Advanced Journal of Microbiology Research Vol. 2017
Available online at http://internationalscholarsjournals.org/journal/ajmr

Full Length Research Paper

Molecular typing of methicilin-resistant *Staphylococcus aureus* isolates at Ain Shams University Hospital, Egypt

H. I. Awadalla[1], I. A. Khalil[2], H. H. Bassim[2], M. N. Ahmed[3] and L. M. Wahba[3]

[1]Institute of Environmental Studies and Research, Ain Shams University, Egypt.
[2]Faculty of Medicine, Ain Shams University, Egypt.
[3]Ain Shams University hospital, Egypt.

Accepted 30 June, 2017

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen and it has been increasingly seen in community settings. The general objective of this study was to characterize by phenotyping and genotyping methods MRSA strains isolated from inpatients, outpatients and health care workers. Specimens were collected from patients in Ain Shams University hospitals. Genotyping is based on polymerase chain reaction (PCR) and restriction fragment-length polymorphism (RFLP), following Hae II digestion of the amplified part of the hyper variable region of *mecA* gene (*mecA* - HVR). The study included 51 phenotypically detected MRSA isolates by conventional methods. PCR revealed the presence of 50 *mecA* positive strains, whereas, one strain was genotypically *mecA* negative. PCR-RFLP revealed three different patterns (A, B and C) which were detected in the three tested groups in patients – outpatients and health care workers (HCWs) in variable percentages. Genotyping using PCR-RFLP of *mecA* -HVR can rapidly demonstrate and discriminate the relatedness of isolates in different hospital wards and also in the community. As the same genotypes (A, B and C) of MRSA were detected in both hospitals and communities as well as in HCWs, therefore it is impossible to decide where they originated.

Key words: Methicillin-resistant *Staphylococcus aureus,* polymerase chain reaction, hospital acquired.

INTRODUCTION

Methicillin resistant *Staphylococcus aureus* (MRSA) constitute a major health care problem with a strong potential for dissemination and high rate of mortality and morbidity. Therefore, the availability of sensitive and specific methods for the accurate detection of antibiotic resistance in these bacteria has become an important tool in clinical diagnosis. Since, phenotypic typing methods are not discriminating enough and are highly dependent on growth conditions (Sinsimer et al., 2005). The *mecA* gene, the structural determinant encoding PBP2a, is therefore considered a useful molecular marker of putative methicillin resistance in *S. aureus* (Felten et al., 2002). As, methicillin resistance in *S. aureus* is primarily mediated by the over production of PBP2a, an additional altered penicillin binding protein with low affinity for β-lactm antibiotics (Perez et al., 2001).

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

Quick and reliable typing methods are required to obtain information on the relatedness among MRSA isolates and to allow faster implementation of appropriate control measures. Numerous methods have been des-cribed as the bacteriophage typing and plasmid analysis which have disadvantages that the reagents are not commercially available and some strains are non-typeable (Hookey et al., 1998).

Also, antibiogram schemes are often uninformative. On the other hand, molecular methods for MRSA typing as pulsed field gel electro-phoresis (PFGE) have good discriminatory power more reproducibility but they are expensive, not widely available and time consuming (Hookey et al., 1998). A developed method for molecular typing of MRSA depends on mecA-hyper variable region length polymorphisms using polymerase chain reaction - restriction fragment-length polymorphism (PCR-RFLP) was considered to be the gold standard method (Wichelhaus et al., 2001).

The objective of this study was to characterize by

phenotyping and genotyping (reliable molecular typing technique, PCR -RFLP) methods MRSA strains isolated from inpatients, health care workers (HCWs) in chest intensive care unit (Chest ICU) and outpatients attending to the central microbiology laboratory in Ain Shams University hospitals.

MATERIALS AND METHODS

Subjects

This cross sectional study was conducted during September 2007 to 2008 at Ain Shams University Hospitals (ASUH). A verbal consent was taken from every participant who agreed to be included in this study. Researchers took data of antimicrobial susceptibility testing from the laboratory, except HCWs). So, verbal consent used only for HCWs because verbal consent already done by doctor in charge or hospital personnel. It involved the following groups:

