concentration supplemented with 2 mg/l oxacillin (Himedia Laboratories, Pvt. Ltd., India). Plates incubated at 35°C for 24 h were examined for evidence of mannitol fermenting growth. The growth of yellow colonies on MSO plates were considered as presumptive MRSA isolates (Simor *et al.*, 2001).

- Mannitol salt agar medium supplemented with Cefoxitin (MSA-Cefox)

S. aureus isolates were subcultured on MSA agar plates (Oxoid Limited, UK) containing 7.5% sodium chloride supplemented with 4 mg/liter cefoxitin (Himedia, Pvt. Ltd., India). Plates were incubated at 35°C for 24 h. The growth of any visible yellow colonies after incubation was recorded as a positive result (MRSA) (Stoakes *et al.*, 2006).

- Oxacillin salt agar screen (OAS)

S. aureus isolates were inoculated on MHA (Himedia, Pvt. Ltd., India) supplemented with 4% NaCl and 6.0 mg/l oxacillin (Himedia, Pvt. Ltd., India). Plates incubated at 35°C for 24 h were examined for evidence of growth. Any visible growth of more than one colony was interpreted as a positive oxacillin salt agar screen result for MRSA (Adaleti *et al.*, 2008).

- Oxacillin and cefoxitin disc diffusion tests (ODD and CDD)

OXA and CEF disc diffusion tests were carried out on overnight *S. aureus* cultures at 37°C according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2006). Isolates were spread to confluence on MHA (Himedia) plates containing 2% NaCl for OXA (one µg) and without NaCl for CEF discs (30 µg). Inhibition zone diameters were read from the back of the agar plate using reflected light and calipers to read to the nearest millimeter at the inner zone edge.

Interpretation criteria of OXA inhibition zone diameter for *S. aureus* were ≥ 13 mm (susceptible), 11-12 mm (intermediate) and ≤ 10 mm (resistant) while that of CEF were ≥ 18 mm (susceptible), 15-17mm (intermediate) and ≤ 14 mm (resistant). Intermediate category was considered as susceptible. In cases of heterogeneous growth, defined as the occurrence of

small colonies in the circular growth inhibition area, the diameter of the inner limit of the small colonies' inhibition zone was taken into account.

MicroScan® conventional system (Pos Combo 10 Panels; Dade Behring, Inc., West Sacramento, Calif.) was used to determine breakpoints of tested isolates according to the manufacturer's instructions. OXA MICs of *S. aureus* ORSAB positive isolates with breakpoints of ≤ 2 µg/ml were considered susceptible (i.e. *mecA* negative) and ≥ 4 µg/ml resistant (i.e. *mecA* positive).

- Detection of *mecA* gene by PCR

Bacterial lysates were made by suspending a single colony in 100 µl of sterile distilled water and boiling for 10 min (Huygens *et al.*, 2002). For amplification we used puReTaq Ready-To-Go PCR Beads (Product code 27-9557-01; GE Health care kit, UK). Oligonucleotides primers used for the detection of *mecA* gene were *mecA-1* (5-AAA ATC GAT GGT AAA GGT TGG C-3) and *mecA-2* (5-AGT TCT GCA GTA CCG GAT TTG C-3) (Louie *et al.*, 2002). The PCR was carried out in 50 µl volumes, (4 µl of each primer, 10 µl of DNA, 36 µl of sterile high quality water and PCR Beads). Using a PCR Express thermal cycler (Hybaid, UK) amplification profile was initial denaturation 5 min at 94°C, followed by 30 cycles of denaturation 30 sec at 95°C, annealing 30 sec at 50°C and extension 30 sec at 72°C followed by final extension 10 min at 72°C for one cycle. Eight µl PCR products mixed with seven µl loading buffer (Bromothymol blue, BDH, England) were electrophoresed in 1% agarose gel in TBE buffer (Tris borate-EDTA; Sigma-Aldrich, USA) at 100 v for 30 min. Gel was stained with ethidium bromide 0.5 µg/ml (Sigma-Aldrich, USA) PCR products (533bp) were sized against a molecular weight marker (0.05 mg DNA ladder 304005, Bioron, Germany) (Figure 1).

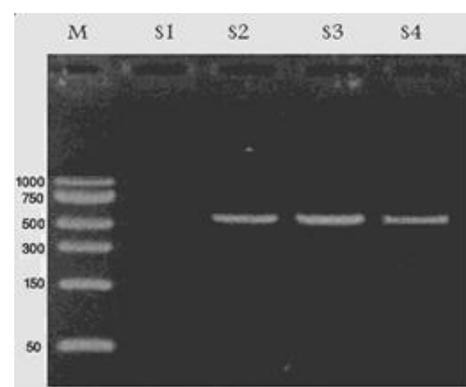


Figure 1. Detection of *mecA* gene by PCR.

