
Frequency of *MecA* gene and Susceptibility Pattern in Airborne Methicillin Resistant *Staphylococcus aureus* Isolated from Different Hospitals in North of Jordan.

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Summary:

Methicillin-resistant *Staphylococcus aureus* (MRSA) is seen with increasing frequency in hospitals and considered as a major cause of hospital acquired infection. Our objective is to evaluate the presence of airborne MRSA in the operating theatres (OT), intensive care units (ICU) and nursery intensive care units (NICU) of six Hospitals, North of Jordan, their susceptibility to different antibiotics and their analysis for the presence of methicillin resistant gene (*mecA*) by PCR. Thirty four air samples of 100 liters volume/min were collected by a microbiological air sampler from the above units during the period June-December/2005. Air samples were impacted on trypticase soy agar (TSA) then incubated at 37 °C for 48 h. Each bacterial colony appeared on agar plates were sub-cultured on TSA or blood agar with incubation at 37 °C for 24-48 h, and then identified by standard methods. From all *S. aureus*, 17.2 % were identified as MRSA which comprised 5.8%, 3.9% and 7.5 % in the ICU, NICU and OT, respectively. MRSA isolates were remarkably susceptible (>80%) to each of amikacin, chloramphenicol, imipenem and rifampin. MRSA isolates were shown in all units with MIC value between 4 to >256µg/l in hospitals units. PCR analysis of 96.8 % MRSA isolates indicated the amplification of the *mecA* gene with size band of 220 bp.

Key Words: Airborne, Antibiotic, MRSA, *mecA* gene

Introduction:

The presence of airborne microorganisms within the indoor environments of hospitals is of increasing concern with respect to many acute diseases, infections, allergies, and types of microorganisms.^{1,2} Different types of Gram positive cocci were reported to be transmitted in hospital environments though the air and cause the infection of the patients. They include methicillin-resistant *Staphylococcus aureus* (MRSA), coagulase-positive staphylococci and beta-hemolytic streptococci.^{3,4}

Methicillin-resistant *Staphylococcus aureus* (MRSA) is of a special concern because they are resistant to numerous antibiotics and associated with community-acquired infection and considered as a major nosocomial pathogen that can have serious consequences in terms of increased patient mortality, morbidity, length of hospital stay and overall costs.^{5,6} MRSA was isolated from hospital air, medical equipment and devices⁷⁻⁹ it also has been reported in ICU, NICU and OT especially in cases where MRSA has colonized the respiratory tract of patients and carriers and

transmission among persons within hospitals or the community.¹⁰⁻¹² MRSA was found to comprise 16 % of all bacterial isolates sampled from the air of hospitals¹¹ and 12.98% from all isolated bacteria from air of ICU.¹³

Most MRSA resistance commonly mediated by the *mecA* gene which is chromosomally encoded. *mecA* is a large element called a resistance island that is only rarely transferred horizontally from one organism to another because of its size and complexity. Harboring *mecA* gene is not sufficient for methicillin resistance; some *S. aureus* (<2%) strains containing the *mecA* gene are susceptible to methicillin.^{14,15} The *mecA* gene is widely distributed in both coagulase positive and coagulase negative staphylococci, is carried on a transposon, and appears to integrate into a single site in the staphylococcal chromosome along with an additional 30 kb of DNA, the *mec* locus.¹⁶

Identification of MRSA by molecular methods is widely used and based in general on the detection of *S. aureus* specific gene target the *mecA* gene.¹⁷

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Several methods have been used to identify and detect the MRSA isolates including the 1µg of oxacillin disk diffusion test, agar plate screen, the micro broth dilution and Epsilometer test (E-test) to evaluate MIC and polymerase chain reaction (PCR) amplification of *mecA* gene. For typical detection of MRSA isolates, most of these methods include media supplemented with NaCl.^{18,19}

This article reports the findings of a study on the measurements of airborne Gram positive cocci present in the indoor environments of the operating theatres (OT), intensive care units (ICU) and nursery intensive care units (NICU) of six Hospitals, North of Jordan. In addition to the presence of airborne MRSA in the above units, their susceptibility to different antibiotics and their content of *mecA* gene were evaluated.

