ORIGINAL ARTICLE

Trends of Antibiotic Resistance in *Staphylococcus aureus* Isolates Obtained from Clinical Specimens

Hadi Sedigh Ebrahim-Saraie[1], Hamid Heidari[1], Reza Khashei[1], Fatemeh Edalati[1], Yalda Malekzadegan[1], Mohammad Motamedifar[1,2]*

[1]Department of Bacteriology and Virology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran, [2] Shiraz HIV/AIDS Research Center, Institute of Health, Shiraz University of Medical Sciences, Shiraz, Iran

Abstract:

Background: Staphylococcus aureus is one of the most common causes of nosocomial infections that cause a broad spectrum of diseases. Increasing antibiotic resistance among *S. aureus* isolates is a serious concern in the treatment and control of staphylococcal infections. The knowledge of *S. aureus* prevalence and the current antimicrobial resistance profile is necessary in selection of appropriate treatment of related infections. *Aim and Objectives:* The present study aimed to determine the frequency and antibiotic resistance profile of *S. aureus* isolates in clinical isolates. *Material and Methods:* This cross-sectional study was performed within six months from October 2012 on 345 *S. aureus* isolates collected from different clinical specimens from two major hospitals in Shiraz, Southwest Iran. *S. aureus* isolates were identified using standard microbiological procedure. Antimicrobial resistance patterns were determined using disk diffusion method in accordance with Clinical & Laboratory Standards Institute (CLSI) recommendation. *Results:* Overall, the most prevalent clinical source of Methicillin-Resistant *Staphylococcus aureus* (MRSA) isolation belonged to respiratory specimens. MRSA isolates significantly showed higher antibiotic resistance rates compared to Methicillin-Sensitive *Staphylococcus aureus* (MSSA) isolates (P <0.001). Despite the differences in relative frequency of MRSA isolates' antibiotic resistance between two hospitals, no statistical differences were estimated. The rate of MSSA isolates' antibiotic resistance between the studied hospitals are close and only significant differences were seen toward co-trimoxazole (P *<0.05*). Moreover, molecular characteristics were associated with specific isolation sites and antibiotic resistance trends. *Conclusion:* Demonstrated association of some clinical sources with higher rates of MRSA isolation and unique antibiotics trends and also association with their molecular background can help healthcare facilities manage efficient infection control policies.

Keywords: *Staphylococcus aureus*, Antibiotic Resistance, MRSA, Molecular Characterization

Introduction:

Staphylococcus aureus is one of the most common causes of nosocomial and community acquired infections that cause a broad spectrum of diseases such as boils, carbuncle, cellulitis, and abscesses [1, 2]. The reaction between *S. aureus* and human host is very complicated because it can colonize in many parts of the host [3]. Although primary colonization of *S. aureus* in these sites is not threatening, the consequence of their entrance into the body via invasive procedures (e.g. cutaneous burns, surgical incisions and indwelling catheters) or trauma may cause infections [4]. Moreover, *S. aureus* can be transmitted easily among patients and their visitors and extend the burden of infections [5].

Since the introduction of beta-lactam antibiotics, *S. aureus* drug-resistant strains have been rapidly developed; thus, the treatment of this organism has become difficult [2]. Increasing antibiotic resistance among *S. aureus* isolates, particularly Methicillin-Resistant *S. aureus* (MRSA) is a serious concern in the treatment and control of staphylococcal infections [6]. MRSA infections are a major cause of increasing morbidity and mortality [2]. Understanding MRSA resistance mechanisms and their current antimicrobial profile can be useful to overcome the higher emergence of MRSA strains from Iran compared to the reports from other countries in the Middle-East [7].

Antibiotic resistance is a growing problem in developing countries; many studies have reported increasing antibiotic resistance from Iran [8-17]. The knowledge of *S. aureus* prevalence and the current antimicrobial resistance profile is necessary in selection of appropriate treatment of *S. aureus* infections in any parts of the world. The present study was carried out to determine the frequency and antibiotic resistance profile of *S. aureus* isolates obtained from clinical specimens at Shiraz teaching hospitals, Southwest of Iran.

Material and Methods:

Study Design and Setting:

This cross-sectional study was performed within six months from October 2012 on 345 *S. aureus* isolates were collected from different clinical specimens from two main teaching hospitals (Shahid Faghihi and Nemazee hospitals) in Shiraz, the southwest of Iran. Nemazee and Faghihi hospitals are two major tertiary care hospitals affiliated to Shiraz University of Medical Sciences, Shiraz, Iran with 1000 beds.

