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Original Article

Prevalence of Methicillin Resistant *Staphylococcus aureus* Carriage Amongst Healthcare Workers of Critical Care Units in Tertiary Hospitals of Jashore, Bangladesh

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***Staphylococcus aureus* is responsible for numerous mild to severe diseases in humans in both hospital and community settings. This problem is getting even worse for multi-drug resistant *S. aureus*. Healthcare workers (HCWs) are in a threat of MRSA carriage and can serve as reservoirs. Therefore, this can lead to subsequent infection and cause a nosocomial outbreak of MRSA. This cross-sectional study involved 85 healthcare workers (HCWs) from surgery units and critical care units of five different tertiary hospitals at Jashore, Bangladesh. Nasal swab samples were collected and inoculated on selective Mannitol Salt Agar (MSA) media. *S. aureus* was confirmed by selective growth on MSA and detection of the presence of the *fem*A gene. Antibiotic susceptibility pattern was determined for all *S. aureus* isolates. Methicillin resistance was detected using both disc diffusion assay and gene specific polymerase chain reaction. *S. aureus* specific staphylococcal protein A (Spa) typing was also done by sequence analyses of PCR products. Among the 85 enrolled HCW personnel, 34 (40%) were carrying *S. aureus* followed by the *S. intermedius* (N=32). An overall prevalence of MRSA was found to be 3.53% (3/85). However, all the strains were susceptible to vancomycin. Additionally, 100% sensitivity was found against Trimethoprim/sulfamethoxazole and Linezolid. Sequence analysis of Spa PCR product revealed that all *S. aureus* isolates were of t304 spa type. None of the *S. aureus* was a biofilm former. This study was only a survey on the prevalence of MRSA among HCWs. Integrated surveillance for MDR MRSA carriage among HCWs is warranted to control bursts of nosocomial infections effectively. It is strongly recommended to maintain good microbiological and personal hygiene practices by HCWs.**

Keywords: *Staphylococcus aureus,* Healthcare Workers, MRSA carriage, MDR MRSA.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become the most eminent etiological agent of hospital-acquired infection (HA-MRSA). HA-MRSA might lead to high morbidity and mortality among patients in hospitals throughout the world[1]. Surgical site infections are a major contributor to hospital-acquired infections. *S. aureus* colonization is associated with severe surgical site infections in high-risk patients, whereas methicillin-resistant *S. aureus* (MRSA) is associated with devastating outcomes[2]. Increased MRSA outbreaks in communal settings as a result of community-associated MRSA (CA-MRSA) have also been noted[3]. The hospital personnel may play significant role in transmitting the infection as they work at the point where the hospital and the community meet. Transmission may be caused in the hospital through hand, clothes, or pieces of equipment[4]. Along with hospital-acquired infection, the burden of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has increased in the community[5]. In recent years, the nosocomial pathogen MRSA has been reported to cause infections in healthcare institutes, and the HA-MRSA and CA-MRSA is highly prevalent in Asia[6]. The surgical site infection rate accounts for almost 17% of hospital-acquired infections by MRSA[6]. Infection caused by *S. aureus* ranges from mild to systemic infection, including cellulitis, impetigo, folliculitis, paronychia, endocarditis, septicemia, toxic shock syndrome, endocarditis, etc.[7]. Multidrug-resistant MRSA is widespread in hospitals throughout Asia, where the prevalence is estimated to range from 28% to >70%[8]. However, studies showed that colonization by MRSA leads to subsequent infection, can cause infection even after 18 months of discharge and accounts for ~30% of MRSA infections after identification[9]. Risk factors for progressive MRSA infection include a history of antibiotic use, intensive care settings, ulcers, surgical wounds, urinary catheterization, and the specific population being studied[10]. Studies have shown that most MRSA carriers (approximately 80-95%) are asymptomatic and could interrupt infection control during hospital admission[3]. However, if multidrug-resistant strains are involved, MRSA infections may have grave complications

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and deadly outcomes. The emergence of multidrug resistance MRSA is now a major concern due to the susceptibility of immunocompromised patients or patients recovering from surgery or a serious disease, rendering the drug's choice less available. Moreover, data regarding the colonization of healthcare workers are scarce, and sometimes proper hygiene is not maintained[10].

