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RESEARCH ARTICLE

Prevalence of MRSA as an Infectious Agent in Sanitary Swimming Pools and Jacuzzis

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Abstract

Introduction Methicillin-resistant *Staphylococcus aureus* (MRSA) is considered resistant to beta-lactam antibiotic groups. Infection caused by this strain is more difficult to treat with antibiotics, and hence, it will be more dangerous. This study focused on detecting the *mecA gene* Staphylococcus in sanitary swimming pools and Jacuzzis in Yazd city, Iran (2019). Also, the relationship between methicillin-resistant Staphylococcus aureus (MRSA) and the water quality standards has been investigated.

Materials and Methods 60 samples were randomly collected in sterile bottles from 20 active pools and Jacuzzis. Quality parameters were analyzed by standard methods. Antibiotic resistance and the mecA gene's presence were detected by the disk diffusion and PCR method, respectively.

Results The results of this study showed that the resistance of *Staphylococcus aureus* isolates was high against erythromycin (41.20%), tetracycline (35.10%), clindamycin (28.90%), and cefoxitin (25.80%). Out of 97 samples, 9 (25.80%) strains of Staphylococcus aureus were identified as MRSA, 30 samples (30.92%) showed multiple patterns of antibiotic resistance, and 9 samples (9.27%) carried the mecA gene. The results revealed that water quality has greatly impacted the *mecA gene* strain presence, especially microbial parameters. On the other hand, in the presence of mecA gene strains, the averages of microbial qualities were higher than standard in Jacuzzis; the latter finding was confirmed for swimming pools due to physicochemical parameters.

Conclusion The number of reported sanitary water is increasing, and this study's results are useful examples of these findings. Therefore, a lack of careful and regular monitoring of swimming pools and Jacuzzis can lead to MSRA prevalence and outbreak sources.

Keywords Swimming pools · Jacuzzis · *Staphylococcus aureus* · Antibiotic Resistance · *mecA* gene · MRSA

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Introduction

Swimming pools and Jacuzzis can act as a potential source of biological contaminants due to direct and continuous contact with different human groups that differ in economic, social, individual, and public health status [1, 2]

Recently, the risk of the occurrence and prevalence of emerging biological agents is increasing, and therefore, it is necessary to consider health measures more than ever. Besides, to control pathogens, using beta-lactam antibiotics such as penicillin, methicillin, oxacillin, and cephalosporins is increasing. Despite the effectiveness of using these drugs, some pathogens have acquired resistance due to continuous contact, and consequently, these drugs could not eliminate them. Humans and animals have been

reported as potential living sources of antibiotic resistance in aquatic environments and can transmit antibiotic resistance genes through transposons, plasmids, and integrons to other pathogens and microbes naturally present in water [3]. The possibility of antibiotic-resistant genes transmission is a concern, especially in the presence of these genes in the water. Many people may be affected by contaminated water containing antibiotic-resistant bacteria [4, 5].

Staphylococcus aureus is one of the pathogens on the list of standard criteria that should be considered in recreational water, including swimming pools and Jacuzzis. *Staphylococcus aureus* (MRSA) and infection sources cannot be recognized, and infection symptoms may appear months after the patient is exposed to the infection. Infected patients may act as reservoirs for further transmission, especially since most of these species contain different types of SCC*mec* (Staphylococcal Cassette Chromosome *mec*) encoded for resistance to methicillin and other beta-lactams [3].

Staphylococcus aureus is an opportunistic bacterium that plays a significant role in recreational water [6]. It is also a halophile bacterium and causes a wide range of human diseases such as skin and soft-tissue infections (SSTIs) and invasive diseases such as bacteremia, sepsis, endocarditis, and pneumonia, which are among the leading causes of deadly infections in developed and developing societies [7–9].

