Iranian Journal of Microbiology
Volume 7 Number 5 (Octobr 2015) 265-272

ORIGINAL ARTICLE

Antimicrobial susceptibility pattern of *Staphylococcus aureus* isolated from clinical specimens in Northern area of Jordan

Mazhar Salim Al-Zoubi[1,2*], Ibrahim Ali Al-Tayyar[3], Emad Hussein[1], Alla Al Jabali[1], Salih Khudairat[4]

[1]Biology Deparrment, Yarmouk University, Irbid, Jordan.
[2]Molecular Pathology Department, University of Pisa, Italy.
[3]Department of Medical Laboratory Science, Faculty of Engineering & Technology, Brack, Sebha University, Lybia.
[4]Hussain Cancer Center, Amman, Jordan.

Received: May 2015**, Accepted:** July 2015

ABSTRACT

Background and Objectives: The global spread of methicillin resistant *Staphylococcus aureus* (MRSA) constitutes one of the most serious contemporary challenges to the treatment of hospital-acquired infections. We aimed to screen and assess the antibiotic susceptibility pattern of *Staphylococcus aureus* isolated from clinical specimens in local hospitals of Northern province in Jordan.
Materials and Methods: *Staphylococcus aureus* was isolated and identified using standard methods from various clinical specimens of different infected body sites from 358 patients during the period from January 2008 to November 2012.
Results: Our analysis showed that 31.6% of *S. aureus* infections were MRSA, while 31% were multidrug resistance (MDR) and 42.7% were Oxacillin-resistant (ORSA). Most of these strains were isolated from wound specimens. All isolates were susceptible to vancomycin (100%). They were also susceptible to chloramphenicol, linezolid, nitrofurantoin, rifampicin and teicoplanin (>80%), but showed resistance to erythromycin and penicillin.
Conclusion: Vancomycin was the most effective antimicrobial agent against *S. aureus*. We recommend regular surveillance of hospital associated infections and monitoring antibiotic sensitivity pattern and strict drug policy for antibiotics used within and outside the hospital environments.

Keywords: *Staphylococcus aureus*, MRSA, MDR,

INTRODUCTION

Staphylococcus genus is a heterogeneous group of bacteria consisting of 30 species. *Staphylococcus aureus* has been found to be the most clinically important species, with broad presence in nature. It is part of the normal flora of human body and commonly carried on the skin or in the nose of healthy individuals, which makes it easy to be transmitted by air or fomites from patients or carriers (1, 2). It been recognized as one of the most common cause of human infections, such as skin infections, wound infections and bacteremia. Nevertheless; the introduction of antibiotics has lowered the mortality rate of *S. aureus* infections. However, the bacteria have rapidly developed resistance mechanisms against many antimicrobial agents (1, 3).

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been isolated and recognized more than 50 year ago. MRSA is a specific strain of the *S. aureus,* which is resistant to methicillin and all β-lactams. Later use of Oxacillin as an alternative to methicillin

*Corresponding author: Dr. Mazhar Salim AL-Zoubi
Address: Biology Department, Yarmouk University, Irbid, Jordan.
E-mail: mszoubi@yu.eudu.jo

in susceptibility tests resulted in the term 'Oxacillin-resistant *S. aureus*' (ORSA) (2), which is resistant to numerous antibiotics. Before the development of antibiotics, invasive infections caused by *Staphylococcus aureus* have often been fatal (4).

The global spread of MRSA constitutes one of the most serious contemporary challenges to the treatment of hospital-acquired infections (5). MRSA carries a uniquely effective antibiotic resistance mechanism that can protect the microorganisms against all members of β-lactam antibiotics. This makes infections caused by these pathogens very difficult to manage and costly to treat (6, 7).

