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ORIGINAL ARTICLE

Increased incidence of methicillin resistant *Staphylococcus aureus* and methicillin resistant *Staphylococcus epidermidis* in the skin and nasal carriage among healthcare workers and inanimate hospital surfaces after the COVID-19 pandemic

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ABSTRACT

Background and Objectives: Healthcare workers in hospitals are exposed to infectious diseases that occur in the hospital making them a source of infection for the patients. It is interfaced as cross-contamination agents for MRSA and MR-CoNS, and preventive measures need to be adapted accordingly. The study aimed to assess Methicillin-Resistant Staphylococcus (MRS) on the skin and nasal cavities of healthcare workers (HCWs) and identifying isolates to the species level.

Materials and Methods: Swab samples were cultured on mannitol salt agar (MSA) to obtain MRS and determine their ability to produce coagulase. Their susceptibility to antibiotics were determined by agar screening and disk diffusion methods and further identification was done at the species level.

Results: The highest percentage of methicillin resistant coagulase positive Staphylococci (MRCoPS) was reported among skins of male HCWs, (71.4%) were identified as MRSA. The highest levels of methicillin resistant coagulase negative Staphylococci (MRCoNS) were mainly detected in both nasal cavities, (75%) were identified as MRSE. MRSA was reported from

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doctors (p-value 0.033), whereas the highest incidence of MRSE was obtained from the nurses (p-value 0.048).
Conclusion: This study highlighted that incidence of MRSA was mainly detected in doctors and MRCoNS in both nasal cavities. The highest percentage of MRCoNS was recovered from the patients' room followed by the reception table. Moreover, vancomycin is suggested to be highly effective in managing and controlling *S. aureus*, MRSA- and MRSE related infections.

Keywords: Healthcare workers; Methicillin resistant staphylococci; Methicillin-resistant *Staphylococcus aureus*; Methicillin-resistant coagulase-negative Staphylococci; *Staphylococcus aureus*; COVID 19

INTRODUCTION

Healthcare workers (HCWs) in hospitals are often exposed to infectious diseases occurring in hospitals making them a source of infection for patients. Healthcare-associated infections (HAIs) have been widely studied for the last few decades (1-3). Bacteria often spread from contaminated surfaces to the hands of HCWs, patients, visitors or other environmental surfaces. The World Health Organization (WHO) estimated that 10 out of every 100 hospitalized patients in developing countries and 7 out of every 100 hospitalized patients in developed countries will acquire at least one HAI (4).

The typical microbiota of skin and mucous membranes of the upper respiratory tract consists of many species of *Staphylococcus.* Coagulase-positive Staphylococci (CoPS) especially *S. aureus* is the major pathogen responsible for a wide range of clinical infections in humans and is a leading cause of bacteremia, endocarditis, and infections associated with invasive medical devices. Coagulase-negative Staphylococci (CoNS), particularly *S. epidermidis*, have emerged as a common cause of nosocomial infections, particularly those involving indwelling devices (5-7).

Methicillin-resistant *S. aureus* (MRSA) strains, first identified in 1961, were sporadically occurring and were resistant only to antibiotics of β-lactam (8); however, in a few years, they spread in hospitals worldwide (9, 10). Besides, Methicillin-resistant CoNS (MR-CoNS) infections have also been reported, posing a serious threat to hospitals; in addition to their potentiality to form biofilms (11). Several studies have reported a high prevalence of nasal colonization by MRSA and MR-CoNS among community members, hospitalized patients, and HCWs (12-14). Furthermore, the COVID 19 pandemic has caused changes in healthcare, impacting patient care and the safety of healthcare workers. One important outcome is the impact on MRSA infections among healthcare workers and in hospital environments. As hospitals and healthcare facilities allocated a number of resources to address the rise in COVID 19 cases, changes were made to infection control practices. There was an increased use of personal protective equipment (15). While these measures were necessary to reduce the spread of the coronavirus, they may have unintentionally affected how MRSA is transmitted. The increased focus on hygiene protocols regarding surface disinfection could have altered the balance, on hospital surfaces and potentially affected the survival and transmission of MRSA (16).

