Annals of Microbiology, **57 (2)** 273-276 (2007)

Detection of methicillin-resistance (*mec-A*) gene in *Staphylococcus aureus* strains by PCR and determination of antibiotic susceptibility

Alireza ZAMANI[1]*, Siavash SADEGHIAN[2], Jalal GHADERKHANI[2], Mohammad Yousef ALIKHANI[2], Mohammad NAJAFIMOSLEH[2], Mohammad TAGHI GOODARZI[3], Hadi SHAHRABI FARAHANI[2], Rasoul YOUSEFI-MASHOUF[2]

[1]Department of Immunology, [2]Department of Medical Microbiology, [3]Department of Biochemistry and Nutrition, School of Medicine, Hamedan University of Medical Sciences, Hamedan, Iran

Received 11 December 2006 / Accepted 30 April 2007

Abstract - Methicillin-Resistant *Staphylococcus aureus* (MSRA) has become a frequent cause of serious infections. Extended hospitalization and antibiotic therapy have been identified as additional risk factors for MRSA carrier and infection. The aim of this study was to determine the incidence of MRSA infections in the hospitals affiliated to Hamedan University of Medical Sciences. Seventy *S. aureus* clinical strains were isolated from patients from June 2005 to June 2006 and examined by PCR and conventional microbiological tests. Then, the antibiotic susceptibility to methicillin/oxacillin and other antibiotics were performed by Disc Diffusion Agar (DDA). The results of this study showed that methicillin resistance gene was detected in 35 (50%) and 22 (31.4%) cases by PCR and DDA, respectively. The results of antibiotic susceptibility assays also showed there were high resistance MRSA strains to penicillin (100%), cloxacillin (91.4%), tetracycline (74.2%), cotrimoxazole (68.5%), erythromycin (68.5%) and less resistance to rifampin (11.4). Two MRSA also had decreased susceptibility to vancomycin. But the strains of Methicillin-Sensitive *S. aureus* (MSSA) showed high sensitivity to all antibiotics profiles except to penicillin (complete resistance). As a conclusion, the resistance to methicillin/oxacillin of *S. aureus* in Hamedan hospitals has reached to 50% and they show multidrug resistance.

Keywords: MRSA, *mec-A*, methicillin/oxacillin.

INTRODUCTION

Staphylococcus aureus is recognized as one of the most important bacterial pathogens seriously contributing to the problem of hospital infections all over the world (Leski *et al.*, 1998). Penicillin was produced in large quantities in the 1940s and many lives were saved. But it was found that some strains of *S. aureus* quickly developed resistance to penicillin by producing an enzyme (β-lactamase) which could break down the penicillin molecule. Therefore the methicillin which is a synthetic derivates of penicillin and resistant to the β-lactamase enzyme, was developed in 1959 to treat infections caused by penicillin-resistant *S. aureus* (Shehab El-Din *et al.,* 2003). In 1961, the first Methicillin-Resistant strains of *S. aureus* (MRSA) were isolated in Europe (Enright *et al.,* 2002). β-Lactam antibiotics like methicillin inactivate penicillin binding proteins (PBP1, 2 and 3) by the acylation of catalytic site of the PBPs. BPs normally possess a high affinity for β-lactam antibiotics; in MRSA this affinity is reduced. MRSA carry the *mec-A* gene which encodes an additional low-antibiotic affinity PBP known as PBP2a (Merlino *et al.,* 2002).

* Corresponding author. Phone: +98 811 8276295-8 ext 338;
Fax: +98 811 8276299; E-mail: zamanialireza@yahoo.com

Standardized methods of the susceptibility test have been used for the detection of MRSA strains. However, phenotypic express of methicillin-resistance is usually heterogeneous (Enright *et al.,* 2002). In addition, methicillin-resistance is influenced by culture conditions such as temperature, medium, pH and NaCl content in the medium. These factors complicate the detection of methicillin-resistance, especially for strains with low level resistance. The PCR methods have high sensitivity, specificity and are independent of the physical and chemical conditions of the culture (Japoni *et al.,* 2004).

Therefore, the aim of this study was to assess the incidence of methicillin resistance in *S. aureus* isolates in Hamedan University by PCR and conventional methods, to compare them and to detect antibiotic susceptibility.

MATERIALS AND METHODS:

Samples collection. A total of 70 *S. aureus* isolates were collected from June 2005 to June 2006 from five hospitals affiliated to Hamedan University of Medical Sciences, Hamedan, Iran. All *S. aureus* isolates were identified by routine laboratory procedures including, Gram stain, catalase test, mannitol salt fermentation, coagulase activity and DNase production (Merlino *et al.,* 2002).

