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Methicillin-resistant *Staphylococcus aureus* in air and surfaces of hospital wards: a comparison between new and old buildings

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Abstract

Background: Airborne particles that contain *Methicillin resistant Staphylococcus aureus* (MRSA) can be transferred from hospital air and environmental surfaces. It presents special risks of infections to patients and personnel and imposes exorbitant financial costs and human losses.

Objective: This research has been done to determine the prevalence of MRSA in the air and on surfaces of different hospitals wards.

Methods: In this cross-sectional study, surfaces and air samples were collected from 12 wards of new and old-building hospitals, following identification of MRSA by detection of *pvl, mecA and vanA* genes using Polymerase chain reaction (PCR) assay in 2017. Both hospitals are located in the north of Qazvin city (population: 596,932), Iran, with 255 and 230 patients' beds respectively. Also, some environmental properties of the sampling areas were measured. The data were analyzed using IBM-SPSS version 23, parametric tests and Pearson product-moment correlation.

Results: *S. aureus* and Gram-negative bacteria were detected in 59.6 and 80% of the samples. The Intensive care unit (ICU) with 7.5% MRSA prevalence was the most contaminated ward. *S. aureus* was detected in 20% of the surface samples while MRSA was isolated in 16.7%. There are positive correlations between bacterial contamination levels of the air, surfaces and the CO_2 concentration of the sampling spaces ($p<0.0001$).

Conclusion: According to the findings of this study, air and surfaces of hospitals are contaminated with MRSA. Because of significant correlation between bioaerosol concentration and fomites, to reduce and control prevalence of MRSA, using air cleaning systems as well as decontamination of surfaces is suggested.

Keywords: Hospital, Cross Infection, Methicillin-Resistant *Staphylococcus aureus*, Air Pollution, Environmental Microbiology

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Abbreviations / Acronyms:
ARNI: antibiotic-resistant nosocomial infections; **GNB:** Gram-negative bacteria; **ICU**: Intensive care unit; **I/O:** Indoor-to-outdoor concentration ratio; **mecA**: Methicillin Resistance Gene; **MRSA:** Methicillin resistant Staphylococcus aureus; **PCR:** Polymerase chain reaction; **vanA:** Vancomycin resistance gene.

1. Introduction

Nosocomial infections have recently been considered as health threatening and economic loss causes in health care systems and medical microbiology (1). Hospital antibiotic overuse leads to increase in the spread of antimicrobial resistance. In the United States, more than thirty billion dollars annually is dedicated to the treatment of antibiotic-resistant nosocomial infections (ARNI). Some pathogens causing ARNI including *Pseudomonas aeruginosa*, Aspergillus species and *Staphylococcus aureus* are transmitted from air, surfaces, workers and faculties of different hospital wards to patients (2). *S. aureus*, a non-spore-forming gram-positive cocci, is a microflora of human skin and mucus membrane. This pathogen is one of the most important causes of ARNI transmitted from the hands of workers and faculty to different parts of medical environments, directly to patients (3). According to a report in the U.S., approximately 120,000 *S. aureus* bloodstream infections and 20,000 associated fatalities occurred in 2017 (2). The principal challenge of this pathogen in hospital infections is antimicrobial resistance properties against methicillin antibiotic family. *Methicillin-resistant Staphylococcus aureus* (MRSA) is an antibiotic-resistant category of *S. aureus* which is also resistant to other antibiotics such as vancomycin, linezolid, trimethoprim/sulfamethoxazole and ceftobiprole as 5th generation of cephalosporine antibiotic. MRSA has been recently identified in clinical samples by molecular techniques as rapid, specific and sensitive methods (4). Resistance to methicillin is characterized in the genome of MRSA isolates by the (Methicillin Resistance Gene) mecA gene. Vancomycin resistance gene (vanA) also was reported positive in MRSA isolates in several studies previously. *S. aureus* is usually identified in clinical and environmental samples by detection of some specific virulence factor encoding genes. A cytotoxin released by *S. aureus*, Panton-Valentine Leukocidin (*pvl*) encoded gene is commonly detected by molecular techniques to identify and confirm the positive coagulase *S. aureus* in clinical samples (5). Consequently, rapid detection and identification of MRSA and determination of antimicrobial susceptibility patterns of this pathogen are effective strategies to decrease the prevalence and the risk of lethality in hospitalized patients (6).