Materials and Methods:

Collection of air samples

During the period between June to December /2005, a total of 258 air samples were collected from operating theatres (OT), intensive care units (ICU) and nursery intensive care units (NICU) of six different hospitals serving a population of about 1.3 million. 128 separated samples were collected from the OT, and 68, 62 other samples were collected from the ICU and the NICU, respectively. Each air sample was collected manually or by a microbiological air sampler (M.A.Q.S.II-90)/ OXOID, UK) that holds 90 mm Petri dishes within an autoclavable anodized aluminum head of 380 holes. The sampler was set at an air-sampling rate 100 L/min for two minutes per sample. Duplicated air samples were collected from each visit at different sites of each unit with a one m elevation from the floor (i.e. the same level of the patient's bed). Samples from the OT were collected before the operation when the room is empty.

Sample processing

After impacting the air samples on Trypticase Soy Agar (TSA) media plates, they were transported to the laboratory and immediately incubated at 37 °C for 48 h to determine the total Gram positive cocci bacterial count. Diversity and total counts of bacteria on TSA plates were recorded by using the colony counter (560, Suntex, Labolan).

Bacterial identification

Based on Gram stain results, each Gram positive cocci bacterial colony was sub-cultured under aseptic conditions on different culture media for isolation pure form,

identification, and testing the susceptibility of the isolates for common used antibiotics. The culture media that have been used were: blood agar with 5-7% defibrinized blood, Mueller-Hinton agar, mannitol salt agar, and nutrient agar. All of the above agar or broth media were obtained from Oxoid Company, UK.

Identification of *Staphylococcus aureus*

Identification of *Staphylococcus* was based on the catalase test which was performed to the standard methods (20) and on the morphology of colonies. They can grow on mannitol and their coagulation of plasma indicates positive results of coagulase production especially for *S. aureus*.

Antimicrobial susceptibility test

Plates and inoculums preparation

Test organism from isolated culture was activated by inoculation into Mueller-Hinton broth or trypticase soy broth (Oxoid, UK) then incubated at 37 °C for 24 h. Turbidity of organisms in the broth was adjusted to be equal to or greater than 0.5 McFarland turbidity standards (1.5×10^8 cfu/ml). Mueller-Hinton agar plates and 0.5 McFarland standards were prepared according to the recommendation of Kirby-Bauer disc diffusion procedure (Bauer *et al.*, 1966) and NCCLS.²¹ The test organisms were homogeneously inoculated by a sterile cotton swab on the surface of two freshly prepared Mueller-Hinton agar plates. Plates were incubated at 37 °C for 24 h.

Application of disks and incubation

Eighteen different antibiotic discs (Oxoid, UK) (Table 1) were used for susceptibility test of *Staphylococcus aureus*. Antibiotic disks were applied to the agar surface under aseptic conditions. Plates were then inverted and incubated at 35°C for 16-18 h. The results of susceptibility test (inhibition zone diameter) for each isolate were measured in (mm) and interpreted according to NCCLS standards on a special data sheet.

Identification of methicillin resistant *S. aureus* (MRSA)

The isolated MRSA were confirmed by widely used standard methods including the positive coagulase reaction and growth on mannitol salt agar for isolation *S. aureus*, disk diffusion (1 µg oxacillin and cefoxitin 30 µg) on Mueller Hinton agar and E-test.²² Any bacterium identified as coagulase positive Gram positive cocci and was able to ferment mannitol by growing on mannitol salt agar was described as *S. aureus*. Methicillin resistant *S. aureus* (MRSA) was detected from all isolated *S.*

aureus by the inoculation of the tested organisms on 4% NaCl Mueller-Hinton agar and then a disc of Oxacillin (OX, 1 µg) and cefoxitin (FOX, 30 µg) was applied to the plates. The inoculated plates were incubated at 37 °C for 24 h, the diameter of the inhibition zone around the Oxacillin disc was measured. The results were interpreted according to NCCLS standards on a special data sheet.²²

E- Test method

The E-test method was performed according to Carolyn *et al.*, (1994) on Mueller Hinton agar plates.²³

Handling and applying of E-test strips

E-test strip (AB Biodisk/Sweden) was applied to the central of the inoculated agar plates by a sterile forceps with complete contact with the agar surface and the MIC scale facing upwards. The plates were incubated at 37 °C for 24 h then the MIC was visually observed by detection of the inhibition ellipse intersects the scale (Fig. 1). The results of MIC of oxacillin and susceptibility were reported according to NCCLS (2005).

Detection of *mecA* gene by PCR

Growth conditions

All of the 31 MRSA isolates were cultured on nutrient broth (Oxoid) at 37 °C with shaking at 140 rev min⁻¹ for 24 h. Purity of the cultures was confirmed by plating 0.1 ml from the broth on TSA plates and incubated at 37 °C for 48 hours.

