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Characteristics of Methicillin Resistant *Staphylococcus aureus* Strains Isolated from Hospital Wastewater in Tehran, Iran

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ABSTRACT

Backgrounds: Hospital sewage is known as an important source of human pathogenic bacteria such as methicillin resistant *Staphylococcus aureus* (MRSA) strains disseminated from hospital to the environment. This study aimed to investigate the presence of MRSA in the treated outgoing wastewater collected from a referral hospital in Tehran, Iran.

Materials & Methods: During 2015, sampling was carried out at two stages from a hospital wastewater. All black colonies with halos on HiCrome aureus agar medium supplemented with oxacillin were collected and identified as MRSA using specific primers for *nuc*A and *mec*A genes. Isolates susceptibility to 18 antibiotics was determined according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI). Bacterial typing was performed for the isolates using a combination of Phene plate (PhP) typing, prophage typing, staphylococcal cassette chromosome *mec* (SCC*mec*) and *ccr* typing methods.

Findings: A total of 79 MRSA isolates were confirmed using specific primers and showed susceptibility to quinupristin-dalfopristin, vancomycin, chloramphenicol, and linezolid. High resistance to penicillin, ciprofloxacin, kanamycin, tobramycin, and erythromycin was reported. Sixteen PhP types consisting of eight common types (CTs) and eight single types (STs) were identified among the strains, among which CT1 was the dominant type. Also, two prophage patterns and four prophage types were identified, and all the strains were positive for SCC*mec* type III and *ccr* type 3.

Conclusion: The results of this study revealed that sewage-treatment process was able to remove community-acquired MRSA (CA-MRSA) strains; however, hospital-acquired MRSA (HA-MRSA) strains were able to survive during the treatment process in this hospital.

Keywords: Methicillin resistant *Staphylococcus aureus*, Wastewater, Hospital, Bacterial typing, Community-acquired MRSA, Hospital-acquired MRSA.

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Introduction

Staphylococcus aureus is known as one of the most common human bacterial pathogen and the cause of a variety of infections such as skin and soft tissue infections, septicemia, fatal necrotizing pneumonia, osteomyelitis, endovascular infections, endocarditis, septic arthritis, and food poisoning [1]. The high potential of *S. aureus* to cause a broad spectrum of important infections in humans is due to the expression of a variety of virulence factors that participate in pathogenesis and allow this bacterium to adhere to surface/tissues, evade from the immune system, and produce different enzymes and lethal toxins [2]. *S. aureus* strains have the ability to acquire mobile genetic elements and show resistance to a variety of antimicrobial agents and different classes of antibiotics.

Methicillin was first introduced in 1960 to treat infections caused by penicillin resistant *S. aureus* strains, and the first methicillin resistant *S. aureus* (MRSA) strain was reported in 1961 in the United Kingdom. Over the past decades, different clonal groups of *S. aureus* have disseminated in different communities and hospitals worldwide [3]. Resistance to methicillin could be due to the presence of *mec*A gene encoding a penicillin binding protein 2a (PBP2a) which has a low affinity for most semi-synthetic penicillins, such as methicillin, oxacillin, and nafcillin [4]. The *mec*A gene and its regulatory genes (e.g. *mec*I and *mec*R) are part of a mobile genetic element called staphylococcal cassette chromosome *mec* (SCC*mec*). Based on the *mec* gene complex classes and the cassette chromosome recombinase (*ccr*) gene types, 13 different types of SCC*mec* have been reported so far among livestock-associated MRSA (LA-MRSA), hospital-acquired MRSA (HA-MRSA), and community-acquired MRSA (CA-MRSA) [5].

