JOURNAL OF PURE AND APPLIED MICROBIOLOGY, April 2011. Vol. **5**(1), p. 11-18

The Role of Health Care Workers and Environment on Transmission of Methicillin–Resistant *Staphylococcus aureus* among Patients in a Medical Intensive Care Unit in a Saudi Hospital

Safaa M. El-Ageery[1], Maha A. Abo-Shadi[2]*, Asmaa M. Elgendy[3], Abdulaziz A. Alghaithy[1] and Amina Y. Kandeel[4]

[1]Medical Laboratories Technology Department, Faculty of Applied Medical Sciences, [2]Department of Biology, Faculty of Science, [3]Microbiology Department, Faculty of Medicine, Taibah University; Al-Madinah Al-Munawwarah, Saudi Arabia.
[4]Department of Medical Microbiology & Immunology, Faculty of Medicine, Mansoura University, Egypt.

(Received: 08 October 2010; accepted: 24 November 2010)

As methicillin -resistant *Staphylococcus aureus* (MRSA) is a virulent pathogen responsible for health care-associated onset disease. In the present study, we have investigated the impact of carriage by health care workers (HCWs) and environment on patient transmission of MRSA in a Medical Intensive Care Unit (MICU), Fahd hospital, Al-Madinah Al-Munawarah, KSA. MRSA isolates from 117 patients, 25 HCWs and 12 environmental sites were collected. Antibiotyping and pulsed-field gel electrophoresis (PFGE) analysis were done to determine the clonal relationship between isolates and potential routes of transmission.

The average nosocomial infection rate of MRSA was 5.98 % (7/117), the overall carriage rate of HCWs was 36% (9/25). Two MRSA was isolated from the 12 environmental sites samples. All MRSA isolates showed multidrug resistance. However, isolates with reduced susceptibility to vancomycin were not detected. Four major MRSA clusters were identified based on the PFGE patterns. Several HCWs were carrying MRSA isolates with the same PFGE patterns (pulsotype A3 and pulsotype C2) as those of isolates from patients (P2, 3, 7), thereby establishing the transmission of MRSA between patients and HCWs. Furthermore, the environmental isolates had PFGE pattern (pulsotype A3) indistinguishable from that of some patients isolates (P2, 7); ensuring that environment may serve as a reservoir for MRSA. The presence of MRSA among HCWs and environment has been associated with increased risk of MRSA nosocomial infection.

Key words: Methicillin-resistant *Staphylococcus aureus* (MRSA); health care workers; intensive care unit; Saudi hospital; pulsed-field gel electrophoresis; environment.

Methicillin-resistant strains of staphylococci were identified immediately upon the introduction of methicillin into clinical practice. Methicillin-resistant *S. aureus* (MRSA) was initially identified for the first time in 1961 by Jevons[1,2]. Since then MRSA have become one of the most significant nosocomial pathogens worldwide, being capable of causing a wide range of hospital

* To whom all correspondence should be addressed.

Maha A. Abo-Shadi, M.Sc., Ph.D.
Assistant Prof. of Microbiology,
Microbiology & Immunology Department,
Faculty of Pharmacy, Al-Azhar University.
E-mail: m_a_shadi@hotmail.com
Tel: +966567717248; Fax: +966-04-8460008-3867.

infections and clinical syndromes associated with severe diseases, including bacteremia, pneumonia, endocarditis, septic arthritis, osteomyelitis, and deep abscess formation. As MRSA strains can disseminate very rapidly, it is necessary to implement effective monitoring programs for the identification and control of epidemic strains[3].

In the late 1960s and 1970s, the prevalence of MRSA was less than 5% in most hospital settings worldwide, but increased in the 1990s to as high as 40% in several hospitals in the United States and Europe[4,5]. In Japan, MRSA comprised 60-70% of *S. aureus* strains isolated from inpatients[6]. In a multicenter surveillance study in Taiwan, threat of methicillin resistance among *S. aureus* collected from inpatients and outpatients combined was nearly 60%, and was highest in isolates from the intensive care unit (ICU) (73%)[7]. The prevalence of MRSA in Malaysia ranged from 17% in 1986 to 40% in 2000[8]. Not only MRSA has become a worldwide problem in hospital but also in community settings[9].

