ORIGINAL ARTICLE

Methicillin-resistant *Staphylococcus aureus* tracking spread among health-care workers and hospitalized patients in critical wards at a university hospital, Tehran, Iran

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Abstract

Health-care workers may serve as a reservoir for dissemination of methicillin-resistant *Staphylococcus aureus* (MRSA) to patients in hospital settings. The present study aimed to screen MRSA in nasal swabs of health-care workers and clinical specimens from patients and investigate the possible relationship between these isolates at a university hospital in Tehran, Iran. Additionally, we aimed to identify potential risk factors for MRSA colonization in health-care workers. *Staphylococcus aureus* strains were isolated from health-care workers and inpatients who completed a questionnaire on risk factors. Cefoxitin disc diffusion test was also used for detection of MRSA. Moreover, all of the MRSA isolates were subjected to pulsed-field gel electrophoresis (PFGE). Colonization rate of MRSA among health-care workers was 22.5%. Furthermore, out of 24 *S. aureus* isolates obtained from patients, nine (37.5%) were MRSA. Regarding risk factors, the prevalence of nasal MRSA carriage among hospital personnel who used masks was significantly lower than in those without masks (p 0.007). Using PFGE, 10 clusters and 14 singletons were identified among the MRSA isolates. In this regard, most of the MRSA isolates recovered from health-care carriers and patients in intensive care wards, especially general intensive care units, were grouped in certain clusters, indicating intra-ward transmission of the mentioned isolates in these restricted areas. We concluded that screening and decolonization of carriers, contact precautions, prudent use of antibiotics and implementation of active surveillance are recommended strategies for the prevention and control of MRSA transmission in hospital settings.

Keywords: Health-care workers, methicillin-resistant *Staphylococcus aureus*, patients, pulsed-field gel electrophoresis, risk factors
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Introduction

Over the past few years, there has been a renewed interest in methicillin-resistant *Staphylococcus aureus* (MRSA), perhaps fuelled in large part due to the pervasiveness of community-acquired MRSA worldwide [1]. Nasal carriage of *S. aureus* is a recognized risk factor for subsequent endogenous infections as well as a vector for pathogen transmission in health-care settings [2]. In this respect, health-care workers, who are at the interface between hospital and community, have the potential to complicate the control of health-care-associated *S. aureus* in medical settings. They may serve not only as vectors but also as reservoirs, or even victims of health-care-associated MRSA cross-transmission [3].

In the early days of the antibiotic era, *S. aureus* was susceptible to almost every class of antibiotic developed at that time. However, many *S. aureus* strains became increasingly resistant to a greater number of antibiotics as time passed. The pivotal point in the evolution of *S. aureus* resistance was the emergence of MRSA clones in the 1960s. Today, MRSA has become pandemic in many medical institutions throughout the

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world [1,4]. According to a report released from the Centers for Disease Control and Prevention in 2013, more than 80 000 people in the USA are infected with MRSA annually, of whom roughly 11 300 die [5]. In Europe, over 171 000 healthcare-associated infections due to MRSA occur annually, which account for 44% of all healthcare-associated infections, resulting in 5400 attributable extra deaths as well as over a million extra days of hospitalization [6]. In Iran, a recent systematic analysis study revealed that the prevalence of MRSA infections has risen in the last decade [7].

With the advent of molecular typing methods, epidemiologists can easily investigate outbreaks of infectious diseases, tracing the transmission of pathogens, and elucidate evolutionary relationships between microorganisms. Several genotyping methods such as amplified fragment length polymorphism [8,9], multiple locus variable-number tandem repeat analysis [8,10,11], multilocus sequence typing [12,13], and pulsed-field gel electrophoresis (PFGE) [10,14–16] have been successfully applied for epidemiological investigations of MRSA infections worldwide. Of these methods, PFGE is still considered the reference standard fingerprinting method for monitoring and tracking MRSA dissemination in the hospital environment [17].

