Advanced Journal of Microbiology Research Volume 2013
Available online at http://advancedresearchjournals.org/AJMR

Full Length Research Paper

Detection of methicillin- resistant *Staphylococcus aureus* in nosocomial infections in Gaza Strip

N.S. Abu Hujier[1*] and F.A. Sharif[2]

[1]Medical Department, College of Science and Technology, Khan Younis, Palestine.
[2]Medical Technology Department, Islamic University of Gaza, Gaza, Palestine.

Accepted August 13, 2011

Methicillin-resistant *Staphylococcus aureus* (MRSA) is considered to be one of the most important causative agents of nosocomial infections. The present study therefore aimed broadly at obtaining a snapshot of MRSA prevalence in Gaza Strip, as well as to examine the antibiotic resistance profile of such isolates. A total of 150 clinical isolates of *S. aureus* were identified from patients. Disk diffusion tests and a polymerase chain reaction (PCR) assay, based on detection of the *mecA* gene, were performed on each the isolates in order to identify MRSA strains. The PCR assay was considered as the "gold standard" and the disk diffusion tests were interpreted by comparison to this standard. The prevalence of methicillin resistance among *S. aureus* isolates was 22% (33 isolates). There were great variations between the antibiograms of methicillin-resistant and -sensitive *S. aureus* isolates. These variations were most evident for the β-lactam antibiotics, although antibiotics other than β-lactams also showed variations among the two types of *S. aureus.* The results showed that the conventional disk diffusion test using methicillin disks is highly reliable for detection of MRSA in hospital laboratories and that it can reach the specificity of the PCR assay.

Key words: Polymerase chain reaction, antibiotic resistance, nosocomial infections, methicillin-resistant *Staphylococcus aureus*.

INTRODUCTION

Methicillin- resistant *Staphylococcus aureus* (MRSA) are isolates of the bacterium *S. aureus* that have acquired genes encoding antibiotic resistance to all -lactam antibiotics, including methicillin (Foster, 1996). However, the term has increasingly been used to refer to multi-drug resistant *S. aureus*, as MRSA isolates also frequently carry resistance genes to other antibiotics that have traditionally been used against *S. aureus* (Franklin, 2003).

MRSA is found worldwide, predominantly in hospitals and institutions such as nursing homes (Goettsch et al., 2000), while, less commonly, in the general community. There are three main reservoirs (and thus sources of spread and infection) for MRSA in hospitals and institutions, i.e. staff, patients and inanimate objects such as beds and linens. By far the most important reservoir is patients who may be colonized with MRSA without evidence of infection, especially since MRSA may be carried for an extremely long period of time (Helisangela et al., 2003; Sachdev et al., 2003). Hospital-acquired infection is often caused by antibiotic-resistant strains (MRSA) and is treated with vancomycin. Until recently, infections acquired outside hospitals have been treated with penicillinase-resistant ß-lactams. However, CA-MRSA isolates have become globally pervasive and reports of serious and rapidly progressive fatal disease due to virulent CA- MRSA have alarmed healthcare professionals and the lay media alike (Boyle-Vavra et al., 2007).

Methicillin resistance is caused by production of an additional penicillin- binding protein (PBP), termed PBP2' or PBP2a, which is encoded by the *mecA* gene (Skov et al., 1999; Wu et al., 2001) . PBPs are membrane-bound enzymes that catalyze the transpeptidation reaction that is necessary for cross-linkage of peptidoglycan chains (Franklin, 2003) . Methicillin-susceptible *S. aureus* (MSSA) isolates produce five PBPs, namely PBP1, PBP2, PBP2B, PBP3 and PBP4, for which the encoding genes have been cloned and sequenced (Boyle-Vavra et al., 2003).

*Corresponding author. E-mail: niddalh@yahoo.com

MRSA isolates have acquired an additional PBP that has a low affinity for ß-lactam antibiotics and substitutes for the other PBPs in cell wall synthesis when they are inhibited by ß-lactams. As a consequence of their ability to express PBP2a, MRSA becomes resistant to β-lactams, including methicillin. It has recently been revealed that the ability of PBP2a to affect cell wall synthesis in the presence of methicillin requires cooperation from the transglycosylase domain of the native PBP2 (Boyle-Vavra et al., 2003).

In the United States, a retrospective analysis showed that the prevalence of MRSA in 33 hospitals participating from 1996 to 2000 increased from 30.1% in 1996 to 45.7% in 2000 in inpatient isolates; among outpatient isolates, the prevalence of MRSA increased from 17.3% to 28.6% (Mark, 2002).