Extraction of genomic DNA from methicillin-resistant *Staphylococcus aureus* (MRSA) isolates.

Genomic DNA extraction was conducted using Wizard Genomic DNA Purification Kit (Promega, USA). One ml of a 24 h broth growth was used. All DNA manipulation, handling and PCR work was conducted using DNase, RNase-Free barrier tips.²⁴

Estimation of the purity and quantity of the extracted DNA.

The isolated DNA was checked for purity and quantitated by spectrophotometric method.²⁵ In each extraction group one sample (selected randomly) was extracted twice, and was used in the estimation process. One sample (50 µl) was diluted in 950 µl TE buffer and measured at 260 and 280 nm wavelengths (Genesys 2, Milton Roy, USA). The readings at 260 nm were used to calculate the DNA concentration in the original sample, where 1 OD corresponds to 50 µg/ml of double-stranded DNA. The ratio between the readings at 260

nm and 280 nm (OD₂₆₀ /OD₂₈₀) was used to estimate the purity of the DNA.

Primers for detection of *mecA* gene (*mecA*).

Two primers (Operon Technologies, USA) were selected,^{22,24} were used in the PCR study. These primers were forward primer (*mecA*₁) of 15 mer long: 5'-GCAATCGCTAAAGAA-3', and reverse primer (*mecA*₂) of 20 mer long: 5'-GGGACCAACATAACCTAATA-3'.

PCR amplification

Amplification reactions were performed in volumes of 20 µl containing 7.76 µl of master mix (Promega, USA), 0.9 µl of each forward and reverse primer (200 pmol/µl), 10.24 µl nuclease free water and 2 µl of template DNA. PCR amplification was carried out in 0.2 ml thin walled, nucleases free water PCR tubes (Treff lab, Switzerland) using iCycler thermocycler (Bio-Rad, USA) programmed as followed: initial denaturizing step at 94°C for 3 min for the first cycle, 94 °C for 10 sec and 53 °C for 20 sec for the next 30 cycles, and 72 °C for 5 min in the last cycle. After that, the tubes were held at 4 °C for direct use, or stored at -20 °C.

Electrophoresis and photography

Electrophoresis was carried out at 100 Volts for 1.5 hours. The size of the PCR products were estimated using 100 bp DNA ladder (Promega, USA) and viewed on 2% agarose (Promega, USA) in 1X TBE buffer and detected by staining with ethidium bromide (EB) (Acros Organic, USA) at 0.5 µl ml⁻¹ final concentration. DNA bands containing-gels were viewed using Gel Doc (Bio-Rad, USA) and photographed using the same instrument.

Results and Discussion:

In this study bacterial count and diversity were variable with wide range in OT, ICU and NICU between the different hospitals this may due to many factors including the number of visitors, cases of patients, opened doors and the amount of material brought from outside, such as flowers and fruits.^{1,2,26} These were recognized as sources of hospital contamination.²⁷ Bacterial counts in OT were less than ICU and NICU. This is due to the high sanitary standards in OT, as compared to other hospital areas.

Diversity of bacteria is usually related to the count. In this study the bacterial diversity was similar in units of each hospital. Ross & Menezes (2004) approved that the high microbial biodiversity is associated with high temperature and relative air humidity that favors microbial growth.²⁸

The results of cultured sample identification show three major groups of bacteria were Gram positive cocci (91 %), *Staphylococcus* was the most isolated Gram positive cocci bacteria (78%) in all units; of which *S. aureus* comprised of (36.2%) of all isolated *Staphylococcus* and (28%) of all Gram positive cocci bacteria, MRSA was comprised of 4.8 % from all isolated Gram positive cocci bacteria and 17.2 % from all isolated *S. aureus* (Table 2). That can be explained by the ability of *Staphylococcus* spp. to survive relatively well in the air compared to many bacteria.^{1,27} The markedly high percentage of the isolated Gram positive bacteria in NICU may be related to the high number of visitors in summer and to the outdoor contamination. In addition the results can be related to the different factors such as human activities, number of visitors and the number of operations.^{11,29}