The aim of the present study is to investigate the incidence of MRSA and MR-CoNS specifically MRSE on the skin and nasal cavities among HCWs and on inanimate hospital surfaces, identify recovered species of MR-CoPs and MR-CoNS adopting phonetic characterization (API system) and confirm results by molecular identification (PCR). Also, in response to the heightened and occasionally inappropriate use of antibiotics during the COVID-19 pandemic, we determined antibiotic susceptibility patterns for eleven types of antibiotics viz. Penicillin, Ceftriaxone, Amoxicillin, Cefotaxime, Gentamycin, Levofloxacin, Ciprofloxacin, Vancomycin, Ceftazidime, Tetracycline, and Erythromycin.

MATERIALS AND METHODS

Sample collection. During a period of two months, starting from September 2021 to the middle of November 2021, one hundred and seventy swab samples were obtained using sterile swabs from HCWs and inanimate hospital surfaces in different wards in Jordan University Hospital. These included 61 samples from nasal cavities (N) and 63 samples from the skin of hand (S) in addition to swabbing of forty-six samples from various environmental sources like elevator (buttons and walls), reception table, medical devices, and patients' room (Walls, bedding doorknobs, curtains etc.) (E). All the workers were divided into two groups based on demographic data of health workers reported through interviewing in addition to the last

time they had taken antibiotics, and these were considered as criteria of exclusion and inclusion in the study. This also included age, gender, occupation, ward type, duration of work, and education level as shown in Table 2.

Culturing of sample and diagnostic. Swabs were put in AMIE's transport media and carried out to the laboratory within one hour (hr). then they were inoculated into brain heart infusion broth (Oxoid) and incubated at 35°C for 24 hrs. Then they were sub-cultured onto Mannitol Salt Agar (MSA) (Oxoid) and incubated at 35°C for 24 hrs. Grown colonies were purified onto nutrient agar plates; cells were examined for Gram staining, production of catalase and coagulase (using rabbit plasma Remel, REF 21060) according to Baron et al. (2013) (17). Susceptibility to methicillin was detected by two methods. First, using agar screening method by culturing isolates onto mannitol-salt-agar supplemented with 6 µg/ml oxacillin (methicillin). Second, by antimicrobial susceptibility test of 175 staphylococcal isolates from 170 samples using the Kirby-Bauer disk diffusion method as per Clinical Laboratory Standard Institute guidelines (18). We took 61 samples from nasal cavities but the number of isolates from nasal cavities was 67. However, 63 samples from skin hand were taken and the number of isolates was 68. Whereas 46 samples from various environmental sources were taken but the number of isolates was 40. As a result, the number of samples was 170 but the account of isolates was 175. This information is now updated in the revised manuscript. The Isolates were tested against eleven antibiotics: Penicillin G - P (10 µg), Ceftriaxone - CRO (30 µg), Amoxicillin – AMC (30 µg), Cefotaxime –CTX (30 µg), Gentamycin –CN (10 µg), Levofloxacin – LEV (5 µg), Ciprofloxacin – CIP (5µg), Vancomycin – VA (30µg), Ceftazidime – CAZ (30 µg), Tetracycline – TE (30 µg), and Erythromycin – E (15 µg). Antibiotics were applied using a dispenser device (Oxoid) to place antibiotics at equal distances from each other on the inoculated plate. Then plates were incubated for 18-20 hrs. at 35°C, zones of inhibition were measured in millimeter using a ruler. Isolates were categorized as sensitive, intermediate, or resistant (intermediate grouped with sensitive) based on standard interpretation tables (18, 19). Identification of methicillin-resistant staphylococci to the species level was detected firstly using the API system (RapID STAPH PLUS System; STAF SYSTEM 18R) and secondly through PCR.