The prevalence of MRSA has also been determined in different European countries. The highest prevalence of MRSA isolates was noted in hospitals in Portugal (54%) and Italy (43 - 58%), while the prevalence of MRSA was only 2% in participating hospitals from Switzerland and The Netherlands (Fluit et al., 2001_a). The major factors associated with MRSA colonization are prolonged hospitalization, burns, surgery that necessitates intensive care and the use of multiple antibiotics, especially during a prolonged course of treatment (Franklin, 2003; Hidron et al., 2005).

Detection of MRSA can be achieved by several methods such as agar and/or broth phenotypic methods and genotypic methods, e.g. PCR assays. PCR assays became broadly used after the introduction of a thermostable DNA polymerase (*Taq* DNA polymerase) and the development of automated oligonucleotide synthesis and thermocyclers. PCR involves cycles of heating the sample for denaturing, annealing of the primers, and elongation of the primers by *Taq* DNA polymerase. In theory, each round of amplification gives a doubling of the number of DNA target molecules, but the process is seldom 100% efficient because of the presence of inhibitors, and in later rounds of amplification *Taq* DNA polymerase may become limited (Fluit et al., 2001_b). Cefoxitin has recently been investigated as an alternative agent for detection of resistance by disc diffusion and all studies indicate that tests are more reliable than those with oxacillin (Brown et al., 2005).

This study was conducted to determine (i) the percentage of MRSA in nosocomial infections among *S. aureus* isolates in Gaza Strip; (ii) the percentage of MRSA isolates for each of the three main hospitals and hospital wards in Gaza Strip, namely Al-Shifa Hospital, Naser Hospital and the Gaza European Hospital; (iii) the susceptibility of the collected isolates to the commonly used antibiotics against *S. aureus* infections; (iv) the correlation between methicillin susceptibility and the presence of *mecA* gene; and (v) the relationship between resistance of the isolates to both methicillin and cefoxitin.

MATERIALS AND METHODS

Study design

One hundred and fifty *S. aureus* isolates were identified from a total of 283 nosocomial infection isolates collected from three hospitals, that is, Al-Shifa, Nasir and Gaza European, during the period of February to September 2006. One isolate per patient was collected in this study. Isolates were selected randomly from routine clinical specimens from different infected sites (deep and superficial wounds, blood, urine and sputum). Statistical analysis was performed by the Chi -square test and P values of 0.05 were considered significant. Nosocomial infection is defined in this study as an infection acquired in hospital by a patient who was admitted for a reason other than that infection (CDC, 1998).

Ethical considerations

An authorization to carry out the study was obtained from the Helsinki Committee, Ministry of Health, Palestinian National Authority. The nature of the work was explained to all participants, and the study was conducted with their informed consent.

Bacterial identification

S. aureus isolates were identified based on colonial morphology on blood agar plates supplemented with 5% sheep blood, CHROMagar *S. aureus* medium (CHROMagar, France), Gram stain characteristics, mannitol fermentation, and tests for catalase, coagulase and DNAse (Olivier et all., 2000; Merlino, 2000).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done on Mueller-Hinton agar (Biomark, India) using the disc diffusion technique, as recommended by the Clinical and Laboratory Standards Institute (CLSI). The following drugs and concentrations were used to determine the antibiogram of the *S. aureus* isolates: methicillin (5 g), cefuroxime (30 g), oxacillin (1 g), ciprofloxacin (30 g), trimethoprim-sulfamethoxazole "SXT" (5 g), vancomycin (10 g), clindamycin (2 g), gentamicin (10 g), tobramycin (10 g), penicillin (10 units), rifampicin (5 g), erythromycin (15 g), and cephalexin (30 g). The plates were incubated in an inverted position overnight at 35°C. The diameter, to the nearest millimeter, was measured for each zone of inhibition that appeared around the disk.

PCR assay

A PCR assay to detect the *mecA* gene was used as the reference method, and PCR-positive isolates were considered as true positives.

Preparation of bacterial DNA

S. aureus isolates were inoculated onto selective and differential media, and following incubation the colonies were lysed as described below. Bacteria were swabbed onto Mueller-Hinton agar and one disk of vancomycin was added. Following incubation overnight, bacteria from the edge of the inhibition zone was taken and suspended in sterile distilled water to a quantity that matches to McFarland standard (10^8 bacteria/ml). The bacterial suspensions were lysed by heating at 95°C for 15 min and then at room temperature. The crude lysate mixture (3.0 μl) was used as DNA

Table 1. Antibiotic sensitivity pattern of the 150 *S. aureus* isolates to the commonly used antibiotics in each hospital.

