Egyptian Journal of Medical Microbiology Volume 27 / No.4 / October 2018 27-35 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Genotyping of Nosocomial Methicillin Resistant *Staphylococcus aureus* with tracing the Source of Infection: A Guideline Step in Infection Control Strategy at General Surgery Department of Tanta University Hospital

[1]Sarra M. Samy, [2]Fazil A.Tkeli, [1]Eman A. Eisa*, [1]Ahmed M. Amin, [3]Hamada H. Dawood, [1]Mohammed Z. Hussein

[1]Department of Microbiology and İmmunology; Faculty of Medicine Tanta University, Egypt,
[2]Department of Medical Microbiology; Faculty of Medicine Ankara University, Turkey;
[3]Department of General Surgery, Faculty of Medicine Tanta University, Egypt

Key words:
MRSA, Epidemiology, Antibiotic susceptibility, typing, PFGE

Corresponding Author:
Eman Eisa
Department of Microbiology and İmmunology; Faculty of Medicine Tanta University, Egypt
Tel : 01276798602
emkhatib20032002@yahoo.com

ABSTRACT

Background: *Methicillin-resistant Staphylococcus aureus (MRSA) represents a serious causal agent in nosocomial infections that are becoming increasingly difficult to cure due to their emerging resistance. Therefore, it becomes essential to understand the epidemiology of MRSA where pulsed field gel electrophoresis (PFGE) is considered to be the gold standard for the this.* ***Objectives:*** *This study aimss to make genotyping for the nosocomial strains of methicillin-resistant Staphylococcus aureus (MRSA) isolated form General Surgery Department of Tanta University Hospitals with tracing the source of infection as a guideline for infection control.* ***Methodology****: 159 different samples were collected from patients and 41 from suspected sources of infection. MRSA isolates were screened by Cefoxitin disk diffusion method then confirmed by detection of MecA gene by PCR. Phenotyping of the isolates was done by using the antibiogram while genotyping was done by using pulsed field gel electrophoresis (PFGE).* ***Results:*** *MRSA isolates were found in 60 patients and 5 health care workers (HCWs). Genotyping revealed 26 patterns (A - N & a - l) where type (A) was the most predominant. Isolates which had identical genotypes had different antiograms .Each ward revealed infection with muliple strains indicating multiple sources of infection while certain strains were found in multiple patients and multiple wards. Genotyping revealed that 2 HCWs were the most probable source of infection in 4 patients.* ***Conclusions:*** *Genotyping using PFGE is highly significant in studying the epidemiology of MRSA. HCWs should be seriously considered, not only as a source of infection, but also as a major cause for transmitting infection between patients in different wards.*

INTRODUCTİON

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major causes of healthcare-associated infections worldwide [1]. MRSA infect by self inoculation or by external contact. The risk of infection is increased after nasal colonization and when a disruption in the skin or mucosal membrane occurs. Host factors, like trauma, implants, and prosthesis, and systemic factors, such as diseases (diabetes and cystic fibrosis) or immunosuppression (cytostoxic drugs, HIV), constitute risks for infection [2,3].

MRSA isolates are resistant to virtually all beta-lactams (except the newer anti-MRSA compounds) due to the expression of low-affinity penicilin binding proteins (PBPs), encoded by the mecA or mecC genes, which can overtake the functions of the other PBPs. The mec genes are carried by particular mobile genetic elements, named staphylococcal cassette chromosome (SCC) element[4].

The most efficient way to prevent the spread of MRSA is controversial. Contact isolation of MRSA patients, hand hygiene increased surface disinfection and topical antibiotic treatment are common measures to reduce transmission [5,6] . The surveillance of MRSA clones (both from infections and colonization) is crucial for the implementation of effective treatment protocols and infection control measures [7].

Various typing techniques have been developed to investigate the spread and evolution of MRSA. Bacterial typing method should be highly discriminatory, reproducible, standardized, widely available and inexpensive. In bacterial strain typing, both phenotyping and genotyping procedures can be used [8].

Phenotyping methods have practical limitations e.g. low discriminatory power which render them largely unsuitable for comprehensive bacterial population

analyses[9,10]. Hence, over the past two decades, phenotyping has been largely replaced by molecular genotyping. The most commonly used techniques for MRSA typing are PFGE, PCR, SCC*mec* typing and sequence-typing including multilocus sequence typing (MLST) and *spa*-typing [11].