Sewage is one of the most important sources for persistence and dissemination of different pathogenic bacteria as well as antibiotic resistance genes in the environment and community. Also, *S. aureus* is released to the environment directly or via the urban and hospital sewage and could persist there for a long time. Hospital sewage plays an important role in the dissemination of clonal groups of MRSA strains from the hospital environment to the community. The presence of MRSA strains in hospital sewage and sewage-treatment plants (STPs) has already been reported in Iran and other countries [5-11]. Therefore, typing of MRSA strains could be a useful method to reveal their clonal dissemination in different geographical regions. Different typing methods such as SCC*mec* typing, prophage typing, pulsed field gel electrophoresis (PFGE), multilocus sequence typing (MLST), *spa* typing, *coa* typing, and Phene Plate (PhP) biochemical fingerprinting are commonly used in epidemiological studies on MRSA [12]. PhP biochemical fingerprinting is a high-resolution powerful method used to type different pathogenic bacteria such as MRSA. This system is based on the measurement of the absorbance value of fermentation reactions at three different intervals, and data are analyzed using PhPWin software (PhPlate Microplates Techniques AB, Sweden) [3, 11]. To date, more than 250 staphylococcal bacteriophages have been identified and classified into SGA, SGB, SGD, SGF (SGFa and SGFb), and SGL prophage types according to their morphology as well as lytic and serological activities. Prophage typing is one of the most common typing method used extensively for different bacterial genera and species. This method is a time-consuming and tedious method needing to an international phage set that is not available in most laboratories. On the other hand, detection of different prophage genes among MRSA strains using specific

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primers is a convenient, reliable, and rapid method that could be employed to detect different prophage types among MRSA strains [13-14].

Objectives: The present study aimed to investigate the presence of different clonal groups of multidrug resistant MRSA strains in the wastewater of a referral hospital in Tehran, Iran.

Materials and Methods

Sampling and isolation of strains: During February to March 2015, two sewage samples were collected from the treated outgoing wastewater of a referral hospital in Tehran, Iran. Samples were collected in sterile (500 mL) bottles and transferred to the laboratory while maintaining cold chain. All samples were five-fold diluted using phosphate buffered saline (PBS). Then 500 mL of each diluted sample was filtered through a 0.45 µm membrane (Millipore Corporation, Burlington, MA, USA) and placed on HiCrome aureus agar medium (Hi Media Ltd, Mumbai, India) supplemented with 1 µg/mL of oxacillin antibiotic (Sigma-Aldrich, Mo, USA) and incubated at 37 °C for 48 hrs [10]. Black colonies with opaque halos were selected and identified as MRSA using specific primers for *nuc*A and *mec*A genes as described previously [3].

Antibiotic susceptibility testing: All oxacillin resistant *S. aureus* strains were examined for susceptibility to amikacin (30 µg), cefoxitin (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), kanamycin (30 µg), linezolid (30 µg), minocycline (30 µg), nitrofurantoin (50 µg), penicillin (10 U), quinupristin-dalfopristin (15 µg), rifampin (5 µg), tetracycline (30 µg), trimethoprim-sulfamethoxazole (1.25-23.75 µg), and tobramycin (10 µg) (Rosco, Denmark) using disc diffusion method according to the recommendations of the Clinical and Laboratory Standards Institute, 2016 (CLSI, 2016) [15]. The minimum inhibitory concentration (MIC) of oxacillin and vancomycin (Sigma-Aldrich, Germany) against MRSA strains was determined using broth microdilution method according to the guidelines of CLSI (2016) [15]. MRSA and vancomycin resistant *S. aureus* (VRSA) strains were resistant to ≥4 and ≥16 µg/mL of oxacillin and vancomycin, respectively.

DNA extraction: DNA of MRSA strains was extracted using a DNA extraction kit from Gene Transfer Pioneers Company according to the instructions of the manufacturer.

Identification of MRSA strains: All suspected colonies were identified as *S. aureus* strains using specific primers for *nuc*A gene (Table 1) encoding thermonuclease enzyme as described previously [3]. Moreover, to confirm MRSA strains, the presence of *mec*A gene was tested by specific primers [3].

Typing of MRSA strains:

Prophage typing. Specific primers (Table 1) and multiplex-PCR reaction were employed to detect SGA, SGB, SGF, SGFa, SGFb, SGD, and SGL prophage types among MRSA strains according to the previously described instructions [13].

