**Accuracy of current oxacillin sensitivity tests routinely used in hospitals in Western Saudi Arabia**

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**ABSTRACT**

**Objective:** To determine the accuracy of the current oxacillin resistant *Staphylococcus aureus* (*S. aureus*) detection test used in Makkah hospitals in comparison with the National Committee for Clinical Laboratory Standards (NCCLS) method.

**Methods:** A total of 500 *S. aureus* clinical isolates and its oxacillin sensitivity patterns were collected from the 4 main hospitals in Makkah, Kingdom of Saudi Arabia between April 2003 and January 2004. The oxacillin sensitivity of these clinical isolates were re-examined using the NCCLS standard method and confirmed using polymerase chain reaction (PCR) technique.

**Results:** Of 500 clinical isolates, 103 (20.6%) were resistant to oxacillin using NCCLS standard method but they were sensitive according to the current hospital routine sensitivity test method. The PCR technique confirmed the presence of mecA gene in 88/103 isolates appeared to be methicillin-resistant *S. aureus* (MRSA) using NCCLS standard technique.

**Conclusion:** A significant percentage of MRSA are currently misdiagnosed in accordance with the current routine sensitivity method. In addition, some mecA negative and oxacillin resistant strains (according to the NCCLS standard method) can be misdiagnosed by using PCR technique. These findings emphasis the urgent need to comply with the recommended NCCLS guidelines for detection of oxacillin resistance. Moreover, the PCR technique can not be used as a single diagnostic tool for detection of MRSA.


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*S. aureus* continue to be among the most common of all nosocomial and community-acquired bacterial pathogens. Both *Staphylococcus aureus* (*S. aureus*) and the coagulase-negative species of *Staphylococcus* may be oxacillin-resistant due to the production of a low-affinity penicillin-binding protein (PBP2a). Production of PBP2a results in broad resistance to semisynthetic penicillins, cephalosporins, and carbapenems.1 Oxacillin-resistant strains are often (but not always) multiply resistant to several other drug classes, including macrolides, clindamycin, aminoglycosides, chloramphenicol, fluoroquinolones, and trimethoprim/sulfamethoxazole.2 Kim et al,3 have calculated the attributable cost of developing an oxacillin resistant *S. aureus* (ORSA) infection in a Canadian tertiary care hospitals. They found that mean hospital stay was increased by 14 days as a result of methicillin-resistant *S. aureus* (MRSA) infection and associated with an excess cost of $CDN 14360 (2000 $US) per patient. The cost of managing colonized patients was calculated at

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$CDN 1363 per admission. Accurate methods for detection of oxacillin resistance have been a particular concern of many laboratories due to the possibility of missing some resistant strains using the standard phenotypic susceptibility testing methods due to the heterogeneous phenomena. This phenomena was recognized soon after the discovery of ORSA; that in cultures of most strains only a small proportion of cells (usually one in 103-106) were highly resistant to oxacillin while the majority expressed resistance levels at or near those of susceptible strains, such heterogeneity of resistance is a characteristic of ORSA in contrast to almost all other bacteria. Currently, the most definitive method for detection of ORSA is the detection of the gene (meCA) that encodes production of PBP2a using polymerase chain reaction (PCR) technique. However, such methods are beyond the scope of many clinical laboratories, and Food and Drug Administration-cleared commercial kits for performing them are not yet available. The most practical and reliable phenotypic test for detection of ORSA appears to be the National Committee for Clinical Laboratory Standards (NCCLS) oxacillin-salt agar procedure. The aim of this study was to determine the accuracy of the current ORSA detection test used in Makkah hospitals in comparison with the NCCLS standard method. The PCR technique was used as a gold standard method to confirm the detection of ORSA.

Methods. A total of 500 S. aureus clinical isolates and its oxacillin sensitivity patterns were obtained from the 4 main hospitals in Makkah, Kingdom of Saudi Arabia. Bacterial strains were stored at -86°C in nutrient broth (Oxoid, UK) containing 15% glycerol (Sigma Ltd., USA) and were sub-cultured onto nutrient agar plates and incubated overnight at 37°C before used. Staphylococcus aureus 25923 strain was used as a control in this work.