Pulsed field gel electrophoresis (PFGE) used to be the "gold standard" of MRSA typing because it can distinguish among several concurrent epidemic strains. PFGE analysis is very convenient and has high discriminatory power [12]. In PFGE for MRSA, the chromosomal DNA is digested with the restriction enzyme SmaI, and the resulting DNA fragments are separated by agarose gel electrophoresis.

METHODOLOGY

Subjects:

This study was conducted over a period of one and half years starting from November, 2013 till May 2015. It was performed on 60 MRSA isolates recovered from (159) different samples collected from Inpatients with nosocomial infections in General Surgery Department of Tanta University Hospitals . Also , 5 MRSA isolates recovered from 41 Suspected sources of infection were included (21 Health care workers (HCWs) nasal swabs and 20 swabs from bed side tables, dressing tables, linens, surgical dressing, surgical instruments and equipments).

Isolation and identification of *Staphylococcus aureus*: This was done according to Cheebrough[14].

Antimicrobial susceptibility testing:

Detection of antimicrobial susceptibilities of the *S.aureus* isolates were performed using disk diffusion method (modified Kirby- Bauer) on Muller-Hinton agar according to The European Committee on Antimicrobial Susceptibility Testing[15].

Identification of Methicillin Resistant *Staph aureus* (MRSA):

Methicillin Resistant *staph aureus* (MRSA) isolates were screened and confirmed by: Cefoxitin disk diffusion susceptibility method according to *EUCAST*[15], isolates with inhibition zone to Cefoxitin less than 22 mm were considered MRSA and Detection of mecA gene by PCR.

Chromosomal DNA extraction *(RibospinTMvRDby Geneall):*

Ribospin™ vRD procedures employed the glassfiber membrane technology for the fastest and the most convenient of high purity DNA isolation.

Nucleic acid amplification:

PCR Master Mix 2X (Thermo Scientific):

PCR Master Mix is a 2X concentrated solution of Taq DNA polymerase, dNTPs (deoxynucleotide triphosphates), reaction buffer, MgCl2 and all other components required for PCR, except DNA template and primers.

Primer solution (Thermo Scientific):

The sequence of the primers used were:

P1:5'-GTA GAA ATG ACT GAA CGT CCG ATA A-3'

P2: 5'-CCA ATT CCA CAT TGT TTC GGT CTA A-3'

Amplification reaction:

A final volume of 50 µl contained:

PCR Master Mix (2X)	25 µL
Forward primer	4 µM
Reverse primer	4 µM
Template DNA	4 µg
Water, nuclease-free	13 µL
Total volume	50 µL

Staphylococcus aureus subsp. *Aureus* (ATCC® BAA-1556™): USA300 FPR3757 was used as positive control strain and water was used instead of the DNA template for the negative control.Thermal cycler *(Techne)* program was adjusted according to Geha et al.[16]

Agarose gel electrophoresis of the amplified DNA was done and ethidium bromide stained bands in gel were visualized on long wave length ultraviolet transilluminator, and photographed (Figure 1).

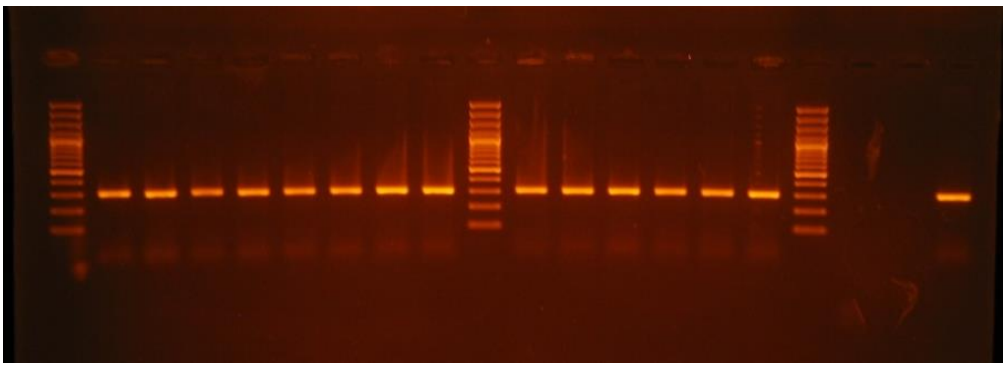

Fig. 1: Agarose gel electrophoresis of amplified *mecA* DNA by PCR.