Previously several nosocomial MRSA outbreaks were caused by the nasal carriage of hospital staff[11,12]. Although there is a lack of information on the carriage of MRSA in critical healthcare workers (HCWs), MRSA carriage among HCWs is being reported from other countries[4,13–15]. Studies from Bangladesh only report on MRSA isolated from clinical samples[16–18]. Therefore, this study aimed to study the prevalence of MRSA carriage among healthcare workers.

Methods and Materials

Ethical approval

Before commencing sample collection, ethical approval was taken from Jashore Science and Technology University ethical review committee and from each selected hospital. Each personnel was briefed about the study, and their written consent was taken before enrollment.

Study design and sampling procedure

This hospital-based cross-sectional study was conducted in five different private hospitals in Jashore city, Bangladesh. The main focus was on hospital personnel, especially those working in the surgery/critical care unit. Before taking their nasal swab, verbal consent was taken from them. The demographic characteristics of the participants were collected with a structured questionnaire. Most of the personnel were between 25-35 years of age. HCW personnel having skin and soft tissue infections, otitis, or rhinitis and/or taking antibiotics at that time or within three weeks, were excluded from our study[4].

Sample Collection and Laboratory Testing

A total of 85 nasal swab samples were collected from five hospitals. Autoclaved cotton swab was dipped into normal saline and swirled in each anterior noses for few seconds and immediately placed in the sterile TSB broth. The samples were transported to the Microbiology Laboratory of Jashore University of Science and Technology. Nasal swabs were inoculated on Mannitol Salt Agar (MSA) within 2 hours from sample collection and incubated at 37ºC for 48 hours. Different colonies were selected from each sample based on colony morphology. Yellow colonies on MSA were primarily identified as *S. aureus,* and white was considered as other Staphylococci. Catalase test was also performed to exclude *Micrococcus* which are catalase-negative. Moreover, coagulase test was also performed to identify coagulase-negative staphylococci (CONS). Other biochemical tests e.g., Gram staining, MR-VP test, MIU test, citrate test, and KIA test was performed., Strains were presumptively identified using the result in an online tool named "ABIS online." (https://www.tgw1916.net/bacteria_logare_desktop.html).

Antibiotic Susceptibility testing

Each *S. aureus* isolate was tested for antimicrobial susceptibility against a pool of ten different antibiotics using the Kirby-Bauer disc diffusion method [19], and the results were interpreted according to the Clinical and Laboratory Standards (CLSI) guidelines M100 [20]. Used antibiotic discs were Amoxicillin (30µg), Chloramphenicol (30µg), Cefoxitin (30µg), Ciprofloxacin (5µg), Cefepime (30µg), Cephradine (30µg), Trimethoprim/sulfamethoxazole (1.25µg/23.75µg), Erythromycin (15µg), Linezolid (30µg), and Vancomycin (30µg). Overnight grown liquid culture was resuspended in normal saline, adjusted with 0.5 McFarland standard turbidity. Then with a sterile cotton swab, the inoculum was swabbed on a Mueller Hinton agar (MHA) plate and incubated at 37! for 24 hours. Zones were subsequently interpreted as sensitive, resistant, and intermediate using CLSI guidelines. To identify Methicillin-resistant *Staphylococcus aureus* (MRSA), cefoxitin (30 µg) disc was used at 35! incubation for overnight. Zones less than or equal to 21 mm were considered MRSA according to the CLSI guideline. Isolates showing resistance to at least three different classes of antibiotics were screened as multi-drug-resistant (MDR) *Staphylococcus*. *S. aureus* ATCC 25625 was used for quality control in all tests.

Molecular detection of femA and mecA gene

Genomic DNA was extracted using the boil DNA extraction method[21]. The *femA* gene encodes a protein precursor involved in peptidoglycan biosynthesis and is thus used for identifying *S. aureus*. *femA*-specific primer pairs (FemA-F CTTACTTACTGCTGTACCTG and FemA-R ATCTCGCTTGTTGTGTGC) were used in a polymerase chain reaction (PCR) to confirm the identification of *S. aureus*[22]. Specific primer pairs (*mecA*-F: 5'-AAAATCGATGGTAAAGGTTGGC-3' and *mecA*-R: 5'-AGTTCTGCAGTACCGGATTTGC -3') were used for the amplification of the 533bp fragment of *mecA* gene responsible for methicillin resistance[23]. PCR conditions were as follows: 5 minutes at 94°C, followed by 40 cycles of denaturation at 94°C for 50 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 60 seconds, and the final extension step at 72°C for 10 minutes. The PCR products were visualized on a 1.5% agarose gel with EtBr (Ethidium Bromide) under a UV transilluminator at 254 nm and 365 nm wavelengths.