Bacterial Isolates and Identification:

S. aureus isolates were recovered from different specimens such as blood, sputum, urine, wound and etc. Primary culture was performed on blood agar and subsequently identification of *S. aureus* isolates was done based on morphological characteristics and standard microbiological tests, including Gram-stain, Catalase, tube Coagulase, DNase and Manitol Salt Agar. The confirmed isolates were stored in Trypticase Soy Broth (TSB) with 15% glycerol at -70^0 C for long preservation.

Antimicrobial Susceptibility Testing:

Susceptibility profiles were determined toward Ampicillin (AP), Gentamicin (GM), Erythromycin (E), Tetracycline (T), Ciprofloxacin (CIP), Clindamycin (CD), Teicoplanin (TEC), Co-trimoxazole (SXT), Chloramphenicol (C), Rifampicin (RP), Linezolid (LZD), Synercid® (SYN), and Vancomycin (VA) antibiotic discs (MAST, UK) by the disc diffusion method according to Clinical & Laboratory Standards Institute (CLSI) guidelines on Muller-Hinton agar (Oxoid, UK). *S. aureus* ATCC 25923 which is a Methicillin-Sensitive *Staphylococcus aureus* (MSSA) was used in this study as the control strain in antibacterial susceptibility testing. MRSA isolates were screened based on resistance to cefoxitin (30µg) discs (MAST, UK) by disc diffusion method according to CLSI guidelines [18]. In results, intermediate-resistant isolate was considered as resistant. The isolates' resistance to cefoxitin was subsequently tested for presence of *mecA* gene by previously described primer [19].

Statistical Analysis:

Data were analyzed using Statistical Package for the Social Sciences (SPSS)™ software, version 19.0. The frequency of bacterial isolation and antibiotic resistance are presented as descriptive

statistics in terms of relative frequency. Chi–square or Fisher's exact tests was used to estimate any statistical association. Statistical significance was regarded as P values less than 0.05.

Results:

Frequency of MRSA Isolation:

The majority of *S. aureus* isolates in the present study were obtained from sputum 93 (27%) and urine 51 (14.8%) specimens. Frequency of MRSA isolation in the sputum specimens was notably high (62.4%); while, urine specimens was one of the lower MRSA isolation sources with 27.7%. The full rates of *S. aureus* isolation sources and MRSA frequency are demonstrated in Table 1.

Antibiotic Susceptibility Profile:

Overall, all of the tested MRSA and MSSA isolates were sensitive to teicoplanin, linezolid, synercid and vancomycin. Among other tested agents, except chloramphenicol, MRSA isolates significantly showed higher resistance rates compared to MSSA isolates (P <0.001) (Data not shown).

Faghihi MRSA Isolates:

Of MRSA isolates recovered from Faghihi hospital, the highest resistance rates were seen against tetracycline, ciprofloxacin and erythromycin each with 88.3% (Table 2). The obtained isolates from the wound and axillary specimens compared to those from other sources showed the lowest resistance rate toward tetracycline with 62.5% and 50%, respectively. MRSA isolates obtained from the superficial specimens showed a remarkable resistance rates to co-trimoxazole (more than 50%). Although the most of MRSA isolates showed maximum up to 50% resistance against rifampicin, the higher resistance rates were observed among the isolates obtained from the throat (100%) and sputum (82.9%). The most sensitive MRSA isolates belonged to those obtained from urine samples.

Nemazee MRSA Isolates:

The highest antibiotic resistance rates among MRSA isolates recovered from Nemazee hospital were seen against tetracycline 91.3%, ciprofloxacin and gentamicin each with 89.8% (Table 3). Moreover, the lowest resistance frequency was against co-trimoxazole (42%). Also, the most sensitive isolates against the tested antibiotics belonged to MRSA isolates obtained from eye specimens.

Faghihi MSSA Isolates:

Out of 114 MSSA isolates from Faghihi hospital, the highest resistance rate was seen against ampicillin 93%, followed by erythromycin and tetracycline with 32.5% and 29.8%, respectively (Table 4). While the most of MSSA isolates were resistant to ampicillin, the isolates obtained from wound and urine specimens each with 84.6% showed a lower resistance rate compared to others. Moreover, the most resistant isolates against the tested antibiotics were seen among those recovered from the throat specimens.