1. Inpatients group: Different clinical specimens were collected from 160 chest ICU patients (sputum – endotracheal tube aspirates, urine, and wound swabs). Date of admission in relation to the onset of infection was considered.
2. Outpatients group: Different clinical specimens of 2506 outpatients were collected from the central microbiology laboratory (sputum, urine, pus, and swabs from skin lesions, eyes and ears). Patient with nosocomial MRSA infection was defined as those from whom MRSA was isolated 48 h after hospitalization. All other MRSA isolates were labeled CA-MRSA. Due to the large number of the outpatients' specimens, the molecular assay was done only for *S. aureus* isolates collected during three months. The included outpatients fulfilled the following criteria during the previous year of the study: not admitted to hospitals, no intravenous drug use or surgery and no percutaneous medical device.
3. HCWs group: Nasal swabs and hand swabs from the anterior nares and both hands of all HCWs working in chest ICU (20 nurses, 20 doctors and 5 workers).

Sample collection and handling

Different swabs were taken by sterile disposable swabs, sputum was obtained by deep cough and midstream urine was collected in sterile containers. The endotracheal aspirates from inpatients and outpatients having pneumonia were collected by disposable sterile catheter connected to a suction apparatus in sterile test tubes. These aspirates were liquefied before processing by incubating them at 37°C for one hour with frequent shaking with sterile glass beads.

Isolation of bacteria

Bacteriological examination was carried out for isolation and identification of *S. aureus* from different specimens, detection of methicillin resistance among *S. aureus* isolates and antibiogram for MRSA strain.

Identification of *S. aureus* isolates and antimicrobial susceptibility testing

MRSA was detected by both disc diffusion method using 1 ug oxacillin discs, and oxacillin resistance screening agar base (ORSAB). MRSA appeared as intense and diffuse blue colonies on ORSAB after incubation at 35°C for 24 h. Antimicrobial susceptibility testing was performed on Muller-Hinton agar and 1 ug oxacillin disc (Oxoid-England) by the disk diffusion method using commercial disks (Oxoid-England) according to the guidelines of the clinical laboratory standard institute (CLSI, 2008). The plates were incubated in ambient air at 35°C for 24 h. Any growth with 13 mm in diameter zone around the disk was considered indicative of resistance. *S. aureus* colonies were preserved in Stuart transport medium at -70°C until further molecular analysis according to Brown et al. (2005).

Molecular assay

Polymerase chain reaction (PCR) technique for detection of hyper variable region of *mecA* (*mecA*-HVR) gene in *S. aureus* isolates. Restriction fragment length polymorphism (RFLP) analysis of *mecA*-HVR.

A- PCR for mec A-HVR

1. DNA extraction: DNA was extracted from *S. aureus* colonies using the high pure PCR template preparation kit supplied from Boehringer Mannheim (Meylan, France).
2. DNA amplification: The primers used were supplied commercially by life technologies, Egynestein, Germany. Their sequences were HVR1: 5'-ACT ATT CCC TCA GGC GTC C-3', HVR2: 5'-GGA GTT AAT CTA CGT CTC ATC-3'. Twenty (20) l of the extracted DNA was added to the PCR amplification mixture which consisted of: 5 l (1.0X) Taq buffer, 1 l (0.2 mm/L) deoxy ribonucleotide triphosphate, 1 l (50 pmol/ml) of each primer and 0.5 l (2.5 U) of Taq DNA polymerase (Boehringer, Mannheim) and 21.5 l nuclease free water. The final volume was 50 l. The amplification was performed by including the reaction mix for 35 cycles in a thermocycler. Each cycle consisted of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min, with a final extension of 10 min at 72°C. The oligonucleotide primers used in this study had been previously described by Wichelhaus et al. (2001).