MRSA is spread by contact and by air and may circulate amongst patients, staff and visitors for several months during an outbreak in hospitals and long-term care facilities[10]. Colonized healthcare providers are generally asymptomatic, but create a potential reservoir of infection[11-12]. Prolonged survival in the hospital environment for up to 10 months is one of the determinants of the spread and persistence of MRSA[13,14]. Furthermore, the continually rising number of hospital acquired infections and particularly MRSA colonization poses a major challenge from both clinical and epidemiological perspectives[15].

Typing plays an important role in understanding the epidemiology of MRSA and evaluating the effectiveness of infection control and antimicrobial prescribing measures. The many different methods employed for MRSA typing include antibiograms and chemical resistograms, phage typing, ribotyping, pulsed-field gel electrophoresis (PFGE)[16], and PCR-based methods[17-19]. Regarding molecular typing of MRSA, pulsed-field gel electrophoresis (PFGE) is often considered to be the "gold standard" and is commonly used in clinical, national, and reference laboratories[20]. Al-Thawadi *et al.*[21] found that PFGE is superior to restriction endonuclease analysis of plasmid deoxyribonucleic acid (REAP) and randomly amplified polymorphic DNA (RAPD) and therefore, more suitable for routine and standardized tracing of nosocomial bacterial isolates.

Although carriage of MRSA by health care workers (HCWs) has been a topic of concern since the 1980s [22,23], the precise role that HCWs play in initiating and maintaining MRSA outbreaks in the hospitals is not well defined. Accordingly in our study, the possible transmission of MRSA between HCWs, the hospital environment, and patients were investigated in the Medical Intensive Care Unit (MICU) of King Fahd Hospital, K.S.A. Conventional and molecular typing was analyzed on MRSA isolates obtained from patients, HCWs, and environmental samples to determine their clonal relationship and the potential routes of transmission.

METHODS

Setting and study design

A four-month prospective study on in-patients, HCWs and environmental sites was done at the MICU of King Fahd Hospital in Al-Madinah Al-Monawarah, KSA. We tested for the presence of *S. aureus* infection or colonization and if present, determined methicillin resistant strains. Surveillance cultures were performed on the HCWs including the nursing personnel and the physicians. In addition, environmental cultures were also performed within the unit at the same time.

Microbiological analysis

Samples from patients were collected according to the site of infection. Samples from HCWs were collected from both nares, right and left hands. The HCW was considered to have persistent colonization if MRSA was cultured from three or more consecutive samples. A single sterile cotton swab was used to sample both nares, which was then plated immediately into 5% sheep blood agar plate, SBA (Oxoid, England). The right and left hand specimens of HCWs were obtained by placing their hands into 2 separate SBA plates directly without washing their hands before culture. Environmental samples were collected before the regular daily cleaning by rolling sterile cotton swabs moistened with sterile saline several times over a surface area of approximately 5×5 cm^2

and then inoculated into 5% SBA plates. The environmental sites cultured included the patients' charts, bed rails, faucets, monitors, oxygen adapters, suction switches, and ventilator buttons. Identification of *S. aureus* was performed according to the standard bacteriological procedures: Gram stain reactions, colony morphology, catalase and slide coagulase (Staphaurex; Bio-Rad Laboratories, USA). Resistance to methicillin was diagnosed using mannitol salt agar with oxacillin (2 µg/ml) (Oxoid, England) incubated at 37°C for up to 48 h.

Antibiotypying

Susceptibilities to 11 antimicrobial agents were determined based on results of disk diffusion method in accordance with the guidelines of the Clinical and Laboratory Standards Institute[24]. The antibiotics (Oxoid, England) tested included chloramphenicol (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), quinupristin/dalfoprestin, rifampin (30 µg), tetracycline (30 µg), trimethoprim-sulfamethoxazole (25 µg), vancomycin (30 µg), and teicoplanin (30 µg).

