Accuracy of current oxacil
routinely used in hospita
Saudi Arabia

Aiman M. Momenah, PhD, ***Atif H. Asghar,*** PhD, ***Syed Z***
Essam I. Azhar, PhD, ***Ahmed M. Ashi,*** PhD, ***Mohammed***

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 it's 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 hospitals' routine sensitivity test method. T... confirmed the presence of mecA ge... appeared to be methicillin-resistant... using PCR technique.

Conclusion: A significant percen... currently misdiagnosed and accordan... routine sensitivity test method. In ad... negative and oxacillin-resistant stra... NCCLS standard method can be m... PCR technique. These findings emp... comply with the recommended N... detection of oxacillin resistance. ... technique can not be used as a sing... detection of MRSA.

Saudi Med J 2005; Vol. 26

Staphylococci continue to be among the most common of all community-acquired bacterial pathogens. Both *Staphylococcus aureus* and coagulase-negative species of staphylococci 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

...be classes, including macrol... aminoglycosides, chloramphenico... and trimethoprim/sulfamethoxaz... (S. aureus)... the attributable... of *Staphylococcus aureus* (... In Canadian tertiary care hospital... mean hospital stay was increase... resulting in methicillin-resistant S... infection associated with... $14360 (2000 US) per p... managing colonized patients ...

From the Department of Medical Microbiology (Momenah), Department of Medic...
Department of Environmental and Health Research (Asghar), The Custodian of th...
University, Hera General Hospital (Bukhari, Ashi), Makkah, and the Faculty of A...
Jeddah, *Kingdom of Saudi Arabia*.

Received 13th September 2004. Accepted for publication in final form 16th January

Address correspondence and reprint request to: Dr. Aiman M. Momenah, Assista...
Medicine and Medical Sciences, Umm Al-Qura University, PO Box 6630, Makk...
aiman34@hotmail.com

$CDN 1363 per admission.[3,4] Accurate methods for detection of oxacillin resistance have been of 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 ...) were highly resistant to oxacillin ... expressed resistance levels ... susceptible strains. Such heteroresistance is a characteristic of ORSA ... other bacteria. Currently, the most definitive method for detection of ORSA is the detection of the gene (mecA) that encodes production ... polymerase chain reaction (PCR). However, such methods are ... many clinical laboratories ... Administration-cleared ... performing them are not widely available ... practical and reliable phenotypic test for detection of ORSA appears to be the ... Clinical Laboratory Standards (NCCLS) oxacillin-salt agar procedure.[5]

The aim of this study was to determine the accuracy of the current ORSA detection ... Makkah hospitals in comparison with the NCCLS standard method. The PCR technique was used as a gold standard method to confirm ... ORSA.

Methods. A total of ... isolates and its oxacillin sensitivity patterns were obtained from the 4 main hospitals in Makkah, Kingdom of Saudi Arabia. ... 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. *Staphylococcus aureus* 25923 strain was used as control in this work.

The identity of all *S. aureus* ... were confirmed following standard procedures: colonial morphology on ... identification of Gram-positive cocci in clusters, catalase and coagulase positivity ... sensitivity patterns of all *S. aureus* ... were confirmed by disc diffusion test (oxacillin 1µg; Oxoid, UK) using 2 methods: ... Muller Hinton agar (Oxoid, UK) plates incubated at 37°C (routine diagnostic method used in the hospitals under study), and the other ... Hinton agar plates supplemented with 4% NaCl ... incubated at 35°C for 24 hours ... NCCLS, any zone of inhibition ... was considered as an oxacillin sensitive ...

Chromosomal staphylococcal DNA was extracted and purified according to the ... Pitcher et al.[8] Primers were designed ... amplify ...