SCCmec and ccr typing. The presence of different SCC*mec* types among MRSA strains was tested using multiplex-PCR assay by employing specific primers (types I-V) (Table 1) according to the protocol previously described by Zhang et al. (2005) [16]. Also, another multiplex-PCR assay was employed for *ccr* typing of MRSA strains as published previously [16].

PhP typing. The high-resolution PhenePlate system (PhPlate AB, Stockholm, Sweden) was employed for prophage typing of MRSA strains according to the guidelines of manufacturer and the protocol described previously [3, 12].

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Table 1) Primers used in this study.

Primer	Sequence (5´ to 3´)	Size (bp)	Reference
*nuc*A-F *nuc*A-R	TAATGTACAAAGGTCAAC TGATAAATATGGACGTGGCT	310	3
*mec*A-F *mec*A-R	TGGCTATCGTGTCACAATCG CTGGAACTTGTTGAGCAGAG	195	3
SCC*mec* type I-F SCC*mec* type I-R	GCTTTAAAGAGTGTCGTTACAGG GTCTCTCATAGTATGACGTCC	613	16
SCC*mec* type II-F SCC*mec* type II-R	CGTTGAAGATGATGAAGCG CGAAATCAATGGTTAATGGACC	398	16
SCC*mec* type III-F SCC*mec* type III-R	CCATATTGTGTACGATGCG CCTTAGTTGTCGTAACAGATCG	280	16
SCC*mec* type IVa-F SCC*mec* type IVa-R	GCCTTATTCGAAGAAACCG CTACTCTTCTGAAAAGCGTCG	776	16
SCC*mec* type IVb-F SCC*mec* type IVb-R	TCTGGAATTACTTCAGCTGC AAACAATATTGCTCTCCCTC	493	16
SCC*mec* type IVc-F SCC*mec* type IVc-R	ACATATTTGTATTATCGGAGAGC TTGGTATGAGGTATTGCTGG	200	16
SCC*mec* type IVd-F SCC*mec* type IVd-R	CTCAAAATACGGACCCCAATACA TGCTCCAGTAATTGCTAAAG	881	16
SCC*mec* type V-F SCC*mec* type V-R	GAACATTGTTACTTAAATGAGCG TGAAAGTTGTACCCTTGACACC	325	16
SGA-F SGA-R	TATCAGGCGAGAATTAAGGG CTTTGACATGACATCCGCTTGAC	744	13
SGB-F SGB-R	ACTTATCCAGGTGGYGTTATTG TGTATTTAATTTCGCCGTTAGTG	405	13
SGF-F SGF-R	CGATGGACGGCTACACAGA TTGTTCAGAAACTTCCCAACCTG	155	13
SGFa-F SGFa-R	TACGGGAAAATATTCGGAAG ATAATCCGCACCTCATTCCT	548	13
SGFb-F SGFb-R	AGACACATTAAGTCGCACGATAG TCTTCTCTGGCACGGTCTCTT	147	13
SGD-F SGD-R	TGGGCTTCATTCTACGGTGA GTAATTTAATGAATCCACGAGAT	331	13
SGL-F SGL-R	GCTTAAAACAGTAACGGTGACAGTG TGCTACATCATCAAGAACACCTGG	748	13

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Table 2) Antibiotic resistance patterns among MRSA strains