Air and surfaces of different wards in the hospital may be subject to transmitted contamination with MRSA the health of the patients may be threatened (7). MRSA has been shown as airborne nosocomial pathogen. Previous studies of air circulation in hospitals and wards revealed that intensive care units and isolation rooms have the transmission potential of carrying MRSA to patients, especially as an airborne pathogen (8). Regarding contaminated surfaces in hospital wards, healthcare workers are the most suspect for transmitting MRSA as confirmed by several researchers (9). However, other surfaces including gowns, gloves, masks, clothes and nursing staff in general have the transmission potential. Nevertheless, MRSA is a principal cause of fatal nosocomial infections; survey and examination of the spreading pattern of this pathogen for air and surfaces of different hospital wards is necessary to be evaluated at regular intervals (10). Iran is faced with significant growth of antibiotic resistance (11). To the best of our knowledge, previous research in Qazvin city, Iran, has tended to focus on MRSA strains that are isolated from patients and personnel and have considered individual factors such as age, gender, type of disease, etc. (12-14), while MRSA transmission factors as contamination of air and fomites in hospitals and some environmental parameters have not been examined. A key drawback with much of the study on MRSA infections is that researchers have described prevalence of this pathogen in clinical isolates, and monitoring of hospital environments as infection prevention and control have not been noted. Therefore, in the present study, we determined the pattern of MRSA prevalence isolated from air and surfaces of different hospital wards using Polymerase chain reaction (PCR) assay with specific primers for detection of virulence factor and antimicrobial susceptibility genes in isolates.

2. Material and Methods

2.1. Subjects

This descriptive-analytical and cross-sectional study was conducted between March and September 2017. Air and surface samples were collected from different wards of two educational hospitals, one new and one old, with 255 and 230 patients' beds respectively. Both hospitals are located in the north of Qazvin city (population: 596,932), Iran (15). The wards of hospitals subjected to sampling included emergency, Intensive care unit (ICU), surgery, coronary care unit (CCU), operating theatres, orthopedic wards and air samples from landscape spaces of each

hospital. For determination of indoor-to-outdoor concentration ratio (I/O), one outdoor station was chosen to compare the measurement of data inside the hospital with those outside the hospital.

2.2. Measurement

2.2.1. Sampling and isolation of S. aureus

Air sampling was conducted based on NIOSH 0800 sampling standard method (16) using a QuickTake 30 sample pump (SKC, Inc. USA) positioned in the center of the rooms, one-meter distance from the wall ledge and the hospital beds. All air samples were taken from an inflow of 28.3 L.min-1 for 10 minutes. Sampling was carried out on two days each week in a time period between 08:00–14:00. All air samples were cultured on Manitol Salt Agar (ProMedia, Spain) and Eosin Methylene Blue Agar (ProMedia, Spain) at 37 °C for 24 h. For surface sampling, swap sampling was performed on a 10×10 cm area for different surfaces consisting of covers, clothes, dining tables, serum holders, computers in nurse stations, and on hospital beds. All swap samples were enriched in tryptone soy broth (ProMedia, Spain) overnight at 37 °C then inoculated on mannitol salt agar (ProMedia, Spain) and eosin methylene blue agar (ProMedia, Spain) and incubated at 37 °C for 48 h. All presumptive colonies were subjected to coagulase test. All coagulase positive isolates were evaluated for methicillin resistance characteristic using minimum inhibitory concentration (MIC) method (17). Antimicrobial resistance properties of strains were detected and confirmed for oxacillin and cefoxitin antibiotics (Oxoid, UK). Temperature and relative humidity were measured using Indoor Air Quality Meter model IAQ55 (Supco, Co. USA) during sampling.