DNA extraction PCR conditions. Genomic DNA of MR-CoPS and MR-CoNS strains were extracted using the i-genomic BYF DNA Extraction Mini Kit, which was further used to identify MR-CoPS clones that harbored the *Coa* gene and to identify MR-CoNS clones that harbored the *SesC* gene. All the primers used are listed in Table 1. MR-CoPS and MR-CoNS isolates were sub-cultured onto nutrient agar and incubated at 37°C for 24 hrs. After that a single colony was picked up with a sterile pipette tip without touching the agar and mixed with 50 µl nuclease free water. For PCR template preparation, mixture was performed in a total volume of 25 µl, which contained 12.5 µl i-Taq master mix (iNtRON), 8.5 nuclease-free water, 1 µl DMSO and 1 µl of each forward and reverse primers; a total of 3 µl of DNA template were added to the mixture. The mixture was then amplified using a PCR cycler (XP Thermal Cycler/TC-XP-*) according to the protocol of the i-Taq master mix sheet with modifications as follows: initial denaturation step at 94°C for 2 min, followed by 35 cycles of each denaturation at 94°C for 20 s, then, annealing at 53°C for 15 s and extension at 72°C for 1 min, followed by a final extension step at 72°C for 3 mins. The presence of PCR products was determined by electrophoresis (PHERO-sub-0710-E) of 15 µl of products in 1.5% agarose gel with TBE buffer and 100 bp DNA ladder as a marker (Promega, Germany). API diagnostic tests and PCR identification of isolates were examined alongside the following reference strains: *S. epidermidis* (ATCC 51625), *S. aureus* (ATCC 29213), and MRSA (ATCC 1026).

Statistical analysis. Statistical Package for Social Sciences (SPSS) software version 27 was used to analyse data. Participants' characteristics were reported using mean (standard deviation (sd)) for continuous variables, and frequencies with percentages were used for categorical variables. One-way analysis of variance (ANOVA) was used to know if there was any significant difference at p-value equal to or less than 0.05 for each MRSA and MRSE in the nasal cavities and skin among healthcare workers as correlated to the demographic variables. A post-test (L.S.D) was conducted to find out the differences that were found by One-way ANOVA. Binary logistic regression analysis was used to identify factors associated with recovery of MRSA and MRSE from skin and

Table 1. Primer sequences used for PCR reaction.

Size	Target gene	Sequence of primers	Species
388 bp	*SesC*	F: 5-'GTTGATAACCGTCAACAAGG -3' R:5'-CATGTTGATCTTTTGAATCCC-3'	*S. epidermidis*
600-850 bp	*Coa*	F:5'ACCACAAGGTACTGAATCAACG3' R: 5'-TGCTTTCGATTGTTCGATGC-3'	*S. aureus*

Source: (Hasan et al., 2014; and Behshood et al., 2020).

nasal cavities. A two-sided $p < 0.05$ was considered statistically significant.

Ethical aqpproval and consent to participate. This study was approved by the research Ethics Committee at University of Jordan Hospital (REF: 285/2021). Informed consent was obtained from all subjects. All experiments were performed in accordance with relevant guidelines and regulations. All methods were carried out in accordance with relevant guidelines and regulations.

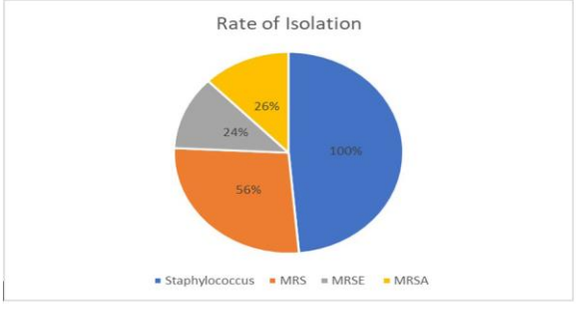

Fig. 1. The rate of *Staphylococcus* isolation, including Methicillin-Resistant *Staphylococcus* (MRS), Methicillin-Resistant *Staphylococcus aureus* (MRSA), and Methicillin-Resistant *Staphylococcus epidermidis* (MRSE).