Genotyping for MRSA strains by Pulsed-field gel electrophoresis (PFGE):

Chromosomal patterns of the MRSA isolates were investigated by Pulsed-field gel electrophoresis (PFGE) as described by Mulvey et al.[17].

Procedure of PFGE:

Preparation the casting agarose plugs from *staph*. cell suspension was done followed by lysis of cells.

For restriction enzyme digestion of agarose plugs, the volume of restriction enzyme buffer (REB) required to digest the plugs was calculated as follow:

Reagent	µl/plug	µl/13 plugs
Sterile water	180	2340
Tango buffer	20	260
Total volume	200	2600

200 µl of REB preparation were added to the tube containing one-third of a plug and the tubes were incubated at room temperature for 30 min.The REB was removed from the tube with pipette tip, taking care not to damage the plug slice. The restriction enzymeSmaI wasprepared and calculated as follow:

Reagent	µl/plug	µl/13 plugs
Sterile water	180	2340
Tango buffer	17	221
SmaI	3	39
Total volume	200	2600

200 µl of the restriction enzyme and buffer preparation were added to each tube and the tubes were incubated at 30°C for 4 h.

Electrophoresis:

Electrophoresis was performed with a CHEF DR-II apparatus *(Bio-Rad)* and stained for 20 min in a covered container with 0.5 mg of ethidium bromide per liter, then UV light transilluminator was used to visualize samples (Figures 2, 3).

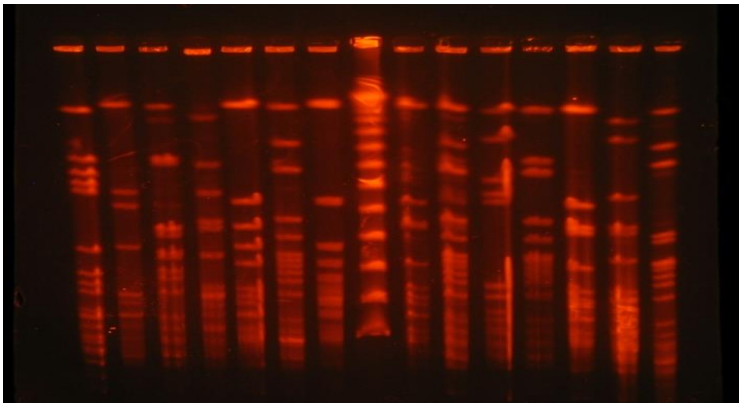

Fig. 2: Most frequent PFGE patterns of MRSA isolates.Lane (1) represents most frequent pattern (13 isolates), Lane (2): 6 isolates, Lane (3,4): 4 isolates for each pattern, Lane (5,6,7,9,10,11): 3 isolates for each pattern, lane (12,13,14,15): 2 isolates for each pattern and lane(8): Lambda ladder PF marker.

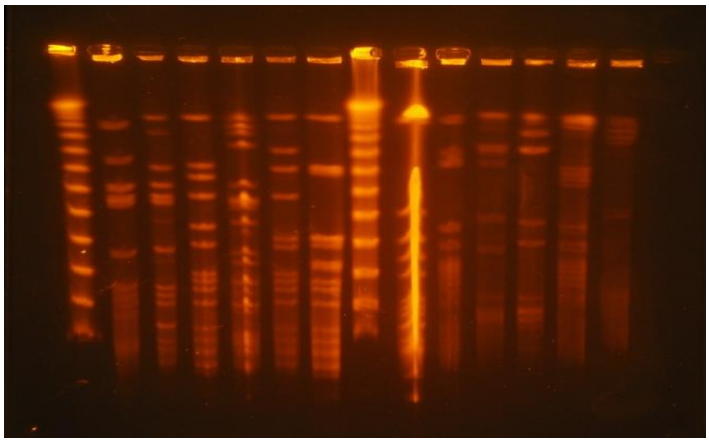

Fig. 3: Varient PFGE patterns of MRSA isolates following DNA digestion with SmaI restriction enzyme.

Computer-aided analysis:

The fingerprints generated were evaluated using Gel-Compar II software version 6.5 (Applied Maths, Sint-Martens-Latem, Belgium). DNA fragments on each gel were normalized using the molecular weight standard run on each gel to allow comparisons between different gels. Cluster analysis and dendrogram were performed by the unweighted pair-group method using (UPGMA), and DNA relatedness was calculated based on the Dice coefficient with 1.0% band tolerance and1.0% optimization.