Nemazee MSSA Isolates:

The highest antibiotic resistance rates among MSSA isolates obtained from Nemazee hospital were against ampicillin (94.1%), tetracycline (41.2%) and erythromycin (31.8%) (Table 5). However, the lowest resistance rate to ampicillin (72.7%) belonged to urine MSSA isolates. Moreover, isolates recovered from ETT specimens showed the highest resistance rate to tetracycline (66.7%). The highest rate of erythromycin resistant isolates belonged to ETT (100%), TJPS (66.7%), and wound (66%) MSSA isolates. Overall, TJPS MSSA isolates showed higher resistance to the tested agents compared to others.

Table 1: Proportion of *S. aureus* and MRSA Isolates with Respect to the Source of Isolation

Samples	Total *S. aureus*		MRSA	
	No.	%	No.	%
Sputum	93	27	58	62.4
Urine	51	14.8	14	27.4
Blood	46	13.3	20	43.5
Wound	41	11.9	18	43.9
Nose	27	7.8	3	11.1
Skin	25	7.2	8	32
Throat	12	3.5	4	33.3
ETT	9	2.6	6	66.7
Eye	9	2.6	3	33.3
Body fluid	8	2.3	4	50
CSF	5	1.4	2	40
Axillary	2	0.6	2	100
Ear	2	0.6	0	0
TJPS	2	0.6	0	0
Plural	1	0.3	1	100
Other	12	3.5	3	25
Total	**345**	**100**	**146**	**42.3**

ETT- Endotracheal Tube; CSF-Cerebrospinal Fluid;
TJPS- Trans Jugular Intrahepatic Portosystemic Shunt

Table 2: Antibiotic Resistance Pattern of MRSA Isolates Obtained from Faghihi Hospital

Samples	No.	Antibiotic								
		RP No. (%)	**AP No. (%)**	**T No. (%)**	**CIP No. (%)**	**SXT No. (%)**	**E No. (%)**	**CD No. (%)**	**GM No. (%)**	**C No. (%)**
Sputum	**35**	29 (82.9)	35 (100.0)	33 (94.3)	33 (94.3)	04 (11.4)	33 (94.3)	33 (94.3)	33 (94.3)	02 (5.7)
Blood	**09**	05 (55.6)	09 (100.0)	08 (88.9)	07 (77.8)	03 (33.3)	06 (66.7)	06 (66.7)	06 (66.7)	0
Urine	**09**	03 (33.3)	09 (100.0)	08 (88.9)	05 (55.5)	02 (22.2)	06 (66.7)	06 (66.7)	04 (44.4)	0
Wound	**08**	03 (37.5)	08 (100.0)	05 (62.5)	08 (100.0)	05 (62.5)	08 (100.0)	08 (100.0)	08 (100.0)	0
Skin	**08**	0	08 (100.0)	07 (87.5)	07 (87.5)	06 (75.0)	07 (87.5)	07 (87.5)	04 (50.0)	0
Body fluids	**03**	01 (33.3)	03 (100.0)	03 (100.0)	03 (100.0)	0	03 (100.0)	03 (100.0)	03 (100.0)	0
Nose	**02**	01 (50.0)	02 (100.0)	02 (100.0)	02 (100.0)	0	02 (100.0)	02 (100.0)	01 (50.0)	0
Axillary	**02**	01 (50.0)	02 (100.0)	01 (50.0)	02 (100.0)	01 (50.0)	02 (100.0)	02 (100.0)	02 (100.0)	01 (50.0)
Throat	**01**	01 (100.0)	01 (100.0)	01 (100.0)	01 (100.0)	0	01 (100.0)	01 (100.0)	01 (100.0)	0
Total	**77**	**44.0 (57.1)**	**77.0 (100.0)**	**68.0 (88.3)**	**68.0 (88.3)**	**21.0 (27.3)**	**68.0 (88.3)**	**68.0 (88.3)**	**62.0 (80.5)**	**03.0 (3.9)**

RP- Rifampicin, AP- Ampicillin, T- Tetracycline, CIP- Ciprofloxacin, SXT- Co-trimoxazole, E- Erythromycin, CD- Clindamycin, GM- Gentamicin, C- Chloramphenicol

Table 3: Antibiotic Resistance Pattern of MRSA Isolates Obtained from Nemazee hospital [a]