Moreover, Staphylococcus aureus infections become more troublesome when they contain MRSA [10]. The MRSA strain includes a group of *Staphylococcus aureus* resistant to a wide range of beta-lactam antibiotics, including penicillins and cephalosporins [11, 12]. *Staphylococcus aureus* species are not inherently antibiotic-resistant, and the development of such resistance does not make them resistant. However, this strain of *Staphylococcus aureus* infection is more difficult to treat with standard antibiotics, and hence, it will be more dangerous [13]. On average, 40% of Staphylococcus aureus strains are resistant to methicillin, increasing every year [14]. In 2014, MRSA was estimated to cause 72,444 clinical infections and 9194 deaths in the United States [15]. Besides, methicillin-resistant Staphylococcus aureus strains became resistant by acquiring the mecA gene, which is scientifically called methicillin resistance [16, 17].

The prevalence of MRSA was initially associated with hospital-associated methicillin-resistant *Sta*phylococcus *aureus* (HA-MRSA) and exposure to an infected patient. However, community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA) has been identified since 1990 [18]. MRSA infection can also be transmitted by using recreational seawater, beaches, and pools that are not properly managed or mineral water bottles scattered around [19]. Sources of *Staphylococcus aureus* and MRSA contamination in the marine environment have not yet been identified [20].

Given that swimming pools are recreational places for public use, it will be necessary to detect this bacterium in these places. This study's main purpose was to detect Staphylococcus containing the *mecA* gene in the sanitary swimming pools' and Jacuzzis' water. Also, the correlation of MRSA and the quality parameters' standards have been investigated.

Material and Methods

Study area and sampling

This study was conducted in Yazd (Iran) cross-sectionally in two seasons, namely summer and autumn (2019). Ten pools and ten Jacuzzis were investigated. Selected pools and Jacuzzis had the same treatment methods. According to Iranian National Standard No. 4208 [21], the sampling method was performed once every 15 days and in a total of 8 stages. Overall, 160 samples were taken (80 pools and 80 Jacuzzis). 10–15% of the total analyses were considered as repeatability.

Physical parameters of water, including residual chlorine, pH, and temperature, were in situ measurements. The Hatch device analyzed the turbidity and electrical conductivity (EC) parameters (2, 28, 29). DPD (Diethyl-p-phenylenediamine) calorimeter kit was used to measure the residual chlorine, and a pH Portable Multiparameter Meter was used. Total and fecal coliforms were performed as standard methods for water and wastewater examination [22]. Materials were purchased from Merck Company.

Staphylococcus aureus laboratory and antibiotic susceptibility test.

The Membrane Filter (MF) and Baird-Parker Agar Base were used to grow and isolate *Staphylococcus aureus* detection. 100 ml of the sample was passed through a membrane filter of 0.45 µm. The filter was then transferred into a plate containing the Baird-Parker Agar Base and placed in an incubator at 37 °C for 24 h. Standard microbial tests such as catalase, coagulase, DNase, and Gram staining were used to identify the bacteria [22, 23].

Antimicrobial agent susceptibility of bacteria was determined by the Kirby-Bauer test [24]. Muller-Hinton agar medium was used as the medium of choice for the experiment. Discs of quinupristin-dalfopristin, cefoxitin, clindamycin, gentamicin, linezolid, erythromycin, tetracycline, vancomycin, mupirocin, ofloxacin, and ciprofloxacin of the Mast UK. of the United Kingdom were used in this study. Freshly purified cultured bacteria were harvested from 3–4 colonies using a swab to prepare the microbial suspension in this experiment. They were suspended in a saline tube to obtain turbidity equal to that of a standard tube (0.5 McFarland solution). It was then spread on the plate's surface at an

angle of 60 degrees toward each other, and finally, the swab was rotated around the inner part of the plate. Antibiotic discs were placed near the flame at the culture medium's surface and held in place with the pence tip to complete the contact. The plates were placed at 35 °C for 18–24 h, after which the growth inhibition zone was measured in millimeters using an accurate ruler, and the susceptibility of the bacteria to antibiotics was reported as sensitive, semi-sensitive, and resistant. This method was performed using a cefoxitin 30 µg disc from Mast UK. The growth inhibition zone of up to 21 mm was considered a methicillin-resistant strain [25].