Spa typing

Spa typing was done by the method described by Harmsen *et al.*[24]. Primers used to amplify the spa regions were spa-F (AGCACCAAAAGAGGAAGACAA) and spa-R (GTTTAACGACATGTACTCCGT). PCR products of the spa gene were sequenced by the Illumina platform (Celemics Inc. Korea). Obtained sequences were edited, and particular spa types were assigned using Ridom SeqSphere+ software (https://www.ridom.de/seqsphere/).

Biofilm formation assay

Each bacterial strain was grown in Tryptic Soy Broth (TSB) supplemented with 0% and 1% glucose at 37°C for 24 hrs. A total of 100 µL of cultured bacteria were inoculated in 3 replicates to wells of a 96-well polystyrene plate and incubated for 48 hours at 37°C. After this time, the medium was removed and non-adherent bacterial cells were discarded by washing the biofilms twice with 250 µL of sterile normal saline. Biofilms were fixed with 100 µL of methanol per well for 15 minutes and stained for 5 minutes with 100 µL of 1% crystal violet per well. After rinsing with distilled water, the plates were air-dried. After that, the colorant was dissolved in 96% ethanol to measure absorbance at 492 nm in a microtiter plate reader [25]. Values of absorbance e" 0.12 were regarded as biofilm positive, < 0.2 was considered weak producers, 0.2-0.4 was a moderate producer, and > 0.4 was considered strong producers [26].

Statistical Analysis

All data were collected in triplicate. Data were tabulated and analyzed using a statistical program for social sciences (SPSS) vs. 24.0 for Windows (SPSS, Inc.). Where applicable, the test for association between categorical variables was done by using the Chi-square test/Fishers Exact test. A P-value of < 0.05 was considered significant. All graphs were prepared using GraphPad Prism 8.0 (GraphPad Software).

Results

A total of 85 healthcare workers (HCWs) working in the critical surgery unit from five different hospitals of Jashore city, Bangladesh were screened for MRSA. Among them, 36 (42%) were male, and 49 (58%) were female. Colonies obtained on the MSA plate were tested for a pool of biochemical properties. By analyzing the biochemical characteristics of different isolates, various bacteria, including *S. aureus*, *S. intermedius*, and *S. pseudintermedius* were obtained from the nasal swab samples (Table 1). None of them were coagulase-negative. All isolates identified as *S. aureus* were catalase positive.

Carriage of *S. aureus* was found in 34/85 (40%) enrolled HCWs. Second highest colonization was obtained for *S. intermedius* 32 (37.65%) enrolled HCWs and 13 isolates was non identifiable from the biochemical tests (Table 2). From the above characterization 34 *S. aureus* were used to determine the prevalence of MRSA and VRSA.

Table 1. *Biochemical identification of the microorganisms*

Microorganism	**Biochemical Characteristics**														
	Colony color	**Catalase**	**Coagulase**	**Gram staining**	**Motility**	**Indole**	**Urease**	**Oxidase**	**Glucose**	**Lactose**	**Gas production**	**Hydrogen sulfide production**	**Methyl red**	**Vogues prosker**	**Citrate utilization**
S. aureus	Yellow	+	+	+	-	-	-	-	+	-	-	-	+	+	+
S. hyicus	Colorless	+	+	+	-	-	-	-	+	+	-	-	+	-	-
S. intermedius	Colorless	+	+	+	-	-	+	-	+	+	-	-	+	-	+
S. lugdunensis	Colorless	+	+	+	-	-	-	-	+	+	+	-	+	+	+
S. massiliensis	Colorless	+	+	+	-	-	-	-	-	-	-	-	+	-	-
S. muscae	Colorless	+	+	+	-	-	-	-	+	-	-	-	+	-	-
S. pseudintermedius	Colorless	+	+	+	-	-	+	-	+	+	-	-	+	+	-

The isolated microorganisms were identified using the result using an online tool named "ABIS online".