Antibiotic	Resistant		Susceptible		Intermediate		Al-Shifa		Nasir		European	
	n	%	n	%	n	%	n	%	n	%	n	%
Methicillin	33	22.0	117	78.0	0	0.0	11	22	6	12	16	32
Cefoxitin	35	23.3	115	76.7	0	0.0	11	22	6	12	18	36
Oxacillin	35	23.3	112	74.7	3	2.0	10	20	9	18	16	32
Penicillin	141	94.0	9	6.0	0	0.0	48	96	46	92	47	94
Trimethoprim-	32	21.3	118	78.7	0	0.0	6	12	6	12	20	40
Cephalexin	24	16.0	126	84.0	0	0.0	10	20	5	10	9	18
Ciprofloxacin	28	18.7	122	81.3	0	0.0	14	28	2	4	12	24
Erythromycin	71	47.3	75	50.0	4	2.7	19	38	22	44	30	60
Cefuroxime	24	16.0	125	83.3	1	0.7	10	20	2	4	12	24
Clindamycin	33	22.0	117	78.0	0	0.0	12	24	6	12	15	30
Gentamicin	58	38.7	91	60.7	1	0.7	17	34	13	26	28	56
Rifampicin	14	9.3	135	90.0	1	0.7	8	16	2	4	4	8
Tobramycin	61	40.6	88	58.7	1	0.7	26	52	13	26	22	44
Vancomycin	1	0.66	149	99.33	0	0.0	0	0	0	0	1	2

2- Stanway A (2004). Methicillin resistant Staphylococcus aureus. New Zealand Dermatological Society Incorporated.1-3.

template for subsequent PCR assays (Japoni et al., 2004).

Amplification reactions

The primers used in the PCR comprised Primer 1 (5'-AAAATCGATGGTAAAGGTTGGC-3') and Primer 2 (5'-AGTTCTGCAGTACCGGATTTGC-3'), which correspond to nucleotides 1282 to 1303 and nucleotides1793 to 1814 of the mecA gene, respectively (Murakami et all., 1991), thus yielding an amplicon 533 bp. Each PCR reaction mixture (25 µl) contained 3.0 µl (ca. 200 ng) of crude lysate, 1 × PCR buffer, 1.5 mM MgCl2, 2.0 µM of each of the primers, 200 µM of each deoxynucleotide triphosphate (dNTP) and 1.25 U is the final concentration of Taq DNA polymerase (Promega, USA). After initial denaturation at 95°C for 1 min, the samples were subjected to 35 cycles of denaturation at 95°C for 1 min, annealing at 54°C for 1 min and extension at 72°C for 1 min. A final extension was performed at 72°C for 5 min. In all PCR assays, negative control (reaction mixtures without DNA template) and positive controls. Control organisms included S. aureus ATCC 43300 (mecA-positive control) and S. aureus ATCC 25923 (mecA-negative control). were included. Following PCR, aliquots (2.0 µ l) of the reaction mixtures were analyzed by electrophoresis on a 2% agarose gel, containing ethidium bromide (0.2 mg/ml), in the presence of an appropriate DNA molecular weight marker (Murakami et al., 1991).

RESULTS

Antibiotic sensitivity profile of the *S. aureus* isolates and distribution of resistant *S. aureus* isolates according to hospital

S. aureus isolates that were resistant, susceptible or displayed intermediate resistance to commonly used antibiotics are shown in Table 1. The results indicated that 33 (22%) of the *S. aureus* isolates were resistant to methicillin and the remainder were methicillin-sensitive (78%). As also shown in Table 1, the frequency of resistant *S. aureus* isolates varied among the hospitals included in this study**.** The results revealed that there were statistically significant differences in MRSA prevalence among the three hospitals in Gaza Strip (P 0.05), where the percentage of methicillin-resistant *S. aureus* isolates in the Gaza European hospital was 32% as compared to 22 and 12% in the Al-Shifa and Nasir hospitals, respecttively. In contrast, there was no statistically significant difference in oxacillin-resistance *S. aureus* isolates among the three hospitals (P ≥ 0.52). Moreover, the results also indicated that there is a significant difference in cefoxitin-resistant *S. aureus* isolates among the three hospitals (P 0.017) (Table 1).