As colonized health-care workers may transfer MRSA strains to hospitalized patients, we tried to screen *S. aureus* in nasal swabs of health-care providers and clinical specimens from patients in a university hospital to provide an added perspective to the Iranian health initiative. To this end, we investigated the probable relationship between nasal and clinical isolates of MRSA using PFGE. Additionally, risk factors for MRSA colonization in health-care workers were evaluated.

Materials and methods

Study design and sampling

This survey was conducted at a university hospital, Tehran, Iran, from April 2016 through to February 2017. A total of 133 nasal swabs were collected from the anterior nares of health-care workers using cotton swabs. These swabs were inserted into the nasal cavities and rotated several times either clockwise or anticlockwise. In addition, 120 clinical specimens (including, blood, cerebrospinal fluid, wound) were obtained from various body sites of hospitalized patients. Samples were immediately transferred to transport medium and taken to the molecular laboratory of the pathobiology department, then sub-cultured on blood agar and incubated for 24 hours at 37°C. Presumptive *S. aureus* colonies were confirmed based on standard biochemical tests as described elsewhere [18].

Health-care workers were interviewed by one of the authors and completed a questionnaire on risk factors. Potential risk factors for MRSA carriage in health-care workers were analysed by chi-square or Fisher's exact test. Values of p less than 0.05 were considered statistically significant. This study was approved by the ethics committee of the Tehran University of Medical Sciences.

Detection of methicillin resistance

Cefoxitin disc diffusion tests for predicting methicillin resistance in *S. aureus* isolates were performed using cefoxitin (30 μg) discs as described previously [19]. Genomic DNA was extracted using a DNA extraction kit (Yekta Tajhiz Azma, Iran) based on the manufacturer's instructions. PCR assays targeting the *mecA* gene using specific primers were also employed [19].

Antimicrobial susceptibility testing

The MRSA isolates were tested for their susceptibility to antibiotics by the disc diffusion method according to CLSI guidelines [20]. The following antibiotics (Oxoid, Basingstoke, UK) were used: gentamicin (10 μg), teicoplanin (30 μg), rifampin (5 μg), doxycycline (30 μg), quinupristin/dalfopristin (15 μg), chloramphenicol (30 μg), clindamycin (2 μg), erythromycin (15 μg), linezolid (30 μg), mupirocin (5 μg), and trimethoprim-sulfamethoxazole (25 μg). ATCC 25923 was also used for quality control.

MICs of vancomycin

Minimum inhibitory concentrations of vancomycin for the MRSA isolates were determined by MIC Test Strips (Liofilchem, Roseto degli Abruzzi, Italy) according to CLSI guidelines [20]. *Staphylococcus aureus* ATCC29213 was used as control strain.

Molecular typing

In this study, we used PFGE following *Sma*I restriction digestion of chromosomal DNA to determine genetic relatedness between MRSA isolates obtained from nasal swabs of health-care workers and clinical samples from hospitalized patients [21]. Briefly, after SeaKem® Gold (Lonza, Rockland, ME, USA) agarose-embedded DNA was digested with 10 units of restriction nuclease *Sma*I (Roche, Mannheim, Germany) for 2 hours in a water bath at 37°C, DNA fragments were electrophoresed in 0.5 × TBE buffer at 14°C for 24 hours in a Chef Mapper electrophoresis system (Biometra, Göttingen, Germany) at 220 Volts with pulse times of 5–60 seconds. *Salmonella* Braenderup strain H9812 was chosen as the molecular size standard for PFGE. The agarose gels were stained with ethidium bromide (0.6 mg/L) and visualized under UV light. The banding patterns were examined using GelCompar II software (Applied Maths, Belgium). Clustering was also performed by the unweighted pair group average method (UPGMA) using Dice coefficient.

Results

Of 133 health-care workers, *S. aureus* was observed in nasal cavities of 53 (39.8%) individuals. Moreover, 24 (20%) *S. aureus* isolates were obtained from clinical specimens of 120 patients. In addition, 30 health-care workers (22.5%) carried MRSA. Notably, the highest rate of MRSA carriers (43.4%) was found in personnel on the Nephrology ward. Of 24 *S. aureus* isolates obtained from patients, 9 (37.5%) were MRSA. Among these MRSA strains, three isolates were recovered from blood and three from wound infections. The prevalence of MRSA isolated from patients in different wards is shown in Table 1.