Antimicrobial susceptibility profiles

The different antibiotics that are commonly used in Jordan were evaluated against all *S. aureus* and MRSA isolates by disc diffusion method (Table 1). *S. aureus* isolates were resistance to cefoxitin 33.1%, oxacillin 40%, azithromycin 70%, clarithromycin 50.9% and erythromycin 58.2% (Table 1). These results are consistent with results reported by Shehabi & Baadran (1996). However, they showed a remarkable susceptibility ($\geq 80\%$) to amikacin, amoxicillin, ampicillin, cefaclor, cefuroxime, chloramphenicol, ciprofloxacin, gentamycin, imipenem, penicillin, rifampin and tetracycline (Fig. 2). This means that these are acceptable alternative antibiotics used for *S. aureus* infection treatments.³⁰

Distribution of methicillin-resistant *Staphylococcus* (MRSA)

Methicillin-resistant *Staphylococcus* (MRSA) was isolated from the hospital units and identified based on the ability of *S. aureus* to resist oxacillin (OX) and cefoxitin (FOX). MRSA was isolated from the environment of ICU,^{12,13} NICU (10, 31) and from OT.^{3,7} From all *S. aureus*, 17.2% (31/180) of them were identified as MRSA because they completely resist both antibiotics (oxacillin and cefoxitin) (Table 2). MRSA comprised of 43.7%, 22.9% and 33.4% in the ICU, NICU and OT from total isolated *S. aureus*, respectively. Shehabi and Baadran (1996) reported that MRSA comprised of 42 % of total isolated *S. aureus* from Jordanian ICU patients. Other studies reported MRSA comprised of 40-50 % and 42 % of total isolated *S. aureus* in hospital patients and

hospital staff, respectively,²⁷ and 38 % in those hospitals with more than 500 beds.¹⁰ The high percentages of MRSA in OT and ICU than the other units were correlated with the excess use of disinfection process antibiotics and patient infection.^{10,32-34}

Data indicated that MRSA isolates are remarkably susceptible ($>70\%$) to each of amikacin, ampicillin, chloramphenicol, ciprofloxacin, gentamycin, imipenem, rifampin and tetracycline (Fig. 2). This pattern of susceptibility is similar to the results of other studies.^{30,32,33}

When *E-test* method was carried out for the methicillin-resistant *Staphylococcus* (MRSA) isolates, data in table 3 indicated that the MIC value of the MRSA isolates was ranged from 4 to >256 $\mu\text{g/l}$ in all studied units. The high resistant MRSA percentage (> 256) was present in ICU isolates 63.6% (7/11) isolates. While, the percentage of high resistant MRSA in OT and NICU was 15.4 % (2/13) and 14.3 % (1/7), respectively. The results presented here are similar to that of Merlino *et al.*, (2002) which may be associated with increased expression of multiple antibiotic resistance genes.³⁵

PCR study for *mecA* gene

A Total of 31 coagulase positive MRSA isolates (table 3) were tested for the presence of *mecA* gene. A DNA fragment of 220 bp was amplified from DNA of all of the tested MRSA isolates (Fig. 3). When the presence of the *mecA* gene is correlated with the resistance level in the aerosol isolates of *S. aureus* and the MIC of oxacillin and cefoxitin data indicated that one (3.2%) of 31 methicillin-resistant *S. aureus* was classified as *mecA* gene negative *S. aureus* according to Merlino *et al.*,³⁵ this negative detection is due to hyperproduction of type A β -lactamase. When the E test was correlation with PCR pattern of the *mecA* gene and the susceptibility to Oxacillin, data indicated that 30 isolates (96.8%) were positive for the *mecA* gene which is similar to the results reported by.^{32,34-37}

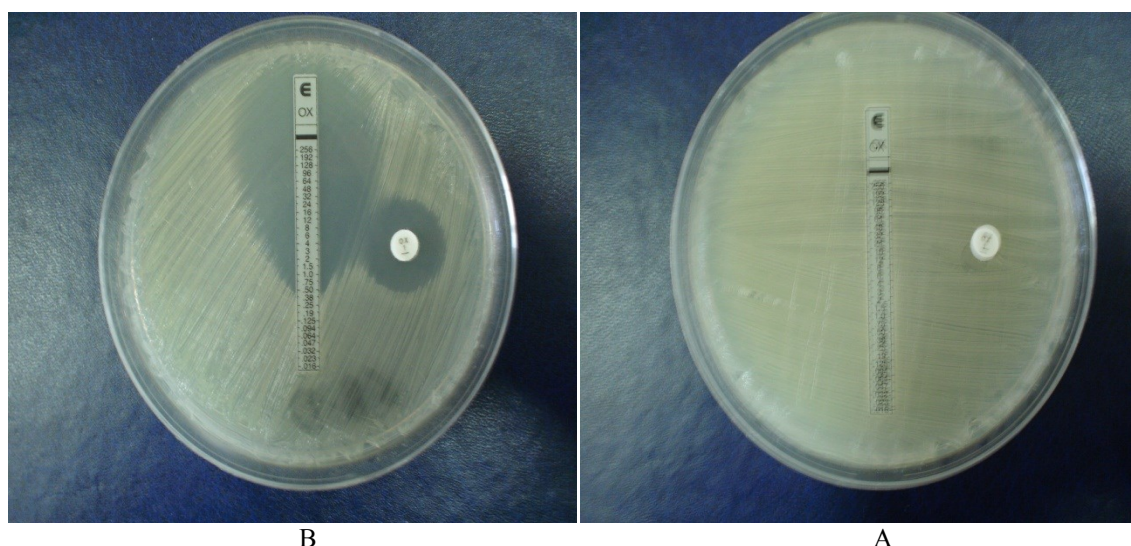
Therefore, continuous monitoring and control of MRSA in hospital environments, carriers and patients, and scientific using of antimicrobial drugs and disinfection process are recommended.