Distribution of multidrug resistance (MDR) among *S. aureus* in Gaza Strip hospitals

S. aureus is considered MDR when they display resistance to at least five of the following antibiotics, that is, oxacillin, penicillin, erythromycin, clindamycin, gentamicin, ciprofloxacin, tetracycline and chloramphenicol (Juuti, 2004). In this study, however, MDR was defined as resistance to at least three of the non-ß-lactam antibiotics; otherwise, the isolates were considered non-multidrug resistant (NMDR).

Although the prevalence of antibiotic resistance among MRSA (*n* = 33) was much higher than that among MSSA (*n* = 117), as indicated in Table 2, the results presented in Table 3 indicated that there was no significant difference in multidrug-resistant *S. aureus* isolates among the three hospitals included in the study (P ≥ 0.05). However, the distribution of MRSA isolates were found to vary widely between hospitals wards. Table 4 indicates that the surgery units of all three hospitals were found to have the greatest burden of MRSA.

Table 2. Distribution of antibiotic resistance among MRSA and MSSA isolates in each hospital

Antibiotic		Al-Shifa		Nasir		European		Total (*n*)	Total % = Total *n*/33
		n	%	n	%	n	%		
cefoxitin	MRSA	11	100	5	83.3	16	100	32	97.0
	MSSA	0	0.0	1	2.3	2	5.9	3	2.6
Oxacillin	MRSA	9	81.8	6	100	15	93.8	30	90.9
	MSSA	1	2.6	3	6.8	1	2.9	5	4.3
Penicillin	MRSA	11	100	6	100	16	100	33	100
	MSSA	37	94.9	40	90.9	31	91.2	108	92.3
SXT	MRSA	3	27.3	3	50.0	8	50.0	14	42.4
	MSSA	3	7.7	3	6.8	12	35.3	18	15.4
Cephalexin	MRSA	9	81.8	2	33.3	9	56.3	20	60.6
	MSSA	1	2.6	3	6.8	0	0.0	4	3.4
Ciprofloxacin	MRSA	9	81.8	1	16.7	7	43.8	17	51.5
	MSSA	5	12.8	1	2.3	5	14.7	11	9.4
Erythromycin	MRSA	10	90.9	4	66.7	14	87.5	28	84.8
	MSSA	9	23.1	18	40.9	16	47.1	43	36.8
Cefuroxime	MRSA	10	90.9	1	16.7	12	75.0	23	69.7
	MSSA	0	0.0	1	2.3	0	0.0	1	0.9
Clindamycin	MRSA	10	90.9	2	33.3	12	75.0	24	72.7
	MSSA	2	5.1	4	9.1	3	8.8	9	7.7
Gentamicin	MRSA	9	81.8	3	50.0	15	93.8	27	81.8
	MSSA	8	20.5	10	22.7	13	38.2	31	26.5
Rifampicin	MRSA	7	63.6	2	33.3	2	12.5	11	33.3
	MSSA	1	2.6	0	0.0	2	5.9	3	2.6
Tobramycin	MRSA	10	90.9	4	66.7	15	93.8	29	87.9
	MSSA	16	41.0	9	20.5	7	20.6	32	27.4
Vancomycin	MRSA	0	0.0	0	0.0	1	6.25	1	3.0
	MSSA	0	0.0	0	0.0	0	0.0	0	0.0

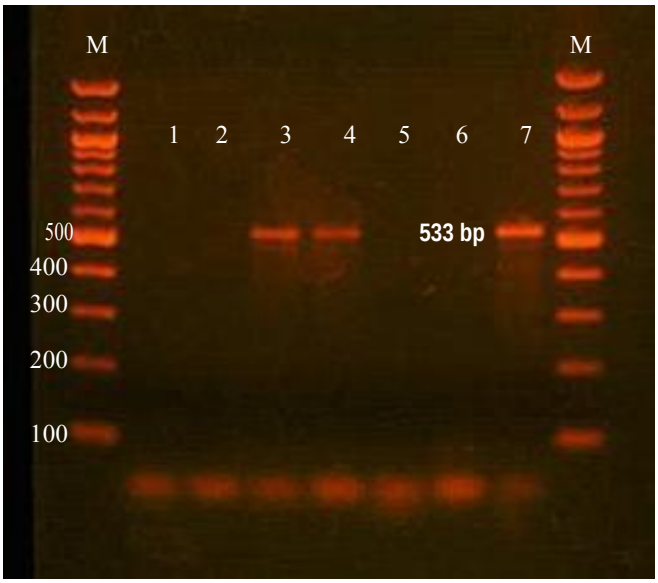

Figure 1. Amplification products of *S. aureus mecA* gene by PCR. M: 100- bp ladder; lanes 1, 2 and 5: negative samples; lanes 3 and 4: *mecA*-positive samples; lane 6: negative control; lane 7: positive control