RESULTS

Culture isolation. Out of one hundred and seventy samples taken from various sources, one hundred and seventy-five isolates of the genus *Staphylococcus* were recovered: 67 isolates from nasal cavities, 68 from the skin and 40 from the environment. Fig. 1 depicts the rate of *Staphylococcus* isolation, including Methicillin-Resistant *Staphylococcus* (MRS), Methicillin-Resistant *Staphylococcus aureus* (MRSA), and Methicillin-Resistant *Staphylococcus epidermidis* (MRSE).

Coagulase-positive Staphylococci (CoPS) and coagulase-negative staphylococci (CoNS) as related to demographic data. Table 2 demonstrates that the highest percentage of CoPS are reported from the nasal cavities of females (66.7%), followed by skins of males (62.5%). Regarding age distribution, for the age ranging from 28-35 years, there was 66.7% and 62.5% recovery of CoPS in nasal cavities and skins, respectively. Doctors and nurses harbored almost similar frequency of CoPS in both nasal cavities and skin ranging from (44.4%-50.0%). Whereas for CoNS, among females, the highest percentage recovery of 61.2% and 60.0% was reported from nasal cavities and skin respectively, however, for the age group of 28-35 years, the percentages was 40.8% and 45.0%, from nasal cavities and skin, respectively. Nurses harbored 55.0% of CoNS in both nasal cavities and skin. Frequency of CoNS from environmental sources exceeded CoPS by almost twenty times (95.0% Vs 5.0%).

Susceptibility to methicillin. Results of susceptibility to methicillin as detected by using oxacillin–mannitol salt agar screening methods was like as obtained by cefotaxime disk diffusion. Table 3 shows the frequency of MRCoPS and MRCoNS from various sources. Out of one hundred and seventy-five *Staphylococcus* isolates ninety-eight (56.4%) were identified as being methicillin resistant (MRS). MRCoNS constituted the highest percentage (n=73; 75.3%). The percentage recovery of both MRCoPS and MRCoNS from nasal cavities was almost twice of the percentage from the skin.

Table 4 displays the frequency of MRCoPS and MRCoNS as related to the demographics. The highest percentage of MRCoPS was reported from HCWs male skins (71.4%) followed by females nasal cavities (66.7%) whereas at the age range 28-35 the percentage of recovery from nasal cavities and skin was 66.7% and 71.4%, respectively. The nasal cavities of doctors

Table 2. Distribution and frequency of coagulase-positive Staphylococci and coagulase-negative Staphylococci as related to demographic data.