2.2.2. DNA extraction and PCR assay

Confirmed colonies of MRSA isolates were subjected to DNA extraction. The total genome of isolates was extracted using Sinaclon commercial kit (Sinaclon, Co. Iran) for DNA extraction of gram positive bacteria according to the manufacturer`s instructions. Quantitative and qualitative of the extracted DNA were measured by NanoDrop spectrophotometer (Thermo Fisher Scientific, USA). Species-specific genes *pvl, mecA and vanA* were detected for identification of MRSA in this study. Specific primers used for detection of these genes have been described in Table 1. PCR mixture consists of 10 µL PCR master mix 2X (Ampliqon, Denmark), 1 µL of each primer, 3 µL of DNA templates and addition of deionized nuclease free water to final volume was carried out in a 25 µL reaction volume. Thermal cycling programs for each primer are described in Table 1. For characterization of PCR products, gel electrophoresis was used on 1.4% agarose gel with 0.01% V/V safe staining dye (Ampliqon, Denmark) (18).

Table 1. Primer sequences and thermal cycling program used for identification of MRSA

Gene	Primer sequence	Thermal cycling program	Product length
mecA	F-GCAAACGTGGCGAAGAAT R-TGATCTTCACCTTCTAATGTTTGAG	Initial denaturation: 6 min at 94°C, followed by 35 cycles: 30 s at 95°C, 30 s at 56°C and 35 s at 72°C; finally, 5 min at 72°C as final extension step	235 bp
pvl	F-CACGATGCGAGCAATCAATC R-ACAGCCGTGGATAACTTCTAAA	Initial denaturation: 6 min at 94°C, followed by 40 cycles: 40 s at 95°C, 40 s at 59°C and 30 s at 72°C; finally, 7 min at 72°C as final extension step	452 bp
vanA	F-CATGGCAAGTCAGGTGAAGA R-TTTCACACCGAAGGATGAGC	Initial denaturation: 6 min at 94°C, followed by 30 cycles: 40 s at 94°C, 61 s at 57°C and 30 s at 72°C; finally, 5 min at 72°C as final extension step	252 bp

2.3. Statistical analysis

For statistical analysis, analysis of variance ANOVA followed by Duncan`s multiple range test for significant difference evaluation ($p<0.05$) was used employing SPSS software version 23.0 (Chicago, IL, USA). The relationships between data were examined by using the Pearson product-moment correlation test. Also, all experimental and statistical measurements were implemented in triplicates.

2.4. Ethical approve

This research was approved by Qazvin University of Medical Sciences ethical committee and registered by code IR.QUMS.REC.1396.286.

3. Results

At the present study, totally, 180 and 322 surface and air samples respectively were collected from 12 internal wards and 2 places located in the landscape space of two hospitals in spring and winter seasons. Gram-negative bacteria

(GNB), *S. aureus* and MRSA were detected in 80, 59.6 and 32.5% of the air samples respectively. Isolation of MRSA was confirmed by specific-primer PCR assay by detection of *pvl, mecA and vanA* genes (Fig. 1A and B). Table 2 provided the prevalence of airborne pathogens and some environmental characteristics of different sampling wards and places. A significant difference ($p<0.002$) was observed for pathogen prevalence between different wards of two hospitals. Bacterial contaminations were observed in more than 18% of the surface samples from which three MRSA strains were isolated (Table 3). Temperature, relative humidity range and carbon dioxide (CO_2) gas concentration average of different internal wards of the hospitals were 5.6-21.28 °C, 17-44% and 612.4 ppm respectively as described. ICU and emergency wards with 1505 and 1421 ppm had highest CO_2 density.