Samples	No.	Antibiotics								
		RP No. (%)	AP No. (%)	T No. (%)	CIP No. (%)	SXT No. (%)	E No. (%)	CD No. (%)	GM No. (%)	C No. (%)
Sputum	**23**	20 (86.9)	23 (100.0)	23 (100.0)	22 (95.6)	03 (13.0)	20 (86.9)	20 (86.9)	22 (95.6)	0
Blood	**11**	10 (90.9)	11 (100.0)	08 (72.7)	11 (100.0)	02 (18.2)	10 (90.9)	10 (90.9)	11 (100.0)	0
Wound	**10**	05 (50.0)	10 (100.0)	10 (100.0)	08 (80.0)	03 (30.0)	08 (80.0)	08 (80.0)	08 (80.0)	0
ETT	**06**	03 (50.0)	06 (100.0)	05 (83.33)	04 (66.67)	01 (16.7)	05 (83.3)	05 (83.3)	04 (66.67)	0
Urine	**05**	02 (40.0)	05 (100.0)	04 (80.0)	04 (80.0)	02 (40.0)	04 (80.0)	04 (80.0)	04 (80.0)	0
Eye	**03**	01 (33.3)	03 (100.0)	02 (66.7)	02 (66.7)	01 (33.3)	01 (33.3)	01 (33.3)	02 (66.7)	0
Throat	**03**	03 (100.0)	03 (100.0)	03 (100.0)	03 (100.0)	0	03 (100.0)	03 (100.0)	03 (100.0)	0
CSF	**02**	01 (50.0)	02 (100.0)	02 (100.0)	02 (100.0)	01 (50.0)	02 (100.0)	02 (100.0)	02 (100.0)	0
Plural	**01**	01 (100.0)	01 (100.0)	01 (100.0)	01 (100.0)	0	01 (100.0)	01 (100.0)	01 (100.0)	0
Body fluids	**01**	01 (100.0)	01 (100.0)	01 (100.0)	01 (100.0)	0	01 (100.0)	01 (100.0)	01 (100.0)	0
Nose	**01**	01 (100.0)	01 (100.0)	01 (100.0)	01 (100.0)	0	01 (100.0)	01 (100.0)	01 (100.0)	0
Other	**03**	03 (100.0)	03 (100.0)	03 (100.0)	03 (100.0)	0	03 (100.0)	03 (100.0)	03 (100.0)	1 (33.3)
Total	**69**	**51 (73.9)**	**69 (100.0)**	**63 (91.3)**	**62 (89.8)**	**13 (18.8)**	**59 (85.5)**	**59 (85.5)**	**62 (89.8)**	**01 (1.4)**

ETT- Endotracheal Tube; CSF-Cerebrospinal Fluid, RP- Rifampicin, AP- Ampicillin, T- Tetracycline, CIP- Ciprofloxacin, SXT- Co-trimoxazole, E- Erythromycin, CD- Clindamycin, GM- Gentamicin, C- Chloramphenicol

Table 4: Antibiotic Resistance Pattern of MSSA Isolates Obtained from Faghihi hospital

Samples	No.	Antibiotics								
		RP No. (%)	AP No. (%)	T No. (%)	CIP No. (%)	SXT No. (%)	E No. (%)	CD No. (%)	GM No. (%)	C No. (%)
Urine	**26**	0	22 (84.6)	09 (34.6)	02 (7.7)	04 (15.4)	06 (23.1)	01 (3.8)	02 (7.7)	0
Skin	**17**	0	17 (100.0)	04 (23.5)	02 (11.8)	0	07 (41.2)	04 (23.5)	01 (5.9)	0
Sputum	**16**	01 (6.3)	16 (100.0)	05 (31.3)	0	03 (18.8)	06 (37.5)	02 (12.5)	01 (6.3)	0
Nose	**16**	0	15 (93.8)	01 (6.3)	0	0	05 (31.3)	02 (12.5)	02 (12.5)	0
Blood	**14**	0	13 (92.9)	07 (50.0)	0	0	05 (35.7)	03 (21.4)	02 (14.3)	0
Wound	**13**	0	11 (84.6)	02 (15.4)	0	0	02 (15.4)	0	01 (7.7)	0
Throat	**04**	0	04 (100.0)	03 (75.0)	04 (100.0)	04 (100.0)	03 (75.0)	0	0	0
Ear	**02**	0	02 (100.0)	0	0	0	01 (50.0)	0	0	0
Eye	**01**	0	01 (100.0)	0	0	0	0	0	0	0
Body fluid	**01**	0	01 (100.0)	01 (100.0)	0	01 (100.0)	0	0	0	0
Other	**04**	0	04 (100.0)	02 (50.0)	0	0	02 (50.0)	0	01 (25.0)	0
Total	**114**	**01 (0.9)**	**106 (93.0)**	**34 (29.8)**	**08 (7.0)**	**12 (10.5)**	**37 (32.5)**	**12 (10.5)**	**10 (8.8)**	**0**