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Tables and figures:**Table 1: Antibiotic panel used for *S. aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) and the percentage resistance of these bacteria to different antibiotics.**

Antibiotic	Symbol	Concentration (ug)	<i>S. aureus</i>	MRSA
Amikacin	AK	3	3.5%	12.9%
Amoxicillin	AX	25	9.4%	51.6%
Ampicillin	AM	10	11.3%	16.1%
Azithromycin	AZM	15	70%	86%
Cefaclor	CEC	30	7.5%	64.5%
Cefotaxime	CTX	30	20%	80.6%
Cefoxitin	FOX	30	33.1%	100%
Cefuroxime	CXM	30	15%	54.8%
Chloramphenicol	C	30	11.3%	12.9%
Ciprofloxacin	CIP	5	6.6%	16.1%
Clarithromcin	CLR	15	50.9%	51.6%
Erythromycin	E	15	58.2%	61.2%
Gentamicin	GN	10	9.4%	16.1%
Imipenem	IPM	10	4.7%	12.9%
Oxacillin	OX	1	40%	100%
Penicillin	P	10	13.2%	61.3%
Rifampin	RA	10	5.2%	12.9%
Tetracycline	TE	30	9.4%	19.4%
Total			18	18

**Figure 1: E-Test Method for a resistant (A) and susceptible (B) *S. aureus* isolate to Oxacillin.****Table 2: the distribution percentages of isolated bacteria from the total isolates.**

	*OT (%)	*ICU (%)	*NICU (%)	*T (%)
<i>Gram positiveve cocci</i>	39	25	27	91
<i>Staphylococcus spp</i>	28	24.5	25.5	78
<i>S. aureus</i>	10.5	8.5	9	28
MRSA	2.1	1.6	1.1	4.8
MRSA from all isolated <i>S. aureus</i>	7.5	5.8	3.9	17.2

*OT: operating theatres, ICU: intensive care units, NICU: nursery intensive care units, T: Percentage from the total isolated bacteria.