DNA extraction and PCR analyses

The boiling method was used to obtain genomic DNA [26]. One milliliter of the bacterial suspension was poured into a sterile microtubule of 0.2 ml and centrifuged at 1000 (× g) for 10 min. After draining the supernatant, one milliliter of sterile PBS buffer was added, and a uniform suspension was obtained. It was then centrifuged at 5232 (× g) for 4 min. After draining the supernatant to the sediment, one milliliter of PBS buffer was added again, and centrifugation was repeated. Finally, the supernatant was discarded, and the precipitate was used for cell lysis. 100 µl of distilled water was added to the precipitate for injection, the microtube lid was closed and sealed by parafilm, and the microtube was boiled at 100 °C. The microtubes were kept in the freezer at -20 °C for 10 min. Subsequently, the microtubes containing the solution were centrifuged at 4000 (× g) for 5 min. The supernatant was transferred into a sterile microtube as a solution containing genomic DNA, and the sample was stored in a freezer at -20 °C until further testing.

Quantitative analysis of the extracted DNA was performed by spectrophotometry. Each sample was examined at wavelengths of 260 and 280 nm, and DNA purity was calculated by obtaining the light absorption ratio of 260 to 280 [27].

16S rRNA gene amplification was used as an internal reaction control. Sterile 0.2 ml microtubes were selected according to the number of samples tested. Also, a 1.5 ml microtube was selected and marked to prepare a master mix. The required amount of PCR solution components including 5.2 µl of sterile distilled water, 10 µl PCR master mix, 5.2 µl primer, and 5 µl of template DNA were prepared in a volume of 20 µl for each sample. According to the predetermined temperature programming, the microtubes prepared in the thermal cycler (Table 1) were used to amplify the desired gene using a specific *mecA* primer with a size of 293 base pairs (bp) in a polymerase chain reaction (PCR) [28].

Table 1 Temperature program for replication with MEC primer

Number of cycles	Temperature (°C)	Time (s)
1 (Denaturing)	94	300
	94	45
30 (Annealing)	52	30
	72	45
1 (Extension)	72	300

Table 2 Frequencies and Antibiotic resistance pattern of Staphylococcus aureus

Antibiotic resistance	Antibiotic concentration (µg)	Antibiotic	
		Number	Percentage (%)
QD	15	**0**	**0**
Linezolid	30	0	0
Mupirocin	20	7.2	7
Cefoxitin	30	25.8	25
Ciprofloxacin	5	1.1	1
Ofloxacin	5	6.2	6
Erythromycin	15	41.20	40
Clindamycin	2	28.9	28
Gentamicin	120	0	0
Vancomycin	30	0	0
Tetracycline	30	35.10	34

Results

Antibiotic resistance pattern

The antibiotic resistance pattern of Staphylococcus aureus isolates as frequencies are presented in Table 2.

As shown in Table 2, the Staphylococcus aureus isolates were resistant to erythromycin (41.20%), tetracycline (35.10%), clindamycin (28.90%), cefoxitin (25.80%), mupirocin (7.20%), ofloxacin (20.6%), and ciprofloxacin (1.10%). Figure 1 shows the antibiotics used and the antibiotic resistance pattern based on the Clinical Laboratory Standards Institute (CLSI) guidlines.

As shown, creating a bright halo around the bacteria indicates susceptibility to an antimicrobial agent. Brighter halo was described as more susceptibility to the antimicrobial agent.