RESULTS

Table (1) shows that *Staphylococcus aureus* represented 93 isolates (46.50%) *:* 50.31% out of 159 patients samples and 31.71% out of 41 suspected sources of infection.

Table 1: Distribution of different bacterial growth found in different specimens from patients and suspected sources of infections:

Bacterial growth		From patients' samples		From suspected sources		Total	
		N	%	N	%	N	%
Staphylococcus aureus		80	50.31	13	31.71	93	46.5
Coagulase negative *Staphylococci* (CoNS)		32	20.13	0	0	32	16.0
Non *Staphylococci*		47	29.56	17	41.46	64	32.0
No bacterial growth		0	0	11	26.83	11	5.5
Total		159	100	41	100	200	100
Chi-square	**X^2**	**95.862**		**20.172**			
	P-value	**P1: 0.001***		**P2: 0.001***			

P-value: P1: 0.001* means there is statistically significant difference between types of bacterial growth isolated from patients samples with significant increase in *S.aureus*.
P2: 0.001* means there is statistically significant difference between types of bacterial growth isolated from suspected sources of infection.

Table 2 shows that among the 65 MRSA isolates, 5 isolates were recovered from suspected sources of infection (nasal swabs from HCWs) out of 13 *S.aureus* (38.5%) while 60 isolates were recovered from patients' samples out of 80 *S.aureus* (75.0%).

Table 2: Distribution of MRSA isolates among whole s.aureus isolated from patients' samples and expected sources of infection:

Specimen		MRSA	MSSA	Total *S.aureus*	P	
From expected sources of infection	**N**	5	8	13	P1	0.239
	%	38.5	61.5	100		
From patients	**N**	60	20	80	P2	0.001*
	%	75	25	100		
Chi-square	**X2**	7.092				
	P-value	0.008*				

P-value: P1: 0.239 means there is statistically insignificant difference between MRSA and MSSA in suspected sources.
P2: 0.001* means there is statistically significant increase in MRSA than MSSA in patients samples.

Table 3 shows that all tested MRSA isolates (recovered from patients and expected sources of infection) that were resistant to Cefoxitin (FOX) disk were examined for detection of *Mec*A gene by PCR and all tested isolates (100%) were *Mec*A positive.

Table (3): Detection of MecA gene in all MRSA isolates by PCR:

All MRSA isolates	Screening by Fox disc		Confirmed by MecA gene	
N (%)	**R (%)**	**S (%)**	**+ve (%)**	**-ve (%)**
65 (100%)	65 (100%)	0 (0%)	65 (100%)	0 (0%)

Table 4 shows that there were 14 different resistance patterns identified in the MRSA isolates according to antibiotics susceptibility testing results. The most common were the first 4 types.

Table 4: Antibiogram typing of all MRSA isolates :

Antibiogram types		Resistance pattern	Number of MRSA isolates	%
1		E+DA+AK+CN+TE+CIP	15	23.08
2		E	13	20.00
3		E+AK+CN	8	12.31
4		AK+CN	6	9.23
5		AK+CN+TE	4	6.15
6		E+DA+TE	4	6.15
7		E+DA+AK+CN+TE+CIP+RD	3	4.62
8		SXT+E+DA+AK+CN+TE+CIP+RD	3	4.62
9		C+E+DA+TE+CIP	2	3.08
10		CN+TE	2	3.08
11		SXT+TE+CIP	2	3.08
12		SXT+E+CN+TE+CIP	1	1.54
13		C+EDA+AK+CN+TE+CIP+RD	1	1.54
14		E+ AK+CN+TE	1	1.54
		Total	65	100.00
Chi-square	X^2	25.324		
	P value	0.001*		

P value:**0.001*** means that there is there is statistically significant difference between different antibiogram types with significant increase in type1&2.

Table (5) shows that there were 14 frequent patterns named in capital letters from (A) to (N) and 12 variant patterns named in small letters from (a) to (l).Pattern (A) was the most frequent pattern represented 13 identical isolates (20.00%).