M: Ladder 50-1000bp; S1:negative control; S2-S4: *mecA* gene positive strains (MRSA).

Statistical Analysis

Data obtained in this study were represented as mean \pm standard deviation (SD) or percentage (%). Sensitivity, specificity, and positive and negative predictive values were all calculated using standard methodology (Stongin, 1992).

Results

In total, 389 specimens from 100 patients were included in this study, their age ranged from 14 to 85 years; age mean of 44.37 \pm 17.44 years. Staphylococcal isolates were identified in 74 patients using MSA. Identification of the *S. aureus* species was confirmed by detection of the coagulase enzyme in 56 out of 74 staphylococcal isolates. Of the 56 *S. aureus* isolates, 41 were found to be *mecA* gene- positive (MRSA) and 15 were found to be MSSA. Overall, MRSA was detected from at least one specimen from the same patient in 41/100 patients and the MRSA percentage was 73.2% of *S. aureus* infections (41/56).

The most frequently reported infection with MRSA was pneumonia (48.8%, n=20/41), followed by bacteraemia (19.5%,

n=8/41), and upper respiratory tract infection (14.6%, n=6/41). MSRA isolates obtained from other sites of infection such as wound, skin and urinary tract infection accounted for less than 10% of total MRSA recovered in this study (Figure 2).

In the present study, we compared the ability and accuracy of four culture media [ORSAB, MSO, MSA-Cefox, OAS]), two disc diffusion methods [ODD and CDD] using OXA and CEF and the MicroScan conventional panels to differentiate and identify MSSA and MRSA clinical isolates. The presence or absence of the *mecA* gene was regarded as the gold standard to detect oxacillin resistance.

The results of testing for 56 *S. aureus* isolates by different methods for identification of MRSA are shown in Table (1) as the number and percentage of correct results as determined by the presence or absence of the *mecA* gene and the performance of those different techniques are displayed in Table (2).

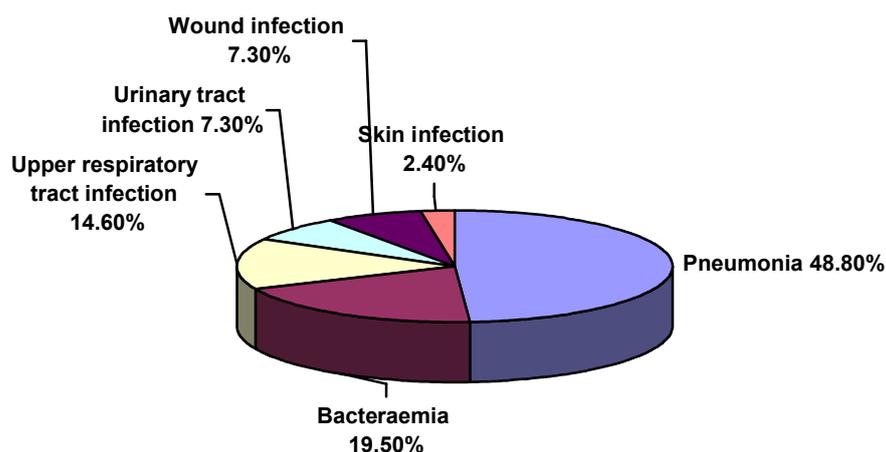


Figure 2. Types of Infections in MRSA-Positive Patients.

Table 1: Results of 56 *S. aureus* isolates testing by different methods for the detection of methicillin resistance as shown by the number (percentage) of strains that gave correct results for each method using the PCR for *mecA* gene as the reference test.

Organism group	MecA-PCR No. (%)	No. (%) of correct results as determined by:							
		ORSAB 24h	ORSAB 48h	MSO	OAS	MSA-Cefox	ODD ^a	CDD ^a	Micro Scan
MRSA n = 41	41 (100)	36 (88)	37 (90)	31 (76)	28 (68)	38 (93)	38 (93)	40 (98)	38 (93)
MSSA n = 15	15 (100)	12 (80)	10 (67)	12 (80)	15 (100)	15 (100)	11 (73)	15 (100)	10 (67)

*Abbreviations: ORSAB, Oxacillin resistance screening agar base; MSO, Mannitol salt agar media with oxacillin; MSA-Cefox, Mannitol salt agar media with Cefoxitin; OAS, Oxacillin salt agar screen; CDD, Cefoxitin disk diffusion, ODD, Oxacillin disk diffusion.