Centre for Disease Control and Prevention (CDC) published a report in 2004 demonstrating that approximately 60% of all healthcare-associated *Staphylococcus aureus* infections in the United States are caused by MRSA. *Staphylococcus aureus* was the second most common organism causing nosocomial blood stream infections, and the proportion of MRSA isolates increased from 22% in 1995 to 57% in 2001(4, 8). Moreover, data from the National Nosocomial Infections Surveillance system suggest that in intensive care units the proportion of *Staphylococcus aureus* isolates that are resistant to methicillin has increased to approximately 60% (1, 6, 8). Recent reports also suggest that community-associated MRSA infections have become the dominant cause of community-associated *Staphylococcus aureus* skin and soft tissue infections (9). About 15% of reported infections have been considered to be community–associated, which means that the infection occurred in people without documented healthcare risk factors (10, 11).

Currently, vancomycin has been accepted worldwide as the last choice against MRSA infections (11). Rarely, clinical isolates of vancomycin-resistant *S. aureus* (VRSA) have been reported recently (8). The emergence of *S. aureus* isolates resistant to vancomycin and other wide range of structurally un-related antibiotics have elevated MRSA into a multi-drug-resistant 'Superbug", making it more and more dangerous than ever in a hospital environment and also recently, in the healthy community (12, 13).

The objectives of the present study were to detect the prevalence and identify the multi-drug and non-multidrug Methicillin/Oxacillin resistant *S. aureus* (MRSA, ORSA& MDR) from clinical specimens in Jordan using common used antibiotics.

MATERIALS AND METHODS

Our study included specimens that are collected between January 2008 and November 2012. All clinical specimens were received and collected by laboratory department of assigned hospitals. 358 isolates of *S. aureus* were isolated from both genders and all age groups of out- or inpatients hospitalized in different wards. Clinical specimens were taken from various body sites of infection including blood, wound, sputum, urine and others.

Clinical specimens were collected using standard collection techniques (14, 15), and inoculated on appropriate bacteriological media, including 10% Sheep Blood agar, Chocolate agar, Thioglycollate, MacConkey Agar Media. The plates were incubated aerobically at 37 °C for 18-24 hours. The blood cultures were performed using an automated blood culture system (BACTEC 9240 and 9050 BD), followed by bacterial growth inspection.

The identification of isolates was made according to standard methods (16) for any potential clinically significant growth appear on the culture media on the base of quantity, feature of growth, source and site of specimens. The primary identification was made with basic microbiological methods using colony morphology, Gram staining, catalase and coagulase tests (14). The final identification and antibiotic susceptibility testing of the bacteria isolated from clinical specimens were obtained using an auto-analyzer system (Biomerieux, VITIC 60, France). The results of VITIC 60 were confirmed manually using biochemical tests and Kirby–Bauer disk diffusion technique according to CLSI guidelines.

The susceptibility of all isolates were determined against penicillin (P, 10ug), azithromycin (AZM, 15ug), chloramphenicol (C, 30ug), gentamicin (GN, 10ug), trimethoprim-sulfamethoxazole (SXT, 1,25/23,75ug), oxacillin (OX, 1ug), erythromycin (E, 15ug), cefoxitin (FOX, 30ug), nitrofurantoin (NIT, 300ug), linezolid (LZD, 30ug), levofloxacin (LEV, 5ug), tetracycline (T, 30ug), teicoplanin (TE, 30ug), clindamycin (CC, 2ug), rifampicin (RA, 5ug) and vancomycin (V, 30ug).

RESULTS

Based on culture, Gram staining, catalase, coagulase and biochemistry tests, 358 *Staphylococci au-*

reus were isolated from different infected body sites. The percentage of MRSA (resistant to both oxacillin and cefoxitin) and MDR (Resistant to four or more than four antibiotics) (17) were 31.6 % and 31 %, respectively (Fig. 1), and the percentage of ORSA was the highest (42.7 %) among the resistant types of *S. aureus*. The remaining strains were considered to be oxacillin susceptible *S. aureus* (OSSA). The OSSA represented more 57.3 % of total isolates.