Table 2. *Microbiological findings*

Organisms	N
Staphylococcus aureus	34
Staphylococcus hyicus	2
Staphylococcus intermedius	32
Staphylococcus Lugdunensis	2
Staphylococcus massiliensis	2
Staphylococcus muscae	1
Staphylococcus pseudintermedius	6
Unidentified	13

Primary screening for MRSA (Methicillin-resistant *Staphylococcus aureus*) found three isolates resistant to cefoxitin (Figure 1). None of the isolates were resistant to Vancomycin, Linezolid, Chloramphenicol, Erythromycin, and Trimethoprim/sulfamethoxazole. All of the isolates (100%) were sensitive against Trimethoprim/sulfamethoxazole and Linezolid. Half of the *S. aureus* isolates were resistant to Cefepime (50%) and Ciprofloxacin (47%).

All MRSA isolates were resistant to â-lactams and fluoroquinolones, with an overall prevalence of 3.53% (3/85). MRSA isolates were 100% resistant to only cefoxitin and cefepime. However, these isolates were 100% sensitive to Trimethoprim/sulfamethoxazole, Chloramphenicol, Vancomycin, and Linezolid.

All *S. aureus* isolates were confirmed using a positive PCR for the *fem*A gene (Figure 2a). All three *S. aureus* isolates, which phenotypically showed methicillin resistance, showed positive amplification for 533 bp fragments specific for the *mec*A gene (Figure 2b).

All 34 *S. aureus* were tested for the presence of the *spa* gene in PCR. Based on the size of the amplified product of the *spa* gene, three different types were found among all the isolates (Figure 3).

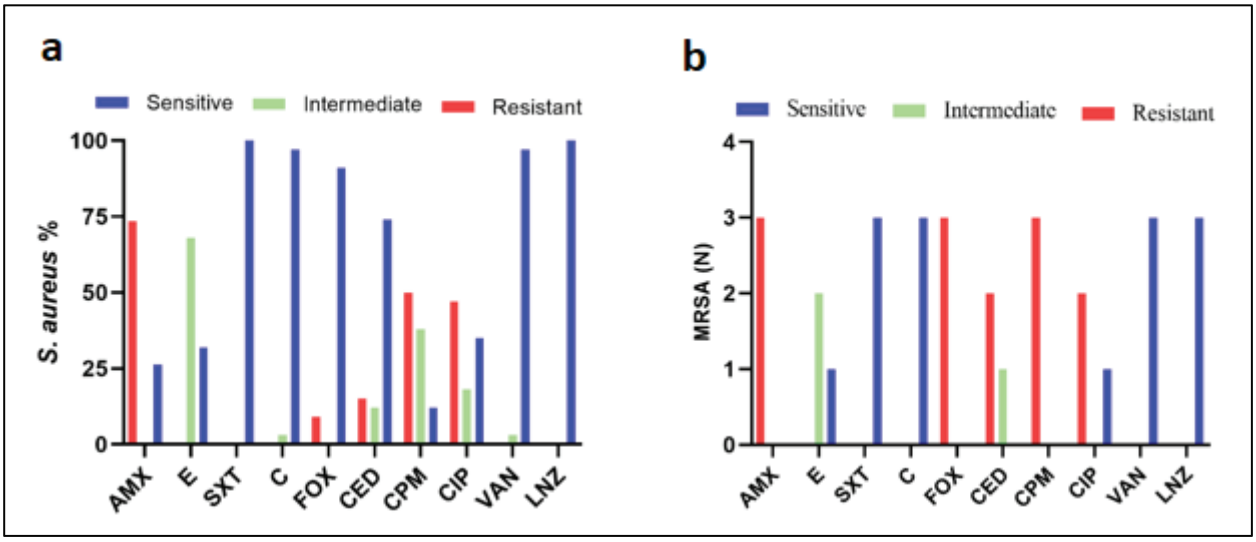

Fig. 1. *Antibiotic susceptibility pattern of (a) S. aureus and (b) methicillin-resistant S. aureus (MRSA) isolates. Abbreviation: AMX, amoxicillin; E, erythromycin; SXT, Trimethoprim /sulfamethoxazole; C, Chloramphenicol; FOX, Cefoxitin; CED, Cefradine; CPM, Cefepime; CIP, Ciprofloxacin; VAN, Vancomycin; and LNZ, Linezolid.*