^aFor MecA-positive strains, results in the "R" category only were considered correct.

Table 2: Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of different methods for detecting methicillin resistance in 56 *S. aureus* isolates using PCR as the reference test.

Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
ORSAB 2 mg/L 24h	87.8	80.0	92.3	70.6
ORSAB 2 mg/L 48h	90.2	66.7	88.1	71.4
MSO 2 mg/L 24h	75.6	80.0	91.2	54.5
OAS 6 mg/L 24h	68.3	100	100	53.6
MSA-Cefox 4 mg/L 24h	92.7	100	100	83.3
ODD 1 µg 24h	92.7	73.3	90.5	78.6
CDD 30 µg 24h	97.6	100	100	93.8
MicroScan	91.6	66.7	97.1	40.0

*Abbreviations: ORSAB, Oxacillin resistance screening agar base, MSO, Mannitol salt agar media supplemented with oxacillin, MSA-Cefox, Mannitol salt agar media supplemented with Cefoxitin, OAS, Oxacillin salt agar screen, CDD, Cefoxitin disc diffusion, ODD, Oxacillin disc diffusion.

For all methods except OAS and MSO media, the sensitivity (percentage of *mecA* gene positive strains correctly identified) was $\geq 85\%$. The CDD yielded the best sensitivity value (97.6%), followed by the ODD and MSA-Cefox medium (92.7%). For all

methods except ORSAB at 48h, ODD and the MicroScan, the specificity (percentage of *mecA* gene-negative strains correctly identified) was $\geq 80\%$. The CDD, and MSA-Cefox and OAS media showed the best specificity values (100%) (Figure 3).

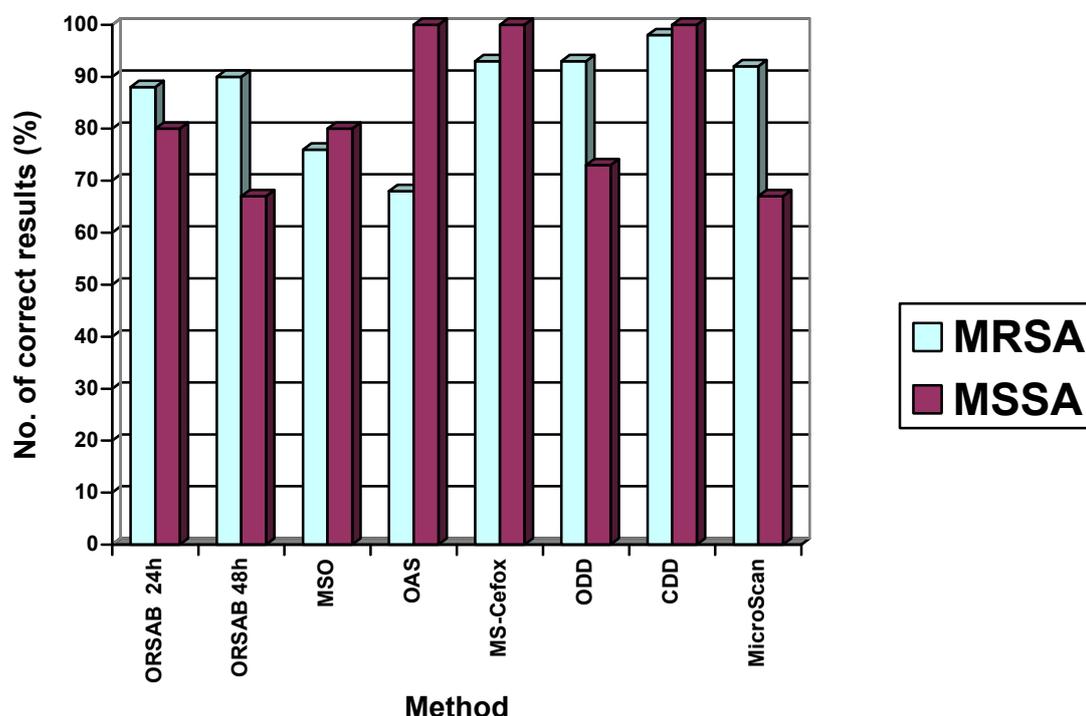


Figure 3. Results of testing of 56 *S. aureus* isolates by different methods for the detection of methicillin resistance as shown by percentage of strains that gave correct results for each method using presence of *mecA* gene as the reference.