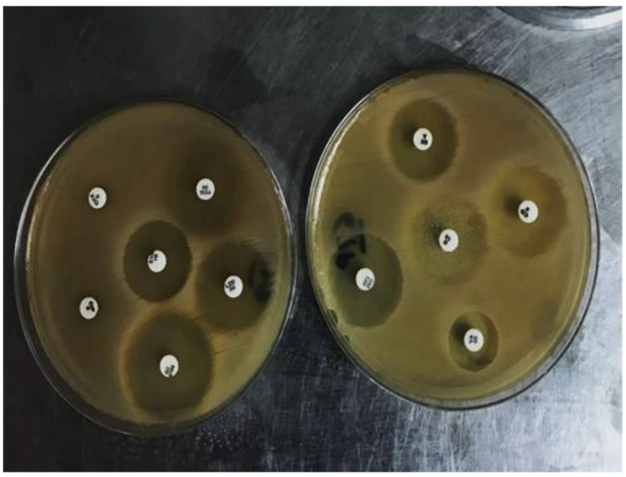

Fig. 1 The antibiotic resistance pattern according to CLSI standard guidelines

Table 3 Frequencies of mecA gene and MDR in Staphylococcus aureus isolates

mecA		MDR	
Percentage (%)	Number	Percentage (%)	Number
9.27	9	30.92	30

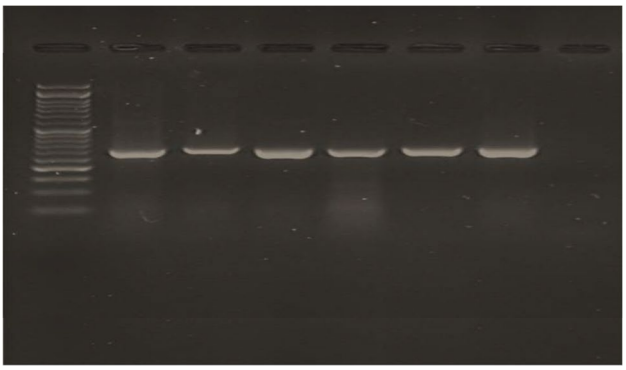

Fig. 2 Electrophoresis of PCR product of the mecA gene

Methicillin-resistant gene *(mecA)* and MDR isolates

Table 3 shows the frequency distribution of strains with methicillin-resistant gene (*mecA*) and multidrug resistance (MDR) in swimming pools and Jacuzzis.

As presented, 30 samples (30.92%) of *Staphylococcus aureus* isolates revealed multidrug resistance (resistance to 3 or more classes of antibiotics studied), and 9 *Staphylococcus aureus* isolates (9.27%) contained methicillin-resistant gene (mecA). Figure 2 demonstrates the electrophoresis of the mecA gene by PCR method in a 1.5% agarose gel with a bandwidth of 293 bp.

Frequency of *mecA* gene strains and physicochemical parameters

A comparison of the frequency of strains with the *mecA* gene concerning physicochemical parameters in the swimming pools and Jacuzzis is presented in Table 4.

As shown, in compression with lack of a resistant gene, in swimming pools with conditions including average residual chlorine of 0.83 ± 0.05, an average temperature of 32 ± 1 °C, average turbidity of 0.52 ± 0.04, and the average EC was equal to 2086.66 ± 201.32 uS/cm. Besides, the frequencies of Staphylococcus aureus strains with *the mecA* gene were higher. These conditions were also similar for Jacuzzis including average residual chlorine of 0.15 ± 0.19, the average temperature of 41.5 ± 0.54 °C, average turbidity of 0.72 ± 0.1, and the average EC was equal to 2230 ± 7100.54 uS/cm.

Frequency of *mecA* gene strains and microbial quality parameters

Table 5 indicates the frequency of *mecA* gene strains considering total coliform, fecal coliform, and heterotrophic bacteria (parameters mentioned in the standard) in the swimming pools and Jacuzzis.

According to Table 5, in the presence of *mecA* gene strains, the averages microbial quality in the Jacuzzi was higher than the microbial standard, so that the average

Table 4 Frequency of *mecA* gene strains and physicochemical parameters

Variables	Pool						Jacuzzis					
	Lack of a resistant gene			Containing a resistant gene			Lack of a resistant gene			Containing a resistant gene		
	N	Average	Sd	N	Average	Sd	N	Average	Sd	N	Average	Sd
cl2	**77**	1.9	0.88	3	0.83	0.05	74	1.18	1.05	6	0.15	0.19
Temperature	**77**	30.63	1.5	3	32	1	74	40.70	1.31	6	41.5	0.54
pH	**77**	7.79	0.14	3	7.73	0.05	74	7.73	0.11	6	7.75	0.10
Turbidity	**77**	0.4	0.21	3	0.52	0.04	74	0.46	0.26	6	0.74	0.34
EC	**77**	1503.59	537.68	3	2086.66	201.32	74	1244.75	568.97	6	2230	710.54