Among MRSA isolates recovered from health-care providers, the highest rates of antimicrobial resistance were observed for cefoxitin (100%), followed by erythromycin (56.6%), clindamycin (53.3%) and gentamicin (36.6%), as shown in Fig. 1. Furthermore, all of the MRSA isolates were susceptible to linezolid, teicoplanin, quinupristin-dalfopristin and vancomycin. Regarding risk factors, the prevalence of nasal MRSA carriage among hospital personnel who used masks was significantly lower than those without masks (p 0.007) (Table 1). Regarding clinical MRSA, high percentages of resistance against cefoxitin (100%) and erythromycin (66.6%) were observed. All of the MRSA isolates recovered from patients were susceptible to linezolid, teicoplanin, mupirocin and rifampin. The ranges of vancomycin MICs for MRSA isolates from patients varied from 0.5 to 1.5 mg/L, which were interpreted as susceptible.

In order to define the clonal patterns of the MRSA isolates, genotyping was performed (Fig. 2). The levels of similarity between the PFGE fingerprints of the isolates range from 50% to 100% (Fig. 3). With an 80% similarity cut-off point, a total of ten clusters (A to J) and 14 singletons were identified. The singletons had unique patterns and were not similar to any of the other isolates. Most of the MRSA isolates recovered from general intensive care units (ICUs) (from either patients or carriers) were distributed in certain clusters (except C, F, H and J clusters). In this respect, similar patterns were observed between two MRSA isolates obtained from nasal swabs of personnel working in general ICUs, and two MRSA isolates from patients who were hospitalized in neurological and general ICUs. However, singletons belonged to different wards. The percentages of antibiotic resistance among singletons were generally less than those of the isolates within clusters.

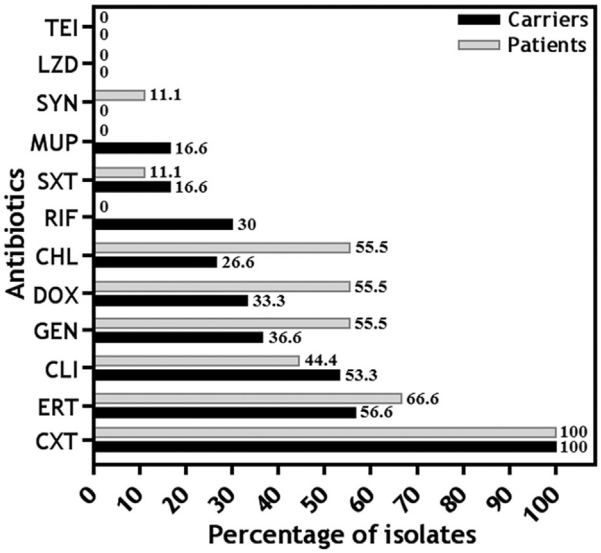

FIG. 1. The prevalence of antimicrobial resistance of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates among personnel carriers and patients. CHL, chloramphenicol; CLI, clindamycin; CXT, cefoxitin; DOX, doxycycline; ERT, erythromycin; GEN, gentamicin; LZD, linezolid; MUP, mupirocin; RIF, rifampin; SXT, trimethoprim-sulfamethoxazole; SYN, quinupristin-dalfopristin (synercid); TEI, teicoplanin.