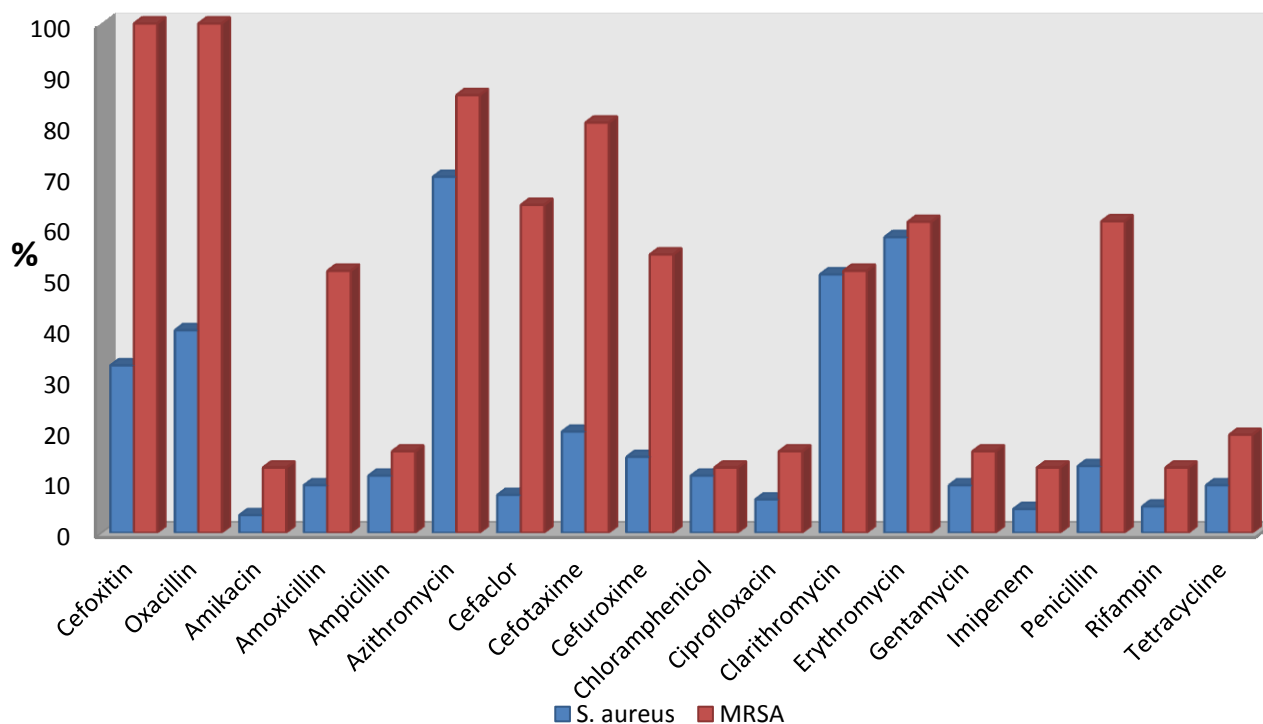


Figure 2: Antimicrobial resistance pattern of (blue) *S. aureus* and (MRSA) Methicillin-resistant *Staphylococcus aureus* (MRSA).

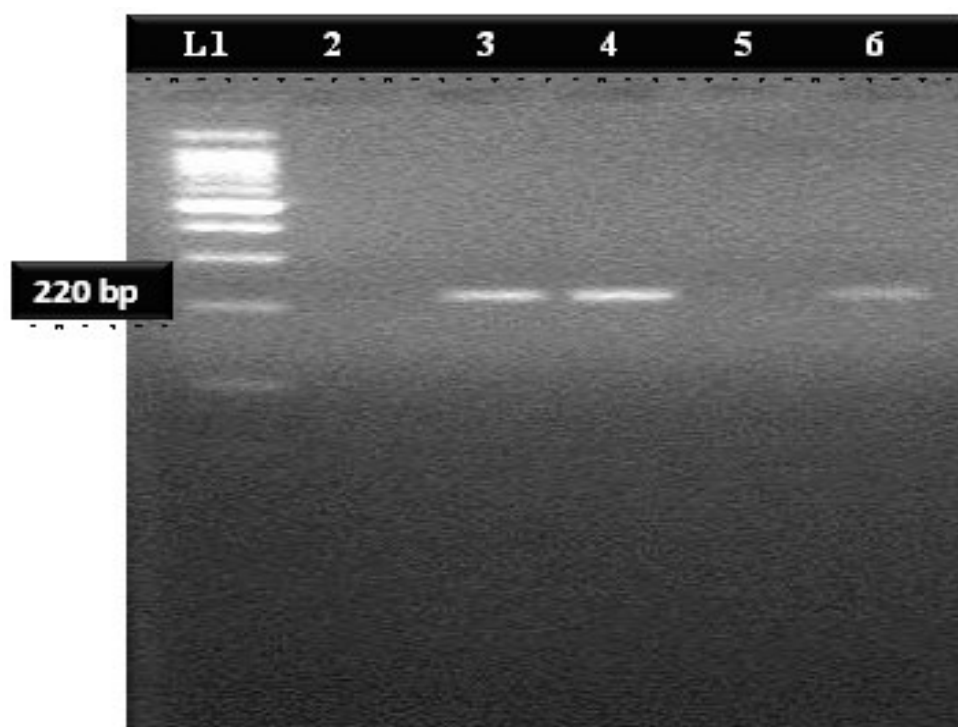


Fig. 3: Agarose gel (2%) electrophoresis of PCR amplification of *mecA* gene from methicillin-resistant *Staphylococcus aureus* (MRSA) with *mecA1* and *mecA2* primers. L1: 100bp DNA ladder, 2: negative control, 3, 4, 6: coagulase positive MRSA (positive *mecA* gene), 5: coagulase positive MRSA (negative *mecA* gene).

Table 3: the distribution of MIC values and *MecA* gene according to hospital units.