RP- Rifampicin, AP- Ampicillin, T- Tetracycline, CIP- Ciprofloxacin, SXT- Co-trimoxazole, E- Erythromycin, CD- Clindamycin, GM- Gentamicin, C- Chloramphenicol

Table 5: Antibiotic Resistance Pattern of MSSA Isolates Obtained from Nemazee hospital

Samples	No.	Antibiotics								
		RP No. (%)	AP No. (%)	T No. (%)	CIP No. (%)	SXT No. (%)	E No. (%)	CD No. (%)	GM No. (%)	C No. (%)
Sputum	**19**	0	19 (100.0)	08 (42.1)	0	0	04 (21.1)	01 (5.3)	0	0
Blood	**12**	0	12 (100.0)	03 (25.0)	02 (16.7)	0	02 (16.7)	01 (8.3)	0	0
Urine	**11**	0	08 (72.7)	03 (27.3)	0	0	04 (36.4)	0	0	0
Wound	**10**	0	10 (100.0)	05 (50.0)	02 (20.0)	0	06 (60.0)	03 (30.0)	02 (20.0)	01 (10.0)
Nose	**08**	0	07 (87.5)	04 (50.0)	0	0	03 (37.5)	0	0	0
EYE	**05**	0	04 (80.0)	02 (40.0)	0	0	01 (20.0)	0	0	0
Throat	**04**	0	04 (100.0)	02 (50.0)	0	0	01 (25.0)	0	01 (25.0)	0
Body fluids	**03**	0	03 (100.0)	01 (33.3)	0	01 (33.3)	0	0	0	0
ETT	**03**	0	03 (100.0)	02 (66.7)	0	0	02 (66.7)	02 (66.7)	01 (33.3)	0
CSF	**03**	0	03 (100.0)	0	0	0	0	0	0	0
TJPS	**02**	0	02 (100.0)	01 (50.0)	0	0	02 (100.0)	02 (100.0)	01 (50.0)	0
Other	**05**	0	05 (100.0)	04 (80.0)	02 (40.0)	0	02 (40.0)	01 (20.0)	0	0
Total	**85**	**0**	**80 (94.1)**	**35 (41.2)**	**06 (7.1)**	**01 (1.2)**	**27 (31.8)**	**10 (11.8)**	**05 (5.9)**	**01 (1.2)**

ETT- Endotracheal Tube, CSF-Cerebrospinal Fluid, TJPS- Trans Jugular Intrahepatic Portosystemic Shunt, RP- Rifampicin, AP- Ampicillin, T- Tetracycline, CIP- Ciprofloxacin, SXT- Co-trimoxazole, E- Erythromycin, CD- Clindamycin, GM- Gentamicin, C- Chloramphenicol

Discussion:

Because of the devastating nature of MRSA infections and their potential ability to acquire antimicrobial resistance over time, nosocomial MRSA will continue to be a problem in the future [20]. Therefore, early detection of MRSA strains is an important step toward decreasing the unwanted consequence of MRSA infections. According to the reports from Iran, the incidence of MRSA is high and the mean prevalence of MRSA is 52.7% ± 4.7 [21]. In this study, the frequency of MRSA among clinical specimens was estimated 42.3% which, as shown before, is slightly higher than that of the north of Iran [1, 7]. However, compared to previous reports from Shiraz (2006-2007), it seems that MRSA rate (43.8%) was steady over these years [22]. Moreover, the frequency of MRSA in Nemazee hospital (44.8%) was more than Faghihi hospital (40.3%); however, as previously shown, there was no significant difference in the rates of MRSA isolation between the studied hospitals [23].

In this study, the most prevalent clinical source of MRSA isolation belonged to respiratory specimens, which may indicate the main route of MRSA transmission in studied hospitals. Moreover, there are some reports showing that human respiratory system is one the favorite sites of MRSA colonization [24, 25]. In our study, blood specimens is another source which has a remarkable rate of MRSA isolation (43.5%), and it is an important finding due to the significant mortality and morbidity of MRSA bloodstream infections compared to MSSA isolates [26]. The observed higher MRSA isolation rates in respiratory and blood specimens may relate to their molecular characterization since we previously showed that most of the respiratory and blood MRSA isolates contain specified Staphylococcal Cassette Chromosome (SCC) types (SCC*mec* types 1 and 2) and these types inclined to specific human sites [7].