The ORSAB medium failed to identify 5 of the 41 *mecA* gene-positive strains as resistant on testing at 24h, and only one of these strains was positive after 48h incubation. The ORSAB medium at 24h showed sensitivity of 87.8% and slightly better sensitivity of 90.2% at 48h. However, the specificity at 24h was 80.0% and lower specificity of 66.7% was obtained at 48h resulting in a positive predictive value of 92.3% and 88.1% and a negative predictive value of 70.6% and 71.4% after 24h and 48h incubation, respectively (Tables 1 and 2).

Disc susceptibility tests (CDD and ODD) were performed on 56 *S. aureus* strains. By CDD method, using the CLSI interpretive zone diameter of $R \leq 14$ mm (CLSI, 2006), all 15 *mecA*-negative isolates were correctly identified as oxacillin susceptible (specificity,

100%), and 40 of the 41 *mecA*-positive isolates were found to be oxacillin resistant (sensitivity, 97.6%), resulting in a positive predictive value of 100% and a negative predictive value of 93.8%. On the other hand, using the CLSI interpretive zone diameter of $R \leq 10$ mm for ODD method, (CLSI, 2006), 11 of 15 *mecA*-negative isolates were found to be oxacillin susceptible (specificity, 73.3%), and 38 of the 41 *mecA*-positive isolates were found to be oxacillin resistant (sensitivity, 92.7%). The positive predictive value was 90.5% and a negative predictive value 78.6% (Tables 1 and 2). We evaluated two disc diffusion methods for the detection of MRSA in 56 *S. aureus* isolates. For *mecA*-positive strains, results in the resistant (R) category only were considered correct; true positive (Table 3).

Table 3: Evaluation of two disc diffusion methods for detection of MRSA in 56 *S. aureus* isolates.

Method	Resistant	Intermediate	Susceptible
Oxacillin disc diffusion (ODD)	42/56	2	12/56
Cefoxitin disc diffusion (CDD)	40/56	1	15/56

For *MecA*-positive strains, results in the resistant (R) category only were considered correct (true positive).

Discussion

In this study, we examined the accuracy of the agar screening tests for the detection of MRSA when compared to *mecA* gene PCR, which is accepted as the gold standard. These media were ORSAB, MSO, OAS, and MSA-Cefox. The performances of oxacillin- and cefoxitin-disc diffusion tests and the MicroScan conventional method for detection of MRSA were also assessed and compared with the agar screening tests.

The sensitivity and specificity of ORSAB after 24h incubation were 87.8% and 80%, and further incubation for up to 48h increased the sensitivity to 90.2%, but the specificity decreased to 66.7%. Simor *et al.* (2001) have also found a sensitivity of 76% after 24h and improved to 98% after 48h incubation for ORSAB and reported that an incubation time of 24h was not sufficient for the detection of MRSA and it should be extended to 48h. Apfalter *et al.* (2002) found a sensitivity of 51% (68%) and a specificity of 96% (95%) after 24h (48h), and within the context of these detected values, they draw attention to the existence of mannitol-negative *S. aureus* strains and mannitol-positive methicillin-resistant *CoNS*. Perry *et al.* (2004), found sensitivities and specificities of 62% (78%) and 98% (93%) after 24h (48h) incubation. Compennolle *et al.* (2007) also reported sensitivities and specificities of 57% (77%) and 92% (83%) after 24h (48h) incubation for ORSAB. In contrast, Velasco *et al.* (2005) found a sensitivity of 100% after 24h incubation and specificities of 92% (67%) after 24h (48h) incubation for ORSAB and

reported that the low specificity of the ORSAB medium prevents its use, at least alone, in predicting methicillin resistance in *S. aureus*.

This study confirms that ORSAB lacks sensitivity for detection of MRSA if they are incubated for only 24h. It may be explained by the frequent inducible and heterogeneous expression of methicillin resistance, which requires an extended incubation period for full expression (Nsira *et al.*, 2006). The presence of 5.5% salt in ORSAB may also have contributed to the failure to isolate some MRSA strains (Perry *et al.*, 2004).

In this study, the sensitivity of MSO medium after 24h incubation was 75.6%, higher than the sensitivities (47%), (64%), (42%), that were yielded by Davies *et al.* (2000); Simor *et al.* (2001) and Zadik *et al.* (2001), respectively. The specificity of the MSO medium was 80%, comparable to 78.1% that was reported by Simor *et al.* (2001). However, Kampf *et al.* (1998), reported higher sensitivity and specificity for MSO medium (98.1% and 95.1%, respectively).