E-Test/MIC value ($\mu\text{g/l}$)	4	6	8	16	24	32	64	96	128	>256	TOTAL
MRSA IN ICU	1	0	0	2	1	0	0	0	0	7	11
MRSA IN OT	4	2	1	0	1	2	0	0	1	2	13
MRSA IN NICU	0	1	0	0	2	1	1	1	0	1	7
<i>MecA</i> gene psitive	4	3	1	2	4	3	1	1	1	10	30/31

References:

- Jaffal, A.A., Nsanze, H., Banat, I.M., Mogheth, A.A., Bener, A. & Ameen, A.S. (1997). Hospital airborne microbial pollution in a desert country. *Environ Int* 23, 167-172
- Saad, S.G. (2003). Integrated Environmental Management for Hospitals. *Indoor Built Environment* 12, 93-98.
- Javed I, Hafeez R, Zubair M, Anwar MS, Tayyib M, Husnain S. (2008). Microbiological Surveillance of Operation Theatres and ICUs of a Tertiary Care Hospital, Lahore. *African Journal of Biotechnology*, 7(20): 3535-3539.
- Newman, M.J. (2002). Neonatal intensive care unit: reservoirs of nosocomial pathogens. *West Afr J Med* 21(4), 310-312.
- Karabay O, Kocoglu E, Tahtaci M. (2007). The role of mobile phones in the spread of bacteria associated with nosocomial infections. *J Infect Developing Countries*, 1:72-73.
- Hoet AE, Johnson A, Nava-Hoet RC, Bateman S, Hillier A, Dyce J, Gebreyes WA, Wittum TE. (2011). Environmental methicillin-resistant *Staphylococcus aureus* in a veterinary teaching hospital during a nonoutbreak period. *Vect Zoonot Dis*, 11:609-615.
- Buemi, M., Floccari, F. & Netto, M. (2000). Environmental air pollution in an intensive care unit for nephrology and dialysis. *J Nephrol* 13(6), 433-6.
- Nzeako BC, Alrashied S, Neilson F, Albalkhair A. (2010). Type of Bacteria on Some Medical Devices Used in Sultan Qaboos University Hospital Wards. *Middle-East J. of Scientific Resarch*. 5(6): 449-453.
- Ekhaize FO, Ighosewe OU, Ajakpovi OD. (2008). Hospital Indoor airborne Microflora in Private and Government Owned Hospitals in Benin City. *Nigeria. World J. of Medical Science*, 3 (1): 19-23.
- Andersen, B.M., Lindemann, R., Bergh, K., Nesheim, B.I., Syversen, G., Solheim, N. & Laugeurd, F. (2002). Spread of methicillin-resistant *Staphylococcus aureus* in a neonatal intensive unit associated with understaffing, overcrowding and mixing of patients. *J Hosp Infect* 50, 18-24.
- Beggs, C.B. (2003). The airborne transmission of infection in hospital buildings: fact or fiction? *Indo Built Environ* 12, 9-18.
- Meyer E, Schwab F, Gastmeier P, Rueden H, Daschner FD. (2006). Surveillance of antimicrobial use and antimicrobial resistance in German intensive care units (SARI): a summary of the data from 2001 through 2004. *Infection*, 34:303-309.
- Cavalcanti, S.M., Franca, E.R., Cabral, C., Vilela, M.A., Montenegro, F., Menezes, D. & Medeiros, A.C. (2005). Prevalence of *Staphylococcus aureus* introduced into intensive care units of a University Hospital. *Braz J Infect Dis* 9(1), 56-63.
- Hiramatsu K. (1995). Molecular evolution of MRSA. *Microbiol Immunol*. 39:531.
- Niemeyer DM, Pucci MJ, Thanassi JA, et al. (1996). Role of *mecA* transcriptional regulation in the phenotypic expression of methicillin resistance in *Staphylococcus aureus*. *J Bacteriol*. 178:5464-71.
- Archer GL, Niemeyer DM, Thanassi JA. (1997). Dissemination among staphylococci of DNA sequences associated with methicillin resistance. *Antimicrob Agents Chenother*. 38:447-54.
- Ahmed S, Edet E. Tulsi DC. (2000). Molecular diagnosis of antimicrobial resistance. *Lebanese Medical Journal*. 48 (4): 203-7.
- Araj GF, Talhouk RS, Simaan CJ, Maasad MJ. (1999;). Discrepancies between *mecA* PCR and conventional tests used for detection of MRSA. *Inter J Antimicrobial Agents*. 11: 47-52.
- Betty AF, Danial FS, Alice SW. *Diagnostic Microbiology* 10th ed. Mospy: Don Ladig; 1998:608.
- Koneman, E.W., Allin, S.D., Janad, W.M., Schreckenberger, P.C. & Winn, W.C.