Comparison between PCR assay results, and disk diffusion tests of methicillin, cefoxitin and oxacillin

The *mecA* PCR amplification results of five of the *S. aureus* isolates are shown in Figure 1. An amplicon of 533 bp was considered indicative for the presence of *mecA* gene (Murakami et al., 1991).

The relationships between the results of the PCR assay and the disk diffusion test results of methicillin, cefoxitin and oxacillin are summarized in Table 5.

As can be deduced from Table 5, the number (n = 33) of MRSA detected by the disk diffusion test and PCR assay were identical. Consequently, the sensitivity and specificity of methicillin disk diffusion test as compared to *mecA* gene PCR are therefore 100%. Similarly, the sensitivity and specificity of cefoxitin disk diffusion method in detecting MRSA as compared to *mecA* gene PCR were 97.0 and 97.4%. Additionally, those of oxacillin disk diffusion method were 90.0 and 95.8%, respectively.

DISCUSSION

According to the results obtained from disk diffusion tests

Table 3. Distribution of MDR/NMDR *S. aureus* in Gaza Strip hospitals.

Hospital	MDR		NMDR		P-value
	n	%	n	%	
Al-Shifa hospital	12	24	38	76	
Nasir hospital	7	14	43	86	0.155
European hospital	15	30	35	70	
Total	34	22.7	116	77.3	

Table 4. MRSA isolates among hospital wards.

Hospital ward	Al-Shifa		Nasir		European	
	n	%	n	%	n	%
Female general surgery	3	27.3	2	33.3	3	18.8
Male general surgery	2	18.2	2	33.3	9	56.3
Burns	1	9.1	2	33.3	3	18.8
ICU	5	45.5	0	0.0	1	6.3
Tumors	0	0.0	0	0.0	0	0.0
Internal medicine	0	0.0	0	0.0	0	0.0
Total	11		6		16	

and PCR assays, MRSA prevalence in Gaza Strip was 22.0%. This finding is close to that obtained by Essawi et al. (1998), who reported 19.1% prevalence of MRSA in East Jerusalem. The occurrence of MRSA in Gaza Strip could be attributed to many factors, despite methicillin not being routinely used against *S. aureus* infections. In addition to antibiotic stress, horizontal gene transfer is considered a contributing factor in the occurrence of antibiotic resistance in clinical isolates. Consequently, it has been suggested that the high prevalence of resistance to a particular antibiotic does not always reflect antibiotic consumption (Ako-Nai et al., 2005; Brown et al., 2005). The use of antimicrobials in animal food is another contributing factor. Antibiotics are commonly added to feed to promote growth in animals, particularly dairy cattle, sheep and poultry (Gin et al., 2001). Frequent traveling is an additional factor for transmitting resistant strains between countries. Misuse of antimicrobials is another contributing factor (Chambers, 1997).

Asensio et al. (1996) identified six factors that were independently associated with MRSA infection and colonization, namely increasing age, ward type (particularly intensive care units), coma, previous hospitalization, invasive procedures and length of hospitalization. The prevalence of MRSA varied among the three hospitals included in the study and the differences were statistically significant. This could be attributed to variations related to the rapid identification and strict policies of isolation of patients with MRSA colonization or infection, combined with the restricted use of antibiotics and the hygiene practices employed in each hospital (Fluit et al., 2001a). In Shifa Hospital, especially in the intensive care unit where the rate of MRSA was 45%, the situation suggests that some patients may have a greater chance of becoming colonized or infected (Fluit et al., 2001a) . The situation in both the Nasir and Gaza European Hospitals was different. The highest level of MRSA was found in the surgical units (both male and female surgery unit) of these hospitals. In the Nasir and Gaza European Hospitals, the rate of MRSA in the surgical unit was 66.6 and 75.0%, respectively. The percentage of MRSA in the surgical unit of the Shifa Hospital was also high (45.4%). The explanation for the high occurrence of MRSA in surgical units could be attributed to the long hospitallization of the patients in these wards. Moreover, most of the isolates that were collected from surgical units in Gaza European and Nasir Hospitals were from aged and diabetic patients, which reflect the lower efficiency of their immune system. This is considered a contributing factor for MRSA colonization, in addition to the long hospital stay and prolonged antibiotic treatment that results in enhanced antibiotic pressure.