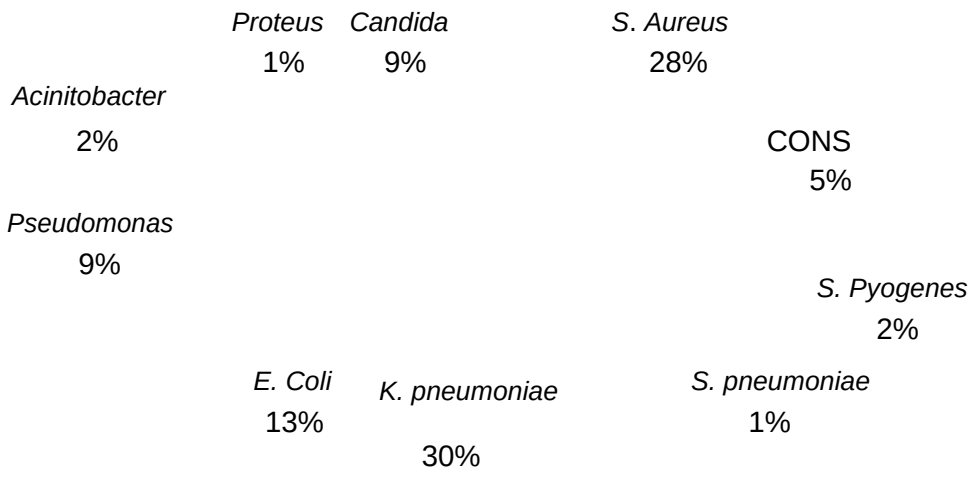
After PCR amplification, 15 l of the PCR product was subjected to Agarose gel electrophoresis (1.8% agarose, 1 x Tris-acetate-EDTA) for 45 min at 100 V in the presence of a molecular size marker 100: 2000 bp supplied by Boehringer-Mannheim for size assessment, stained with ethidium bromide and photographed under ultra-violet (UV) illumination. The positive samples yielded a 575 base pair (bp) amplicon corresponding to HVR *mecA* positive control used according to Sambrook et al. (1989).

B- RFLP

Ten (10) l of each amplified product were mixed and digested with 10 U of Hae II restriction enzyme (Ferments, USA), and incubated at 37°C for 3 h. The sizes of the restriction fragments were documented by electrophoresis, ehtidium bromide, UV transillumination and photography. The interpretation criterion for identifying different strains was a single band difference. Three different patterns A, B and C could be identified with PCR end product at 575 bp, 519 - 56 bp and 519 - 42 - 14 bp respectively (Wichelhaus et al., 2001).

RESULTS

The age of the inpatients ranged from 18 to 70 years with mean age 45 years. HCWs ages ranged from 16 to 50



Graph 1. Prevalence of isolates in inpatients specimens. CoNS: Coagulase test used for *Staphylococcus* sp.

Table 1. Distribution of MRSA* and MSSA* among the total isolates in different groups.

Organism / Group	MRSA*		MSSA*		Other organisms		Total isolates	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Inpatients	24	24	4	4	72	72	100	100
Outpatients	44	2.8	139	8.9	1377	88.3	1560	100
HCWs	10	22.2	5	11.1	30	66.7	45	100

MRSA*: Methicillin-resistant *S. aureus* , MSSA*: Methicillin-susceptible *S. aureus.*

Table 2. Distribution of MRSA* and MSSA* among the total number of *S. aureus* isolates in different groups.

Organism / Group	MRSA*		MSSA*		Total *S. aureus*	
	No.	(%)	No.	(%)	No.	(%)
Inpatients	24	85.7	4	14.3	28	100
Outpatients	44	24	139	76	183	100
HCWs	10	66.7	5	33.3	15	100

MRSA*: Methicillin-resistant *S. aureus* , MSSA*: Methicillin-susceptible *S. aureus.*

years with mean age of 22 years. The outpatient's ages ranged from 15 to 60 years with mean age of 24 years. One hundred out of 160 inpatient specimens and 1560 out of 2506 outpatient specimens gave growth of different organisms while the rest of the specimens gave no growth or growth of normal flora. The prevalence of MRSA among different organisms in the inpatients group was 24.0% while in the outpatients group, it was 2.8%. The percentage of MRSA colonization among HCWs was 22.2%.

Graph 1 shows that the most common causative agents recovered from clinical specimens of the chest ICU inpatients were *Klebsiella* followed by *S. aureus* then *Esherieshia coli.* The prevalence of MRSA among different isolated organisms in the inpatients and outpatients groups was 24 and 22.2% respectively (Table 1).

The distribution of MRSA isolate of the total *S. aureus* organisms were higher among the inpatients group (85.7%) and HCWs (66.7%) than MSSA isolate, while among outpatients the distribution of MSSA isolate was higher (76%) than MRSA isolate (Table 2).

Due to the large number of the outpatients specimens (183) of *S. aureus* isolates which were collected during the whole year, the molecular assay was done only for a subsample of *S. aurues* isolates collected during three months (48 specimens). Molecular assay for the three studied groups showed that 51 specimens of them were MRSA. Table 3 revealed that 50 out the 51 phenotypically detected MRSA were genotypially *mecA* positive. Whereas one strain was only genotypially *mecA*

Table 3. Phenotypic and molecular characterization of *S. aureus* isolates (MRSA* and MSSA*) in different groups.