Variable	Nasal Cavities n= 67 (%)		Skin n= 68 (%)		p-value	
	CoPS n (%)	CoNS n (%)	CoPS n (%)	CoNS n (%)	Nasal Cavities	Skin
Gender:						
Male	6 (33.3%)	19 (38.8%)	5 (62.5%)	24 (40.0%)	0.689	0.233
Female	12 (66.7%)	30 (61.2%)	3 (37.5%)	36 (60.0%)		
Age (Years):						
18-27	6 (33.3%)	17 (34.7%)	3 (37.5%)	19 (31.7%)	0.101	0.509
28-35	12 (66.7%)	20 (40.8%)	5 (62.5%)	27 (45.0%)	0.212	0.285
36-50	-	11 (22.4%)	-	13 (21.7%)	0.094	0.558
Above 50	-	1 (2.0%)	-	1 (1.7%)	-	-
					-	-
Ward :						
Cardiac care unit	1 (1.2%)	5 (5.1%)	1 (1.3%)	5 (4.2%)		
Delivery room	3 (3.5%)	4 (4.1%)	2 (2.6%)	5 (4.2%)		
General surgery ward	3 (3.5%)	7 (7.1%)	2 (2.6%)	5 (4.2%)		
Internal medicine ward	5 (5.9%)	10 (10.2%)	1 (1.3%)	16 (13.3)	-	-
Maternity room	1 (1.2%)	-	-	1 (0.8%)		
Operation room	1 (1.2%)	4 (4.1%)	1 (1.3%)	3 (2.5%)		
Pediatric ward	-	6 (6.1%)	-	8 (6.7%)		
Pediatric ICU	1 (1.2%)	2 (2.0%)	-	3 (2.5%)		
Pediatric surgery ward	1 (1.2%)	4 (4.1%)	-	5 (4.2%)		
Recovery ward	-	1 (1.0%)	-	1 (0.8)		
Surgical ICU	2 (2.4%)	6 (6.1%)	1 (1.3%)	8 (6.7%)		
Type of occupation:						
Doctor	10 (55.6%)	15 (30.6%)	4 (50.0%)	22 (36.7%)	0.173	0.806
Nurse	8 (44.4%)	27 (55.1%)	4 (50.0%)	33 (55.0%)	0.032	0.330
Auxiliary nurse	-	-	-	-	-	-
Non-medical personnel	-	5 (10.2%)	-	4 (6.7%)	0.851	0.991
Pharmacist	-	2 (4.1%)	-	1 (1.7%)	-	-
Duration of work:						
Less than 1 year	1 (5.6%)	10 (20.4%)	1 (12.5%)	7 (11.7%)	0.315	0.928
1-3 years	7 (38.9%)	11 (22.4%)	2 (25.0%)	20 (33.3%)	0.868	0.931
4-8 years	6 (33.3%)	13 (26.5%)	3 (37.5%)	16 (26.7%)	0.175	0.800
Above 8 years	4 (22.2%)	15 (30.6%)	2 (25.0%)	17 (28.3%)	-	-
Level of education:						
University graduate	18 (100%)	44 (89%)	8 (100%)	56 (93.3%)	0.382	0.762
High school graduate	-	2 (4.1%)	-	2 (3.3%)	0.188	0.487
Secondary school	-	3 (6.1%)	-	2 (3.3%)	0.673	0.811
Last time antibiotic taken:						
None	9 (50.0%)	32 (65.3%)	6 (75.0%)	35 (58.3%)	0.804	0.692
Less than 1 week	2 (11.1%)	3 (6.1%)	1 (12.5%)	3 (5.0%)	-	-
1-2 weeks	1 (5.6%)	1(2.0%)	-	2 (3.3%)	-	-
1-6 months	5 (27.8%)	11 (22.4%)	1 (12.5%)	17 (28.3%)	0.400	0.234
1 year or above	1 (5.6%)	2 (4.1%)	-	3 (5.0%)	0.824	0.851

CoPS: coagulase-positive staphylococci, CoNS: coagulase-negative staphylococci

Table 3. Distribution frequency of methicillin-resistant coagulase-positive (MRCoPS) and methicillin-resistant coagulase-negative (MRCoNS) Staphylococci from various sources.

MRS (N= 98)	MRCoPS (N=25) n (%)	MRCoNS (N=73) n (%)
Nasal cavities	16 (16.4%)	31 (31 %)
Skin	7 (7.2%)	20 (20.7%)
Environment	2 (2.0%)	22 (22.7%)

revealed higher percentages of MRCoPS compared to nurses (60.0% Vs 40.0%). In contrast, the skin of nurses showed a higher percentage of MRCoPS (57.1%) than doctors (42.9%).

Working period of 4-8 years in hospital, reported the highest frequency of MRCoPS from both nasal cavities and skin 40.0% and 42.0%, respectively, compared to percentage of recovery from other working periods. MRCoNS were mainly detected in both nasal cavities (75.0%) and skin (67.7%) of nurses. Additionally, in both nasal cavities and skin of university graduate healthcare workers, the percentage of recovery was 95.0% and 93.5%, respectively. Whereas the percentage recovery was 70.0% and 64.5% from nasal cavities and skin, respectively among those not taking antibiotics.

Regarding environmental samples, only two MRCoPS isolates were detected, one from medical devices and the other from the rooms of patients. But, twenty-two MRCoNS were detected, where patients' rooms revealed the highest percentage of recovery (63.7%) followed by the reception table (22.7%).