In present study, 57% of *S. aureus* were isolated from males and 43 % from females. The percentage of MRSA, ORSA and MDR strains were similar in both genders, while the percentage of *S. aureus* and OSSA were higher in males than females (Fig. 2).

According to age (Table 1), the high percentages of *S. aureus* strains (30.2% - 35.2%) were isolated from

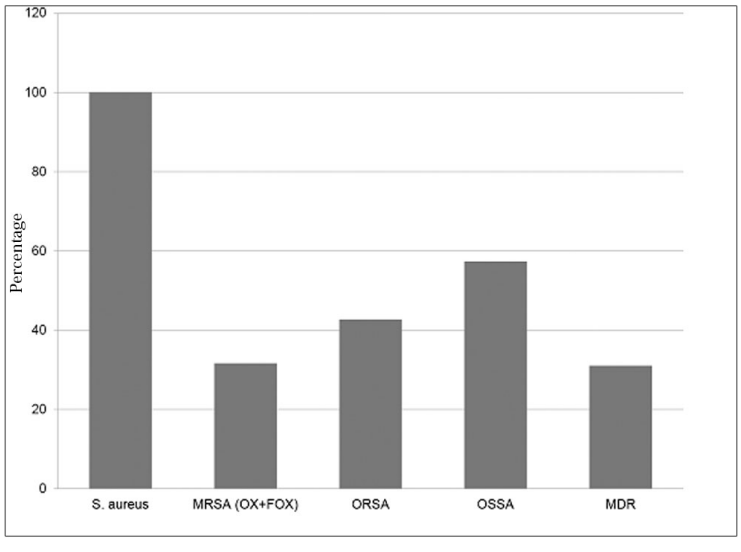

Fig. 1. Percentage of all isolated *S. aureusstrains.*

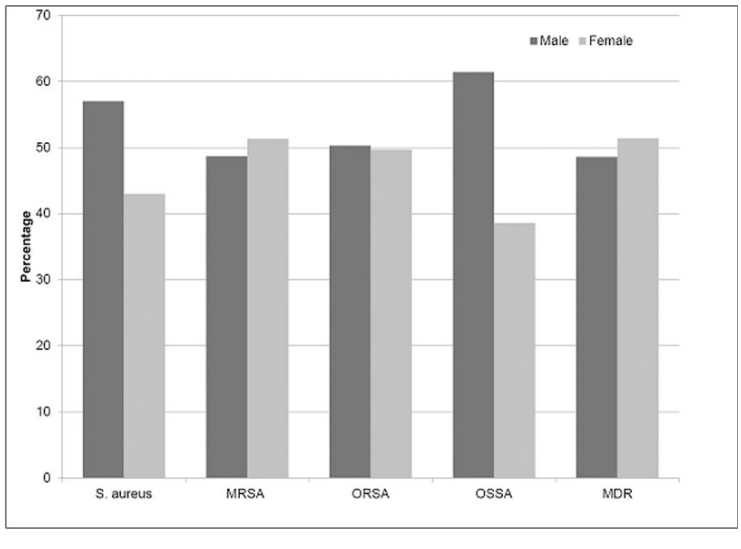

Fig. 2. 2009 Percentage of all of isolated *S. aureus* strains according to gender. (MRSA: Methicillin resistant *S. aureus* (Resistant to both Oxacillin and Cefoxitin). ORSA: Oxacillin resistant *S. aureus*. OSSA: Oxacillin susceptible *S. aureus*. MDR: Multidrug resistant *S. aureus.*)

20 to 39 years age group, and the percentages of these strains (21 % - 30.2 %) were nearly equal for each age group from 40 to 79 years. In the age group less than 19 years the isolated percentage were slightly higher (9.9 % - 16.3 %), while the lowest percentage (2.5 % - 6.3 %) were isolated from >80 years of age group.