Table 5: Genotyping of all MRSA isolates according to PFGE patterns:

PFGE Pattern		No of isolates	%
A		13	20.00
B		4	6.15
C		4	6.15
D		6	9.23
E		3	4.62
F		3	4.62
G		3	4.62
H		3	4.62
I		3	4.62
J		3	4.62
K		2	3.08
L		2	3.08
M		2	3.08
N		2	3.08
(a)		1	1.54
(b)		1	1.54
(c)		1	1.54
(d)		1	1.54
(e)		1	1.54
(f)		1	1.54
(g)		1	1.54
(h)		1	1.54
(i)		1	1.54
(j)		1	1.54
(k)		1	1.54
(l)		1	1.54
Total		65	100
Chi-square	X^2	15.012	
	P value	0.001*	

P value: **0.001*** means that there is there is statistically significant difference between different PFGE patterns with significant increase in pattern (A).

It was found that that the isolates which were genotypically identical could have different antibiogram types. Table (6) shows the distribution of different genotypes of MRSA isolates among patients in different wards.

Table 6: Distribution of different genotypes of MRSA isolates among patients in different wards:

Ward	PF genotyping pattern
male/A	A, B, D, E, K, (c)
male/B	A, C, D, H N, (d), (f), (g)
male/C	A, D, F, J, I, M, (h), (i), (l)
female/A	A,D, G, F, M, K
female/B	A,D, H, (e)
female/C	B, E, J, (a), (j), (k)

Table (7) shows antibiogram typing and genotyping patterns by PFGE to the 5 MRSA isolates recovered from the suspected sources of infection in this study.

Table 7: Antibiogram typing and genotyping of the 5 MRSA isolates recovered from the suspected sources of infection:

No	Sample	PF pattern	Antibiogram typing
1	Nasal swab	E	6
2	Nasal swab	L	6
3	Nasal swab	L	6
4	Nasal swab	F	1
5	Nasal swab	(b)	7

DISCUSSION

In this study out of 92 isolates proved to be *S.aureus*, 65 (69.89%) isolates were MRSA. This was similar to the result of Mendes et al.[18] who demonstrated that (73%) of the clinical *S. aureus* isolates from two hospitals in Korea were MRSA in 2011, but higher than that obtained by Rahima et al [19] (44.6 %) , Falagas et al[20] (24%), Havaei et al [21] (16%) and Jana et al.[22] (18%). Silveira et al[23] reported that several European countries have national surveillance data with very low rates of MRSA. These low prevalence rates in their studies may be probably due to proper hand hygiene, antibiotic stewardship and surveillance programs.

Detection of the *mec*A gene by PCR is considered the gold standard for MRSA confirmation. The molecular detection methods are more preferable, favorable and accurate than the phenotypic method[24,25].

In our study, *Mec*A gene was detected by PCR in which (100%) of MRSA isolates were *MecA* positive. Ak et al.[26] found the gene in (94%) of MRSA while Makgotlho et al.[27] demonstrated it in 99% of cases. On the other hand, García-Garrote et al.[28] found that five MRSA isolates showing resistance to Cefoxitin and *mec*A negative were carrying a new *mec*A gene variant, *mec*C.

Antibiotic resistance pattern is a limited typing method because genetically and epidemiologically unrelated strains can show the same pattern of sensitivity and resistance[29-31].

Among 21 nasal swabs in our study, 13 (61.90%) were *S. aureus* nasal carriers out of which 5 (38.46%) were MRSA. On the other hand, Conceicao et al[32] reported that (15.7%) were *S. aureus* nasal carriers with MRSA. Potential risk factors for MRSA infections including history of MRSA colonization or infection, the presence of surgical wound, urinary, intravenous catheterization, nasogastric or endotracheal tubes, prolonged hospital stay, intensive care setting or burn units, weakened immune system and previous intake of antibiotics, mainly the quinolones and beta-lactams [33].

Antibiogram typing of MRSA isolates in the present study revealed 14 different resistance patterns and all of types were resistant to Penicillin, Cefoxitin and Oxacillin. Janwithayanuchit et al[34] from Thailand identified 9 different antibiotypes. On the other hand, Omar et al.[35] reported 32 antibiotypes. These variations could be explained by the numbers of antimicrobial agents used.

Antibiogram type (1) was the most common (23.08%) which was in agreement with Janwithayanuchit et al[34] and Omar et al[35], where type (1) represented (44.2%) and (16%) respectively.

Epidemiological studies involve different typing methods to explore the epidemiology of MRSA and pulsed field gel electrophoresis (PFGE) has been recommended as a 'gold standard' for typing MRSA isolates because it can distinguish among several concurrent epidemic strains[36].