Test / Group		Disk diffusion for oxacillin (n = 91)		PCR for mec A- HVR (n = 91)	
		Resistant	Susceptible	+ve (575 bp)	-ve
In patients	**(n = 28)**				
MRSA*		24	-	23	1
MSSA*		-	4	-	4
Outpatients	**(n = 48)**				
MRSA*		17	-	17	-
MSSA*		-	31	-	31
HCWs	**(n = 15)**				
MRSA*		10	-	10	-
MSSA*		-	5	-	5
Total 91		51	40	50	41

MRSA*: Methicillin-resistant *S. aureus*, MSSA*: Methicillin-susceptible *S. aureus.*

Table 4. Antibiotic susceptibility pattern of MRSA* isolates in different groups.

Group / Drug susceptibility (g)		Inpatients n = 24		Outpatients n = 17		HCWs n = 10		^{2} and *p*
		No.	(%)	No.	(%)	No.	(%)	
Ciprofloxacin (5)	R*	15	62.5	3	17.6	4	40	^{2} = 8.21
	S*	9	37.5	14	82.41	6	60	*p* = 0.016
Clindamycin (2 g)	R*	6	25	2	11.8	2	20	^{2} = 1.11
	S*	18	75	15	88.2	8	80	*p* = 0.574
Gentamicin (1 g)	R*	9	37.5	2	11.8	3	30	^{2} = 3.35
	S*	15	62.5	15	88.2	7	70	*p* = 0.187
Trimethoprim/ sulfamethoxazote	R*	14	58.3	2	11.8	3	30	^{2} = 9.51
	S*	10	41.7	15	88.2	7	70	p = 0.008
Erythromycin (15 g)	R*	17	70.8	10	58.8	6	60	^{2} = 0.75
	S*	7	29.2	7	41.2	4	40	*p* = 0.687
Tetracycline (30 g)	R*	21	87.5	12	70.6	7	70	^{2} = 2.21
	S*	3	12.5	5	29.4	3	30	*p* =0.332
Oxacillin (1 g)	R*	24	100	17	100	10	100	-
	S*	-	0	-	0	-	0	
Vancomycin (30 g)	R*	-	0	-	0	-	0	-
	S*	24	100	17	100	10	100	

MRSA*: Methicillin-resistant *S. aureus,* R*: Resistant, S*: Susceptible.

negative. All the phenotypically detected MSSA isolates were genotypically *mecA* negative. Table 4 shows that all MRSA isolates were resistant to oxacillin and susceptible to vancomycin but show variable patterns to other antibiotics. The most effective drugs on MRSA isolates in our study were vancomycin followed by clindamycin, gentamicin and trimethoprim / sulfamethoxazole. The multi-drug resistance of MRSA isolates (MDR-MRSA)

Table 5. Frequency of MRSA/ PCR-RFLP patterns in different groups.

Group	PCR-RFLP *mecA*-HVR pattern					
	A (575 bp)		B (519-56 bp)		C (519-42-14 bp)	
	No.	(%)	No.	(%)	No.	(%)
In patients n = 23 (100%)	9	39.1	8	34.8	6	26.1
Outpatients n = 17 (100%)	10	58.8	5	29.4	2	11.8
HCWs n = 10 (100%)	5	50	3	30	2	20
Total n = 50 (100%)	24	48	16	32	10	20