Table 2 shows that *S. aureus* was isolated from 10 body sites and were predominat in wound, abscess and blood specimens. The MRSA, ORSA and MDR strains were mainly present in wound specimens.

The susceptibility pattern of *S. aureus* strains is presented in Table 3. The OSSA isolates were susceptible to the majority of tested antibiotics. The MRSA, ORSA and MDR strains showed high rates of susceptibility (>80%) to chloramphenicol, linezolid, nitrofurantoin (for urine samples), rifampicin and teicoplanin, but high resistance to erythromycin and penicillin. All isolate (100 %) were susceptible to vancomycin.

DISCUSSION

Staphylococcus aureus has been recognized as a major human pathogen. The pathogenicity and virulence of *S. aureus* are associated with its capacity to produce several virulence factors (4). MRSA is a bacterium that has been found to be resistant to antibiotics such as methicillin, oxacillin, penicillin and amoxicillin. This organism had become widespread in healthcare settings globally causing different type of infections. Nosocomial infections represent a burden for both patients and the healthcare system because of their association with increased hospitalization costs and high mortality/morbidity rate (2, 8).

Detection of MRSA by accurate and rapid methods

Table 1. Number of patients reverted to %.

Age group (year)	*% S. aureus*	% MRSA	% ORSA	% OSSA	% MDR
0-19	16.2	12.4	16.3	16.1	9.9
20-39	32.1	34.5	34.6	30.2	35.2
40-59	21.5	25.7	22.3	21	27
60-79	27.2	23	22.9	30.2	21.6
up to 80	3	4.4	3.9	2.5	6.3

Table 2. Percentage of isolated S. aureus strains according to source of specimens.

Type of specimens	% S. aureus	% MRSA	% ORSA	% OSSA	% MDR
Blood	25.7	22.1	22.9	27.8	23.5
Ear	1.1	NA	0.6	1.5	NA
Eye	2.5	2.65	2	2.9	0.9
Nasal Swab	0.3	NA	NA	0.5	NA
Abscess	25.7	21.25	24.9	26.3	22.5
Semen	0.3	NA	0.6	NA	NA
Sputum	8.1	10.6	8.5	7.8	9.9
Catheter tip	3.4	2.65	4.5	2.4	3.6
Urine	3.6	2.65	2	5	3.6
Wound	29.3	38.1	34	25.8	36

MRSA: Methicillin resistant *S. aureus* (Resistant to both Oxacillin and Cefoxitin).
ORSA: Oxacillinresistant *S. aureus.*
OSSA: Oxacillin susceptible *S. aureus.*
MDR: Multidrug resistant *S. aureus.*

Table 3. The susceptibility pattern of isolated *S. aureus* strains.

	S. aureus strains				
Types of Antibiotics	**% S. aureus**	**% OSSA**	**% MRSA**	**% ORSA**	**% MDR**
Oxacillin	57.3	100	0	0	9
Cefoxitin	63.7	91.2	0	26.8	9.9
Azithromycin	74.3	92.2	37.2	50.3	29.7
Chloramphenicol	94.4	98.5	86.7	88.9	82
Clindamycin	81.8	95.1	54.9	64.1	50.4
Erytromycin	58.9	78.5	23	32.7	7.2
Gentamicin	79.9	96.6	47	57.5	41.4
Levofloxacin	80.2	97	42.5	57.5	39.6
Linezolid	96.6	98.5	96.5	94.1	90.1
Nitrofurantin*	95.8	98.5	90.3	92.2	90
Penicillin G	37.2	52.2	5.3	17	9.9
Rifampin	92.7	95.6	85.8	88.9	82
Tetracycline	81.8	94.1	58.4	65.4	51.4
Trimeth/Sulpha	83.2	97	58.4	64.7	57.7
Teicoplanin	98	98.5	97.3	97.4	96.4
Vancomycin	100	100	100	100	100

*Nitrofurantin: Used for urine samples.

is important to choose the best antibiotic for the individual patient and for control of the endemicity of MRSA (18). In present study, we found that the prevalence of MRSA and MDR were equal. The percentage of ORSA was found to be higher than MRSA and MDR. The overall results of MRSA and MDR is lower than the percentage reported by Borg and Al-Zu'bi in 2003 and 2004, and similar to another study in 2005 (19, 20). Moreover, the percentage of OSSA was less than percentage cited in other studies (21-24).