In the present study, genotyping of MRSA isolates was performed by PFGE in which 14 frequent patterns named from (A) to (N) and 12 variant patterns named from (a) to (l) were detected .Similarly, Rajaduraipandi et al[5] reported the SmaI restricted genomic DNA of 19 MRSA identified 11different PFGE patterns and also Bazzoun et al[37] reported PFGE revealed fourty-six types . However, Carles-Nurit et al [38] found that the SmaI restriction pattern in each of 20 MRSA strains collected from a single hospital over a period of 4 months was unique.

PFGE type (A) in the present study was found to be the most common type circulating and was observed in 13 identical isolates (20.00%) which may indicate the endemicity of these strains in the hospital. Similarly, Conceicao et al[32] found that PFGE patterns A, B, C, and E were found in more than three different wards and no ward-specific clonal type was established which suggested cross-transmission.

The detection of identical MRSA strains among patients in different wards could indicate the intradepartmental spread of these strains and the need of implementation of additional infection control measures in this hospital.The distribution of MRSA strains with different PFGE pattern in the hospital presumably occurred by cross-infection from patient to patient because of increased frequency of patients transfer from ward to ward and the extensive movement of surgeons, physicians, and other HCWs among wards.

In agreement, regarding the correlation between PFGE patterns and antibiogram types of MRSA isolates, Alfizah et al[30] and Norazah et al [39], found that strains with the same PFGE-pattern had different in antibiotic resistant patterns and strains with different PFGE patterns had similar antibiotic susceptibility pattern. In the present study , the most frequent PFGE pattern (A) including 13 isolates was subdivided by antibiogram typing into 3 types

HCWs are known to be major reservoirs of *S. aureus* and regarding the tracing the source of infection, this study revealed that PF pattern (E) was isolated from the nasal swab of a HCW and from post operative wound swab of patient at the same ward and the same time which may indicate the HCW was the source of the infection. Also it was isolated from another patient after a long period which may indicate the persistence of the source of infection. PF pattern (F) was isolated from the nasal swab of a HCW in January 2014 and also was isolated from tracheal aspirate samples in October and November 2014.

In agreement, Conceicao et al[32] described isolates colonizing nurses or nurse-aids and patients in the same wards. The World Health Organization claims that hand

hygiene among HCWs is the leading measure to prevent the spread of health care-associated infections [40].

Regarding the distribution of different genotypes of MRSA isolates among patients in different wards, different PF patterns were detected in each Ward in the present study which indicated multiple sources of infection. Also, the detection of multiple patients infected with the same genotype within the same ward , indicated that infection could be transmitted between patients, most probably through HCWs or dressing tables.

On the other hand, the detection of identical MRSA genotypes among patients in different wards within the same unit (pattern A and D in 5 wards) and (pattern B, E, F, H, J and K in 2 wards), indicated the intradepartmental spread of these strains between different wards and the need of implementation of additional infection control measures in this unit.

CONCLUSION

HCWs are considered the most important source of infection through the nasal carriage of MRSA. The detection of multiple patients infected with the same genotype within the same ward indicates the spread of infection between patients, while the detection of different PF patterns in each ward indicates multiple sources of infection. Detection of identical MRSA genotypes among patients in different wards within the same unit indicates the intradepartmental spread of these strains between different wards. PFGE has high discrimination ability that makes it the gold standard method for the accurate epidemiologic study of MRSA.

RECOMMENDATIONS

- All HCWs should be periodically screened for nasal carriage of MRSA.
- Implementation of strict infection control measures by HCWs is highly recommended.
- PFGE typing method could improve greatly the guide lines for infection control in hospitals.