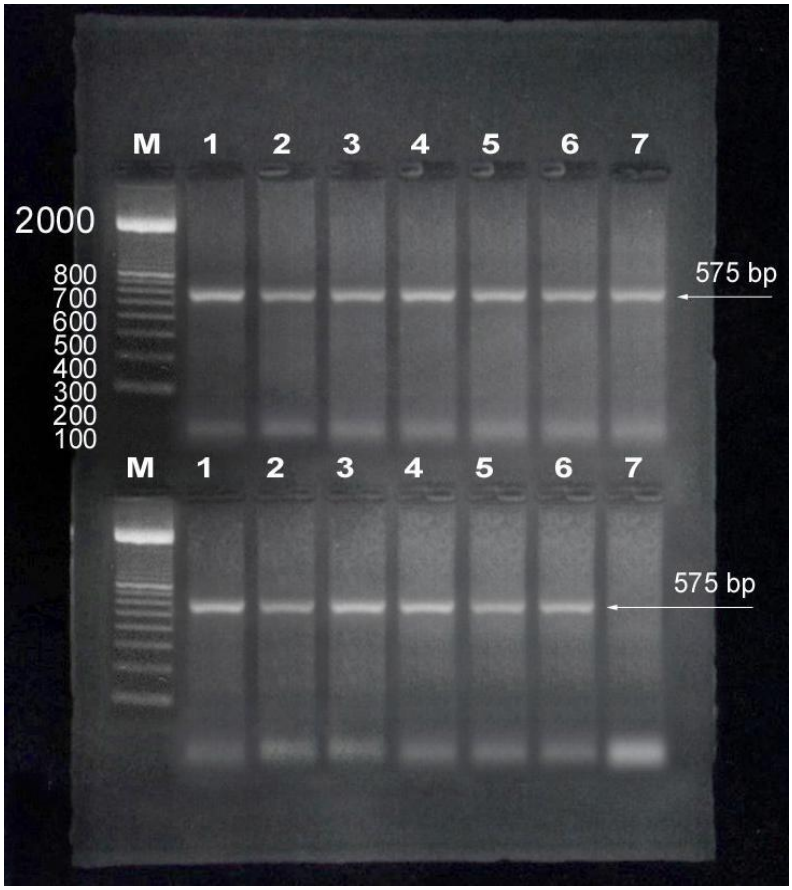


Photo 1. PCR patterns of mec A-HVR of MRSA isolates. * Lane M: Molecular size marker. * Upper lane 1: Positive control for *mecA*. * Lower lane 7: Negative control.

that is, resistant to ≥ 3 antibiotic groups, was more common in the in patients group 62.5% (15/24). While it was less common in the outpatients and HCWs groups 11.8% (2/17) and 20% (2/10) respectively. Non-multidrug resistant (NMDR) MRSA isolates i.e. resistant to 2 non β-lactam antibiotics were more prevalent among outpatients and HCWS groups. Table 5 shows that the 3 patterns were detected in the three tested groups in different percentages. Type A constituted the higher percentage (48%) of the total isolates. Photo 1 shows electrophoric separation of PCR amplification products of *mecA*-HVR of MRSA isolates. Photo 2 shows PCR-RFLP pattern of MRSA isolates.

DISCUSSION

Nosocomial infection is a major problem in the world today. MRSA strains usually resistant to several anti-biotics (Kumari et al., 2008). It is the major cause of