Table 5 Frequency of *mecA* gene strains and microbial parameters

Variables	Pools						Jacuzzis					
	Lack of a resistant gene			Containing a resistant gene			Lack of a resistant gene			Containing a resistant gene		
	N	Average	Sd	N	Average	Sd	N	Average	Sd	N	Average	Sd
Total coliform	**77**	17	126.58	3	13	3.46	74	35	181.22	6	165	228.61
Fecal coliform	**77**	15	125.66	3	8	1.15	74	20	130.74	6	50	93.33
Heterotrophic	**77**	562	2028.06	3	1210	1398	74	1016	1918	6	2599	2026

number of total coliforms, fecal coliform, and heterotrophic bacteria in the presence of mecA gene strains were 165 ± 228.61, 50 ± 93.33 and 2599 ± 2026, respectively. On the other hand, in the absence of mecA gene strains, the average resistance of these bacteria was 53 ± 181.22, 20 ± 1918.74, and 165 ± 228.61, respectively. However, this situation was not the case in the swimming pools.

Discussion

This study aimed to determine the presence of *Staphylococcus aureus* with *mecA* gen and its frequencies with physicochemical and microbial standard values. The results of this study indicated that the highest drug resistance of Staphylococcus aureus isolates was to erythromycin (41.20%), tetracycline (35.10%), clindamycin (28.90%), and cefoxitin (25.80%). A similar study on public recreational water and coastal sands in the Eastern Cape Province of South Africa has shown *Staphylococcus aureus* resistance to erythromycin (70%) and clindamycin (80%) [3]. Staphylococcus aureus isolated from treated wastewater and surface water in Durban, South Africa, was resistant to erythromycin (40%) and cefoxitin (96.25%) [29]. The *Staphylococcus aureus* antibiotic susceptibility test in water resources from different regions in Al Anbar Province, Iraq, showed complete resistance to methicillin, erythromycin, and doxycycline [4].

In this study, cefoxitin disc was used to confirm the resistance of *Staphylococcus aureus* isolates to methicillin, and as mentioned, 25.80% of Staphylococcus aureus isolates were MRSA (Table 2). In a similarly conducted study by Puma et al., 67% of domestic water samples contained staphylococci, and 30.7% were MRSA [12]. In the study of Masoud et al., nine strains of Staphylococcus aureus were identified as MRSA out of 18 samples collected from swimming pool water in Alexandria [2]. Also, the result of a study performed by Sinigalliano et al. showed that 1% of MRSA was in seawater [30]. The results of a performed study by Sina et al. revealed that 53.85% of the groundwater with irrigation purpose in Cotonou contained S. aureus [31]. Some factors could be effective in causing MRSA, such as changes in the geographical location of the study areas and the type of water resource. The resistance may be due to the accidental use of antibiotics or drugs over an incomplete period, leading to bacterial multidrug resistance (MDR) [4]. In this study, 30 isolates of Staphylococcus aureus (30.92%) were MDR. Another study conducted by Messi et al. showed 55% of bacterial isolates with MDR in the mineral water [32]. In the Aluva River, Nigeria, MDR (100%) was established to all antibiotics by all Bacillus strains, Micrococcus, and Pseudomonas, and therefore the Aluva River was not safe for drinking [33]. In health centers and hospitals, the high frequency of multidrug resistance of isolated *Staphylococcus aureus* may lead to failure of the patient's treatment process and the possibility of transmitting plasmid resistance to pathogenic bacteria. As a result, the health of people who come in contact with polluted recreational water will also be endangered.

The results of the current study showed that nine *Staphylococcus aureus* strains (9.27%) were methicillin-resistant (*mecA*) genes (Table 3). Tiao found that none of the 20 strains of Staphylococcus aureus tested in his study had the *mecA* gene, and one case showed methicillin resistance phenotypically [34]. All 30 (100%) *Staphylococcus aureus* isolates of recreational water and coastal sand in the Eastern Cape Province of South Africa exhibited multiple antibiotic resistance patterns (resistant to three or more antibiotics). Meanwhile, the mecA gene was detected in only five samples (22.7%)[13].