Regarding the differences in MRSA prevalence between the three hospitals, the lower prevalence was in Nasir hospital. The surgical unit in Nasir hospital was the less crowded unit, which may effectively reduce the transmission of MRSA among patients. It is also important to note that this hospital recently underwent complete renovation of all the buildings, which may have decreased the chance of MRSA to colonize places such as sinks and bathrooms. In contrast, in the Gaza European hospital, which has the highest patient population, MRSA occurred at the highest rate. Regional hospital variation may also be explained by differences in diagnostic practice, culturing activity and random errors, which may artificially increase variation. Additionally, the high MRSA prevalence in the Gaza European hospital, especially in the surgical units, may be attributed to the low level of cooperation between the hospital wards and the microbiology laboratory. As a result, there was a complete dependence on the use of wide-spectrum antibiotics, which could lead to an increase in the antibiotic pressure on the bacteria.

The results of the present study suggested that some strains were resistant to cefoxitin or oxacillin, but that they were mecA-negative by PCR analysis. Most of these isolates expressed resistance at the borderline of the inhibition zone and were thus termed ‘moderately resistant *S. aureus*‘ (MODSA). Under some test conditions, low-level resistance may also be seen in isolates, which produce large amounts of penicillinase (penicillinase hyperproducers), and these isolates have been referred to as ‘borderline oxacillin-resistant *S. aureus*‘ (Fluit et al., 2001b) . They can be difficult to distinguish from resistant strains that carry the mecA gene by routine tests. Many laboratories still prefer using oxacillin for detection of MRSA, because oxacillin maintains its activity during storage better than methicillin, and is more likely to detect heteroresistant strains (CDC, 2005). Oxacillin is less resis-tant to hydrolysis by staphylococcal -lactamases, so

Table 5. Comparison between PCR results and methicillin, cefoxitin and oxacillin disk diffusion tests

PCR result		Methicillin		Cefoxitin		Oxacillin		
		R	S	R	S	R	S	I
mecA gene-positive	No.	33	0	32	1	30	3	0
	%	100	0.0	97.0	3.0	90.9	9.1	0.0
mecA gene-negative	No.	0.0	117	3	114	5	109	3
	%	0.0	100	2.6	97.4	4.3	93.2	2.6
Total	No.	33	117	35	115	35	112	3
	%	22.0	78.0	23.3	76.7	23.3	74.7	2.0

Footnote: R: resist, S: susceptible, I: intermediate

so problems with penicillinase hyperproducers are reduced with methicillin (Brown, 2001). This may explain why the largest false-positive results were obtained with oxacillin. The oxacillin disc diffusion test has previously been found to be less reliable, with high numbers of both false-susceptible and false-resistant results (Skov et all., 1999).

Cefoxitin, however, is an even better inducer of the *mecA* gene, and disk diffusion tests using cefoxitin give clearer endpoints and are easier to read than tests with oxacillin. This explains the higher level of sensitivity and specificity to cefoxitin than oxacillin. The gold standard method for the detection of resistance mediated by *mecA* is the PCR, which is commonly used as a reference method (Fluit et al., 2001b). The results of this study showed that only 3.4% of MSSA isolates were MDR; however, 90.9% of the MRSA isolates were MDR. The glycopeptide agent vancomycin is still the drug of choice for treatment of life threatening infections caused by MDR strains.

The results of the present study indicated that the accuracy of methicillin disk diffusion test for detection of MRSA approaches the accuracy of PCR assay and is more accurate than any susceptibility testing method used alone for the detection of MRSA. The presence of the *mecA* gene correlated 100% with the methicillin phenotypic resistance in all MRSA isolates. This was not the situation, however, concerning results of the PCR assay and cefoxitin disk diffusion test. The sensitivity and specificity of the cefoxitin disk diffusion test were 97.0% and 97.4%, respectively. One disadvantage of the cefoxitin disk diffusion test is that the gap between the inhibition zones of isolates with and without the *mecA* gene was very narrow (sensitive $\geq$22, resistant 21), and this might affect the results of the cefoxitin disk diffusion test. The narrow gap was due to the high concentration of cefoxitin in the disk (30 μg). The oxacillin disk diffusion test had slightly lower values of sensitivity and specificity, that is, 90.9%, and 95.7%, respectively.