DNA extraction. A single colony was taken from each blood agar plate which had been incubated overnight and emulsified into 100 µl of phosphate buffer salt. After incubation for 10 min at 95 °C, 50 µl of proteinase K (100 mg/l) and 150 µl of TE (1 mM EDTA/10 mM Tris, pH 7.5) were added to the suspension and incubated for a further 20 min at 37 °C. Then each tube was used directly for PCR reactions (Merlino *et al.,* 2002).

PCR for *mec-A* gene detection. The *mec-A* gene was amplified with the following two oligonucleotides: forward primer *1276* (*5´-AAA ATC GAT GGT AAA GGT TGG C-3'*) and backward primer *1787* (*5'-AGT TCT GGA GTA CCG GAT TTG C -3'*) which gave a PCR product of 533 bp (Merlino *et al.,* 2002). The PCR was performed with an initial denaturation step of 5 min 94 ºC, followed by 35 cycles of 45 s 94 ºC, 45 s 50 ºC, 60 s 72 ºC, and the extension step of 5 min 72 ºC. Agarose gels were prepared with TAE buffer (Tris, glacial acetic acid, EDTA, pH 8) and added ethidium bromide (1 µg/15 ml gel). PCR product (5 µl) of each sample was mixed with 1 µl of sample buffer (6X: 0.25% bromophenol, 0.25% xylen cyanol, 15% ficol 400) and loaded on 1% agarose and electrophoresed in 80 volt for 25-30 min. The band of fragment was observed by UV transilluminator and documented by gel analyser machine (Zamani *et al.,* 2006).

Antibiotic-resistance tests. The isolated *S. aureus* strains were tested for resistance to antimicrobial agents including methicillin/oxacillin (1 µg), ciprofloxacin (5 µg), erythromycin (15 µg), tetracycline (30 µg), gentamicin (10 µg), cotrimoxazole (12.5 µg), vancomycin (30 µg), cephalothin (30 µg), ceftazidime (30 µg), cloxacillin (5 µg), rifampin (30 µg) and penicillin (10 U) by Disc Diffusion Agar (DDA) method using commercial discs (Patanteb Company, Iran) and according to the Clinical Laboratory Standards Institute (CLSI) guidelines on Muller Hinton Agar. The plates were used duplicated and incubated in ambient air at 35 °C for 24 hours (Merlino *et al.,* 2002).

RESULTS

The results of our study revealed that the *S. aureus* infections were seen frequently in urine culture (62.9%) and then in wounds (21.5%), blood culture (5.7%), pleural fluid (5.7%), cerebrospinal fluid (1.4%), sputum (1.4%) and urethra (1.4%) of the patients.

The PCR analysis of 70 clinical *S. aureus* isolates for *mec-A* showed 35 (50%) cases were positive and 35 (50%) negative and known as MRSA and MSSA strains, respectively (Fig. 1). But only 22 (31.4%) isolates were methicillin resistant when we used the DDA method (Table 1). These isolates were all resistant to penicillin and more susceptible to vancomycin, rifampin and ciprofloxacin. Also MRSA and MSSA had variable antibiotic resistance profiles. The MRSA isolates were resistant to cloxacillin (91.4%), tetracycline (74.2%) and less resistance to rifampin (11.4%). But about all MSSA isolates were more susceptible to antibiotics (Table 1).

DISCUSSION

Rapid and accurate detection of methicillin resistance *S. aureus* strains is imperative for appropriate patients treatment and implementation of institutional programs for recognition and management of MRSA outbreaks and cross infections (Boyce, 1998; Sakoulas *et al.,* 2001). Consequently, many methods were developed over recent years to provide more rapid identification and susceptibility results. Only a few studies have determined whether those rapid but expensive could improve patient care (Merlino *et al.,* 2002). We examined how a PCR assay that discriminates MRSA from MSSA can improve patients care by tracking the following two parameters of turn around time to results by PCR versus conventional tests and accuracy of diagnosis. In addition, routine culture method required two sequential steps, one to isolate *S. aureus* and the second to determine antibiotic susceptibility (Hallin *et al.,* 2003).

According to literatures, the first methicillin-resistant strain of *S. aureus* (MRSA) was isolated in Europe in 1961 (Enright *et al.,* 2002). Then, there has been a steady increase in the prevalence of MRSA isolated from hospital in the world. For example in the United States approximately 50% of nosocomial isolates of *S. aureus* were methicillin-resistant in 1997 (Jenison *et al.,* 2000). Two studies in Shiraz, Iran, showed MRSA had risen up from 33% (Alborzi *et al.,* 2000) to 43% (Japoni *et al.,* 2004).