The relationship between the sex and the infection of *S. aureus* types were not clear. The percentage of isolated *S. aureus* types varied from males to females in different studies (21, 25). Some retrospective case-control studies have found that males were one of the risk factors for community-acquired *S. aureus* infections (26, 27). In present study, we did not find any significant difference between both sexes that were infected with resistance types (MRSA, ORSA and MDR), while the percentages of isolated *S. aureus* and OSSA were high in males than females. This percentage may change depending on different factors such as the microbial community in patients bodies, method and the distribution of specimens collection during the period of study.

Nasal and skin carriage has been identified as an important risk factor for the development of *S. aureus* infections. The carriage rate depends on different factors such as sex and age (28, 29). The patients, whose age over 60 or very young age is susceptible to these infections (30). Hafeez et al. have shown that the distributions of *S. aureus* were similar among different age groups with the exception of newborns and 75 years and older (31). While Jessica et al. reported that the active age group (15 to 60 years old) was more susceptible to *S. aureus* infections to others (32). In this study the highest percentages isolated from (20 to 39 years) age group and the results were nearly equal in two age group between (40 to 79 years) the percentage were declined after 80 years of age (Table 1). Similar findings have been reported by others (16, 31, 33).

It has been reported that 80% of infections with *S. aureus* are endogenous, caused by the colonizing strain when enter the body by any way in both hospitals and the community (34). *S. aureus* has been found to be the most frequent cause of nosocomial pneumonia and surgical-wound infections and the second most common cause of nosocomial bloodstream infections (35). In this study, the majority of *S. aureus* strains of MRSA, ORSA and MDR (34 % to 38.1 %) were isolated from wound specimens. The second higher percentages were isolated from blood and ab-

scess specimens, while the bacterial strains in all other specimens were lower (Table 2). These results are compatible with different previous results reported in other parts of world (21, 23, 33, 36, 37).

Hafeez et al. (31) detected higher percentage of MRSA (40 %) in ear swabs. However, in this study MRSA were not detected in the same specimens, but ORSA were isolated in lower percentage. In some studies the MRSA have been isolated in lower percentage from blood specimens (24, 36). These variations may related to many reasons such as the patients population, types of skin normal flora, specimens collection procedures and number of specimens.

S. aureus can cause various types of human infections, the treatment of these infections and those resulted from the drug resistance strains of *S. aureus* is worldwide problem (9, 38). The prevalence of MRSA isolates have increased over the years (11, 39). In our study, isolated *S. aureus* and OSSA showed high rate of susceptibility (more than 80%) for 10/16 and 14/16 of all used antibiotic, respectively (Table 3). Other strains (MRSA, ORSA and MDR) showed higher sensitivity pattern (> 80%) to 6 out of 16 of the used antibiotic. These results may give a potential improvement of therapeutic options to treat the affected people by MRSA infection (1, 12, 28).

Susceptibility testing of MRSA now includes the more stable oxacillin instead of methicillin disk to test the susceptibility of the isolated strains to all β-lactam agents and cefoxitin for cephalosporin (2, 4, 15). The sensitivity pattern showed almost similar differences between the MRSA, ORSA and MDR strains. Consistent with other studies, these three strains were highly susceptible to glycopeptides (90.1 %) and linezolid, 100 % (31, 40). Like previous studies (16, 31, 35), these strains showed higher rates of resistance to macrolides (erythromycin and azithromycin). Lower sensitivity (14.6%) for erythromycin (21) and higher sensitivity (62 %) for azithromycin have also been reported (40).