From the above results and the results of other antibiotics in MRSA and MSSA, it can be noted that there is a strong relationship between resistance to methicillin and resistance to most other antibiotics. This is due to the presence of other resistant genes with the *mecA* gene on the same DNA segment. For example, the *aadD* gene, which encodes an enzyme for tobramycin resistance, is located on plasmid pUB110, which has integrated into *mec*-associated DNA within IS*431mec*. The ability of IS*431* elements, through homologous recombination, to trap and cluster resistance determinants with similar IS elements explains the multiple drug resistance phenotype that is characteristic of methicillin-resistant staphylococci (Chambers, 1997).

The data shows that the resistance of erythromycin was higher than that of clindamycin among both MRSA and MSSA. Staphylococcal strains which possess the *erm* gene prevent macrolides and lincosa-mides (erythromycin and clindamycin) from binding to their target site. Clindamycin is a poor inducer of the *erm* gene compared to erythromycin, (Joseph et al., 2005), which explains the higher resistance rate to erythromycin compared with that to clindamycin observed in our study. According to the physicians in the three hospitals many critical cases that were infected with *S. aureus* are treated with vancomycin, which may lead to creation of new strains that are resistant to this antibiotic. Of particular concern are strains of MRSA that are beginning to develop resistance to vancomycin, which is currently the most effective antibiotic against MRSA. This new resistance has arisen because enterococci relatively commonly express vancomycin resistance. In the laboratory, enterococci are capable of transferring the gene for vancomycin resistance to *S. aureus* (Stanway, 2004) . A vancomycin-resistant isolate was collected from the Gaza European hospital from a 55-year diabetic patient who had been hospi-talized for a long time. He was treated for a foot ulcer with multiple antibiotics, including vancomycin.

The prevalence of MRSA documented by this study should be alarming and strict MRSA guidelines and antimicrobial prescription policies should be applied. Further studies are needed for molecular typing of the MRSA isolates and tracking the origin of these isolates.

ACKNOWLEDGEMENTS

A great deal of this work has been carried out in the Medical Technology Laboratories at the Islamic University of Gaza, Palestine. I am deeply grateful to Prof. Fadel A. Sharif, who has served as my light during the course of this study, and Dr. Abbood Kichaoi, without whose sacrifices none of this would be possible. I would also like to thank the staff in the Al-Shifa Hospital Central Laboratory, especially Miss Najah Eliwa and Mr. Farid Abu Elamreen. I am deeply grateful to the group of the European hospital laboratory, especially Mr. Roshdi Rusrus, Ms. Sofia Zorob and Osama El-jorani, all of whom gave up valuable time for this work.

REFERENCES

Ako-Nai A, Adeyemi F, Aboderin O, Kassim O (2005). Antibiotic resistance profile of staphylococci from clinical sources recovered from infants. Afr. J. Biotechnol. 4 (8): 816-822.

Asensio A, Guerrero A, Quereda C (1996). Colonization and infection with methicillin-resistant Staphylococcus aureus: associated factors and eradication. Infection Control and Hospital Epidemiology. 17(1): 20-8.

Boyle-Vavra S, Daum RS (2007). Community-acquired methicillin-resistant Staphylococcus aureus: the role of Panton–Valentine leukocidin. Laboratory Investigation. 46(9): 3104-3105.

Boyle-Vavra S, Yin S, Challapalli M, Daum RS (2003). Transcriptional Induction of the Penicillin-Binding Protein 2 Gene in *Staphylococcus aureus* by Cell Wall-Active Antibiotics Oxacillin and Vancomycin. Antimicrobial Agents and Chemotherapy. 47(3): 1028-1036.

Brown DF (2001). Detection of methicillin/oxacillin resistance in staphylococci. J. Antimicrobial Chemotherapy. 48:65-70.

Brown DF, Edwards DI, Hawkey PM, Morrison D, Ridgway GL, Towner K J (2005). Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus* (MRSA). Journal of Antimicrobial Chemotherapy.56(6):1000-1018.

CDC (1998). guidelines for infection control in hospital personnel. Am. J Infect. Control. 26: 289-354.

Centers for Disease Control and Prevention (2005). Detection of: oxacillin/methicillin-resistant *Staphylococcus aureus*.