Identification tests. The twenty-five isolates (23 from healthcare workers and 2 from the environment) of MRCoPS and seventy-three isolates of MRCoNS from various sources were identified at the species level by RapID STAPH PLUS System and STAF SYSTEM 18R and confirmed by PCR targeting *coa* gene-specific for *S. aureus* and *SesC* gene-specific for *S. epidermidis.*

PCR amplification for *S. aureus.* The size of *coa* gene-specific for *S. aureus* gives specificity to PCR products that ranged from approximately 600 to 850 bp compared to the DNA marker (Fig. 2). All MRCoPS isolates were characterized as *S. aureus* which means that MRSA shares 26% of ninety-eight isolates as shown in Table 5. The table displays the MRSA samples, each identified by a unique code, and their corresponding numbers have been recorded in the NCBI database.

PCR amplification for *S. epidermidis*. The size of *SesC* gene-specific for *S. epidermidis* gives specificity to PCR products that ranged approximately 388 to 400 bp compared to the DNA marker (Fig. 3). Twenty-four isolates of MRCoNS from various sources (twenty-three from healthcare workers and one from the environment) were characterized as *S. epidermidis* which means that MRSE makes 24% out of ninety-eight isolates as shown in Table 5. The table displays the MRSE samples, each identified by a unique code, and their corresponding numbers have been recorded in the NCBI database.

Incidence of MRSA and MRSE among HCWs. Out of one hundred and thirty-five isolates from HCWs, twenty-three isolates (25%) were identified as MRSA, and twenty-three isolates (24%) were identified as MRSE. To determine if there is any significant difference at p=0.05 for each MRSA and MRSE recovered from nasal cavities and skin among HCWs as correlated to the demographic variables, analysis of variance (ANOVA) test was used.

Table 6 illustrates the incidence of MRSA in nasal cavities among healthcare workers according to the demographic data. The analysis identified that type of occupation is associated with a higher risk of having MRSA, where the value "F" equals 4.729 which is significant, at a p-value (0.033).

Out of twenty-three MRSA, the highest incidence was obtained from the doctors. Further, a post-test (L.S.D) was conducted to find out differences as illustrated in Fig. 4.

Table 7 illustrates incidence of MRSE in skin among healthcare workers according to the demographic data. The analysis revealed that type of occupation and age are associated with a higher risk of having MRSE, where the value "F" equals 4.073 and 4.165, respectively which is significant, at a p-value 0.048 and 0.045, respectively.

Out of twenty-three MRSE, the highest incidence was obtained among the nurses, besides, the age ranging from 28-35 years is associated with a higher risk of having MRSE. A post-test (L.S.D) was conducted to find out differences as illustrated in Figs. 5 and 6.

However, no statistically significant difference was

Table 4. Distribution and percentage of methicillin resistance coagulase-positive (MRCoPS) and methicillin resistance coagulase-negative (MRCoNS) Staphylococci as related to demographic data.