Antibiotics	Pattern	No	%
One antibiotic		6	8
P	1	6	8
Three antibiotics		3	4
P, CIP, TS	2	1	1
P, CIP, RP	3	2	3
Four antibiotics		2	3
P, CIP, E, T	4	2	3
Five antibiotics		8	10
P, CIP, K, TN, RP	5	3	4
P, CIP, K, AN, TN	6	5	6
Six antibiotics		1	1
P, CIP, K, AN, TN, RP	7	1	1
Seven antibiotics		4	5
P, CIP, E, K, AN, TN, CD	8	4	5
Eight antibiotics		1	1
P, CIP, E, K, AN, TN, CD, TS	9	1	1
Nine antibiotics		9	11
P, CIP, E, K, AN, TN, CD, T, RP	10	7	9
P, CIP, E, K, AN, TN, CD, RP, GM	11	2	3
Ten antibiotics		17	22
P, CIP, E, K, TN, T, CD, TS, RP, MN	12	3	4
P, CIP, E, K, AN, TN, T, CD, RP, MN	13	6	8
P, CIP, E, K, AN, TN, T, CD, TS, MN	14	1	1
P, CIP, E, K, AN, TN, T, CD, TS, RP	15	1	1
P, CIP, E, K, AN, TN, T, CD, TS, GM	16	2	3
P, CIP, E, K, AN, TN, CD, TS, RP, GM	17	2	3
P, CIP, E, K, AN, TN, T, TS, MN, GM	18	2	3
Eleven antibiotics		25	32
P, CIP, E, K, AN, TN, T, CD, TS, MN, GM	19	11	14
P, CIP, E, K, AN, TN, T, CD, TS, RP, GM	20	6	8
P, CIP, E, K, AN, TN, T, CD, RP, MN, GM	21	3	4
P, CIP, E, K, AN, TN, T, CD, TS, RP, MN	22	3	4
P, CIP, E, K, AN, TN, T, CD, TS, RP, NI	23	1	1
P, CIP, E, K, AN, TN, T, CD, RP, MN, NI	24	1	1
Twelve antibiotics		3	4
P, CIP, E, K, AN, TN, T, CD, TS, RP, GM, MN	25	3	4

P: penicillin, CIP: ciprofloxacin, K: kanamycin, TN: tobramycin, E: erythromycin, AN: amikacin, CD: clindamycin, T: tetracycline, RP: rifampin, TS: sulfamethoxazole-trimethoprim, MN: minocycline, GM: gentamicin, NI: nitrofurantoin.

Findings

Isolation and identification of strains: A total of 79 black colonies, surrounded by halos and suspected to MRSA, were isolated from HiCrome aureus agar plates supplemented with oxacillin. All the isolates were positive for *nuc*A gene and harbored *mec*A gene and identified as MRSA. Of 79 MRSA strains, 37 (47%) strains were isolated in the first sampling stage in February, and 42 (53%) isolates were isolated in the second sampling stage in March.

Antibiotic susceptibility testing: All *mec*A positive strains showed resistance to cefoxitin and penicillin. Moreover, all 79 MRSA strains were susceptible to quinupristin-dalfopristin, vancomycin, chloramphenicol, and linezolid. The highest resistance (78-92%) was observed to ciprofloxacin, kanamycin, tobramycin, erythromycin, amikacin, tetracycline, and clindamycin (Figure 1). On the other hand, the rate of resistance to rifampin, trimethoprim-sulfamethoxazole, minocycline, gentamicin, and nitrofurantoin was also 3-62%.

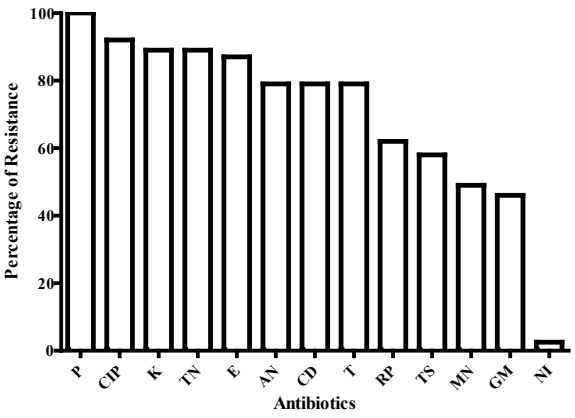

Figure 1) Resistance to antibiotics among MRSA strains isolated from hospital sewage
P: penicillin, CIP: ciprofloxacin, K: kanamycin, TN: tobramycin, E: erythromycin, AN: amikacin, CD: clindamycin, T: tetracycline, RP: rifampin, TS: sulfamethoxazole-trimethoprim, MN: minocycline, GM: gentamicin, NI: nitrofurantoin.