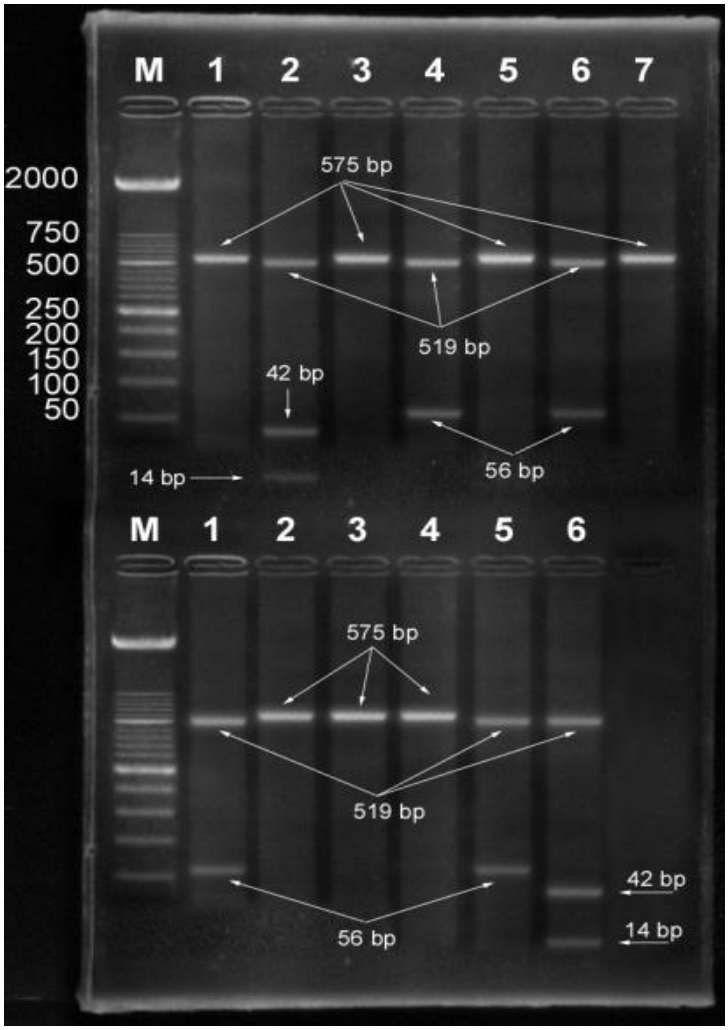


Photo 2. PCR-RFLP patterns of MRSA isolates. * Lane M: Molecular size marker; * Type A (575 bp): detected at upper lanes 1, 3, 5, 7 and lower lanes 2, 3, 4. * Type B (519-56 bp): detected at upper lanes 4, 6 and lower lanes 1, 5. * Type C (519-42-14 bp): detected at upper 2 and lower 6.

nosocomial mortality and morbidity, it is commonly found in the community and hospital especially in the ICU (Davis et al., 2007) . Special attention has therefore been directed to specific nosocomial surveillance systems and strict infection control measures for this microorganism in which the microbiological laboratory plays an important role by applying phenotypic and genotypic methods (Velazco et al., 2008). Prevention of MRSA nosocomial infections in ICU has been recommended for several years. However, the workload and the costs of these programs are to be weighed against the benefit obtained in terms of reduction of morbidity and costs induced by the infection (Larue et al., 2008).

Results of this study revealed that the prevalence of *S. aureus* in chest ICU patients was 28% (24% MRSA, 4% MSSA), which was not far from 35% (29 MRSA - 6% MSSA) detected by Sharaf et al. (2006) in Medical ICU in ASUH. Also, MRSA prevalence in ICU patients in Saudi Arabia was 21.4% (Al-Borhamy and Anoose, 2004) and higher results 35% obtained in New Zealand by Fridkin et al. (2001). MRSA constituted 85.7% of total *S. aureus* isolates in chest ICU patients, this figure was near to that obtained by Sharaf et al. (2006) 82.1% and El-Kholy et al. (2003) 71% in Egypt and Vincent et al. (1995) in Europe (81%). Thus indicating that hospitalization in ICU is an important risk factor for MRSA colonization and infection (Vincent et al., 1995). As regard the community acquired infection, the prevalence of *S. aureus* isolates among outpatient specimens yielding bacterial growth was 11.7% (2.8% MRSA, 8.9% MSSA). MRSA constituted 24% of C.A. *S. aureus* infections which was near that obtained by Al-Ghaithy et al. (2000) in Saudi Arabia who reported it 25.4% of C.A. *S. aureus* infection. In New Zealand it was 29% as reported by Gosbell et al. (2001).

In Japan, it was 27% (Akiyama et al., 2000). Smaller figure was reported in Poland 15.3% (Jesus et al., 2003). This might be attributed to different population studied, infection control measures or different study designs.

In this study, the overall (nasal and hand) MRSA colonization rate among HCWs was 22.2% (10/45) which was near that detected in (HCWs) in chest diseases department in Ankara University hospital 25% (Cesur et al., 2004). This may lead to spread among patients. So, screening of HCWs and decolonization of those linked with transmission may thus need to be considered (Rahbar et al*.,* 2003). The findings of the current study showed that many MRSA strains were multidrug-resistant but all were sensitive to Vancomycin, Kumari et al. (2008) confirmed that no MRSA strains were resistant to Vancomycin.

This study revealed that MDR-MRSA was more common in the inpatients isolates. While NMDR-MRSA was more prevalent among isolates from outpatients and HCWs, this result was in agreement with Merlino et al. (2002). Variations in antibiotic susceptibility profiles of MRSA makes phenotypic interpretation difficult (Merlino et al., 2002). PCR-RFLP is a preliminary screening method for epidemiological study of nosocomial infection caused by MRSA (Mitani et al*.,* 2005). For routine detection of MRSA, Felten et al. (2002) proved that 30 mg cefoxitin disk was superior and easier to interpret than the oxacillin disk in disk diffusion tests. As it correctly identified all low level resistant MRSA which dis-play intermediate resistance. The PBP2a agglutination by the MRSA latex screening test using induced bacteria was as reliable as the *mecA* PCR, even for the phenotypically oxacillin susceptible MRSA (Brown *et al.,* 2005). In the present study, PCR of HVR of *mecA* of 51 phenotypically detected MRSA was positive in 50 MRSA isolates while one phenotypic MRSA was genotypically negative. Similar results were detected by Swenson et al. (2001) who found that 4 out of 36 *mecA* –ve *S. aureus* were phenotypically MRSA. This might be attributed to modification of normal PBPs genes, over expression of normal PBPs, or overproduction of staphylococcal β-lactamases (Chambers et al., 1997). Other reports detected bacteria with positive results in genotypic tests but remain phenotypically susceptible to relevant antibiotics, this might be due to unexpressed or non functional *mecA* gene (Brown et al., 2005).