Molecular typing

Clonal relationship of all MRSA isolates was determined by pulsed field gel electrophoresis (PFGE) which was performed according to the manufacturer's instructions of the reagents used (Bio-Rad Laboratories, U.S.A.). Single colonies of MRSA were used to inoculate brain heart infusion medium, and the cultures were incubated at 37°C overnight. The bacteria were embedded in a plug with an embedding agarose and treated with lysostaphin and proteinase K. The chromosomal DNA in the plug was digested with *Sma*I (25 units/plug) at 25°C overnight. The plugs treated with the restriction enzyme were loaded into 1% agarose gel and electrophoresed with a CHEF-DR® III Variable Angle System (BioRad) at a constant 170V, with switching times ranged from 5 to 80 s, for a total running time of 30 h. Following the electrophoresis, the gel was stained with nucleic acid stain (Takara Shuzo Co. Ltd, Japan) and photographed under ultraviolet light. Isolates with ≥ 80% similarity were considered to belong to the same pulsotype and subtypes were assigned to isolates having ≤3 DNA band differences within the same pulsotype[25].

RESULTS

Nosocomial infection rate of MRSA

Nosocomial infection rate of MRSA was defined as the number of infections acquired during a period by the number of patients discharged during that period[26]. MRSA was isolated from 7 patients of the 117 patients. So the average nosocomial infection rate of MRSA was 5.98%.

Clinical data of the 7 patients are listed in Table 1. MRSA was isolated from sputum of patients P1, 2, 3, 5 & 6; from pus of patient P4 and from blood of patient P7.

MRSA colonisation among HCWs

Of the twenty five HCWs studied, nine (36%) had MRSA colonized in nares and/or hands (Table 2).

PFGE & antibiotyping of MRSA isolates

The total MRSA isolates from the patients[7], HCWs[9] and the environment[2] isolates were subjected to antibiotyping and molecular typing by PFGE. All MRSA isolates were susceptible to quinupristin/dalfoprestin, rifampin, teicoplanin, and vancomycin. The PFGE results susceptibility profiles of the other 7 antimicrobials and of the MRSA isolates are presented in Table 3. Based on the PFGE patterns, as shown in Fig. 1, the MRSA isolates could be divided into 4 Pulsotypes (patterns A - D). Pulsotype A isolates were resistant to erythromycin, tetracycline, gentamicin, ciprofloxacin, and clindamycin but had variable susceptibilities to trimethoprim-sulfamethoxazole, and chloramphenicol.

Isolate obtained from patient (P5) was designated PFGE pattern A1, isolate obtained from patient (P6) was designated PFGE pattern A2, while isolates obtained from patients (P2,P7), HCWs (H1,H9) and environmental sites (V1&V2) were designated PFGE pattern A3.

As regard PFGE pattern B (pulsotype B); isolates were resistant to erythromycin, gentamicin,and chloramphenicol but had variable susceptibility patterns to trimethoprim-sulfamethoxazole, tetracycline, ciprofloxacin and clindamycin. Pulsotype B had 3 subtypes and isolated only from HCWs (H2, 3, 6, 8).

Pulsotype C isolates were resistant to erythromycin, ciprofloxacin, trimethoprim sulfamethoxazole,and clindamycin but had variable

Table 1. Clinical data of patients with MRSA infection

Patient	Age (Y)	Sex	Underlying disease	Specimen
P1	65	M	Myocardial infarction	Sputum
P 2	42	F	Congestive heart failure	Sputum
P 3	45	M	Pulmonary embolism	Sputum
P 4	79	M	Empyema	Pus
P 5	48	M	Recurrent pneumonia	Sputum
P6	76	F	Respiratory failure	Sputum
P7	82	F	Respiratory failure	Blood

P= patient, M= male, F= female

susceptibility patterns to tetracycline, gentamicin and chloramphenicol. Pulsotype C had 2subtypes; C1 subtype was isolated from one patient (P1) and C2 subtype was isolated from one patient (P3) and three HCWs (H4, H5, H7).