The variability of MRSA antibiotic resistance patterns shown at different places and times around the world is one of the main reasons for the success spread of this pathogen. Therefore, understanding new and emerging resistance trends depend on periodic monitoring of MRSA resistance patterns [22].

As seen in our results, MRSA isolates showed higher antibiotic resistance compared to MSSA isolates which according to previous reports is not surprising [2, 24, 27, 28]. Compared to previous reports from Shiraz by Japoni *et al.* [22], there are no remarkable changes in antibiotic resistance pattern of MSSA isolates except susceptibility to gentamicin, which increased from 19% to more than 90% in our results. In MRSA isolates changes are more significant since toward rifampicin, co-trimoxazole and clindamycin we see differences in the rates of antibiotic resistance with Japoni *et al*.'s findings [22]. The higher susceptibility toward rifampicin in our results mainly comes from isolates recovered from Faghihi hospitals, while the study of Japoni *et al*. was performed in Nemazee hospital [22]. The differences in sensitivity to co-trimoxazole may originate from differences in molecular characterization of our isolates compared to theirs [22]. We showed SCC*mec* type 1 as the predominant type in our MRSA isolates [7], while Japoni *et al*. reported SCC*mec* type 3 [29]. Also, such high resistance rate against co-trimoxazole was mentioned in MRSA isolates containing SCC*mec* type 3 by Fatholahzadeh *et al.* from Tehran, the north of Iran [30]. It was indicated that each MRSA clone may have expected antibiotic susceptibility patterns

[31]. The highest resistance rate against clindamycin in our results may be due to inducible clindamycin resistance [7], which was not detected in Japoni *et al*.'s survey [22]. However, antibiotic susceptibility patterns are multi-factorial and as seen in previous reports could be very variable [2, 32].

Overall, there were no significant differences between MRSA isolates of the two studied hospitals in terms of antibiotic resistance patterns; however, there were unique patterns in each hospital. The relative frequency of co-trimoxazole resistance was higher in Faghihi MRSA isolates, which mainly originates from skin samples, while Nemazee hospital did not have skin ward. The isolates at superficial sites are more likely to have the chance to acquire antibiotic resistance, because of more exposure to the environment [33]. Moreover, relative frequency of rifampicin was lower in Faghihi MRSA isolates; it may be from different usage or prescription of this antibiotic in related hospitals.

The rate of MSSA isolates' antibiotic resistance between the two studied hospitals is close and only significant differences were seen toward co-trimoxazole, which was higher in Faghihi isolates (P <0.05). MSSA isolates obtained from Trans-Jugular Intrahepatic Portosystemic Shunt (TJPS) specimens showed remarkable antibiotic resistance, which may originate from high-dose chemotherapy administration in liver transplant recipients. However, lower rates of antibiotic resistance in MSSA compared to MRSA isolates could not rule out their potential risk in nosocomial infections, since they could be more pathogenic than MRSA isolates in term of virulence factors [23].

In summary, the lack of MIC breakpoints for those antibiotics which were sensitive to all tested isolates was one of the main limitations of our study. However, beside the limitations, we have some valuable outcomes. Firstly, we showed association of some clinical sources with higher rates of MRSA isolation and unique antibiotics trends. Second, we showed that antibiotic resistance may arise from a molecular background. Observed diversity in antibiotic susceptibility profile of *S. aureus* isolates obtained from studied hospitals with less than 2 km distance and even diversity with results of the same hospital during time highlights the importance of periodic surveillance for more effective infection control policies.

Acknowledgements

The authors would like to thank Mr. Abasian for their kind assistance to provide control strains. This study was supported by Shiraz University of Medical Sciences grant no. 92-6514 and is a part of an MSc thesis by Hadi Sedigh Ebrahim-Saraie.

References

1. Hoseini Alfatemi SM, Motamedifar M, Hadi N, Sedigh Ebrahim Saraie H. Analysis of Virulence Genes among Methicillin Resistant *Staphylococcus aureus* (MRSA) Strains. *Jundishapur J Microbiol* 2014; 7(6):e10741.
2. Shittu AO, Lin J. Antimicrobial susceptibility patterns and characterization of clinical isolates of *Staphylococcus aureus* in KwaZulu-Natal province, South Africa. *BMC Infect Dis* 2006; 6:125.
3. Talebi-Satlou R, Ahmadi M, Dastmalchi Saei H. Restriction Fragment Length Polymorphism Genotyping of Human *Staphylococcus aureus* Isolates From Two Hospitals in Urmia Region of Iran Using the coa Gene. *Jundishapur J Microbiol* 2012; 5(2):416-420.