REFERENCES

1. Bari F, Wazir R, Haroon M, et al.: Frequency of antibiotic susceptibility profile of MRSA at Lady Reading Hospital, Peshawar. Gomal J Med Sci 2015; 13: 62-65.
2. Crossley KB, Jefferson KK, Archer GL, et al.: Staphylococci in human disease. In: Barun Mathema, José R. Mediavilla, Liang Chen and Barry N. Kreiswirth (Ed.): Evolution and Taxonomy of Staphylococci. Ch (3). 2nd ed. Wiley-Blackwell; Oxford, UK 2010.
3. Blomqvist S, Leonhardt A, Arirachakaran P, et al.: Phenotype, genotype, and antibiotic susceptibility of Swedish and Thai oral isolates of Staphylococcus aureus. J Oral Microbiol 2015; 7: 26250.
4. Bartoloni A, Riccobono E, Magnelli D, et al. (2015): Methicillin-resistant Staphylococcus aureus(MRSA) in hospitalized patients from the Bolivian Chaco. Int J Infect Dis; 30: 156–160.
5. Rajaduraipandi K, Morrison D, Mani M, et al.: Typing of methicillin resistant and sensitive Staphylococcus aureus isolated from Tamilnadu, India using DNA fingerprints by pulsed-field gel electrophoresis. Acta Biol Szeged 2014; 58(2):171-177.
6. Schulz M, Nonnenmacher C and Mutters R: Cost-effectiveness of rapid MRSA screening in surgical patients. Eur J Clin Microbiol Infect Dis 2009; 28: 1291–1296.
7. Uhlemann AC, Otto M, Lowy FD, et al.: Evolution of community- and healthcare-associated methicillin-resistant Staphylococcus aureus. Infect Genet Evol 2014; 21: 563–574.
8. Ostojić M and Hukić M: Genotypic and phenotypic characteristics of Methicillin-resistant Staphylococcus aureus (MRSA) strains isolated on three different geography locations. Bosn J Basic Med Sci 2015; 15 (3):48-56.
9. Budimir A (2012): Detection and typing methods of methicillin-resistant Staphylococcus aureus strains. Med Sci; 37: 73-88.
10. Spinali S, van Belkum A, Goering RV, et al.: Microbial typing by matrix-assisted laser desorption ionization–time of flight mass spectrometry: do we need guidance for data interpretation? J Clin Microbiol 2015; 53(3): 760 – 765.
11. Aguadero V, González-Velasco C, Vindel A, et al.: An analysis of the association between genotype and antimicrobial resistance in methicillin-resistant Staphylococcus aureus clinical isolates. Rev Esp Quimioter 2015; 28(2): 79-85.
12. Seidl K, Leimer N, Marques MP, et al.: Clonality and antimicrobial susceptibility of methicillin-resistant Staphylococcus aureus at the University Hospital Zurich, Switzerland between 2012 and 2014. Ann Clin Microbiol Antimicrob 2015; 14: (14): 1-7
13. Deurenberg RH and Stobberingh EE: The evolution of Staphylococcus aureus. Infect Genet Evol 2008; 8 (6): 747–763.
14. Cheesbrough M: Biochemical tests to identify bacteria in District laboratory practice in tropical

countries. 2nd ed. part (2): 63-70.Cambridge University Press. Cambridge, UK 2007.