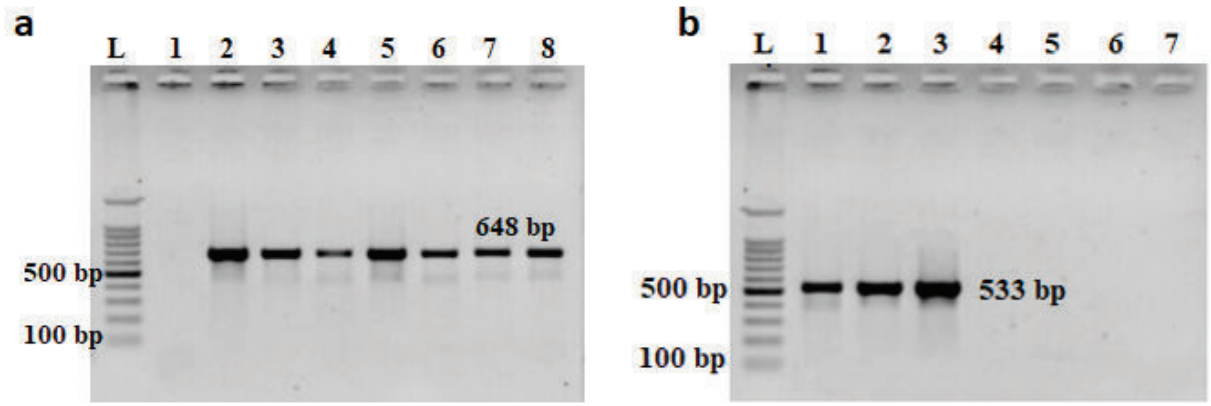

Fig. 2. *Polymerase Chain Reaction result for femA and mecA gene. a) Lane L, 100 bp DNA Ladder; Lanes 1, negative control; Lane 2-7, the PCR product of femA gene (648 bp); Lane 8, positive control. b) Lane L, 100 bp DNA Ladder; Lanes 1, positive control; Lane 2 and 3, the PCR product of mecA gene (533 bp); Lane-7, negative control.*

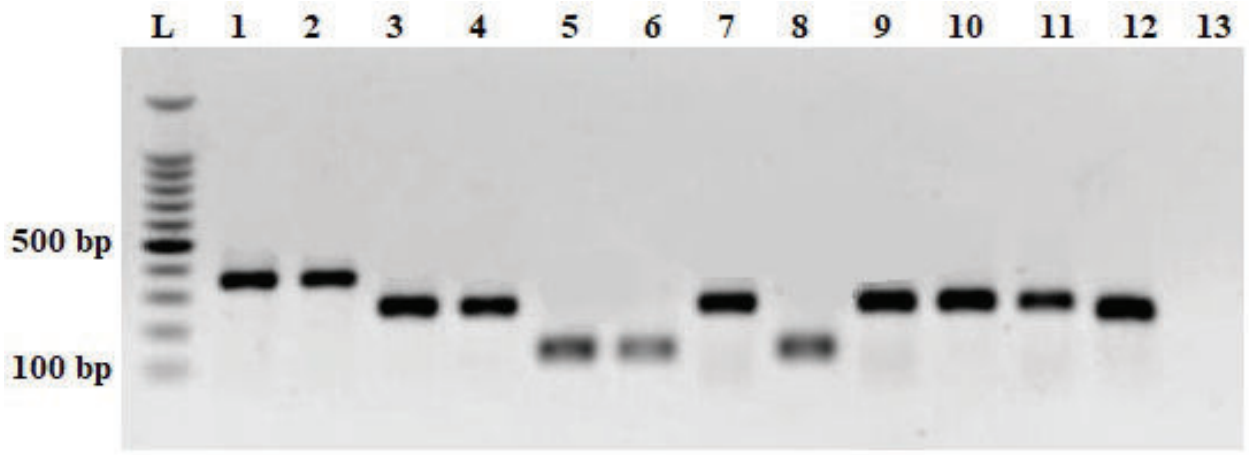

Fig. 3. *Polymerase Chain Reaction result for spa gene. Lane L, 100 bp DNA Ladder; Lanes 1-11, the variable PCR products of spa gene; Lane 12, positive control; Lane 13, negative control*.

As a result, *spa* PCR products of three different *S. aureus* isolates (one from each product size) were sent for sequencing along with the positive control. Sequence analysis revealed that these isolates all belonged to *spa* type t304.