TABLE 1. **Potential risk factors associated with nasal carriage of methicillin-resistant *Staphylococcus aureus* isolates among health-care workers**

Risk factors	Total personnel (n = 133)	MRSA carriage (n = 30), n (%)	Neither MRSA nor *S. aureus* carriage (n = 103), n (%)	p value
Age groups (years)				0.75
20–30	59	15 (25.4)	44 (74.5)	
31–40	61	12 (19.6)	49 (80.3)	
41–60	13	3 (23.1)	10 (76.9)	
Gender				0.40
Male	49	13 (26.5)	36 (73.4)	
Female	84	17 (20.2)	67 (79.7)	
Underlying diseases including diabetes and hypertension				0.89
Yes	5	1 (20)	4 (80)	
No	128	29 (22.6)	99 (77.4)	
Shift work				0.60
Morning	36	7 (19.4)	29 (80)	
Night	97	23 (23.7)	74 (76.2)	
Mask wearing				0.007*
Yes	55	6 (10.9)	49 (89)	
No	78	24 (30.8)	54 (69.2)	
Recent antibiotic intake				0.29
Yes	12	0 (0)	12 (100)	
No	121	29 (24)	92 (76)	
Smoking				0.69
Yes	11	3 (27.2)	8 (72.7)	
No	122	27 (22.1)	95 (77.9)	
Dermatitis				0.86
Yes	8	2 (25)	6 (75)	
No	125	28 (22.4)	97 (77.6)	
Rhinitis and sinusitis				0.32
Yes	27	8 (29.6)	19 (70.3)	
No	106	22 (20.7)	84 (79.3)	
Wards				0.086
General ICU	67	13 (19.4)	54 (80.5)	
Neurological ICU	12	2 (16.6)	10 (83.3)	
Emergency ward	20	3 (15)	17 (85)	
Haemodialysis ward	11	2 (18.1)	9 (81.8)	
Nephrology ward	23	10 (43.4)	13 (56.5)	

Abbreviations: ICU, intensive care unit; MRSA, methicillin-resistant *Staphylococcus aureus*; p values < 0.05 were considered statistically significant.

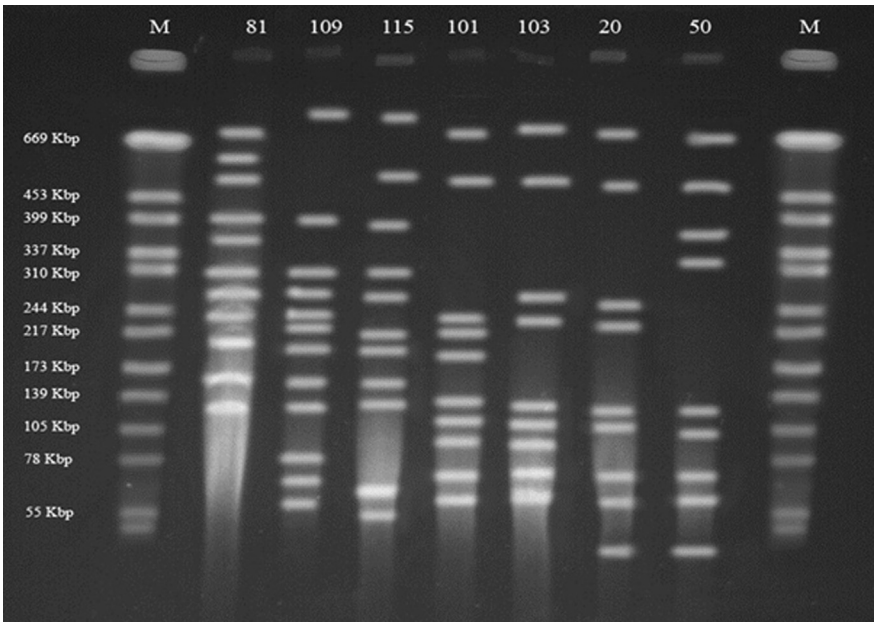

FIG. 2. *Sma*I macrorestriction fragments of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates on pulsed-field gel electrophoresis (PFGE) gels. Lanes 2 to 8, different PFGE patterns of MRSA isolates; M, PFGE marker.

Discussion

There are several molecular methods for genotyping MRSA strains for epidemiological studies. Chromosomal digestion with PFGE is considered a powerful technique for the characterization of epidemiological features of MRSA as well as of outbreaks and their sources.