In the current study, PCR- RFLP of *mecA*-HVR revealed 3 different restriction profile patterns A, B and C, this fin-ding provides support for the postulate of El-Borhamy and Anoose (2004). The three types were detected in the 3 groups involved in this study (in patients – outpatients – HCWs) but with variable percentages. This pattern was consistent with the description of Anne-Merethe et al. (2005) and Patel et al. (2008) that the same clonal types were present in both hospitals and communities. So, the true site of acquisition of MRSA is rarely known with certainty (Salgado et al., 2003). In contrary to this, another study carried out by Wylie et al. (2005) reported that CA-MRSA is genetically distinct from HA-MRSA.

Conclusion and Recommendation

PCR-RFLP represents a powerful, rapid and reliable molecular typing system and an alternative tool for routine epidemiological surveillance and infection control measures. Restriction analysis by PCR-RFLP permitted discrimination of the tested strains by exhibiting a specific PCR-RFLP pattern. We could rapidly demonstrate the prevalence of different patterns in various locations (hospital wards or communities), as well as the similarity or dissimilarity between the tested isolates. As the same genotypes (A, B and C) of MRSA were detected in both hospitals (inpatients) and communities (outpatients) as well as in HCWs who may act as vectors for transmission, this makes comparisons between isolates impossible. Therefore, it is impossible to decide where these genotypes originated, this can be overcome by combined analysis of other genes (spa gene and coa gene) which contain repeat units and thus are highly polymorphic with regard to the number and sequence of repeats.

REFERENCES

Akiyama H, Yamasaki O, Tada J, Arata J (2000). Adherence characteristic and susceptibility to antimicrobial agents of *S. aureus* strains isolated from skin infections and atopic dermatitis. J. Dermatol. Sci., 23: 155-160.

Al-ghaithy A, Bilal E, Gedebou M, Weily H (2000). Nasal carriage and antibiotic resistance *S. aureus* isolates from hospital and non hospital personnel in Abba, Saudi Arabia. Trans. R. Sos. Trip. Med. Hyg., 94: 504-507.

Anne-Merethe H, Aina F, Thomas LM (2005). Dissemination of CA-MRSA clones in Northern Norway. J. Clin. Micro., 43(5): 2118-2124.

Brown DF, Davis SL, Jeff HK (2005). Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant *S. aureus* (MRSA). J. Antimicrobial. Chemotherapy., 56(6): 1000-1018.

Cesur S, Cokca F, Marples RR (2004), Nasal carriage of MRSA among hospital staff and outpatients. Infec. Control Hospital Epidemiol., 25: 169-171.

Chambers HF, Witte WC, Cuny C (1997). Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. Clin. Microbiol. Rev., 10(4): 781-791.

Clinical and Laboratory Standards Institute (CLSI) (2008). Performance standards for antimicrobial susceptibility testing, CLSI approved standards M100-S15., Wayne, PA.

Davis SL, Perri MB, Judith FR (2007). Epidemiology and outcomes of CA-MRSA infection. J. Clin. Micro., 45(6): 1705-1711.

El-Borhamy MI, Anoose FA (2004). Rapid molecular typing of MRSA in routine epidemiological surveillance with special emphasis on PCR-RFLP. Egypt. J. Med. Lab. Sci. (ESIC), 13: 1-15.

El-Kholy A, Baseem H, Hall GS (2003). Antimicrobial resistance in Cairo, Egypt 1999-2000: A survey five hospitals. J. Antimicrob. Chemother, 51: 625-630.

Felten A, Grandry B, Langrange PH, Casin I (2002). Evaluation of three techniques for detection of low level MRSA. : a disk diffusion method with cefoxitin and moxalactam, the Vitek 2 system, and the MRSA-screen latex agglutination test., J. Clin. Microbiol., 40(8): 2766-2771.

Fridkin SK, Soussy C, Carret G (2001). Vancomycin intermediate and resistant *S. aureus*: what the infections disease specialist needs to know. Clin. Infect. Dis., 32: 108-113.