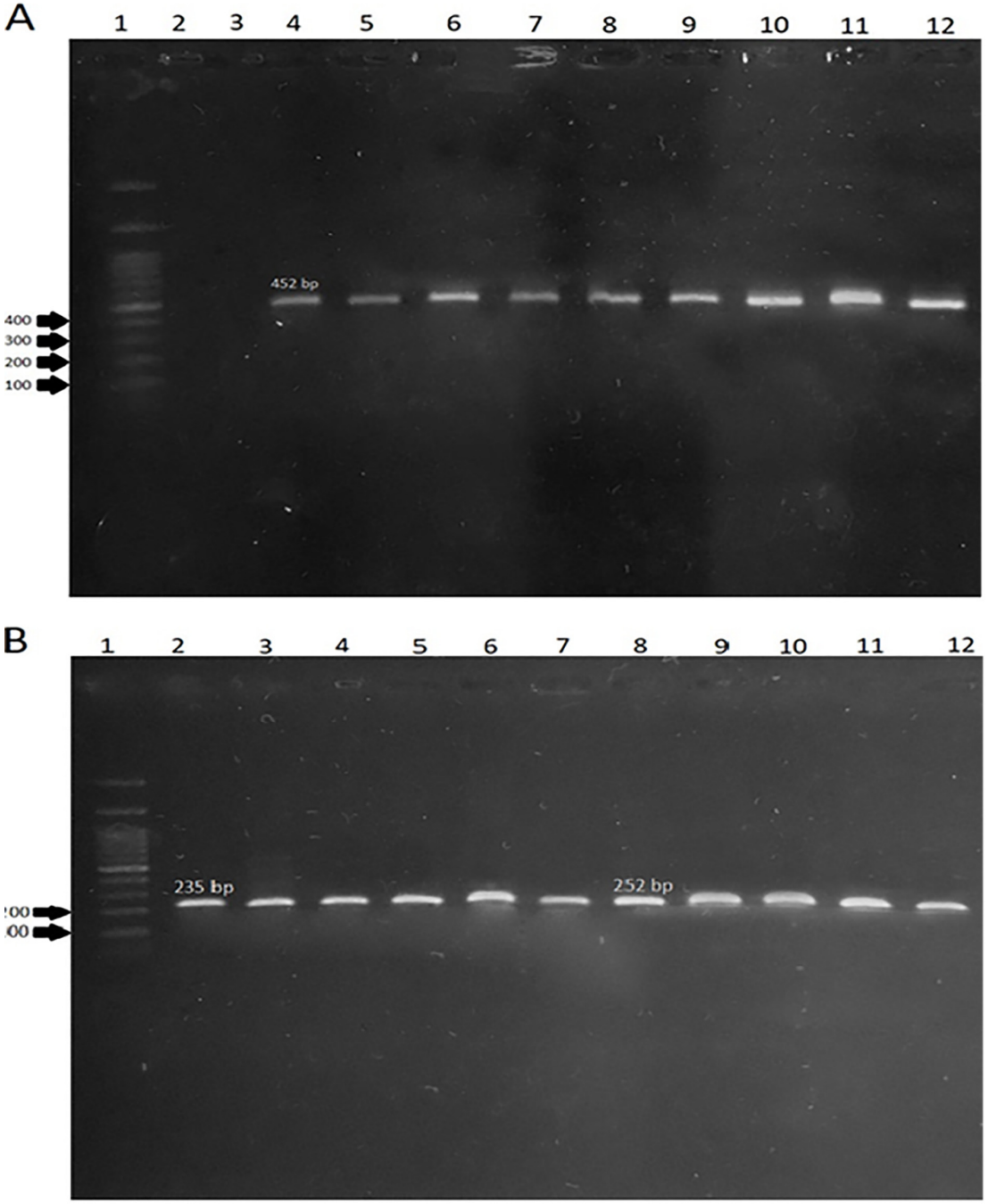

Figure 1 Gel electrophoresis of PCR products for detection of *pvl* (A; lane 1 as marker, lanes 2 and 3 as negative results and lanes 4-12 as positive results), *mecA* and *vanA* (B; lane 1 as marker, lanes 2-7 as positive results for *mecA* and lanes 8-12 as positive results for *vanA*) genes

Table 2. Bacterial density (CFU/m^3), MRSA frequency and environmental characteristics of the wards studied during the two sampling seasons