The contribution of healthy carriers in the dissemination of *S. aureus* in both hospital and community settings is well documented in the literature, though more so for methicillin-sensitive *S. aureus*. In our study, the prevalence of MRSA colonization among hospital personnel was 25.5%, which is comparable to the findings of other studies in developing countries such as Palestine (25.5%), Saudi Arabia (18%) and Ethiopia (12.7%) [22–24]. However, based on a comprehensive review article by Albrich and Harbarth [3] the average MRSA prevalence among health-care providers was 4.6% in 127 studies. In Iran, a recent meta-analysis study showed that the frequency of MRSA among hospital personnel was 32.8%, which is almost consistent with the results of the current study [25]. Generally, these contrasting results may be due to differences in sample sizes, sampling techniques, culture media, interpretation guidelines, and even infection control policies [3].

Several individual risk factors including co-morbidities (e.g. skin and upper respiratory infections), occupational variables (e.g. longer duration of service, service areas, close contact with patients, and poor hand hygiene), and recent antibiotic consumption have been found to be associated with MRSA colonization in hospital personnel [3,7,25]. Hence, we tried to explore different potential risk factors associated with nasal carriage of MRSA. In this regard, the frequencies of nasal MRSA carriage among hospital personnel who wore masks were significantly lower than those without masks (p 0.007), which is in line with the findings of some previous studies showing that mask usage can potentially diminish inadvertent transmission of the pathogen in the hospital environment [3,26]. However, contrary to our initial expectations, we did not find any significant associations between nasal carriage of MRSA and certain risk factors such as dermatitis, sinusitis or rhinitis, and recent antibiotic intake [3].

MRSA infections pose a formidable challenge for physicians, as these strains have gradually developed resistance to different classes of antibiotics. Similar to the present findings, some studies reported high rates of resistance against erythromycin and clindamycin among MRSA from health-care workers [23,27–29]. Regarding gentamicin, 36.6% of the MRSA isolates were resistant in this study, which is higher than in some previous reports [23,28]. However, we showed that all of the MRSA isolates from health-care workers still remained susceptible against linezolid (an oxazolidinone), teicoplanin (a glycopeptide), and quinupristin-dalfopristin (a streptogramin). These findings corroborate the results of other previous studies from Iran, Nepal, India, Oman and Taiwan, in which the frequencies of resistance against these agents were almost nil [27–31]. Fortunately, none of the clinical MRSA isolates exhibited resistance toward linezolid and teicoplanin, which is consistent with the findings of previous studies from Iran [32–35]. On the whole, the inevitable rise of drug resistance in