According to the results of the antibiotic susceptibility testing, 25 resistance patterns were identified among MRSA strains, and strains showed resistance to 1-12 antibiotics, of which 6 strains were only resistant to penicillin (Table 2). Moreover, most of the MRSA strains (n=25, 32%) were resistant to 11 antibiotics. On the other hand, 17 (22%) and 3 (4%) strains showed resistance to 10 and 12 antibiotics, respectively. Totally, 92% of MRSA strains were classified as multidrug resistant (MDR) strains and showed resistance to at least one antimicrobial agent in three or more antibiotic categories. The results of MIC of oxacillin revealed that 38% (n=30) and 30% (n=24) of MRSA strains were resistant to 2048 and 1024 µg/mL of oxacillin (Figure 2), respectively. Furthermore, 6 MRSA strains (8%) which were only resistant to penicillin showed resistance to 32 µg/mL of oxacillin. Moreover, MIC of vancomycin for all MRSA strains was ≤ 1 µg/mL, and 24 (30%), 37 (47%), and 8 (10%) strains showed resistance to 1, 0.25, and 0.5 µg/mL of vancomycin, respectively.

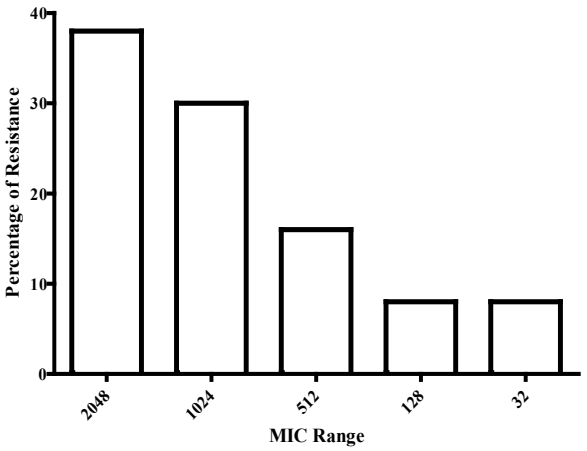

Figure 2) The range of oxacillin MICs among MRSA strains: MRSA had MIC≥4 µg/mL

Prophage typing: The results of prophage typing of MRSA strains showed that none of the strains were positive for SGA, SGD, and SGL prophage types, and the prevalence of prophage types was only limited to SGB, SGF, and two SGF subtypes (SGFa and SGFb). SGF prophage type and its two subtypes were

Table 3) Frequency of different prophage patterns among MRSA isolates

Prophage Pattern	Prophage Types							Frequency
	SGA	SGB	SGF	SGFa	SGFb	SGD	SGL	
1	-	+	+	+	+	-	-	57 (72%)
2	-	-	+	+	+	-	-	22 (82%)

Table 4) Clonality of MRSA strains isolated from hospital sewage in Tehran during 2015

PhP Types	No	Month of Sampling	Prophage Pattern	SCCmec Type	ccr Type	MIC (µg/mL)
CT1	1	February	1	III	3	64
	2	February	1	III	3	64
	3	February	1	III	3	64
	4	February	1	III	3	512
	5	March	2	III	3	1024
	6	March	2	III	3	256
	7	March	2	III	3	256
	8	February	1	III	3	1024
	9	February	2	III	3	1024
	10	February	2	III	3	2048
	11	February	2	III	3	2048
	12	February	2	III	3	1024
	13	March	2	III	3	2048
	14	March	2	III	3	512
	15	February	2	III	3	512
	16	March	1	III	3	512
	17	February	1	III	3	512
	18	February	1	III	3	2048
	19	February	2	III	3	1024
	20	March	1	III	3	2048
	21	March	1	III	3	2048
	22	March	1	III	3	2048
	23	March	2	III	3	512
	24	March	1	III	3	2048
	25	March	1	III	3	2048
	26	February	1	III	3	2048
	27	February	1	III	3	512
	28	March	1	III	3	2048
	29	March	1	III	3	1024