4. Spellberg B, Daum R. Development of a vaccine against *Staphylococcus aureus*. *Semin Immunopathol* 2012; 34(2):335-348
5. David MZ, Daum RS. Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. *Clin Microbiol Rev* 2010; 23(3):616-687.
6. Orrett FA, Land M. Methicillin-resistant *Staphylococcus aureus* prevalence: current susceptibility patterns in Trinidad. *BMC Infect Dis* 2006; 6:83.
7. Ebrahim-Saraie HS, Motamedifar M, Sarvari J, Hoseini Alfatemi SM. Emergence of SCC*mec* Type I Obtained From Clinical Samples in Shiraz Teaching Hospitals, South-West of Iran. *Jundishapur J Microbiol* 2015; 8(6):e16998.
8. Heidari H, Hasanpour S, Ebrahim-Saraie HS, Motamedifar M. High Incidence of Virulence Factors among Clinical *Enterococcus faecalis* Isolates in Southwestern Iran. *Infect Chemother* 2017; 49(1):51-56.
9. Haghighatpanah M, Mozaffari Nejad AS, Mojtahedi A, Amirmozafari N, Zeighami H. Detection of extended-spectrum beta-lactamase (ESBL) and plasmid-borne blaCTX-M and blaTEM genes among clinical strains of *Escherichia coli* isolated from patients in the north of Iran. *J Glob Antimicrob Resist* 2016; 7:110-113.
10. Zalipour M, Sedigh Ebrahim-Saraie H, Sarvari J, Khashei R. Detection of Biofilm Production Capability and icaA/D Genes Among Staphylococci Isolates from Shiraz, Iran. *Jundishapur J Microbiol* 2016; 9(12):e41431.
11. Motamedifar M, Sedigh Ebrahim-Saraie H, Mansury D, Nikokar I, Hashemizadeh Z. Prevalence of Etiological Agents and Antimicrobial Resistance Patterns of Bacterial Meningitis in Nemazee Hospital, Shiraz, Iran. *Arch Clin Infect Dis* 2015; 10(2):e22703.
12. Kazemian H, Heidari H, Ghanavati R, Mohebi R, Ghafourian S, Shavalipour A, *et al*. Characterization of Antimicrobial Resistance Pattern and Molecular Analysis among Extended Spectrum β-Lactamase-Producing *Escherichia coli*. *Pharm Sci* 2016; 22(4): 279-284.
13. Heidari H, Emaneini M, Dabiri H, Jabalameli F. Virulence factors, antimicrobial resistance pattern and molecular analysis of Enterococcal strains isolated from burn patients. *Microb Pathog* 2016;90:93-97.
14. Amini V, Kazemian H, Yamchi JK, Feyisa SG, Aslani S, Shavalipour A, et al. Evaluation of the Immunogenicity of Diphtheria Toxoid Conjugated to Salmonella Typhimurium-Derived OPS in a Mouse Model: A Potential Vaccine Candidate Against Salmonellosis. *Iran Red Crescent Med J* 2016; 18(7): e34135.
15. Kazemian H, Ghafourian S, Heidari H, Amiri P, Yamchi JK, Shavalipour A, et al. Antibacterial, anti-swarming and anti-biofilm formation activities of Chamaemelum nobile against Pseudomonas aeruginosa. *Rev Soc Bras Med Trop* 2015;48(4):432-36.
16. Seifi K, Kazemian H, Heidari H, Rezagholizadeh F, Saee Y, Shirvani F, *et al*. Evaluation of Biofilm Formation Among Klebsiella pneumoniae Isolates and Molecular Characterization by ERIC-PCR. *Jundishapur J Microbiol* 2016; 9(1):e30682.
17. Kaveh M, Bazargani A, Ramzi M, Sedigh Ebrahim-Saraie H, Heidari H. Colonization rate and risk factors of vancomycin-resistant enterococci among patients received hematopoietic stem cell transplantation in Shiraz, Southern Iran. *Int J Organ Transplant Med* 2016; 7(4): 197-205.
18. CLSI (2014). Performance Standards for Antimicrobial Susceptibility Testing; 24th Informational Supplement. M100-S24. Wayne, PA: Clinical and Laboratory Standards Institute.
19. Zhang K, McClure JA, Elsayed S, Louie T, Conly JM. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant Staphylococcus aureus. *J Clin Microbiol* 2005; 43(10):5026-33.
20. Chambers HF, Deleo FR. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nat Rev Microbiol* 2009; 7(9):629-41.
21. Askari E, Soleymani F, Arianpoor A, Tabatabai SM, Amini A, Naderinasab M. Epidemiology of mecA-Methicillin Resistant *Staphylococcus aureus* (MRSA) in Iran: A Systematic Review and Meta-analysis. *Iran J Basic Med Sci* 2012; 15(5):1010-19.
22. Japoni A, Ziyaeyan M, Jmalidoust M, Farshad S, Alborzi A, Rafaatpour N, *et al*. Antibacterial susceptibility patterns and cross-resistance of methicillin resistant and sensitive *Staphyloccus aureus* isolated from the hospitalized patients in Shiraz, Iran. *Braz J Microbiol* 2010; 41(3):567-73.