As shown in the Table 1, vancomycin has remained the mainstay of treatment for serious MRSA infections. But with more extensive use of this antibiotic the likelihood of resistance emerging increases. All of the isolates were resistance to the penicillin thus all of the *S. aureus* isolates must have got the penicillin resistant gene by plasmid transformation (Phillips *et al.,* 1992). In addition, the MRSA were more resistance to cloxacillin, tetracycline and more sensitive to the rifampin.

Two MRSA isolates showed a decreased susceptibility to vancomycin which is called vancomycin-intermediate *S. aureus* (VISA) strain. For the first time VISA strain has been described for clinical isolates of MRSA in Japan in 1996 (Hiramatsu *et al.,* 1997) then in United States (CDC, 1997) and Europe (Ploy *et al.,* 1998) and mainly from patients on vancomycin therapy. It is recommended, alternative antibiotic regimes can be advocated to treat MRSA infections, in particular, rifampin which is highly bactericidal for MRSA but up to 11.4% of MRSA isolates have been resistant to this antibiotic in our finding. According to our study, no MSSA from clinical samples with reduced susceptibility vancomycin and many other antibiotics was detected (Table 1).

In conclusion, MRSA is increasing and reaching to the critical situation and continues to be a major cause of serious infection, both in hospital and in the community in Iran and maybe in the rest of the world. Therefore, it would be wise to change the patterns of antibiotic usage to reduce selective pressure upon sensitive strains. However, we need reliable methods such as PCR for differentiation of sensitive and resistant strains that are independent of physical and chemical conditions of bacterial culture. This would be a guide for the clinician to use appropriate antibiotics.

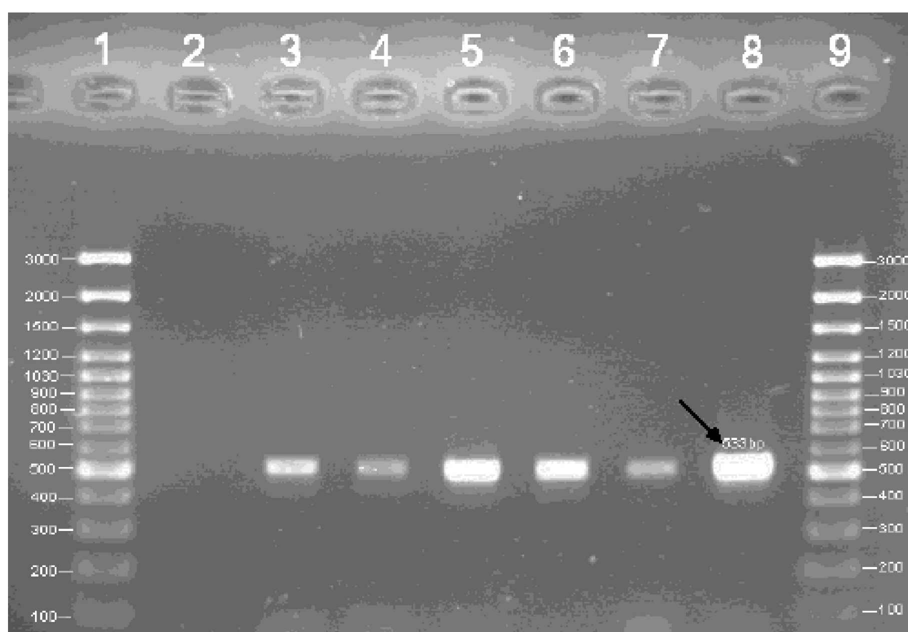

FIG. 1 - Agarose gel electrophoresis analysis for the *mec-A* gene in *Staphylococcus aureus* isolates. Lane 1 and 9, molecular size markers, expressed in base pairs; lane 2, Methicillin-Sensitive *S. aureus* (ATCC 25923*,* negative control); lane 3, Methicillin-Resistant *S. aureus* (ATCC 43300, positive control); lanes 4, 5, 6, 7 and 8 *S. aureus* isolates. Arrow indicates the 533 bp amplicon. Presence of the 533 bp shows the *mec-A* gene existence in the isolates.