Discussion

Most of the literature and studies focus on the prevalence and carriage of MRSA among hospital patients worldwide. Although there is a paucity of information about MRSA prevalence among HCWs, there are few recent reports on MRSA carriages among healthcare workers (HCWs) thus facilitating the spreading of nosocomial infections in many countries [13,27]. However, to our knowledge there are no reports on MRSA prevalence among HCWs in Bangladesh. This study aimed to determine the prevalence rate of MRSA in HCWs, particularly those working in surgery units and critical care units in tertiary hospitals in Bangladesh. HCWs have a great possibility of causing a nosocomial outbreak.

This study reports 40% nasal carriage of *S. aureus* among HCWs. Most importantly, this study provides information on the prevalence of MRSA among HCWs and their antibiotic susceptibility patterns. According to this study MRSA prevalence was 3.53% among HCWs. A similar MRSA prevalence (3.7%) was observed among HCWs in a tertiary referral hospital in Dublin, Ireland [28]. Another study in India also reported a 2.5% prevalence of MRSA in HCWs of critical care units [13]. This prevalence indicates a possibility of cross-transfer between personnel and patients as they could contribute to causing infection as a reservoir. A higher carriage of MRSA (13%) was reported by Buenaventura-Alcazaren *et al*. in HCWs of a tertiary hospital in the Philippines [4]and other studies conducted among patients in some studies, 17.2%, and 24.7% [2,3]. The current study also reports no VRSA or VISA prevalence among HCWs.

All of our *S. aureus* isolates had *spa* type t304, irrespective of being MRSA or MSSA. In recent years clinical MRSA isolates of *spa* type t304 have emerged in many European countries. *Bartels et al.*, also found *spa* type t304 most prominent in Northern Europe [29]. Another study reported t304 as the second most prominent *spa* type and was found to be associated with the colonization site[30]. Spa type t304 is believed to be the community-associated methicillin-resistant *S. aureus* (CA-MRSA)[29,31].

Upon our detection of MRSA prevalence among HCWs, we also detected the antibiotic resistance profile of these MRSA isolates. These MRSA isolates were resistant to two classes of antibiotics (â-lactams and fluoroquinolones). Similar multi-drug resistance properties of MRSA were also reported by Mojaheri *et al*., (80.5% MRSA were MDR)[32], and Tiwari *et al*., (72.1% were MDR)[33]. However, these isolates showed 100% sensitivity towards Trimethoprim/sulfamethoxazole, Chloramphenicol, Vancomycin, and Linezolid. HA-MRSA commonly exhibits multidrug resistance, whereas CA-MRSA is mainly susceptible to antibiotics[34].

Personnel working in a long-term care facility could be transient or persistent MRSA carriers. Baldwin *et al*., demonstrated that HCWs residing in nursing homes had higher chances (OR = 1.91, 95% CI = 1.21-2.03) of MRSA carriage than those residing in individual houses[14]. A similar observation was also reported by Cesur and Çokça who reported a 2.3-fold likelihood of MRSA carriage among HCWs compared with outpatients [15]. Therefore, infection control strategies should be taken seriously in intensive care and surgery units of hospitals.

An increasing reservoir of MRSA strains among HCWs working in critical care units/surgery units might lead to bursts of outbreaks. The personnel should be routinely screened for identifying MRSA. The CDC has recommended culturing personnel based on epidemiological data to identify potential reservoirs. Routine screening is needed to detect MRSA colonization or infection, but the high cost hinders it. Several strategies are being implemented to screen for colonized patients and decolonize them in hospital settings[35–37]. These strategies should be taken for regular decolonization of *S. aureus* in HCWs.

Conclusion

There was the colonization of MRSA in the anterior nasal cavity of HCWs in critical care units of hospitals. Carriage of *S. aureus* was 40% among HCWs in tested units. However, MRSA prevalence was 3.53%. A higher prevalence of MRSA among HCWs might facilitate the mass spread of nosocomial infections among admitted patients. The finding of this study could be used as a reference to screen for the carriage of MRSA in non-outbreak settings, which could lead to an outbreak of nosocomial infections. However, this study was only a survey on the prevalence of MRSA among HCWs. Integrated surveillance for MDR MRSA carriage among HCWs is warranted to control bursts of nosocomial infections effectively.

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Data Availability Statement: Data available on reasonable request from the authors.

Conflict of Interest: The authors declare that there is no conflict of interest.

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