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Table 4) Clonality of MRSA strains isolated from hospital sewage in Tehran during 2015

PhP Types	No	Month of Sampling	Prophage Pattern	SCCmec Type	ccr Type	MIC (µg/mL)
CT2	30	March	1	III	3	256
	31	March	1	III	3	64
	32	March	2	III	3	256
	33	February	1	III	3	1024
	34	February	1	III	3	1024
	35	February	1	III	3	1024
	36	February	1	III	3	512
	37	February	1	III	3	2048
	38	February	1	III	3	2048
	39	February	1	III	3	512
	40	February	1	III	3	2048
	41	March	1	III	3	1024
	42	March	1	III	3	1024
	43	March	1	III	3	512
	44	March	1	III	3	2048
	45	March	1	III	3	1024
	46	February	1	III	3	2048
	47	February	1	III	3	2048
	48	February	2	III	3	1024
	49	February	1	III	3	2048
	50	March	1	III	3	2048
	51	March	1	III	3	2048
	52	March	2	III	3	1024
	53	March	1	III	3	512
	54	February	1	III	3	64
CT3	55	February	1	III	3	256
	56	February	2	III	3	2048
CT4	57	March	1	III	3	1024
	58	March	2	III	3	2048
	59	February	1	III	3	2048
	60	February	1	III	3	2048
	61	March	1	III	3	2048
	62	March	1	III	3	2048

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Table 4) Clonality of MRSA strains isolated from hospital sewage in Tehran during 2015

PhP Types	No	Month of Sampling	Prophage Pattern	SCCmec Type	ccr Type	MIC (µg/mL)
CT5	63	March	1	III	3	512
	64	February	2	III	3	64
CT6	65	March	1	III	3	1024
	66	March	2	III	3	1024
CT7	67	March	1	III	3	2048
	68	February	1	III	3	2048
CT8	69	March	1	III	3	1024
	70	March	1	III	3	1024
	71	March	1	III	3	1024
ST1	72	March	1	III	3	256
ST2	73	February	2	III	3	1024
ST3	74	March	2	III	3	1024
ST4	75	February	2	III	3	1024
ST5	76	March	1	III	3	2048
ST6	77	March	1	III	3	512
ST7	78	February	1	III	3	2048
ST8	79	March	1	III	3	1024

common among all 79 MRSA strains, while SGB prophage type was present only in 72% (n=57) of the strains (Table 3). Moreover, two prophage patterns were also detected among all MRSA strains, and pattern 1 consisting of SGB, SGF, SGFa, and SGFb prophage types was the dominant one.

SCC*mec* and *ccr* typing: Among all *mec*A positive MRSA strains, only one SCC*mec* type and one *ccr* type were detected; all the strains harbored SCC*mec* type III and *ccr* type 3 and were classified as HA-MRSA strains (Table 4).

PhP typing: The results of PhP typing of 79 MRSA strains showed the presence of 16 PhP types among the strains, consisting of 8 common types (CTs) and 8 single types (STs); among which CT1 was the dominant type consisting of 29 strains (37%) (Table 4 and Figure 3), followed by CT2, CT4, and CT8 containing of 25 (32%), 6 (8%), and 3 (4%) MRSA strains, respectively. CTs 1, 2, 4, 5, and 7 were common among MRSA strains isolated in both sampling stages, while CT6 as well as CT8 and CT3 were only prevalent among the strains isolated in the second sampling stage in March and in the first sampling stage in February, respectively. On the other hand, different antibiotic resistance patterns and different prophage patterns were identified in a certain PhP type.