The identity of all S. aureus clinical isolates were confirmed following standard procedures by colonial morphology on nutrient agar plates, identification of Gram-positive cocci in clusters, catalase and coagulase positivity. Oxacillin sensitivity patterns of all S. aureus clinical isolates were confirmed by disc diffusion test (oxacillin 1µg; Oxoid, UK) using 2 methods: first, using Muller Hinton agar (Oxoid, UK) plates incubated at 37°C (routine diagnostic method used in hospitals under study), and the other method used was Muller Hinton agar plates supplied with 4% NaCl and incubated at 35°C for 24 hours as recommended by NCCLS, any zone of inhibition of 13 mm or more was considered as an oxacillin sensitive result. Chromosomal staphylococcal DNA was extracted and purified according to the method detailed in Pitcher et al. Primers were designed to amplify 533bp product within the meCA gene (Table 1). All PCRs were carried out adhering to the standard precautions to avoid contamination. A 5ml of each 2 PCR primers meCA(1) and meCA(2) (0.025 µM final concentration) (TIB Molbiol, Germany, Table 1) plus 5 ml of the extracted DNA were added to a PCR master mix (100 mM Tris-HCl, and 500 mM KCl at pH 8.3 at 20°C, 1.5 mM MgCl2, 200 µM each deoxyribonucleoside triphosphate, 0.025U Taq polymerase) (Qiagen, UK). This mixture was then heated to 94°C for 5 minutes and then subjected to 45 cycles each of 94°C for 30 seconds (denaturation step), 55°C for 30 seconds (annealing step), and 72°C for one minute (extension step) with a final extension of 72°C for 7 minutes using the Eppendorf Mastercycler (Eppendorf, Germany).

A10 µl aliquot of each PCR product was loaded onto the ethidium bromide (final concentration 0.5 µg/ml; Sigma Ltd., USA) stained with 1% agarose (Sigma) in Tris Borate EDTA (TBE), with 174 HaeIII marker (Sigma) and run at 90V for approximately one hour prior to viewing under UVP BioDoct-It digital imaging system (UVP, Inc., Cambridge, UK) to check for the presence of 533bp PCR product.

Results. Oxacillin sensitivity results showed that 103 out of 500 clinical isolates (20.6%) were resistant to oxacillin using NCCLS standard method but were sensitive according to the currently routine diagnostic method used in the hospitals under study (Figure 1). Polymerase chain reaction amplification results with meCA primers on chromosomal DNA extracted from 103 oxacillin resistant staphylococcal clinical isolates confirmed the oxacillin resistance in 88 of the tested isolates (17.6% from 500 isolates) (Figure 2).

Discussion. These findings emphasis the need to review the current laboratory methods used to detect ORSA, with follow-up audit on their implementation. Additional information may be needed in some laboratories to comply with the recommended NCCLS guidelines. A simple ORSA screening test using oxacillin agar screening plate is recommended by the NCCLS to enhance the isolation of heteroresistant ORSA and can be used by laboratories to screen large number of samples. This technique is more practical to use in diagnostic laboratories since it is easy to perform and can screen larger number of samples in the same plate. Another new approach for screening ORSA is to use cefoxitin disk instead of oxacillin, which has been reported to be more specific and sensitive screening method. Several studies have highlighted the problem of detecting heterogeneous MRSA strains using phenotype methods and the usefulness of direct detection of the meCA gene as the gold standard method for detection of oxacillin resistance, mainly due to (i) phenotypic methods
may be difficult to interpret and (ii) some isolates do not express their mecA gene unless selective pressure via antibiotic treatment is applied. 10-17 However, in our study the PCR technique confirmed the presence of mecA gene in 88/103 samples confirmed to be MRSA using NCCLS standard technique. The discrepant findings in our study cannot be attributed to technical problems related, such as colony selection, inoculum size, or incubation time, as repeat testing yielded the same results in each of the tests. Nevertheless, these findings are in agreement with the findings of other researchers who suggested that there are other minor resistance mechanisms involved in mediating oxacillin resistance in MRSA beside the expression of mecA gene. For example, oxacillin resistance in mecA-negative strains of S. aureus can arise due to hyperproduction of ß-lactamase, production of normal PBP with altered binding capacity, or other as unidentified factors. 18 Using the PCR-based amplification technique Araj et al18 detected mecA gene in 13 out of 31 (42%) isolates initially characterized by the 1 µg oxacillin disk diffusion test as oxacillin resistant. Unal et al19 using microdilution testing, reported that 186 of 1450 tested S. aureus clinical isolates were oxacillin resistant (minimal inhibitory concentration [MIC] ≥4 mg/ml). Fifteen of these isolates contribute conflicting results by alternative methods and were classified further. Only 2 of these (MIC ≥4 mg/ml) were mecA positive; 13 were inhibited by oxacillin at 4 mg/ml.

Investigators concluded that significant numbers of S. aureus strains classified as resistant with an oxacillin MIC of 4 mg/ml may prove susceptible by other methods. A similar finding obtained by Bignardi et al,20 who evaluated several phenotypic methods for determining resistance to oxacillin. They found that, out of 44 mecA negative strains 27 were oxacillin resistant according to agar dilution test. Finally, Knapp et al21 noted that MRSA lacking the mecA gene could be classified as false resistant isolates by the oxacillin disk and plate methods, and attributed this to hyper-production of ß-lactamase.

In conclusion, this work clearly demonstrates that a significant percentage of ORSA are currently missed diagnosed using the current sensitivity routine method which may lead to a wrong treatment choice. In addition, some mecA negative strains and oxacillin resistant can be missed diagnosed using PCR technique. This emphasis the urgent need to comply with the recommended NCCLS guidelines.

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