Pulsotype D was isolated from only one patient (P4) and was resistant to erythromycin, tetracycline, gentamicin, ciprofloxacin and chloramphenicol.

Table 2. MRSA colonisation among HCWs

Specimen	No. of MRSA isolates (%)
One hand	2 (8)
Both hands	1 (4)
Nares	3 (12)
One hand & Nares	1 (4)
Both hands & Nares	2 (8)
Total	9 (36)

Table 3. PFGE & antibiotyping of MRSA isolates.

Isolate	Specimen	PFGE pattern	Antimicrobial susceptibility						
			ERY	SXT	TCY	GEN	CIP	CHL	CLI
P5	Sputum	A1	R	S	R	R	R	S	R
P6	Sputum	A2	R	R	R	R	R	R	R
P2	Sputum	A3	R	R	R	R	R	R	R
H1	Nose	A3	R	R	R	R	R	S	R
P7	Blood	A3	R	R	R	R	R	R	R
H9	Right hand	A3	R	R	R	R	R	R	R
V1	Monitor	A3	R	R	R	R	R	R	R
V2	Ventilator	A3	R	R	R	R	R	R	R
H2	Left hand	B1	R	R	R	R	R	R	R
H3	Right hand	B2	R	R	S	R	R	R	R
H6	Nose	B3	R	S	R	R	R	R	R
H8	Nose	B3	R	S	R	R	S	R	S
P1	Sputum	C1	R	R	R	S	R	R	R
P3	Sputum	C2	R	R	R	R	R	S	R
H4	Right hand	C2	R	R	R	R	R	R	R
H5	Right hand	C2	R	R	S	R	R	R	R
H7	Left hand	C2	R	R	R	R	R	S	R
P4	Pus	D	R	S	R	R	R	R	S

P= patient isolates, H= health care worker isolates, V= environmental isolates, PFGE= pulsed-field gel electrophoresis, ERY= erythromycin, SXT= trimethoprim-sulfamethoxazole, TCY= tetracycline, GEN= gentamicin, CIP= ciprofloxacin, CHL= chloramphenicol, CLI= clindamycin, S= sensitive, R= resistant.

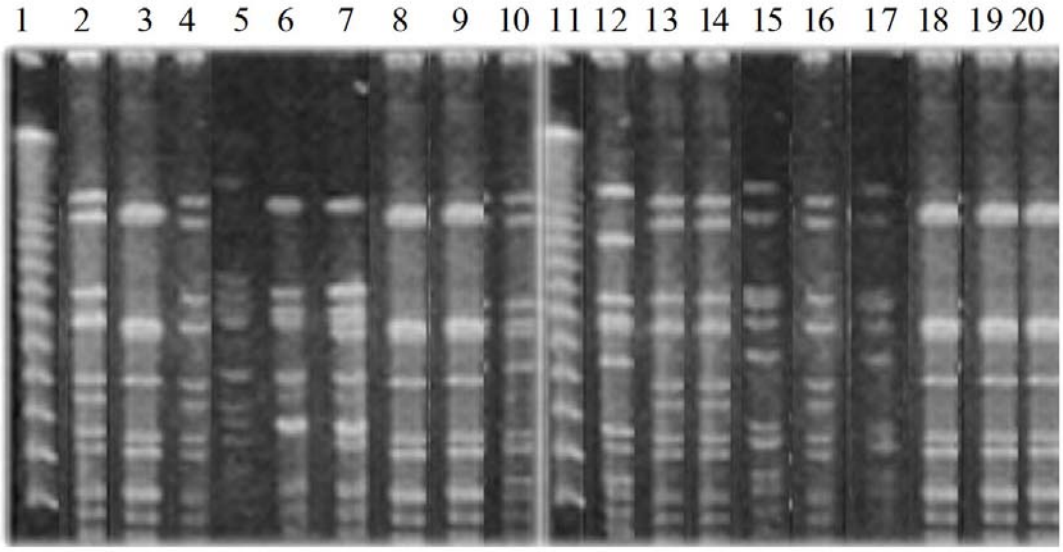

Fig. 1. PEGE profiles of Smaai macrorestriction fragments of the 18 MRSA isolated Lane 1 & 11: Standard marker (molecular size in kilobases), Lane 2 to 8: P1-7 respectively, Lane 9 to 18 (excpet 11): H 1-9 respectively, Lane 19 & 20: V1 -2