Discussion

In this study, the clonal dissemination of MRSA strains in a hospital sewage in Tehran was assessed using a combination of PhP typing, prophage typing, and SCC*mec* typing methods. In this research, during 2 sampling stages in 2015 from the outgoing wastewater of a referral hospital in Tehran, a total of 79 MRSA strains were isolated, indicating the high prevalence of MRSA strains in this hospital. As far as we know, there is no information about the prevalence of MRSA in hospital sewage in Iran, and this is probably the first report on MRSA strains in hospital

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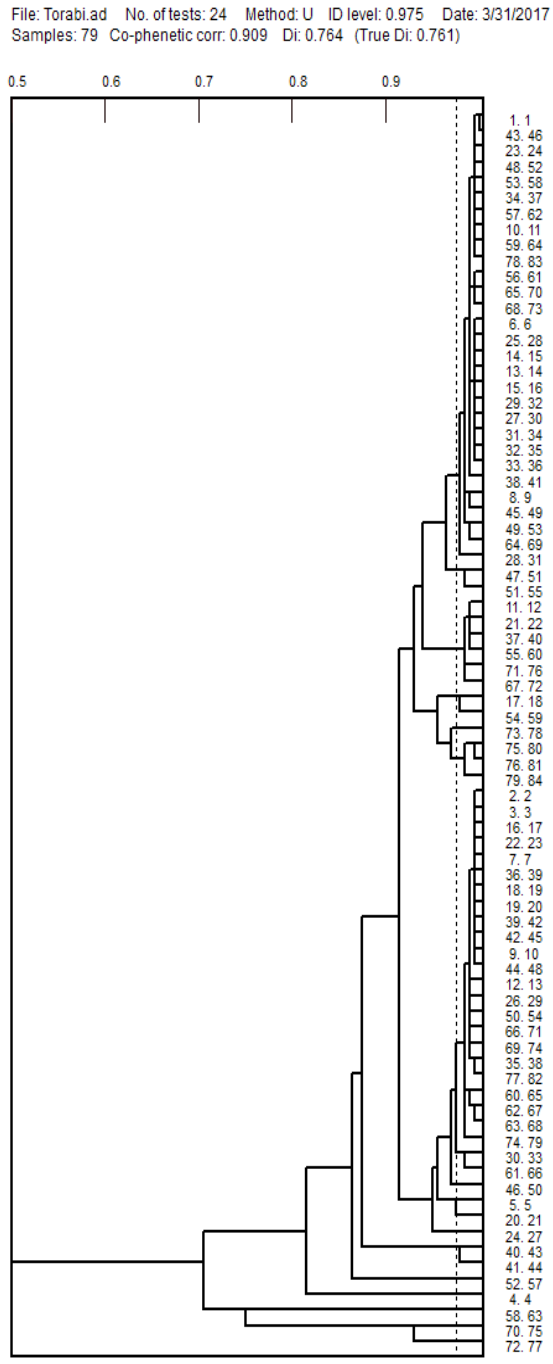
Figure 3) Dendrogram showing the clonality of MRSA strains isolated from hospital sewage in Tehran during 2015



sewage in Iran; however, in different studies in Iran, the prevalence of MRSA strains in clinical, environmental, animal, and meat samples has been reported [2-3, 5, 17-21]; thus the presence of MRSA strains in hospital sewage was not surprising. Although in a study in Germany, no MRSA was detected among urban and hospital sewage [22], in different studies in Iran [5, 10] and other countries [7-9, 11], the prevalence of MRSA strains in municipal sewage has been reported. The prevalence of MRSA strains in Tehran urban sewage has been reported to be 15.3% [5, 10]. Börjesson et al. (2010) in Sweden could isolate 189 MRSA strains from municipal wastewater [7]. Moreover, the presence of MRSA strains in hospital wastewater in Australia was also reported to be 27% (n=61) by Thompson et al. (2013) [11]. The differences between these studies results could be due in part to the differences in the prevalence of MRSA strains in different hospitals, the efficiency of the treatment process used, and the method employed for the isolation of MRSA strains. Furthermore, the prevalence of MRSA strains in some countries is very low (less than 1%), compared to other countries with high prevalence of MRSA (e.g. Iran≥20%); therefore, it is not possible to accurately estimate the prevalence of MRSA strains in hospital and urban sewage in countries with high or low prevalence of MRSA strains.