TABLE 1 - Antibiotic resistance profiles of MRSA and MSSA by DDA

Isolates	Ox/Met	Cip	Eryt	Tet	Gent	STX	Van	Cf	Cz	Cx	Ra	P
MRSA (n=35)	22 (62.8%)	14 (40%)	24 (68.5%)	26 (74.2%)	15 (42.8%)	24 (68.5%)	2 (5.7%)	16 (45.7%)	18 (51.4%)	32 (91.4%)	4 (11.4%)	35 (100%)
MSSA (n=35)	0 (0%)	0 (0%)	6 (17.1%)	6 (17.1%)	1 (2.9%)	4 (11.4%)	0 (0%)	2 (5.7%)	1 (2.9%)	3 (8.9%)	1 (2.9%)	35 (100%)
Total (n=70)	22	14	30	32	16	28	2	18	19	35	5	70

Ox/Met, oxacillin/methicillin; Cip, ciprofloxacin; Eryt, erythromycin; Tet, tetracycline; Gent, gentamicin; STX, cotrimoxazole; Van, vancomycin; Cf, cephalothin; Cz, ceftazidime; Cx, cloxacillin; Ra, Rifampin; P, Penicillin.

REFERENCES

Alborzi A., Pourabbas B.A., Salehi H., Pourabbas B.H., Obiidi B., Panjehshahin M.R. (2000). Prevalence and pattern of antibiotic sensitivity of methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* in Shiraz, Iranian. J. Med. Sci., 25 (1, 2): 1-8.

Boyce J.M. (1998). Are the epidemiology and microbiology of methicillin-resistance *Staphylococcus aureus* changing? J. Am. Med. Associat., 279 (8): 623-624.

CDC - Centers for Disease Control and Prevention (1997). *Staphylococcus aureus* with reduced susceptibility to vancomycin - United States. JAMA, 278 (11): 891-892.

Enright M.C., Robinson D.A., Randle G., Feil E.J., Grundmann H., Spratt B.G. (2002). The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). PNAS, 99 (11): 7687-7692.

Hallin M., Maes N., Byl B., Jacobs F., De Gheldre Y., Struelens M.J. (2003). Clinical impact of a PCR assay for identification of *Staphylococcus aureus* and determination of methicillin resistance directly from blood cultures. J. Clin. Microbiol., 41 (8): 3942-3944.

Hiramatsu K., Hanaki H., Ino T., Yabuta k., Oguri T., Tenover F.C. (1997). Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. J. Antimicrob. Chemother., 40 (1): 135-136.

Japoni A., Alborzi A., Rasouli M., Pourabbas B. (2004). Modification DNA extraction for rapid PCR detection of methicillin-resistant Staphylococci. Iranian Biomed. J., 8 (3): 161-165.

Jenison R., Heaberil A., Yang S., Polisky B., Ostrosf R. (2000). Thin film bio senser for rapid detection of mec-A from methicillin-resistant *Staphylococcus aureus*. Clin Chem., 46 (9): 1501-1504.

Leski T., Oliveira D., Trzcinski K., Sanches I.S., Aires de Sousa M., Hryniewicz W., De Lencastre H. (1998). Clonal distribution of methicillin-resistant *Staphylococcus aureus* in Poland. J. Clin. Microbiol., 36 (12): 3532-3539.

Merlino J., Watson J., Rose B., Beard-Pegler M., Gottlieb T., Bradbury R., Harbour C. (2002). Detection and expression of methicillin/oxacillin resistance in multidrug-resistant and non-multidrug-resistant *Staphylococcus aureus* in Central Sydney, Australia. J. Antimicrob. Chemother., 49 (5): 793-801.

Phillips L.G., Heggers J.P., Robson M.C. (1992). Burn and trauma units as sources of methicillin-resistant *Staphylococcus aureus*. J. Burn Care Rehabil., 13 (2): 293-297.

Ploy C.M., Grelaud C., Martin C., De Lumley L., Denis F. (1998). First clinical isolate of vancomycin-intermediate *Staphylococcus aureus* in a French hospital. Lancet 351 (9110): 1212.

Sakoulas G., Cold H.S., Vencataraman L., Degirolami P.C., Eliopouls G.M., Qian Q. (2001). Methicillin- resistant *Staphylococcus aureus* comparison of susceptibility testing methods and analysis of mec-A positive susceptible strains: J. Clin. Microbiol., 39 (11): 3946-3951.

Shehab El-Din S.A., Shafey S.I., Hadidy M.R., Bahaa El-Din A., Hadidy M.M., Zaghloul H.A. (2003). Methicillin-resistant *Staphylococcus aureus*: A problem in the burns unit. Egypt. J. Plast. Reconstr. Surg., 27 (1): 1-10.

Zamani A.R., Tavakkol Afshari J., Alikhani M.Y. (2006). Molecular cloning and expression of human gamma interferon full cDNA in Chinese Hamster Ovary (CHO) cells. Iranian J. Immunol., 3 (1): 1-7.