Variable	Nasal Cavities n= 67		Skin n= 68		p-value	
	MRCoPS n (%)	MRCoNS n (%)	MRCoPS n (%)	MRCoNS n (%)	Nasal Cavities	Skin
Gender:						
Male	5 (33.3%)	9 (45.0%)	5 (71.4%)	12 (38.7%)	0.404	0.107
Female	11 (66.7%)	11 (55. %)	2 (28.8%)	19 (61.3%)		
Age (Years):						
18-27	5 (33.3%)	4 (20.0%)	2 (28.6%)	7 (22.6%)	0.184	0.051
28-35	11 (66.7%)	11 (55.0%)	5 (71.4%)	14 (45.2%)	0.274	0.042
36-50	-	5 (25.0%)	-	10 (32.3%)	0.161	0.151
Above 50	-	-	-	-	-	-
Ward:						
Cardiac care unit	-	1 (5.0%)	1 (14.3%)	2 (6.5%)		
Delivery room	3 (20.0%)	1 (5.0%)	2 (18.6%)	2 (6.5%)		
General surgery ward	2 (13.3%)	3 (15.0%)	1 (14.3%)	5 (16.1%)		
Internal medicine ward	6 (33.3%)	5 (25.0%)	1 (14.3%)	7 (22.6%)		
Maternity room	1 (6.7%)	-	-	-	0.258	0.427
Operation room	-	2 (10.0%)	1 (14.3%)	1 (3.2%)		
Pediatric ward	-	4 (20.0%)	-	4 (12.9%)		
Pediatric ICU	1 (6.7%)	1 (5.0%)	-	3 (9.7%)		
Pediatric surgery ward	1 (6.7%)	2 (10.0%)	-	3 (9.7%)		
Recovery	-	-	-	1 (3.2%)		
Surgical ICU	2 (13.3%)	1 (5.0%)	1 (14.3%)	3 (9.7%)		
Type of occupation:						
Doctor	10 (60.0%)	4 (20.0%)	3 (42.9%)	7 (22.6%)	0.048	0.081
Nurse	6 (40.0%)	15 (75.0%)	4 (57.1%)	21 (67.7%)	0.104	0.046
Auxiliary nurse	-	-	-	-	-	-
Non-medical personnel	-	1 (5.0%)	-	2 (6.5%)	0.847	0.241
Pharmacist	-	-	-	1 (3.2%)	-	-
Duration of work:						
Less than 1 year	1 (6.7%)	2 (10.0%)	1 (14.3%)	2 (6.5%)	0.192	0.636
1-3 years	6 (40.0%)	5 (25.0%)	1 (14.3%)	10 (32.3%)	0.717	0.268
4-8 years	7 (40.0%)	8 (40.0%)	3 (42.9%)	9 (29.0%)	0.101	0.795
Above 8 years	2 (13.3%)	5 (25.0%)	2 (28.6%)	10 (32.3%)	-	-
Level of education:						
University graduate	16 (100%)	19 (95.0%)	7 (100%)	29 (93.5%)	0.443	0.791
High school graduate	-	1 (5.0%)	-	1 (3.2%)	0.409	0.519
Secondary school	-	-	-	1 (3.2%)	0.331	0.824
Last time antibiotic taken:						
None	9 (53.3%)	14 (70.0%)	6 (85.7%)	20 64.5%)	0.516	0.789
Less than 1 week	2 (13.3%)	-	1 (14.3%)	1 (3.2%)	0.368	0.368
1-2 weeks	1 (6.7%)	-	-	1 (3.2%)	-	-
1-6 months	3 (20.0%)	5 (25.0%)	-	7 (22.6%)	0.073	0.556
1 year or above	1 (5.0%)	1 (5.0%)	-	2 (6.5%)	0.370	0.669

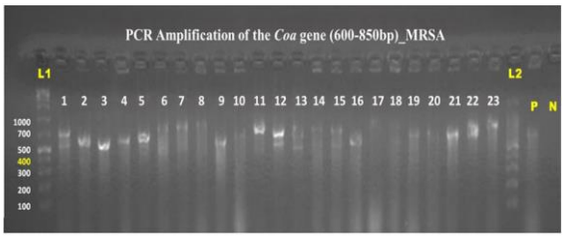

Fig. 2. PCR amplification of *coa* gene in *S. aureus*, (1-23) *S. aureus* isolates, (L1) Ladder 1000bp, (L2) Ladder 100bp, (P) Positive control (*S. aureus* ATCC 29213), (N) Negative control (Nuclease-free water)

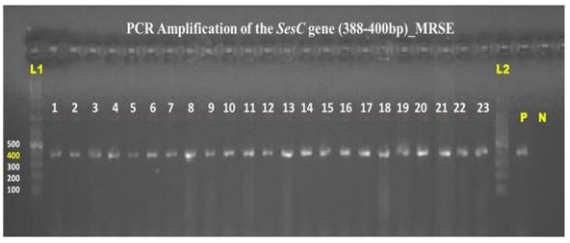

Fig. 3. PCR amplification of *SesC* gene in *S. epidermidis*, (1-23) *S. epidermidis* isolates, (L1) Ladder 1000bp, (L2) Ladder 100bp, (P) Positive control (*S. epidermidis* ATCC 51625), (N) Negative control (Nuclease-free water)

Table 5. Displays the numbers and codes of MRSA and MRSE samples which were recorded in the NCBI database that were recovered from various sources.