DISCUSSION

The prevalence of MRSA in the hospitals has been steadily increasing in the past decade[27]. A four-fold increase in the annual prevalence of MRSA causing nosocomial infection was noted from 1990 to 2000. This is parallel to the increasing consumption of extended-spectrum cephalosporins, carbapenems, ciprofloxacin, and glycopeptides in the hospitals[28]**.**

The few studies that have reported the incidence of MRSA in Saudi Arabia have indicated that a diverse number of circulating MRSA strains have been detected in several major hospitals[29]. This leads us to examine its epidemiology in an ICU in a hospital in Al-Madinah Al-Munawarah, KSA. Our study recorded the nosocomial infection rate of MRSA; 5.98 %.

MRSA is often resistant to all penicillins, penems, carbapenems, and cephalosporins. Antimicrobial agents that are effective against MRSA vary between hospitals, as each hospital has its own unique resistance pattern[30]. Thus, knowledge of the clonal relationship of MRSA and its antimicrobial susceptibility patterns can contribute to hospital infection control efforts in monitoring and limiting the spread of MRSA in and between hospitals[31]. Multidrug-resistant MRSA, defined as isolates with resistance to three or more drug classes other than β-lactam antibiotics, accounted for 95% of all MRSA isolates[27]. All MRSA isolates in this study showed multidrug resistance. However, isolates with reduced susceptibility to vancomycin (VISA), quinupristin/dalfoprestin, rifampin and teicoplanin, were not found in our study. Similarly, no such isolates have been detected nor have they been reported by other researchers in Saudi Arabian hospitals. This is reassuring and indicating that VISA has not yet set foot in the Saudi hospitals studied[32]. Similar results have also been reported as regard these antibiotics except quinupristin/ dalfopristin; less than 5% of isolates from patients were non susceptible in National Taiwan University Hospital[27].

Some isolates within the same pulsotype had varied antibiotic susceptibility pattern,such as pulsotype A3 had varied susceptibility to chloramphenicol and pulsotype B3 had varied susceptibility to ciprofloxacin. Also, there was varied susceptibilities to chloramphenicol and tetracycline within patterns C2. The molecular basis for the varied susceptibilities to these antibiotics is not clearly uknown at the present time. Horizontal

gene transfer may play a role as resistance genes for these classes of antibiotics are often carried on transferable genetic elements[33,34].

Our data provide support that colonization by MRSA among HCWs has been associated with increased risk of MRSA nosocomial infection and could potentially facilitate transmission. Medical staff can be a vehicle in the spread of MRSA within a hospital, as direct person-to-person contact contributes to the transmission of MRSA. It is generally believed that MRSA may spread from colonized HCWs to patients and vice versa[11,35].

In our study, it was alarming to find several HCWs whose isolates demonstrated the same PFGE patterns (pulsotype A3 and pulsotype C2) as those isolates from patients (P2,3,7), thereby establishing the transmission of MRSA between patients and HCWs. The fact that several HCWs also carried MRSA with indistinguishable PFGE patterns from that of the patients' isolates is a great concern, how and when these HCWs and patients acquired these MRSA strains is not known. However, this finding further emphasizes the need for routine surveillance of HCWs and patients at risk for MRSA carriage. Furthermore, the antibiograms of patient isolates and the other MRSA strains carried by HCWs showed that they might be closer to the community acquired MRSA reported in other countries[33,34]. So, further molecular characterization of the isolates in this study and circulating strains of community-acquired MRSA may clarify its origin and identify factors allowing its persistence in the hospital environment.