533bp product within the mecA ... PCR ... have been carried out adhering ... precautions to avoid contamination ... primers mecA(1) and m... concentration ... TIB Molbio ... plus 5 ml of the extracted DN... Roche ... 10 mM Tris- ... KCl, 1.5 mM ... deoxynucleoside triphos... polymerase (Qiagen, UK). This ... heated to 94°C for ... minutes an... 40 cycles of 94°C for 30 sec... 55°C for 30 seconds (an... 72°C for 1 minute (extension ... extension at 72°C for ... minutes u... Mastercycler Personal, German... each PCR p... onto ... ethidium bromide (fina... Sigma and ... USA) staine... (Sigma) in Tris Borate EDTA ... DNA ladder marker (Sigma) and ... approximately one hour prior to v... Molecular imaging sy... Sambrook, UK) to check for the ... PCR product.

Results. ... oxacillin sensitivit... of 500 clinical isol... resistance was tested using NCCL... were detected according to th... diagnostic method used in the ho... (**Figure 1**). Polymerase chain rea... results with mecA primers on ... *S. aureus* clinical ... 503 ... ox... extracted from ... staphylococcal isolate... Bacterial strains were 88 of ... (17.6%) ... isolates) (**Figure**

Discussion. These findings ... to review the current laboratory ... detect ORSA with a follow-up ... implementation. Additional inf... laboratories to ... recommended NCCLS guideline... screening ... oxacillin aga... recommended by ... NCCLS ... isolation of heteroresistant ORS... laboratories ... large n... practical ... since it is easy to ... large number of samples ... Another ... hospitals for screen... Muller ... oxacilli... reported to be more specific and ... studies have ... problem of detecting heterogene... methods and ... the mecA ... detecti... to (i) pl...

Table 1 - Properties of oligonucleotides primers.

Primer designation	Sequences (5-3)	Tm	Position	Amplification size (bp)
mecA(1)	AAA ATC GAT GGT AAA GGT TGG C	64.7	1828-1303	
mecA(2)	AGT TCT GCA GTA CCG GAT TTG C	662	1814-1793	533

Tm - melting temperature, bp - base pair

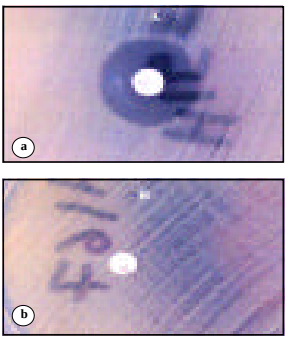

Figure 1 - Oxacillin sensitivity results for the same clinical isolates showing **a)** a sensitive result when bacteria inoculated on Muller Hinton agar plate incubated at 37ºC and **b)** a resistant result on Muller Hinton agar plate supplied with 4% NaCl and incubated at 35ºC.

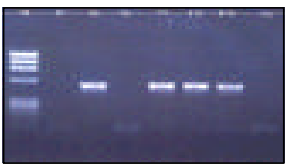

Figure 2 - Polymerase chain reaction (PCR) amplification of mecA gene demonstrating the expected 533 bp products for some of the tested samples. Lane 1 - pUC18 DNA Hae III Digest marker, Lane 2 - clinical isolate No. 51, Lane 3 - clinical isolate No. 56, Lane 4 - clinical isolate No. 76, Lane 5 - clinical isolate No. 77, Lane 6 - clinical isolate No. 471, Lane 7 - clinical isolate No. 479, Lane 8 - PCR negative (sterile water) control.

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 al[18] 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 al[19] 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 al[21] 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.

References

1. Quintiliani JR, Sahm DF, Courvalin P. Mechanisms of resistance to antimicrobial agents. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, editors. Manual of clinical microbiology. 7th ed. Washington (DC): American Society for Microbiology; 1999. p. 1505–1525.

2. Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: special needs for fastidious organisms and difficult-to-detect resistance mechanisms. ***Clin Infect Dis*** 2000; 30: 799-808.
3. Kim T, Oh PI, Simor AE. The economic impact of oxacillin-resistant Staphylococcus aureus in Canadian hospitals. ***Infect Control and Hosp Epidemiol*** 2001; 22: 99-104.
4. Tenover FC, Rasheed JK. Genetic methods for detecting antibacterial and antiviral resistance genes. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, editors. Manual of Clinical Microbiology. 7th ed. Washington (DC): American Society for Microbiology; 1999. p. 1578–1592.
5. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A5. Wayne, Pennsylvania: National Committee for Clinical Laboratory Standards; 2000.
6. Kloos WE, Bannerman TL. *Staphylococcus* and *Micrococcus*. In: Murray PR, Barons EJ, Pfaller MA, Tenover FC, Yolken RH, editors. Manual of Clinical Microbiology. 7th ed. Washington (DC): American Society for Microbiology 1999; p. 264-282.
7. Jorgensen JH, Turnidge JD, Washington JA. Antimicrobial susceptibility tests: dilution and disk diffusion methods. In: Murray PR, Barons EJ, Pfaller MA, Tenover FC, Yolken RH, editors. Manual of Clinical Microbiology. 7th ed. Washington (DC): American Society for Microbiology; 1999. p. 265-281.
8. Pitcher DJ, Saunders NA, Owen RJ. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. ***Letters in Applied Microbiology*** 1989; 8: 151-156.
9. Kwok S, Higuchi R. Avoiding false positive with PCR. ***Nature*** 1989; 339: 237-238.
10. Murakami K, Minamide W, Wada K, Nakamura E, Teraoka H, Watanabe S. Identification of oxacillin-resistant strains of staphylococci by polymerase chain reaction. ***J Clin Microbiol*** 1991; 29: 2240-2244.
11. Predari SC, Ligozzi M, Fontana R. Genotypic identification of methicillin-resistant coagulase-negative staphylococci by polymerase chain reaction. ***Antimicrob Agents Chemother*** 1991; 35: 2568-2573.
12. Ünal S, Hoskins J, Flokowitsch JE, Wu CYE, Preston DA, Skatrud PL. Detection of methicillin-resistant staphylococci by using the polymerase chain reaction. ***J Clin Microbiol*** 1992; 30:1685-1691
13. Barski P, Piechowicz L, Galinski J, Kur J. Rapid assay for detection of methicillin-resistant Staphylococcus aureus using multiplex PCR. ***Mol Cell Probes*** 1996; 10: 471-475.
14. Brakstad OG, Maeland JA, Tveten Y. Multiplex polymerase chain reaction for detection of genes for Staphylococcus aureus thermonuclears and methicillin resistance and correlation with oxacillin resistance. ***APMIS*** 1993; 101: 681-688.
15. Geha DL, Uhl JR, Gustaferro CA, Persing DH. Multiplex detection for identification of methicillin-resistant staphylococci in the clinical laboratory. ***J Clin Microbiol*** 1994; 32:1768-1772.
16. Salisbury SM, Sabatini LM, Spiegel CA. Identification of methicillin-resistant staphylococci by multiplex polymerase chain reaction. ***Am J Clin Pathol*** 1997; 107: 368-373.
17. Vannuffel P, Gigi J, Ezzedine H, Vandercam B, Delmee M, Wauters G, et al. Specific detection of methicillin-resistant Staphylococcus species by multiplex PCR. ***J J Clin Microbiol*** 1995; 33: 2864-2867.
18. Araj GF, Talhouk RS, Simaan CJ, Maasad MJ. Discrepancies between mecA PCR and conventional tests used for detection of methicillin resistant Staphylococcus aureus. ***Int J Antimicrob Agents*** 1999; 11: 47-52.
19. Ünal S, Hoskins J, Flokowitsch JE, Wu CYE, Preston DA, Skatrud PL. Detection of methicillin-resistant staphylococci by using the polymerase chain reaction. ***J Clin Microbiol*** 1992; 30: 1685-1691.
20. Bignardi GE, Woodford N, Chapman A, Johnson AP, Speller DC. Detection of the mecA gene and phenotypic detection of resistance in Staphylococcus aureus isolates with borderline or low-level methicillin resistance. ***J Antimicrob Chemother*** 1996; 37: 53-63.
21. Knapp CC, Ludwig MD, Washington JA, Chambers HF. Evaluation of Vitek GPS-SA card for testing of oxacillin against borderline-susceptible staphylococci that lack mecA. ***J Clin Microbiol*** 1996; 34: 1603-16035.