Hospital	Ward	Bioaerosol	Season					
			Winter			Spring		
			Bacterial density (CFU/m^3)	I/O	MRSA (%)	Bacterial density (CFU/m^3)	I/O	MRSA (%)
			Mean±SD			Mean±SD		
A*	Women's Surgery	*S. Aureus*	5.6±4.1	0.9	0	24.4±16.9	6.9	5
		GNB	9.2±5.3	3.3		11.5±4.3	1.5	
	Men's Surgery	*S. Aureus*	5.7±5.9	0.9	0	10.2±8.1	2.9	0
		GNB	10.1±10.1	3.6		13.5±18.5	1.8	
	ICU	*S. Aureus*	2.3±3.9	0.4	2.5	20±17	5.7	5
		GNB	12.2±12.7	0.4		14.7±21	1.9	
	Women's Orthopedic	*S. Aureus*	17.7±0	2.8	0	3.5±5	1	0
		GNB	10.6±5	3.8		7.07	0.9	
	Men's Orthopedic	*S. Aureus*	15.9±2.5	2.5	2.5	7.06±10	2	0
		GNB	14.1±4.9	5		8.8±7.5	1.2	
	Emergency	*S. Aureus*	17.7±0	2.8	2.5	3.5	1	0
		GNB	30±7.5	10.7		17.7	2.3	
	Mean	*S. Aureus*	6.8±6.5	1.1	1.2	16.4±15	4.6	1.7
		GNB	10.7±9.8	3.8		11.5±12.3	1.5	
	Outdoor	*S. Aureus*	6.4±12.3	-	2.5	3.5±1.4	-	2.5
		GNB	2.8±2.9	-		7.6±11.9	-	
B**	Women's Surgery	*S. Aureus*	1.5±2.8	0.05	0.05	6.2±5.9	0.3	2.5
		GNB	10.6±10	0.4		6.6±6.9	1.1	
	Men's Surgery	*S. Aureus*	4.7±7.3	0.2	0.2	4.7±2.9	0.2	2.5
		GNB	7.1±4.5	0.3		7.8±10.1	1.3	
	ICU	*S. Aureus*	5.3±7.3	0.2	0.2	7.4±6.3	0.3	2.5
		GNB	11.2±13.5	0.4		6.1±8.9	1	
	Operating room	*S. Aureus*	0	0	0	9.7±11.3	0.4	0
		GNB	1.8±2.5	0.07		2.6±5.3	0.4	
	CCU	*S. Aureus*	5.3±2.5	0.2	0.2	10.6±5	0.5	0
		GNB	3.5±0	0.1		3.5±0	0.6	
	Emergency	*S. Aureus*	5.3±7.4	0.2	0.2	7.07±0	0.3	0
		GNB	1.7±2.5	0.07		3.5±3.5	0.6	
	Mean	*S. Aureus*	7.4±14	0.2	0.2	9.5±20.2	0.4	0.4
		GNB	10.7±12.4	0.4	0	5.7±7.1	0.9	
	Outdoor	*S. Aureus*	29.1±28.3	-	-	23±51.1.1	-	0
		GNB	24.7±21.2	-		5.9±5.8	-	-

*old hospital, ** new hospital

There is a significant correlation between the environmental characteristics and the prevalence of the MRSA and GNB for collected air samples; however, no significant relationship was found between the number of staff and workers and the prevalence of airborne pathogens (Table 4). I/O ratio commonly was observed as was found for the old hospital observed in the present study where the air is circulated by windows; however, more declination in I/O ratio was observed in the spring season for this hospital. The prevalence of GNB was 9.4 $CFU.m^{-3}$. The number of workers in each ward, I/O ratio and environmental properties are the main factors for bacterial contamination of the hospital air. GNB was observed in about 15% of the surface samples, principally detected from the surface of the ICU bed covers.

Table 3. Distribution of positive surface samples by internal wards of each hospital

Hospital	Ward	Surface	S. Aureus Dencit (CFU/cm^2)	Dencity GNB (CFU/cm^2)	MRSA (%)
Old	Women's Surgery	Food Table 2	0.02	0	0
		Bed Handle 2	0.08	0.02	0
		Central Hall Rail	0.06	0	0
	Men's Surgery	Nurse Station Desk	0.02	0.06	0
		Bed sheet 2	0	0.08	0
		Bed Handle 2	0.06	0	1
		Food Table 12	0.04	0.46	0
		Serum Leg 12	0.02	0	0
	ICU	Nurse Station Desk 1	0.02	0	1
		Keyboard 5	0	0.04	0
		Food Table 8	0.02	0	0
		Bed Handle	0.02	0.36	0
	Emergency	Bed sheet 3	0.02	0	0
		Food Table 8	0	0.02	0
	Men's Orthopedic	Bed Handle 1	0.04	0	0
		Trolley	0.02	0	0
	Women's Orthopedic	Trolley	0	0.02	0
New	Women's Surgery	Bed sheet 1	0.14	0.3	0
		Keyboard	0	0.52	0
	Men's Surgery	Keyboard 1	0	0.02	0
		Bed sheet 8	0.02	0	0
		Bed sheet 18	0	0.24	0
	ICU	Bed Handle 11	0	0.04	0
		Bed sheet 4	0.2	0	1
	Emergency	Food Table 2	0.02	0	0
		Trolley	0.02	0	0