Environment may serve as a reservoir for MRSA, in addition to the person-to-person transmission observed, as *S. aureus* can survive for relatively long survival periods on inanimate surfaces. *S. aureus* is stable in dry environments with a survival time of 12 days (1 to >60 days) on inanimate surfaces in ICUs. In comparison, the survival time of other important pathogens, such as *Pseudomonas aeruginosa*, is around 1.5 days in ICU [36,37].

We found that MRSA isolates obtained from the environmental samples were shared the same PFGE pattern (pulsotype A3) as that isolated from two patients (P2,7) and two HCWs (H1,9). So, our finding confirmed the role of the environment in maintaining MRSA and thus facilitating its transmission. MRSA from patients can contaminate the environment, being transmitted back to other patients through HCWs due to unsatisfactory infection control measures[38,39].

CONCLUSION

In our study, person-to-person and environment-to-person (or vice versa) transmission has been documented. The increasing rate of nosocomial MRSA infections and colonization indicated that the infection control program needs much strengthening. Strict hand washing before and after patient contact must be enforced and monitored, as it remains the key infection control measure for controlling the spread of MRSA. To prevent the emergence of vancomycin-resistant SA and the transmission of multidrug-resistant organisms. Implementation of periodic or routine active surveillance cultures and antibiotic policy monitoring may also be considered as part of the infection control measures.

ACKNOWLEDGMENTS

This work was supported by grant No.482/430 from Deanship of scientific Research in Taibah University, KSA. The authors are also thankful to Dr. Mohamad Abd Al-Rahman; consultant and head of Microbiology Laboratory and Dr. Khaled Shaaban; Director of Lab.and Blood Bank in King Fahd Hospital, Al-Madinah Al-Monawarah, KSA.

REFERENCES

1. Barber, M. Methicillin-resistant *Staphylococci. J. Clin. Pathol.*, 1961; **14**: 385-393.
2. Jevons, M. P. Celbenin-resistant *Staphylococci. Br. Med. J.*, 1961; **1**: 124-125.
3. Cury, G., Mobilon, C., Stehling, E. G., Lancellotti, M., Romas, M., Martinez, R., Brocchi, M. *et al*. Molecular typing of methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated in two metropolitan areas of São Paulo State, southeast Brazil. *Braz. J. Infect. Dis.*, 2009; **13**: 165-169.
4. Panlilio, A. L., Culver, D. H., Gaynes, R. P. et al. Methicillin-resistant *Staphylococcus aureus* in US hospitals 1975-1991. *Infect. Control.*

Hosp. Epidemiol., 1992; 13: 582-586.