Vancocomycin was the only antibiotic that was effective against all isolates in this study (100%), which makes it a drug of choice for treating multi-drug resistant MRSA. Nitrofurantoin was the second effective antibiotic after vancomycin (90 % to 92.2 %), similar to the report from Iraq (41). It followed by rifampicin and chloramphenicol (80 % to 88.9 %, respectively). Lower sensitivity to rifampicin has been reported by other authors (43% and 43.7%), (38, 39) and chloramphenicol (6.1 %) (34).

Medium sensitivity percentages were found to levofloxacin, gentamicin, clindamycin trimethoprim-sulfamethoxazole and tetracycline (Table 3). Different sensitivity percentages have been reported to these five antibiotics worldwide (21, 31, 41). These differences might be due to prolonged antibiotic treatment, age, type of infection and geographical variation.

In conclusion, the results of this study showed the importance of regular surveillance of hospital associated infections including monitoring antibiotic sensitivity pattern and strict drug policy for antibiotics used within and outside the hospital environments. Moreover, *in-vitro* susceptibility testing of every isolate of MRSA in the clinical laboratories may be helpful for reducing the incidence of these infections.

ACKNOWLEDGMENT

We would like to thank all King Abdullah University Hospital Laboratory staffs for their co-operation during the study period. We really appreciate and thank Prof. Abdulhafeez Khanfor his help in this study.

This research was supported by the Deanship of Scientific Research and Graduate Studies at Yarmouk University.

REFERENCES

1. Hardy KJ, Hawkey PM, Gao F, Oppenheim BA. Methicillin resistant *Staphylococcus aureus* in the critically ill. *Br J Anaesth* 2004;92:121-130.
2. Brown DF, Edwards DI, Hawkey PM, Morrison D, Ridgway GL, Towner KJ, et al. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus* (MRSA). *J Antimicrob Chemother* 2005;56:1000-1018.
3. Anupurba S, Sen MR, Nath G, Sharma BM, Gulati AK, Mohapatra TM. Prevalence of methicillin resistant *Staphylococcus aureus* in a tertiary referral hospital in eastern Uttar Pradesh. *Indian J Med Microbiol* 2003;21:49-51.
4. Palavecino E. Clinical, epidemiological, and laboratory aspects of methicillin-resistant *Staphylococcus aureus* (MRSA) infections. *Methods Mol Biol* 2007;391:1-19.
5. Szczepanik A, Koziol-Montewka M, Al-Doori Z, Morrison D, Kaczor D. Spread of a single multiresis-