Gosbell IB, Mercer JL, Neville SA, Crone SA, Chant KG, Jalaludin BB,

Munro R (2001). Non-multidrug and multiresistant MRSA in community acquired infections. Med. J. Aust., 174: 627-630.

Hookey JV, Richardson JF, Cookson BD (1998). Molecular typing of *S. aureus* based on PCR-RFLP and DNA sequence analysis of the coagulase gene. J. Clin. Microb., 36: 1083-1089.

Jesus ME, Marcus LB (2003). Preliminary study of community acquired *S. aureus* infection in Manaus hospital, Amazonia Region, Brazil. Rev. Soc. Bras. Med. Trop., 36(6): 12-16.

Kumari N, Mohapatra TM, Singh YI (2008). Prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) in a Tertiary-Care Hospital in Eastern Nepal. JNMA J. Nepal Med. Assoc., 47(170): 53-60.

Larue A, Loos-Ayav C, Jay N, Commun N, Rabaud C, Bollaert PE (2008). Impact on morbidity and costs of methicillin-resistant *Staphylococcus aureus* nosocomial pneumonia in intensive care patients.] Presse Med. 2008 [Article in French]

Merlino J, Jason W, Cookson BD (2002). Detection and expression of methicillin resistance in multidrug-resistant and non multidrug resistant *S. aureus* in central Sydney, Aust. J. Antimicrobial Chemothe., 49: 793-801.

Mitani N, Koizumi A, Sano R (2005). Molecular typing of MRSA by PCR-RFLP and its usefulness in an epidemiological study of an outbreak. J. Infect. Dis., 58(4): 250-261.

Patel M, Hoesley CJ, Moser SA (2008), Dissemination of CA-MRSA in a tertiary care hospital. South Med. J. 101(1): 40-50.

Perez RE, Claverie MF, Villar J, Mendez AS (2001). Multiplex PCR for simultaneous identification of *S. aureus* and detection of methicillin and mupirocin-resistance. J. Clin. Microb., 39(11): 4037-4041.

Rahbar M, Karamiyar M, Gra-Agji R (2003). Nasal carriage of MRSA among HCWs of an Iranian hospital. Infec. Control Hospital Epidemiol., 24: 236-237.

Salgado CD, Farr BM, Calfee DP (2003). Community acquired MRSA: a meta analysis of prevalence and risk factors. Clin. Infect. Dis., 36: 131-139.

Sambrook J, Fritsch EF, Maniatis T (1989). Molecular cloning in: A Laboratory manual 2nd edition. Cold Spring Harbor Press, NY, pp. 89-96.

Sharaf H, Badawi D, El-Kinawy N (2006). Simultaneous identification, detection of S. aurues and methicillin resistnce by PCR amplification of FemB and mec A genes. Egypt J. Med. Lab. Sci., 15(2): 1-10.

Sinsimer D, Fred CT, Jana MS (2005). Use of a multiplex molecular beacon platform for rapid detection of methicillin and vancomycin resistance in staphylococcus aureus. Clin. Microb., 43(9): 4585-4591.

Sinsimer D, Leekha S, Park S, Marras SA, Koreen L, Willey B, Naidich S, Musser KA, Kreiswirth BN (2001). Performance of eight methods including two new rapid methods for detection of oxacillin resistance in a challenge set of *S. aureus* organisms. J. Clin. Microbiol., 39(10): 3785-3788.

Velazco E, Nieves B, Vindel A, Alviarez E, Gutierrez B, Bianchi G (2008) Molecular study of methicillin-resistant Staphylococcus aureus isolates at a neonatal high-risk unit in Merida, Venezuela. Med Sci. Monit., 14(9): I25-31.

Vincent JL, Bihari DJ, Suter PM, Bruining HA, White J, Nicolas-Chanoin MH, Wolff M, Spencer RC, Hemmer M (1995). The prevalence of nosocomial infection in intensive care units in Europe. Results of the European Prevalence of Infection in Intensive Care (EPIC) Study. EPIC International Advisory Committee. JAMA. 23-30; 274(8):639-644.

Wichelhaus T, Hunfeld K, Boddinghaus B (2001). Rapid molecular typing of MRSA by PCR-RFLP. Infec. Control Hospital Epidemiol., 22: 294-298.

Wylie JL, Deborah LN, John VH (2005). Molecular epidemiology of community and health care associated MRSA in Manitoba, Canada. J. Clin. Micr., 43(6): 2830-2836.