5. Voss, A., Milatovic, D., Wallrauch-Schwarz, C., Rosdahl, V. T., Braveny, I. Methicillin-resistant *Staphylococcus aureus* in *Europe. Eur. J. Clin. Microbiol. Infect. Dis*., 1994; **13**: 50-55.
6. Kikuchi, K. Genetic basis of neonatal methicillin-resistant *Staphylococcus aureus* in Japan. *Pediatr. Int.*, 2003; **45**: 223-239.
7. McDougal, L. K., Steward, C. D., Killgore, G. E., Chaitram, J. M., McAllister, S. K., Tenover, F. C. Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. *J. Clin. Microbiol*., 2003; **41**: 5113-5120.
8. Rohani, M. Y., Zainuldin, M. T., Koay, A. S., Lau, M. G. Antibiotic resistance patterns of bacteria isolated in Malaysian hospitals. *Int. Med. J.*, 1999; **6**: 47-51.
9. Shittu, A. O., Udo, E. E., Lin, J. Phenotypic and molecular characterization of *Staphylococcus aureus* isolates expressing low- and high-level mupirocin resistance in Nigeria and South Africa. *BMC. Infect. Dis.*, 2009; **9**: 10-19.
10. Andersen, B. M., Lindemanny, R., Berghz, K., Nesheimx, B. I., Syversen, G., Solheim, N., Laugerud, F. Spread of methicillin-resistant *Staphylococcus aureus* in a neonatal intensive unit associated with understaffing, overcrowding and mixing of patients. *J. Hosp. Infect.*, 2002; **50:** 18-24.
11. Lessing, M. P., Jordens, J. Z., Bowler, I. C. Molecular epidemiology of a multiple strain outbreak of methicillin-resistant *Staphylococcus aureus* amongst patients and staff. *J. Hosp. Infect*., 1995; **31**: 253-260.
12. Meier, P. A., Carter, C. D., Wallace, S. E., Hollis, R. J., Pfaller, M. A., Herwaldt, L. A. A prolonged outbreak of methicillin-resistant *Staphylococcus aureus* in the burn unit of a tertiary medical center. *Infect. Control. Hosp. Epidemiol.*, 1996; **17**: 798-802.
13. Wagenvoort, J. H. T., Penders, R. J. R. Long-term in-vitro survival of an epidemic MRSA phage-group III-29 strain. *J. Hosp. Infect.*, 1997; **35**: 322-324.
14. Neely, A. N., Maley, M. P. Survival of enterococci and staphylococci on hospital fabrics and plastic. *J. Clin. Microbiol.*, 2000; **38**: 724-726.
15. Kupfer, M., Jatzwauk, L., Monecke, S., Möbius, J., Weusten, A. MRSA in a large German University Hospital: Male gender is a significant risk factor for MRSA acquisition. GMS. *Krankenhaushygiene Interdisziplinär.,* 2010; **5**(2): 1-8.
16. Tenover, F. C. Comparison of traditional and molecular methods of typing isolates of *Staphylococcus aureus. J. Clin. Microbiol.,* 1994; **32**: 407-415.
17. van Belkum, A. W., van Leeuwen, M. E., Kaufmann, B., Cookson, F., Foery, J., Etienne, R., et al. Assessment of resolution and intercenter reproducibility of results of genotyping *Staphylococcus aureus* by pulsed-field gel electrophoresis of *Sma*I macrorestriction fragments: a multicenter study. *J. Clin. Microbiol*., 1998; **36**: 1653-1659.
18. Deplano, A., Schuermans, A., Van Eldere, J., Witte, W., Meugnier, H., Etienne, J., et al. Multicenter evaluation of epidemiological typing of methicillin-resistant *Staphylococcus aureus* strains by repetitive-element PCR analysis. *J. Clin. Microbiol.*, 2000; **38**: 3527-3533.
19. van Belkum, A. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* strains: state of affairs and tomorrow's possibilities. *Microb. Drug. Resist*., 2000; **6**: 173-188.
20. Murchan, S., Kaufmann, M. E., Deplano, A., de Ryck, R., Struelens, M., Zinn, C. E., et al. Harmonization of pulsed-field gel electrophoresis protocols for epidemiological typing of strains of methicillin-resistant *Staphylococcus aureus*: a single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strains. *J. Clin. Microbiol*., 2003; **41**: 1574-1585.
21. Al-Thawadi, S. I., Kessie, G., Dela Cruz, D., Al-Ahdal, M. N. A comparative study on the application of 3 molecular methods in epidemiological typing of bacterial isolates using MRSA as a prototype. *Saudi. Med. J*., 2003; **24**(12): 1317-24.
22. Cookson, B., Peters, B., Webster, M., Phillips, I., Rahman, M., Noble, W. Staff carriage of epidemic methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol., 1989; **27**: 1471-1476.
23. Eveillard, M., Martin, Y., Hidri, N., Boussougant, Y., Joly-Guillou, M. L. Carriage of methicillin-resistant *Staphylococcus aureus* among hospital employees: prevalence, duration, and transmission to households. *Infect. Control. Hosp. Epidemiol.,* 2004; **25**: 114-20
24. National Committee for Clinical Laboratory Standards. Methods for Antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard, 2000, 5th edn. Wayne: NCCLS M7-A5.
25. Tenover, F. C, Arbeit, R. D., Goering, R. V., Mickelsen, P. A., Murray, B. E., Persing, D. H.,

et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J. Clin. Microbiol.*, 1995; **33**: 2233-2239.