- (1997). Color Atlas and Textbook of Diagnostic Microbiology, 5th ed. USA: J. B. Lippincott Company.
21. National Committee for Clinical Laboratory Standards. (2005). Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically: Approved standards M7-A6 and N2-A8, NCCLS.
22. Smyth, R.W., Kahlmeter, G., Olsson, B.L. and Hoffman, B. (2001). Methods for identifying methicillin resistance in *Staphylococcus aureus*. J. Hos. Infect. 48: 103-07.
23. Carolyn, N.B., Ming, B.H. & Fred, CT. (1994). Optimizing testing of MRSA species. *Diagn Microbiol Infect Dis* 19, 167-70.
24. Promega cooperation. Technical manual genomic DNA purification part TM0580. USA: Madison; 2004: 12.
25. Araj GF, Talhouk RS, Simaan CJ, Maasad MJ. (1999). Discrepancies between *mecA* PCR and conventional tests used for detection of MRSA. *Inter J Antimicrobial Agents*. 11: 47-52.
26. Sessa, R., Di, P.M., Schiavoni, G., Santino, I., Altieri, A., Pinelli, S. & Del, P.M. (2002). Microbiological indoor air quality in healthy buildings. *New Microbiol* 25(1), 51-56.
27. Kalliokoski, P. (2003). Risks caused by airborne microbes in hospitals-source control is important. *Indoor Built Environment* 12, 41-46.
28. Ross, C., Menezes, J.R. (2004). Studies on fungal and bacterial population of air-conditioned environments. *Braz arch Biol Technol* 47, no.5.
29. Obbard, J.P. & Fang, L.S. (2003). Airborne concentrations of bacteria in a hospital environment in Singapore. *Water Air and Soil Pollution* 144, 333-341.
30. Shehabi, A.A. & Baadran, I. (1996). Microbial infection and antibiotic resistance patterns among Jordanian intensive care patients. *Eastern Mediterranean Health Journal* 2, 515-520.
31. Chandrashekar MR, Rathish KC, Nagesha CN. (1997). Reservoirs of nosocomial pathogens in neonatal intensive care unit. *J Indian Med Assoc*. 95(3):72-4, 77.
32. Szczepanik, A., M. Koziol-Montewka, Z. Al-Doori, D. Morrison and D. Kaczor. (2007). Spread of a single multiresistant methicillin-resistant *S. aureus* clone carrying a variant of staphylococcal cassette chromosome *mec* type III isolated in a university hospital. *European J. Clin. Microbiol. Infect. Dis.*, 26: 29–35.
33. Fridkin SK, Steward CD, Edwards JR. (1999;). Surveillance of antimicrobial use and antimicrobial resistance in United States hospitals. Project ICARE phase 2. *Clin Infect Dis*. 29:245-52.
34. Khorvash F, Mostafavizadeh K and Mobasherizadeh. (2008). Frequency of *mecA* Gene and Borderline Oxacillin Resistant *Staphylococcus aureus* in Nosocomial Acquired Methicillin Resistance *Staphylococcus aureus* Infection. *Pakistan Journal of Biological Sciences*. 11(9): 1282-1285.
35. Merlino J, Watson J, Rose B, Pegler MB, Gottlieb T, Bradbury B, Harbour C. (2002). Detection and expression of methicillin/oxacillin resistance in multidrug-resistant and non-multidrug-resistant *Staphylococcus aureus* in Central Sydney, Australia. *Journal of Antimicrobial Chemotherapy*. 49, 793-801.
36. Frebourg NB, Nouet D, Lemée L, Martin E, Lemeland JF. (1998). Comparison of ATB Staph, Rapid ATB Staph, Vitek, and E-Test Methods for Detection of Oxacillin Heteroresistance in *Staphylococci* Possessing *mecA*. *J Clin Microbiol*. 36: 52–57.
37. Khan AU, Sultan A, Tyagi A, Zahoor S, Akram M, Kaur S, Shahid M, Vaishnavi CV. (2007). Amplification of *mecA* gene in multi-drug resistant *Staphylococcus aureus* strains from hospital personnel. *Infect Developing Countries*. 1(3):289-295.