Consistent with other studies in Iran, none of the MRSA strains were resistant to vancomycin, quinupristin-dalfopristin, linezolid, and chloramphenicol [2-5, 10, 12, 14, 17, 19-20, 23-24], which could be due to the low or no consumption of these antibiotics for the treatment of staphylococcal infections in Iran. Moreover, a high rate of resistance to ciprofloxacin, amikacin, tobramycin, erythromycin, amikacin, clindamycin, and tetracycline was observed among MRSA strains, which is consistent with other reports on clinical, environmental, and meat samples in Iran [2-5, 10, 12, 14, 17, 19-20, 23-24].

In the present study, all MRSA strains were resistant to cefoxitin and also were positive for *mec*A gene. Several studies have already reported similar results among MRSA strains [3, 5, 20, 25]. Although some MRSA strains are negative for *mec*A gene and instead harbor *mec*C gene, but since there is very

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low or no report on *mec*C gene in Iran, it could be concluded that detection of *mec*A gene could be a rapid and specific method for detecting MRSA strains in this country. All MRSA strains harbored SCC*mec* type III and *crr* type 3 and were classified as HA-MRSA strains. This finding is similar to the finding of another study on MRSA strains of poultry origin, in which only SCC*mec* type III was detected [17]. In other studies, different SCC*mec* types (III, IV and V) have been reported, and type III has been reported as the dominant one [2-3, 5, 19-20]. Moreover, the present study result is different from that of another research in the United States, in which MRSA strains in STPs harbored SCC*mec* types II and IV [9]. It seems that the sewage treatment process in the hospital under study could eliminate MRSA strains with SCC*mec* types IV, V, and VII (CA-MRSA), while strains with SCC*mec* type III (probably other SCC*mec* types) could survive during the treatment process.

In this study, SGB, SGF, SGFa, and SGFb prophage types as well as two prophage patterns were detected among MRSA strains, and all the strains were negative for SGA, SGD, and SGL prophage types. In different studies in Iran, SGA and SGL prophage types have also been identified among CA-MRSA strains, and diverse prophage patterns have also been reported among MRSA strains of clinical, meat, and sewage origins [2-3, 5, 14, 20, 26-27]. These differences in the prevalence of prophage types among MRSA strains in different samples in Iran could be due to the dissemination of different strains in various cities and hospitals worldwide.

High-resolution PhP typing method is a powerful and specific method used to type *S. aureus* strains, and the usefulness and efficiency of PhP typing for MRSA strains have already been reported [3, 5, 10, 12, 20]. In this study, diverse PhP types consisting of 8 CTs and 8 STs were reported. More diverse PhP types (33 and 21 PhP types) have already been reported in strains isolated from clinical and urban sewage samples [3, 5, 12]. CT1 was the dominant PhP type in this study, followed by CT2, CT4, and CT8. Compared to our previous studies [3, 5, 10, 12, 17, 19, 20], it was found that certain clonal PhP types are disseminating and circulating in different regions in Tehran. Moreover, the presence of MRSA strains with different antibiotic resistance and prophage patterns in a certain PhP type in this study indicated independent evolution of such clones in the community. On the other hand, the presence of some strains, isolated during two sampling stages, in CT7 with similar prophage pattern and antibiotic resistance patterns indicated the presence and persistence of this clone type in this hospital over two months of sampling.

Conclusions

The results of this study indicated the presence and persistence of different clonal groups of MDR MRSA strains in the outgoing sewage of the studied hospital in Tehran. These clone types, which harbor SCC*mec* type III as well as SGF prophage type and its two subtypes (SGFa and SGFb), have a high potential to produce a variety of virulence factors, such as enterotoxins (A, G, K, P and Q), leukocidin, staphylokinase, and β-lysine, and could enter the environment or sewage-treatment systems and survive there for a long time. These strains could also survive during the sewage treatment process, as the sewage treatment process has already been shown to be unable to remove all MRSA strains, and some MRSA strains (with SCC*mec* type III) could survive during the sewage treatment process and enter the environment [5]. The high prevalence of such strains in hospitals and consequently in the community could be considered as a potential threat to public health.

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