Table 4. Relationships[a] between variables according to Pearson product-moment correlation test [b]

Sampling Type	Variable	Air Samples			Surface Samples			Temperature	Relative Humidity	CO_2 Concentration	n [c]	Bed sheets change	Cleaning	Season	Temperature
		S. Aureus	GNB	MRSA	*S. Aureus*	GNB	MRSA								
Air Samples	*S. Aureus*	-	<0.0001	<0.0001	<0.0001	<0.0001	0.02	0.47	0.23	<0.0001	0.86	0.26	0.38	0.06	-
	MRSA	<0.0001	0.002	-	0.01	0.002	0.09	0.37	0.24	0.02	0.7	0.33	0.79	0.08	<0.0001
	GNB	<0.0001	-	0.002	0.24	-	0.11	0.80	0.03	<0.0001	0.62	0.64	0.13	0.16	<0.0001
Surface Samples	*S. Aureus*	0.62	<0.0001	0.98	0.10	-	<0.0001	0.46	0.81	0.88	0.49	-	0.49	0.48	0.62
	MRSA	<0.0001	0.24	0.01	-	0.10	0.47	0.25	0.36	0.11	0.87	-	0.33	0.16	<0.0001
	GNB	0.65	0.11	0.07	0.47	<0.0001	-	0.46	0.81	0.88	0.02	-	-	0.49	0.65

[a] Statistical significant was set at $p<0.05$, [b] Numbers are presented in p-values, [c] Number of persons

4. Discussion

In this study, total air contamination rate of two hospitals were observed at about 60% which was higher than that reported by Azarian et al. (2015) in India (19). *S. aureus* was detected in 20% of the surface samples while MRSA was isolated in 16.7%. There are many research that isolated varied prevalence levels of MRSA from air and surfaces of the different hospital wards (20). Mukhyia et al. observed 14.3% prevalence of *S. aureus* as an airborne pathogen in hospital samples while MRSA was isolated in 40.7% of samples which is higher than that detected in our study (32.5%) (21). Creamer et al. and Mirzaii et al. isolated MRSA in 14.4 and 66.7% of the samples collected from different wards of the hospitals respectively (10, 22). We detected the most prevalence of MRSA in the ICU ward. Wagenvoort et al. isolated MRSA from air samples from the ICU ward even eight weeks after the patients had been discharged (23). As previously described by researchers; higher prevalence of MRSA are in the ICU and CCU wards of the hospitals as the principal causes of the nosocomial infections in these places (24). Due to the fact that

antibiotic treatment with different consumption levels is carried out in the hospital wards (25) a significant difference of MRSA average emerged. Because of long hospitalization period and strong antibiotic treatment, patients in the ICU have more potential to be subjected to MRSA (26). Therefore, microbial environment quality will need to be more considered in intensive care units.

The frequency of GNB in surfaces was 15% which is consistent with previous results (27). The ability of adaptation to environmental conditions in GNB is the reason for survival of these bacteria in surfaces (28). Several outbreaks have demonstrated that surface contamination by GNB and MRSA plays a role in continued transmission in hospitals (29-31). As shown in Table 3, areas around patients such as bed sheets and bed handles were contaminated with MRSA that are touched frequently by workers and patients. Significant correlation was found between MRSA contamination in surfaces and number of persons ($p=0.02$). This can be attributed to the traffic of people in a hospital environment that can lead to an increase in the number of surfaces touched by hands. Thus, improved efficiency of disinfection and routine environmental sampling can be taken to prevent or reduce the incidence of contamination of surfaces and hospital acquired infections.