Chambers HF (1997). Methicillin resistance in Staphylococci: molecular and biochemical basis and clinical implications. Clinical Microbiol. Rev. 10(4): 781-791.

Essawi T, Na'was T, Hawwari A, Wadi S, Doudin A, Fattom AI (1998). Molecular, antibiogram and serological typing of *Staphylococcus aureus* isolates recovered from Al-Makased hospital in East Jerusalem. Tropical Medicine and International Health. 3(7): 576-583.

Fluit AC, Maarten R, Visser, Schmitz FJ (2001b). Molecular Detection of Antimicrobial Resistance. Clinical Microbiology Reviews. 14:836-871.

Fluit AC, Wielders C L C, Verhoef J, Schmitz FJ (2001a). Epidemiology and susceptibility of 3,051 *Staphylococcus aureus* isolates from 25 university hospitals participating in the European SENTRY study**.** J. Clin. Microbiol. 39(10):3727-3732.

Foster T (1996). Staphylococcus. In: Barron's Medical Microbiology (Barron S et al, eds.), 4th ed., University of Texas Medical Branch. ISBN 0-9631172-1-1.

Franklin D (2003). Low Antimicrobial resistance: the example of *Staphylococcus aureus*. The Journal of Clinical Investigation.111(9): 1265-1273.

Gin AS, Zhanel GG (2001). Antimicrobial resistance. Canadian Pharml J. Therapeutics for Pharmacists' Care. 6: 2-8.

Goettsch W, Geubbels E, Wannet W, Hendrix MGR, Wagenvoort J.H.T, Neeling AJ (2000). MRSA in nursing homes in the Netherlands 1989 to 1998: a developing reservoir. Euro Surveill. 5(3): 28-31.

Helisangela AS, Vania O, Steffen A, Claudia LC, Paulo PG (2003). Infection and colonization by *Staphylococcus aureus* in a high risk nursery of a Brazilian teaching hospital. Brazilian J. Infect. Diseases. 7(6): 381-386.

Hidron AI, Kourbatova EV, Halvosa JS, Terrell BJ, McDougal LK, Tenover FC, Blumberg HM, King MD (2005). Risk factors for colonization with methicillin-resistant Staphylococcus aureus (MRSA) in patients admitted to an urban hospital: emergence of community-associated MRSA nasal carriage. Clinical infectious diseases. 41(2): 159-66.

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

Joseph WS, Kosinski M (2005). The widening challenge: how to manage MRSA. Continuing Medical Education 2006. www.podiatrym.com.

Juuti K (2004). Surface protein Pls of methicillin-resistant *Staphylococcus aureus* _ role in adhesion, invasion and pathogenesis, and evolutionary aspects. Faculty of Biosciences of the University of Helsinki.

Mark JE (2002). Prevalence of Oxacillin Resistance in *Staphylococcus aureus* among Inpatients and Outpatients in the United States during 2000. Antimicrob Agents Chemother. 46(9): 3104-3105.

Merlino J, Marcel L, Ross B, Duncan V, Colin H (2000). New Chromogenic Identification and Detection of Staphylococcus aureus and Methicillin-Resistant S. aureus. J. Clin. Microbiol. 38(6): 2378-2380.

Murakami K, Minamido W (1991). Identification of methicillin-resistant strains of Staphylococci by polymerase chain reaction. J. Clin. Microbiol. 29(10): 2240-2244.

Olivier G, Muriel W, Nicolas F, Patrick B (2000). Evaluation of CHROMagar *Staph. aureus*, a New Chromogenic Medium, for Isolation and Presumptive Identification of *Staphylococcus aureus* from Human Clinical Specimens. J. Clin. Microbiol. 38(4): 1587-1591.

Sachdev D, Amladi S, Natraj G, Baveja S, Kharkar V, Mahajan S, Khopkar U (2003). An outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA) infection in dermatology indoor patients. Indian J. of Dermatol. 69(6): 377-380.

Skov LR, Pallesen LV, Poulsen RL, Espersen F (1999). Evaluation of a new 3-h hybridization method for detecting the mecA gene in *Staphylococcus aureus* and comparison with existing genotypic and phenotypic susceptibility testing methods. J. Antimicrobial Chemotherapy. 43(4): 467-475.

Stanway A (2004). Methicillin resistant *Staphylococcus aureus*. New Zealand Dermatological Society Incorporated.

Wu SW, Lencastre H, Tomasz A (2001). Recruitment of the *mecA* gene homologue of *Staphylococcus sciuri* into a resistance