In this study, OAS medium was less sensitive than all other culture media after 24 hr incubation. The sensitivity of OAS was 68.3%, comparable to 65% reported by Safdar *et al.* (2003), and lower than the sensitivities (99% 96.0% and 99.5%) yielded by Becker *et al.* (2002), Velasco *et al.* (2005) and Adaleti *et al.* (2008), respectively. Although OAS medium was the least sensitive method, its specificity was excellent (100%) and correlated exactly with that reported by Velasco *et al.* (2005) and Adaleti *et al.*

(2008), and higher than the specificity 88% reported by Safdar *et al.* (2003).

In this work, MSA-Cefox medium used cefoxitin as a selective agent at a concentration of 4 mg/l. It exploits the apparent advantages of cefoxitin and it shows to be an effective medium for the isolation of MRSA. It inhibited the growth of all MSSA and CoNS strains and allowed the majority of MRSA strains to grow, demonstrating a specificity of 100% and a sensitivity of 92.7% after only 24 hr incubation. Felten *et al.* (2002) also reported that a concentration of 4 mg/l of cefoxitin allowed the growth of all MRSA strains and the inhibition of all MSSA strains. Perry *et al.* (2004) indicated that this medium showed a sensitivity of 89% and a specificity of 99.5% for the detection of MRSA in clinical material. Smyth and Kahlmeter (2005) proposed that agar containing cefoxitin supported the growth of 96.6% of the *mecA*-positive strains and inhibited the growth of 100% of the *mecA*-negative strains. They concluded that selective media containing cefoxitin was superior to those containing oxacillin for the detection of MRSA.

With reference to oxacillin or cefoxitin agar screening methods, the agar plate with 6 mg/l oxacillin (OAS) and MSA-Cefox medium with 4 mg/l cefoxitin showed excellent specificity (100%) that was higher than that of the plates with 2 mg/l oxacillin (ORSAB and MSO), and therefore selected MRSA strains. Moreover, MSA-Cefox method was more sensitive than the oxacillin agar screening methods (ORSAB, OAS and MSO). This result is in accordance with Smyth and Kahlmeter (2005), who demonstrated that cefoxitin is far superior to oxacillin when it is used in this particular mannitol salt medium and they found that the sensitivity and specificity of the cefoxitin MSA plate method were 96% (compared to 85% for oxacillin) and 100% (compared to 96% for oxacillin), respectively.

In this work, with respect to Oxacillin disc diffusion method (ODD) using the CLSI guidelines (2006), a sensitivity of 92.7% was obtained by ODD method. This result is in accordance with Boutiba–Ben Boubaker *et al.* (2004), Velasco *et al.* (2005) and Anand *et al.* (2009) who reported a sensitivity of 94.1%, 90.4%, and 87.5%, respectively, and higher than the sensitivities (41% and 78%) that were reported by Felten *et al.* (2002) and Skov *et al.* (2003), respectively. While Rahbar *et al.* (2006) and Adaleti *et al.* (2008) reported a sensitivity of 98% and 100%, respectively.

The low specificity of ODD method (73.3%) was comparable to (88.8%) that was reported by Adaleti *et al.* (2008). However, Velasco *et al.* (2005), Rahbar *et al.* (2006) and Anand *et al.* (2009) reported a specificity of 100% and Boutiba –Ben Boubaker *et al.* (2004), reported a specificity of 99% for the 1 µg oxacillin disc for detection of MRSA.

In this study, the rate of false susceptibility associated with the oxacillin-disc diffusion test has been noted to be as high as 5.4%, which is comparable to that recorded in some studies (4.4%) (Pottumarthy *et al.*, 2005, Broekema *et al.*, 2009), well above the CLSI-recommended acceptability limit of $\leq 1.5\%$.

Cefoxitin has been described as an inducer of methicillin resistance by production of the PBP2a (Swenson *et al.*, 2007). Our observations with the use of cefoxitin-discs were very encouraging. Using the CLSI (2006) cefoxitin-disc diffusion criteria, a sensitivity of 97.6% and a specificity of 100% were obtained. This is in accordance with Roisin *et al.* (2008) who reported a sensitivity of 96.4% and a specificity of 100%, using the 30 µg cefoxitin disc. Anand *et al.* (2009) have also found that the sensitivity and specificity were 100% using the NCCLS (2003) and CLSI (2006) disc diffusion criteria to define resistance. They reported that the cefoxitin disc diffusion method is very suitable for detection of MRSA and the test can be an

alternative to PCR for detection of MRSA in resource constraint settings.