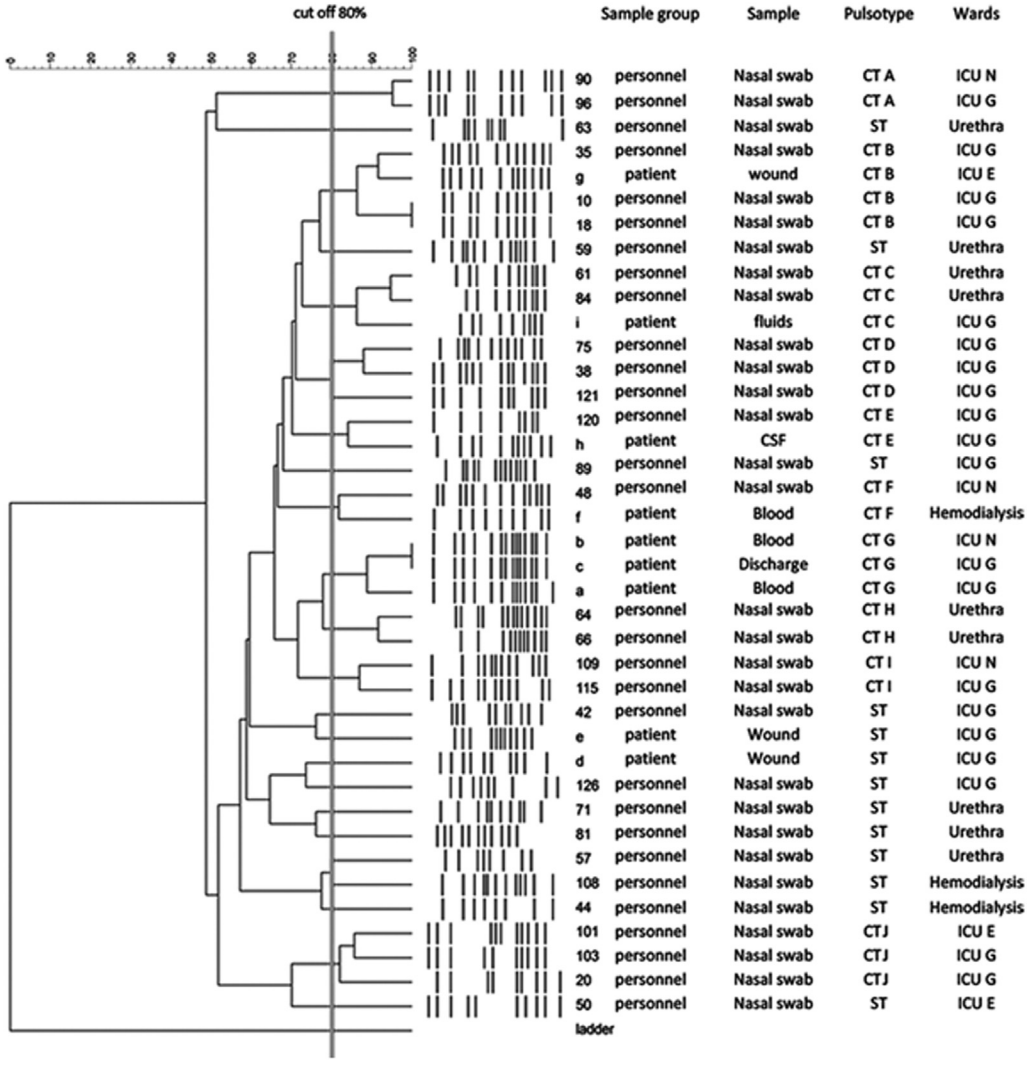

FIG. 3. The UPGMA dendrogram of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates based on pulsed-field gel electrophoresis profiles. Isolate code, source of sample and the related ward are also given for each MRSA isolates.

MRSA has left fewer effective antibiotic options to cure serious infections.

PFGE is still regarded as the reference standard fingerprinting method to assess dissemination of MRSA strains in local outbreaks and surveillance of MRSA infections in hospital settings [17]. In our survey, most of the MRSA isolates recovered from health-care carriers and patients in ICU wards, especially general ICUs, were grouped in certain clusters, indicating intra-ward transmission of the mentioned isolates in these restricted areas. Indeed, some of these MRSA isolates exhibited indistinguishable patterns, confirming that person-to-person transmission has occurred within the ICU wards. However, MRSA isolates from other wards (i.e. emergency, haemodialysis and nephrology wards) revealed higher heterogeneity in their pulsotypes, which may be the result of movement of personnel and/or patients between hospital departments. Crowded hospital wards and overflowing waiting rooms greatly facilitate the spread of infections through coughing, sneezing, physical contact and contaminated fomites (such as gloves, attire and surgical instruments) from personnel to patients or vice versa [26,28]. In this regard, several well-documented instances of person-to-person spread of MRSA in the hospital environment are recorded in the literature [13,36–38].

Our study has a number of limitations that should be addressed in future works. First, larger sample sizes from different hospitals would strengthen the external validity of these findings. Second, as *S. aureus* can survive on inanimate objects for prolonged periods, they can easily disseminate through contaminated fomites. Therefore, these objects such as

gloves, masks, surgical instruments, attire and blankets are potential reservoirs for MRSA transmission [39,40].

Conclusion

In our study, we demonstrated that health-care workers can serve as a vehicle for the transmission of MRSA in the hospital environment. Overall, screening and decolonization of carriers, contact precautions, prudent use of antibiotics, and implementation of active surveillance are recommended strategies for the prevention and control of MRSA transmission in hospital settings.

Transparency declaration

The authors declare no conflict of interest.

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