26. Madison, R., Afifi, A. A. Definition and comparability of nosocomial infection rates. *Am. J. Infect. Control.*, 1982; **10**: 49-52.
27. Hsueh, P. R., Teng, L. J., Chen, W. H., Pan, H. J., Chen, M. L., Chang, S. C., et al. Increasing Prevalence of Methicillin-Resistant *Staphylococcus aureus* Causing Nosocomial Infections at a University Hospital in Taiwan from 1986 to 2001. *Antimicrob. Agent. Chemother*., 2004; **48**: 1361-1364.
28. Huang, A. H., Yan, J. J., Wu, J. J. Rapid dissemination of *Staphylococcus aureus* with classic oxacillin resistance phenotype at a new university hospital. *J. Hosp. Infect.*, 2000; **44:** 309-315.
29. Baddour, M. M., Abuelkheir, M. M., Fatani, A. J., Bohol, M. F., Al-Ahdal, M. N. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from major hospitals in Riyadh, *Saudi Arabia. Can. J. Microbiol.*, 2007; **53**(8): 931-6.
30. Sanches, I. S., Mato, R., Lancastre, HDe., Tomaz, A. Patterns of multidrug resistance among methicillin-resistant hospital isolates of coagulase-positive and coagulase-negative staphylococci collected in the International Multicenter Study RESIST in 1997 and 1998. *Microb. Drug. Resist.*, 2000; **6**: 199-211.
31. Lin, T. K., Junnie, J., Liew, F. Y., Yusof, M. Y., Hanifah, Y. A. Antibiograms and Molecular Subtypes of Methicillin-Resistant *Staphylococcus aureus* in Local Teaching Hospital, Malaysia. *J. Microbiol. Biotechnol.*, 2009; **19**(10): 1265-1270.
32. Baddour, M. M., Abuelkheir, M. M., Fatani, A. J. Trends in antibiotic susceptibility patterns and epidemiology of MRSA isolates from several hospitals in Riyadh, *Saudi Arabia. Ann. Clin. Microbiol. Antimicrob.*, 2006; 5: 3040.
33. Chambers, H. F. The changing epidemiology of *Staphylococcus aureus*? Emerg. Infect. Dis., 2001; **7**: 178-182.
34. McDougal, L. K., Steward, C. D., Killgore, G. E., Chaitram, J. M., McAllister, S. K., Tenover, F. C. Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. *J. Clin. Microbiol.*, 2003; **41**: 5113-5120.
35. Wang, J. T., Chang, S. C., Ko, W. J., Chang, Y. Y., Chen, M. L., Pan, H. J. *et al.* A hospital-acquired outbreak of methicillin-resistant *Staphylococcus aureus* infection initiated by a surgeon carrier. J. *Hosp. Infect.*, 2001; **47**: 104-109.
36. Neely, A. N. A survey of Gram-negative bacteria survival on hospital fabrics and plastics. *J. Burn. Care. Rehabil.*, 2000; **21**: 523-527.
37. Neely. A, Maley, M. Survival of *enterococci* and *staphylococci* on hospital fabrics and plastic. *J. Clin. Microbiol.*, 2000; **38**: 724-726.
38. Asoh, N., Masaki, H., Watanabe, H., Watanabe, K., Mitsusima, H., Matsumoto, K. *et al*. Molecular characterization of the transmission between the colonization of methicillin-resistant *Staphylococcus aureus* to human and environmental contamination in geriatric long-term care wards. *Intern. Med.*, 2005; **44**: 41-45.
39. Sexton, T., Clarke, P., O'Neill, E., Dillane, T., Humphreys, H. Environmental reservoirs of methicillin-resistant *Staphylococcus aureus* in isolation rooms: correlation with patient isolates and implications for hospital hygiene. *J. Hosp. Infect.*, 2006; **62**: 187-194.