On the other hand, one strain which was considered as MSSA in CDD method (had inhibition zone of 16 mm) should have been interpreted as intermediate resistance to methicillin (Table 3). This isolate that tested intermediate to ceftazidime and resistant to oxacillin were found to be positive for *mecA* gene. If we consider the intermediate resistance to methicillin as resistant category or using an interpretive zone diameter of <18 mm for resistance, the sensitivity of CDD method would have increased to 100%.

In comparison between the CDD and the ODD tests, we found that the CDD method showed higher sensitivity and excellent specificity than the ODD method (Tables 1 and 2). These results suggest that MRSA strains grow much more readily in the presence of ceftazidime than oxacillin, possibly due to the enhanced induction of PBP2a by ceftazidime (Rohrer *et al.*, 2001).

The present study demonstrated that the microdilution method with the MicroScan conventional panels showed a sensitivity of 91.6% and a specificity of 66.7%. Farrell, (1997) reported that conventional and rapid MicroScan panels both had a sensitivity of 100% and a specificity of 92% for detection of oxacillin resistance in 335 isolates of *S. aureus* compared with a *mecA* gene PCR test. Swenson *et al.* (2001) showed that MicroScan conventional panels had a sensitivity of 74% and a specificity of 100% for detection of methicillin resistance in a challenge set of 55 *S. aureus* isolates enriched for difficult-to-detect heteroresistant strains.

In conclusion, none of the compared phenotypic techniques showed 100% sensitivity and specificity. MSA-Cefox agar and the CDD method showed improved sensitivity and excellent specificity for detection of MRSA. Data obtained in this study suggest the use of both methods to detect MRSA in diagnostic laboratories, in

particular where PCR to detect *mecA* gene is not available.

References

1. Adaleti R, Nakipoglu Y, Karahan ZC, Tasdemir C, Kaya F (2008). Comparison of polymerase chain reaction and conventional methods in detecting methicillin-resistant *Staphylococcus aureus*. J Infect Developing Countries, 2 (1): 46-50.
2. Anand KB, Agrawal P, Kumar S, Kapila K (2009). Comparison of ceftazidime disc diffusion test, oxacillin screen agar, and PCR for *mecA* gene for detection of MRSA. Indian J Med Microbiol., 27: 27-29.
3. Apfalter P, Assadian O, Kalczyk A, Lindenmann V, Makristathis A, Mustafa S, Rotter M, Hirschl AM (2002). Performance of a new chromogenic oxacillin resistance screen medium (Oxoid) in the detection and presumptive identification of methicillin-resistant *Staphylococcus aureus*. Diagn Microbiol Infect Dis., 44: 209-211.
4. Becker A, Forster DH, Kniehl E (2002). Oxacillin resistance screening agar base for detection of methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol., 40: 4400-1.
5. Boutiba –Ben Boubaker I, Ben Abbes R, Ben Abdallah H, Mamlouk K, Mahjoubi F, Kammoun A, Hammami A, Ben Redjeb S (2004). Evaluation of a ceftazidime disk diffusion test for the routine detection of methicillin resistant *Staphylococcus aureus*. Clin Microbiol Infect., 10: 762-765.
6. Broekema NM, Van TT, Monson TA, Marshall SA and Warshauer DM (2009). Comparison of Ceftazidime and Oxacillin Disk Diffusion Methods for Detection of *mecA*-Mediated Resistance in *Staphylococcus aureus* in a Large-Scale Study. J Clin Microbiol., 47 (1): 217-219.
7. Chambers HF (1997). Methicillin resistance in *staphylococci*: molecular and biochemical basis and clinical implications. Clin Microbiol Rev., 10: 781-791.
8. Clinical and Laboratory Standards Institute (CLSI) (2006). Performance standards for antimicrobial disk susceptibility tests. Approved standard M2–A9, ninth ed. CLSI, Wayne, PA.
9. Compennolle V, Verschraegen G., Claeys G (2007). Combined use of Pastorex Staph-Plus and either of two new chromogenic agars, MRSA ID and CHROMagar MRSA, for detection of

- methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol., 45: 154-158.
10. Dancer SJ, White LF, Lamb J, Girvan EK, Robertson C (2009). Measuring the effect of enhanced cleaning in a UK hospital: a prospective cross-over study BMC Medicine, 7: 28-40.
 11. Davies S, Zadik PM, Mason CM, Whittaker SJ (2000). Methicillin-resistant *Staphylococcus aureus*: evaluation of five selective media. Br J Biomed Sci., 57: 269-72.
 12. Diab M, El-Damarawy M, Shemis M (2008). Rapid Identification of Methicillin-Resistant *Staphylococci* Bacteremia Among Intensive Care Unit Patients. Medscape J Med., 10 (5): 126.
 13. Farrell DJ (1997). The reliability of Microscan conventional and rapid panels to identify *Staphylococcus aureus* and detect methicillin resistance: an evaluation using the tube coagulase test and *mecA* PCR. Pathology, 29: 406-10.
 14. Felten A, Grandry B, Lagrange PH, Casin I (2002). Evaluation of three techniques for detection of low-level methicillin-resistant *Staphylococcus aureus* (MRSA): a disc diffusion method with cefoxitin and moxalactam, the Vitek 2 system, and the MRSA-screen latex agglutination test. J Clin Microbiol., 40: 2766-71.
 15. French GL (2006). Bactericidal agents in the treatment of MRSA infections-the potential role of daptomycin. J Antimicrobial Chemotherapy, 58: 1107-1117.
 16. Huygens F, Nimmo R, Schooneveldt J, Munckhof W, Giffard P (2002). Genotyping of Methicillin - resistant *S. aureus* by assaying for the presence of variable elements associated with *mecA*. J Clin Microbiol., 40 (8): 3093-3097.
 17. Kampf G, Lecke C, Cimbala A, Weist K, Ruden H (1998). Evaluation of mannitol salt agar for detection of oxacillin resistance in *Staphylococcus aureus* by disk diffusion and agar screening. J Clin Microbiol. 36 (8): 2254-57.
 18. Kerttula AM, Lyytikäinen O, Karden-Lilja M, Ibrahim S, Salmenlinna S, Virolainen A, Vuopio-Varkila (2007). Nationwide trends in molecular epidemiology of methicillin-resistant *Staphylococcus aureus*, Finland, 1997-2004. BMC Infect. Dis., Aug 14; (7): 94.
 19. Louie L, Goodfellow J, Mathieu P, Glatt A, Louie M, Simor AE (2002). Rapid detection of methicillin-resistant *Staphylococci* from blood culture bottles by using a multiplex PCR assay. J Clin Microbiol., 40: 2786-90.
 20. Louie L, Matsumura SO, Choi E, Louie M, Simor AE (2000). Evaluation of three rapid methods for detection of methicillin resistance in *Staphylococcus aureus*. J Clin Microbiol., 38: 2170-3.
 21. Louie L, Soares D, Meaney H, Vearncombe M and Simor AE (2006). Evaluation of a new chromogenic medium, MRSA select, for detection of Methicillin - resistant *Staphylococcus aureus*. J Clin Microbiol., 44: 4561-4563.
 22. Matouskova I, Janout V (2008). Current knowledge of methicillin-resistant *Staphylococcus aureus* and community-associated methicillin-resistant *Staphylococcus aureus*. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub., 152 (2): 191-202.
 23. Nakatomi Y, Sugiyama J (1998). A rapid latex agglutination assay for the detection of penicillin-binding protein 2'. Microbiol Immunol., 42: 739-43.
 24. National Committee for Clinical Laboratory Standards (1997). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 4th ed., vol. 17, no. 2. Approved standard M7-A4. National Committee for Clinical Laboratory Standards, Wayne, Pa, USA.
 25. National Committee for Clinical Laboratory Standards (2003). Performance Standards for Antimicrobial Disk Susceptibility Tests-Eighth Edition: Approved Standard M2-A8. NCCLS, Wayne, PA, USA.
 26. Nsira SB, Dupuis M, Leclercq R (2006). Evaluation of MRSA Select, a new chromogenic medium for the detection of nasal carriage of methicillin-resistant *Staphylococcus aureus*. Inter J Antimicrobial Agents, 27: 561-564.
 27. Perry JD, Davies A, Butterworth LA, Hopley AL, Nicholson A, Gould FK (2004). Development and evaluation of a chromogenic agar medium for Methicillin - resistant *Staphylococcus aureus*. J Clin Microbiol., 42: 4519-23.
 28. Pottumarthy S, Fritsche TR, Jones RN (2005). Evaluation of alternative disk diffusion methods for detecting *mecA*-mediated oxacillin resistance in an international collection of *staphylococci*: validation report from the SENTRY antimicrobial surveillance program. Diagn. Microbiol. Infect. Dis., 51: 57-62.