The concentration of isolated *S. aureus* was between 2.24-3.40 $CFU.m^{-3}$ higher than that observed by Ho et al. (32) and lower than Nandalal and Somashekar's report (33). We detected higher levels of airborne pathogens in the old hospital. The most contaminated place with *S. aureus* was the ICU ward in the old hospital with 15.9 $CFU.m^{-3}$ bacterial load. The most important causes of difference in *S. aureus* contamination levels between old and new hospitals were air purification systems. There had been no suitable ventilation or air purification system in the old hospital for more than forty years; In light of the reported indoor/outdoor ratio $I/O>1$, it could be reasonably described as entering ambient air inside the old hospital building. Hence the doors and windows need to be kept closed or the use of purified outdoor air by filtration is needed, to minimize airborne spread of MRSA. The new hospital is equipped with a modern central air circulation and purification system that dilutes airborne microbial concentration and removes microbes via an outside air exhaust. There was a strong correlation between CO_2 concentration, bacterial bioaerosols and the seasons, which is similar to the study of Hwang et al. (34). Peak of CO_2 density was observed in ICU and emergency wards, which is in result of inadequate air movement and overcrowding in limited spaces. Then higher CO_2 concentration of the hospitals' air indicates lower efficiency of the air ventilation and more airborne bacterial load. In addition, an increase of CO_2 concentration up to 1000 ppm leads to some diagnostic medical errors (35). Many studies have suggested the use of ventilation and purification systems to reduce infectious agents in hospitals (36, 37). Some classic strategies including washing hands, and hygienic and sterilization procedures could be effective in reducing airborne pathogens (38). As *S. aureus* is carried by one third of the human population (25), an effective monitoring program should be established to detect, identify and control this pathogen transmitted from the staff and patients to the air and surfaces of the sensitive hospital wards.

Our results have been in agreement with other researchers regarding the correlation between air and surface contamination of MRSA in hospitals (10, 22, 39). This correlation can be due to activities by colonized or infected patients; aerosols are produced and disseminated during talking, coughing and sneezing and creating infectious droplet nuclei. Droplets may remain suspended and spread for some time or settle on surfaces and equipment and can be picked up by the hands of health care workers or patients, which is considered as subsequent transmission. As a matter of fact, microbial air quality is significantly related to contamination of the environment and surfaces. Considerable attention must be paid to air cleaning systems as well as decontamination of surfaces and a more rigorous and scientific strategy to assessing healthcare facilities is needed.

5. Study strength and Limitations

This paper has highlighted the importance of the link between air and surface contamination of MRSA in hospitals. However, this study was carried out in one city in Iran and due to the limited sample size, the findings cannot be generalized to other hospitals in Iran, which should be targeted in future works. It should be noted that in spite of the existence of a ventilation and filtration system in the new hospital, I/O ratio was below 1. This indicates contamination accumulation inside the building is due to low performance of ventilation dilution. Thus assessment of ventilation effectiveness, for controlling air quality in hospitals is necessary. One of the limitations of this study is the lack of measurement of ventilation air system parameters. Prospective investigations should examine efficiency factors of ventilation systems, such as air exchange rate, filtration and disinfection systems, airflow, and pressure.

6. Conclusions

The present study has shown prevalence of MRSA in air and surface samples collected from different wards of new and old building hospitals in Qazvin city, Iran. Environmental contamination can be proposed as a source for subsequent transmission of pathogens. Correlation between MRSA contamination in air and surfaces and also CO_2 concentration implies insufficient ventilation systems in both hospitals. In conclusion, the control of airborne MRSA in hospitals depends not only on air disinfection but also on surface decontamination. On a wider level, research is also needed to assess the relationship between environmental MRSA and isolates from patients and staff to investigate the role played by environmental contamination in the risk of transmission of this pathogen in healthcare facilities.

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Conflict of Interest:

There is no conflict of interest to be declared.

Authors' contributions:

Conception or design of the work (MC, SM, BR, AN); Acquisition of data (MC, ZN, AN); Analysis or interpretation of data (MC, BP, AN); Drafting the manuscript (MC, SM, BP, ZN); Revising the manuscript (MC, SM, BP, BR, AN); Accountable for all aspects of the work (All author). All authors read and approved the final manuscript.

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