tant methicillin-resistant *Staphylococcus aureus* clone carrying a variant of staphylococcal cassette chromosome mec type III isolated in a university hospital. *Eur J Clin Microbiol Infect Dis* 2007;26:29-35.
6. Hiramatsu K, Katayama Y, Yuzawa H, Ito T. Molecular genetics of methicillin-resistant *Staphylococcus aureus. Int J Med Microbiol* 2002;292:67-74.
7. Aires de Sousa M, de Lencastre H. Bridges from hospitals to the laboratory: genetic portraits of methicillin-resistant *Staphylococcus aureus* clones. *FEMS Immunol Med Microbiol* 2004 8;40:101-11.
8. National Nosocomial Infections Surveillance S. National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004. *Am J Infect Control* 2004;32:470-485.
9. Klein E, Smith DL, Laxminarayan R. Hospitalizations and deaths caused by methicillin-resistant *Staphylococcus aureus*, United States, 1999-2005. *Emerg Infect Dis* 2007;13:1840-1846.
10. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, et al. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA* 2007 17;298:1763-1771.
11. Robinson DA, Enright MC. Multilocus sequence typing and the evolution of methicillin-resistant *Staphylococcus aureus. Clin Micrbiol Infect* 2004;10:92-97.
12. Norazah A, Lim VK, Munirah SN, Kamel AG. *Staphylococcus aureus* carriage in selected communities and their antibiotic susceptibility patterns. *Med J Malaysia* 2003;58:255-2561.
13. Lu PL, Chin LC, Peng CF, Chiang YH, Chen TP, Ma L, et al. Risk factors and molecular analysis of community methicillin-resistant *Staphylococcus aureus* carriage. *J Clin Microbiol* 2005;43:132-139.
14. Cheesbrough MC (2006). District Laboratory Practice in Tropical Countries. 1st Edn., Cambridge University Press, Cambridge, UK.
15. Clinical and Laboratory Standards Institute (2009). Performance Standards for Antimicrobia Susceptibility Testing: Nineteenth Informational Supplement. CLSI document M100-S19.
16. Layer F, Ghebremedhin B, Moder KA, Konig W, Konig B. Comparative study using various methods for identification of Staphylococcus species in clinical specimens. *J Clin Microbiol* 2006;44:2824-2830.
17. Muto CA, Jernigan JA, Ostrowsky BE, Richet HM, Jarvis WR, Boyce JM, et al. SHEA guideline for preventing nosocomial transmission of multidrug-resistant strains of *Staphylococcus aureus* and enterococcus. *Infect Control Hosp Epidemiol* 2003;24:362-86.
18. L'Heriteau F, Alberti C, Cohen Y, Troche G, Moine P, Timsit JF. Nosocomial infection and multidrug-resistant bacteria surveillance in intensive care units: a survey in France. *Infect Control Hosp Epidemiol* 2005;26:13-20.
19. Borg MA, de Kraker M, Scicluna E, van de Sande-Bruinsma N, Tiemersma E, Monen J, et al. Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in invasive isolates from southern and eastern Mediterranean countries. *J Antimicrob Chemother* 2007;60:-1310-1315.
20. Al-Zu'bi E, Bdour S, Shehabi AA. Antibiotic resistance patterns of mecA-positive *Staphylococcus aureus* isolates from clinical specimens and nasal carriage. *Microb Drug Resist* 2004;10:321-324.
21. Rajaduraipandi K, Mani KR, Panneerselvam K, Mani M, Bhaskar M, Manikandan P. Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus*: a multicentre study. *Indian I Med Microbiol* 2006;24:34-38.
22. Pai V, Rao VI, Rao SP. Prevalence and Antimicrobial Susceptibility Pattern of Methicillin-resistant *Staphylococcus aureus* [MRSA] Isolates at a Tertiary Care Hospital in Mangalore, South India. *J Lab Physicians* 2010;2:82-84.
23. Chelliah A, Thyagarajan R, Katragadda R, Leela KV, Babu RN. Isolation of MRSA, ESBL and AmpC - beta -lactamases from Neonatal Sepsis at a Tertiary Care Hospital. *J Clin Diagn Res* 2014;8:DC24-27.
24. P RV, M J. A comparative analysis of community acquired and hospital acquired methicillin resistant *Staphylococcus aureus.* Journal of clinical and diagnostic research : JCDR. 2013;7:1339-42.
25. Coombs GW, Goering RV, Chua KY, Monecke S, Howden BP, Stinear TP, et al. The molecular epidemiology of the highly virulent ST93 Australian community *Staphylococcus aureus* strain. PloS one. 2012;7:e43037.
26. Laupland KB, Gregson DB, Zygun DA, Doig CJ, Mortis G, Church DL. Severe bloodstream infections: a population-based assessment. Critical care medicine. 2004;32:992-7.
27. Roder BL, Wandall DA, Frimodt-Moller N, Espersen F, Skinhoj P, Rosdahl VT. Clinical features of *Staphylococcus aureus endocarditis*: a 10-year experience in Denmark. Archives of internal medicine. 1999 Mar 8;159:462-469.
28. Davis KA, Stewart JJ, Crouch HK, Florez CE, Hospenthal DR. Methicillin-resistant *Staphylococcus aureus* (MRSA) nares colonization at hospital admission and its effect on subsequent MRSA infection. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2004 Sep 15;39:776-72.
29. Peacock SJ, Justice A, Griffiths D, de Silva GD, Kantzanou MN, Crook D, et al. Determinants of acquisition and carriage of *Staphylococcus aureus* in in-