Comparing the frequency of mecA gene strains and physicochemical parameters (Table 4) showed that when the physicochemical parameters of water were higher than standard, the frequency of mecA gene-containing *Staphylococcus aureus* was also high. In the study done by Masoud et al., MRSA did not survive long in hot water pools or Jacuzzis using the proper disinfectant (chlorine) and pH [2]. Gregg and Robin found that chlorine significantly reduced MRSA growth and eliminated all MRSA after one hour [35]. In the study of Geyse et al., the bacteriological water quality criteria of drinking water in four urban parks of Sao Paulo were according to Brazilian regulations, but no residual chlorine was found in the samples (< 0.1 mg/l). These data were significantly correlated with the prevalence of Staphylococcus aureus found in 25.2% of the samples. The mecA gene was detected in 36.7% of the isolates, indicating its potential for resistance to several antimicrobials. In addition,

27.3% of isolates carrying the mecA gene had MRSA phenotypic potential [36].

Water temperature was another factor influencing the frequency of the mecA gene strains in the way that increasing the average temperature of swimming pools, 32 °C, compared to a temperature of 30.36 °C, increased the frequency of strains with the mecA gene. Moreover, the number of strains (6 cases) was more in swimming pools than in Jacuzzis (3 cases), which can be related to their higher temperature. When the water temperature was higher in Jacuzzis, more frequencies of mecA gene strains were observed. As reported by Leoni et al. [37] and Osei-Adjei et al. [38], microbial growth increases when the swimming pool water temperature rises. Turbidity and EC were the other influential physical parameters assisting the prevalence of strains with the mecA gene, so their increase has risen mecA. Other studies have revealed that *Staphylococcus aureus* and MRSA can survive for days in seawater. They can even survive better in seawater due to their higher salinity preference [5, 20, 39]. Kloos et al. [39] and Tolba et al. [40] reported that higher salt concentrations were more desirable for staphylococci and microscopes. Levin-Edens et al. reported that salinity is an important factor in MRSA and MSSA. This study indicated that the MRSA/MSSA ratio in freshwater versus seawater was higher in the Pacific Northwest [19], which is consistent with the present study's findings. The results of the frequency of mecA gene strains and microbial contamination also showed that the average number of all three types of bacteria is higher in the presence of strains containing the mecA gene in Jacuzzis (Table 5). However, this latter finding was not applicable for swimming pools, which may be due to the much higher pollution of the Jacuzzis.

The prevalence of *Staphylococcus aureus* and MRSA is increasing, leading to the inclination of hospital-associated and community-acquired infections worldwide, which is a major public health concern [3]. It may be hypothesized that recreational water, contaminated water, acts as a transient environmental reservoir for MRSA. Thus, dangerous crowds may use these resources for recreational purposes, especially those with open wounds or skin abrasions [40]. However, MRSA can be spread by direct and indirect contact with infected people in swimming pools, Jacuzzis, and other places [2, 41]. Studies conducted in previous years have also confirmed the presence of this microorganism containing resistant genes. As observed in reports, with time and conducting further studies, the number of reported cases, even in sanitary water, is increasing. So, the result of this study is a useful example of these findings.

Finally, the results demonstrated that the poor quality of physicochemical and microbial parameters of water increases the abundance of the *mecA* gene; therefore, there is an urgent need for regular controlling and strict monitoring of the implementation of regulations to ensure that standards are met in various pools, especially in public swimming pools.

Conclusion

The results of this study revealed that the resistance of *Staphylococcus aureus* isolates against antibiotics of erythromycin (41.20%), tetracycline (35.10%), clindamycin (28.90%), and cefoxitin (25.80%) was high. Considering 97 samples, 9 (25.80%) strains of *Staphylococcus aureus* were identified as MRSA, 30 samples (30.92%) showed multiple patterns of antibiotic resistance, and 9 samples (9.27%) carried the mecA gene. Hence, it can be concluded that despite the development of health considerations, sanitary recreational water can be a source for the presence and spread of this pathogen due to various reasons such as negligence of executives, lack of proper management of treatment plants, and lack of accurate monitoring. If no measures are taken, infectious diseases, especially pathogens resistant to strong antibiotics, will occur and spread quickly in huge reservoirs, and consequently, controlling those infections will be excruciating.

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Declarations

Conflict of Interest The authors have no conflict of interest to declare.

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