29. Rahbar M, Moulanaee S, Islami P, Atifeh R, Pirayesh K (2006). Evaluation of cefoxitin disk for detection of Methicillin - resistant *Staphylococcus aureus*. Indian J Pathol. 1 (4): 145-148.
30. Ribeiro J, Vieira FD, King T, D'Arrezo JB, Boyce JM (1999). Misclassification of susceptible strains of *Staphylococcus aureus* as methicillin-resistant *S. aureus* by a rapid automated susceptibility testing system. J Clin Microbiol., 37: 1619-20.
31. Rohrer S, Tschierske M, Zbinden R, Berger-Bachi B (2001). Improved methods for detection of methicillin-resistant *Staphylococcus aureus*. Eur. J. Clin. Microbiol. Infect. Dis., 20: 267-270.
32. Roisin S, Claire N, Olivier D, Marc J (2008). Evaluation of new Vitek 2 Card and disk diffusion method for determining susceptibility of *Staphylococcus aureus* to oxacillin. J Clin Microbiol., 8: 2525-28.
33. Safdar N, Narans L, Gordon B, Maki DG (2003). Comparison of culture screening methods for detection of nasal carriage of Methicillin resistant *Staphylococcus aureus*: a prospective study comparing 32 methods. J Clin Microbiol., 41: 3163-6.
34. Shittu AO, Udo EE, Lin J (2007). Insights on Virulence and Antibiotic Resistance: A Review of the Accessory Genome of *Staphylococcus aureus*. Wounds, 19 (9): 237-244.
35. Simor AE, Goodfellow J, Louie L, Louie M (2001). Evaluation of new medium, Oxacillin Resistance Screening Agar base, for the detection of methicillin-resistant *Staphylococcus aureus* from clinical specimens. J Clin Microbiol., 39: 3422.
36. Skov R, Smyth R, Clausen M, Larsen AR, Frimodt-Møller N, Olsson-Liljequist B, Kahlmeter G (2003). Evaluation of a cefoxitin 30 µg disc on IsoSensitest agar for detection of methicillin-resistant *Staphylococcus aureus*. J. Antimicrob. Chemother., 52: 204-207.
37. Smyth RW, Kahlmeter G (2005). Mannitol salt agar-cefoxitin combination as a screening medium for methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol., 43:3797-3799.
38. Stoakes L, Reyes R, Daniel J, Lennox G, John MA, Lannigan R, Hussain Z (2006). Prospective comparison of a new chromogenic medium, MRSASelect, to CHROMagar MRSA and mannitol-salt medium supplemented with oxacillin or cefoxitin for detection of methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol., 44: 637-639.
39. Stongin W (1992). Sensitivity, specificity and predictive value of diagnostic tests: definition and clinical applications. In: Lemette EH (ed.) Laboratory diagnosis of viral infections. New York, Marcel Dekker, pp. 210-218.
40. Struelens MJ, Hawkey PM, French GL, Witte W, Tacconelli E (2009). Laboratory tools and strategies for methicillin-resistant *Staphylococcus aureus* screening, surveillance and typing: state of the art and unmet needs. Clin Microbiol Infect., 15 (2): 112-9.
41. Swenson J M, Williams PP, Killgore G, O'Hara CM and Tenover FC (2001). Performance of eight methods, including two new rapid methods, for detection of oxacillin resistance in a challenge set of *Staphylococcus aureus* organisms. J. Clin. Microbiol., 39: 3785-3788.
42. Swenson JM, Lonsway D, McAllister S, Thompson A, Jevit Lt, Zhu W, Patel JB (2007). Detection of mecA-mediated resistance using reference and commercial testing methods in a collection of *S. aureus* expressing borderline oxacillin MICs. Diagn. Microbiol. Infect. Dis., 58: 33-39.
43. Velasco D, del Mar Tomas M, Cartelle Beceiro A, Perez A, Molina F, Moure R, Villanueva R, Bou G (2005). Evaluation of different methods for detecting methicillin (oxacillin) resistance in *Staphylococcus aureus*. J. Antimicrob. Chemotherap., 55: 379-82.
44. Zadik PM, Davies S, Whittaker S, Mason C (2001). Evaluation of a new selective medium for methicillin-resistant *Staphylococcus aureus*. J Med Microbiol., 50: 476-9.