23. Motamedifar M, Ebrahim-Saraie HS, Alfatemi SM, Zalipour M, Kaveh M, Khoshkharam-Roodmajani H. Frequency of the toxic shock syndrome toxin-1 gene in methicillin-susceptible and -resistant *Staphylococcus aureus* isolates from teaching hospitals in Shiraz, Iran. *Rev Soc Bras Med Trop* 2015; 48(1):90-93.
24. Dibah S, Arzanlou M, Jannati E, Shapouri R. Prevalence and antimicrobial resistance pattern of methicillin resistant Staphylococcus aureus (MRSA) strains isolated from clinical specimens in Ardabil, Iran. *Iran J Microbiol* 2014; 6(3):163-168.
25. Korn GP, Martino MD, Mimica IM, Mimica LJ, Chiavone PA, Musolino LR. High frequency of colonization and absence of identifiable risk factors for methicillin-resistant *Staphylococcus aureus* (MRSA) in intensive care units in Brazil. *Braz J Infect Dis* 2001; 5(1):1-7.
26. Naves KS, Vaz da Trindade N, Gontijo Filho PP. Methicillin-resistant *Staphylococcus aureus* bloodstream infection: risk factors and clinical outcome in non-intensive-care units. *Rev Soc Bras Med Trop* 2012; 45(2):189-193.
27. Kejela T, Bacha K. Prevalence and antibiotic susceptibility pattern of methicillin-resistant *Staphylococcus aureus* (MRSA) among primary school children and prisoners in Jimma Town, Southwest Ethiopia. *Ann Clin Microbiol Antimicrob* 2013; 12(11):1476-0711.
28. Rahimi F, Bouzari M, Katouli M, Pourshafie MR. Antibiotic Resistance Pattern of Methicillin Resistant and Methicillin Sensitive *Staphylococcus aureus* Isolates in Tehran, Iran. *Jundishapur J Microbiol* 2013; 6(2):144-149.
29. Japoni A, Jamalidoust M, Farshad S, Ziyaeyan M, Alborzi A, Japoni S, *et al*. Characterization of SCC*mec* types and antibacterial susceptibility patterns of methicillin-resistant *Staphylococcus aureus* in Southern Iran. *Jpn J Infect Dis* 2011; 64(1):28–33.
30. Fatholahzadeh B, Emaneini M, Gilbert G, Udo E, Aligholi M, Modarressi MH, et al. Staphylococcal cassette chromosome mec (SCC*mec*) analysis and antimicrobial susceptibility patterns of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in Tehran, Iran. *Microb Drug Resist* 2008; 14(3):217–220.
31. Oliveira DC, Tomasz A, De-Lencastre H. Secrets of success of a human pathogen: molecular evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*. Lancet *Infect Dis* 2002; 2(3):180–189.
32. Graffunder EM, Venezia RA. Risk factors associated with nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) infection including previous use of antimicrobials. *J Antimicrob Chemother* 2002; 49(6): 999-1005.
33. Khashei R, Sarie HSE, Alfatemi MH, Zomorodian K. Antimicrobial resistance patterns of colonizing microflora on the personnel hands and noses working in the Neonatal Intensive Care Unit (NICU). *World Appl Sci J* 2014; 30(10):1232-37.

[*] ***Author for Correspondence:*** *Mohammad Motamedifar, Department of Bacteriology and Virology, School of Medicine, Shiraz University of Medical Sciences, Zand st., Imam Hossein sq., Shiraz, Iran.*
Email: motamedm@yahoo.com & motamedm@sums.ac.ir Tel/Fax: +98-71 32304356