15. EUCAST: The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters - Version 5.0. Available at: www.eucast.org., 2015.
16. Geha DJ, Uhl JR, Gustaferro CA, et al.: Multiplex PCR for identification of methicillin resistant Staphylococci in the clinical laboratory. J Clin Microbiol 1994; 32: 1768-1772.
17. Mulvey MR, Chui L and Canadian Committee for the Standardization of Molecular Methods: Development of a Canadian standardized protocol for subtype of MRSA using pulsed field gel electrophoresis. J Clin Microbiol 2001; 39: 3481-3485.
18. Mendes RE, Mendoza M, Banga Singh KK, et al.: Regional resistance surveillance program results for 12 Asia-Pacific nations (2011). Antimicrob Agents Chemother 2013; 57: 5721–5726.
19. Rahima T, Nafissa B and Abdelghani D: Prevalence of methicillin-resistant Staphylococcus aureus and/or intermediate susceptibility to vancomycin isolated from private laboratories in Annaba "Algeria". J Chem Pharm Res 2015; 7 (5):780-786.
20. Falagas ME, Karageorgopoulos DE, Leptidis J, et al.: MRSA in Africa: Filling the global map of antimicrobial resistance. PLoS ONE, 2013; 8 (7): e68024.
21. Havaei SA, Ghanbari F, Rastegari AA et al.: Molecular typing of hospital-acquired Staphylococcus aureus isolated from Isfahan, Iran. Int Sch Res Notices; 2014: 1-6.
22. Jana H, Roy T, Dey R et al.: Prevalence and antimicrobial susceptibility pattern of different clinical isolates of HA-MRSA and CA-MRSA in a tertiary care rural hospital, Bankura, West Bengal, India. Sch J App Med Sci 2015; 3 (2F): 944-948.
23. Silveira AC, Cunha GR, Caierão J et al.: MRSA from Santa Catarina State, Southern Brazil: intriguing epidemiological differences compared to other Brazilian regions. Braz J Infect Dis 2015; 19 (4): 384–389.
24. Kumurya AS, Gwarzo MY and Uba A: One step PCR for detection of Staphylococcus aureus specific sequence gene and MecA gene. Inter J Adv Mat Res 2015; 1 (3): 73-79.
25. Kalhor H, Sharuati L, Validi M, et al.: Comparison of Agar Screen and duplex-PCR methods in determination of methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from nasal carriage. Afr J Microbiol Res 2012; 6 (16): 3722-3726.
26. Ak SK, Shetty PJ, Chidambaram A, et al.: Detection of mecA genes of Methicillin-Resistant Staphylococcus aureus by Polymerase Chain Reaction. Int J Health Rehabil Sci 2012; 1(2): 64-68.
27. Makgotlho PE, Kock MM, Hoosen A, et al.: Molecular identification and genotyping of MRSA isolates. FEMS Immunol Med Microbiol 2009; 57: 104–115.
28. García-Garrote F, Cercenado E, Marín M, et al.: Methicillin-resistant Staphylococcus aureus carrying the mecC gene: emergence in Spain and report of a fatal case of bacteraemia. J Antimicrob Chemother 2014; 69: 45–50.
29. Montesinos I, Salido E, Delgado T, et al.: Epidemiologic genotyping of methicillin-resistant Staphylococcus aureus by pulsed-field gel electrophoresis at a University Hospital and comparison with antibiotyping and protein A and coagulase gene polymorphisms. J of Clin Microbiol 2002; 40 (6): 2119–2125.
30. Alfizah H, Norazah A, Nordiah J, et al.: DNA Fingerprinting of methicillin- resistant Staphylococcus aureus (MRSA) by pulsed-field gel electrophoresis (PFGE) in a teaching hospital in malaysia. Med J Malaysia 2002; 57(3): 319-328.
31. Kuo SC, Chiang MC, Lee WS, et al.: Comparison of microbiological and clinical characteristics based on SCCmec typing in patients with community-onset meticillin-resistant Staphylococcus aureus (MRSA), 2011.
32. Conceicao T, Silva IS, de Lencastre H, et al.: Staphylococcus aureus nasal carriage among patients and health care workers in Sao Tome and Prıncipe. Microb Drug Resist 2014; 20 (1): 57-66.
33. Batabyal B, Kundu GK and BiswasSh: Methicillin-resistant Staphylococcus aureus: A brief review. Inter Res J Biol Sci 2012; 1(7): 65-71.
34. Janwithayanuchit I, Ngam-Uluert S, Paugmoung P, et al.: Epidemiological study of methicillin-resistant Staphylococcus aureus by coagulase gene polymorphism. Sciences Asia 2006; 32: 127-132.
35. Omar NY, Ali HA, Harfoush RA, et al.: molecular typing of methicillin resistant Staphylococcus aureus clinical isolates on the basis of protein A and coagulase gene polymorphisms. Int J Microbiol; Article ID: 650328, 2014.
36. Mehndiratta PL and Bhalla P: Typing of Methicillin resistant Staphylococcus aureus : A technical review . Indian J Med Microbiol 2012; 30: 16-23.
37. Bazzoun DA, Harastani HH, Shehabi AA et al.: Molecular typing of Staphylococcus aureus collected from a Major Hospital in Amman, Jordan. J Infect Dev Ctries 2014; 8 (4):441-447.

38. Carles-Nurit MJ, Christophle B, Broche S, et al. (1992): DNA polymorphisms in methicillin-susceptible and methicillin-resistant strains of Staphylococcus aureus. J Clin Microbiol; 30: 2092-2096.
39. Norazah A, Liew SM, Kamel AGM, et al.: DNA fingerprinting of methicillin-resistant Staphylococcus aureus by Pulsed-Field Gel Electrophoresis (PFGE): comparison of strains from 2 Malaysian hospitals. Singapore Med 2001; 42 (1): 15-19.
40. Bagheri-Nejad S, Allegranzi B, Syed SB, et al.: Health-care-associated infection in Africa: a systematic review. Bull World Health Organ 2011; 89(10):757–765.