fancy. Journal of clinical microbiology. 2003;41:5718-5725.
30. Marshall C, Wolfe R, Kossmann T, Wesselingh S, Harrington G, Spelman D. Risk factors for acquisition of methicillin-resistant *Staphylococcus aureus* (MRSA) by trauma patients in the intensive care unit. The Journal of hospital infection. 2004 Jul;57:245-252.
31. Hafeez R CA, Aslam M. Prevalence and Antimicrobial Susceptibility of Methicillin Resistant *Staphylococcus aureus* (MRSA). IJP 2004;2(1).
32. Bauer KA, West JE, Balada-Llasat JM, Pancholi P, Stevenson KB, Goff DA. An antimicrobial stewardship program's impact with rapid polymerase chain reaction methicillin-resistant *Staphylococcus aureus/S. aureus* blood culture test in patients with *S. aureus* bacteremia. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2010;1;51:1074-80.
33. Gayathri VR PP, Pazhanimuthu A and Prakash B. Epidemiology and Molecular Variations in Methicillin Resistant *Staphylococcus aureus* Isolated from Different Clinical Samples of Private Hospitals of Salem District, India. *Glob J Pharmacol* 2004;7:81-86.
34. Sakwinska O, Blanc DS, Lazor-Blanchet C, Moreillon M, Giddey M, Moreillon P. Ecological temporal stability of *Staphylococcus aureus* nasal carriage. *J Clin Microbiol* 2010;48:2724-2728.
35. Harbarth S, Fankhauser C, Schrenzel J, Christenson J, Gervaz P, Bandiera-Clerc C, et al. Universal screening for methicillin-resistant *Staphylococcus aureus* at hospital admission and nosocomial infection in surgical patients. *JAMA* 2008 ; 299:1149-1157.
36. Rao BN SB. A prospective study of Methicillin Resistant *Staphylococcus aureus* (MRSA) in a teaching hospital of rural setup. *JPSI* 2012;1:37- 40.
37. Anagaw B SY, Biadglegne F, Moges F, Kassu A, UnakalC,Mulu A. Frequency of Methicillin-resistant *Staphylococcus aureus* isolates from Clinical Specimens in Gondar University Hospital, Northwest Ethiopia. *Asian J Med Sci* 2013;5:59-64.
38. Akhter R KK, Hasan F. Isolation and antimicrobial susceptibility pattern of Methicillin Resistant and Methicillin sensitive *Staphylococcus aureus*. *Journal of Surgery Pakistan (International)* 2009;14:161-5.
39. Anbumani N WA, Kalyani J, Mallika M. Prevalence of methicillin-resistant *Staphylococcus aureus* in a Tertiary Referral Hospital in Chennai, South India. *Ind J Pract Doc* 2006;3:8-9.
40. Kaleem F UJ, Hassan A, Omair M, Khalid A, Roz U. Sensitivity pattern of methicillin resistant *Staphylococcus aureus* isolatedfrom patients admitted in a tertiary care hospital of Pakistan. *Iran J Microbil* 2010;2:141-3.
41. Babakir-Mina M, Othman N, Najmuldeen HH, Noori CK, Fatah CF, Perno CF, et al. Antibiotic susceptibility of vancomyin and nitrofurantoin in *Staphylococcus aureus* isolated from burnt patients in Sulaimaniyah, Iraqi Kurdistan. The new microbiologica. 2012;35:439-446.