Number of samples	Approved code of MRSA	Approved code of MRSE
1.	IHN1A	IHN1B
2.	IHN2A	IHN2B
3.	IHN3A	IHN3B
4.	IHN4A	IHN4B
5.	IHN5A	IHN5B
6.	IHN6A	IHN6B
7.	IHN7A	IHN7B
8.	IHN8A	IHN8B
9.	IHN9A	IHN9B
10.	IHN10A	IHN10B
11.	IHN11A	IHN11B
12.	IHN12A	IHN12B
13.	IHN13A	IHN13B
14.	IHN14A	IHN14B
15.	IHN15A	IHS1B
16.	IHN16A	IHS2B
17.	IHS1A	IHS3B
18.	IHS2A	IHS4B
19.	IHS3A	IHS5B
20.	IHS4A	IHS6B
21.	IHS5A	IHS7B
22.	IHS6A	IHS8B
23.	IHS7A	IHS9B
24.	IHE1A	IHE1B
25.	IHE2A	-

observed in the risk of developing MRSA and MRSE among different environmental factors.

Antimicrobial susceptibility pattern of MRSA and MRSE isolates. The result of antimicrobial susceptibility of MRSA (N=25) and MRSE (N=24)

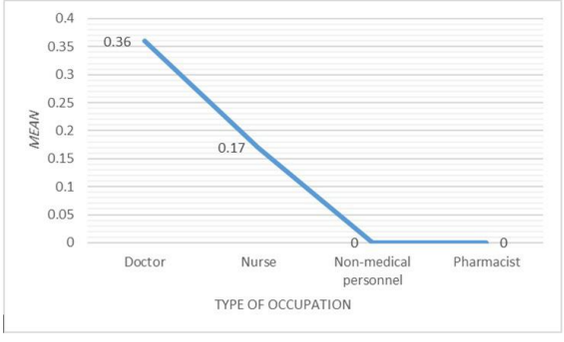

Fig. 4. Incidence of MRSA in nasal cavities among HCWs.

isolates from all sources against eleven different antibiotics as tested on MHA, is presented in Fig. 7. The highest resistance of both MRSA and MRSE was shown against penicillin (100%) and cefotaxime (100%), followed by levofloxacin (95.84%) for MRSA and ceftazidime (70.83%) for MRSE. In contrast MRSA and MRSE were susceptible to gentamycin (4.16% and 8.33%), respectively, and to tetracycline (4.16% and 8.33%), respectively. All the MRSA and MRSE isolates were susceptible to vancomycin.

DISCUSSION

MSA is a medium that selects organisms able to live in a high concentration of salt (sodium chloride). Therefore, in our study, the isolates were sub-cultured on MSA for selective isolation of *Staphylococcus* from clinical and other samples. Also, MSA can differentiate those able of fermenting mannitol as demonstrated by changing the red appearance of MSA to a yellow, pH indicator (phenol red) and therefore, makes it possible to guide the diagnosis of the two main subgroups of Staphylococci man-

Table 6. Incidence of MRSA in nasal cavities among HCWs according to the demographic data.

Variables		Sum of Squares	DF	Mean Square	F	p-value
Gender	Between Groups	0.031	1	0.031	0.127	0.722
	Within Groups	15.641	65	0.241		
	Total	15.672	66			
Age	Between Groups	0.655	1	0.655	1.187	0.280
	Within Groups	35.853	65	0.552		
	Total	36.507	66			
Ward	Between Groups	1.437	1	1.437	0.134	0.716
	Within Groups	699.041	65	10.754		
	Total	700.478	66			
Duration of work	Between Groups	0.145	1	0.145	0.127	0.723
	Within Groups	74.273	65	1.143		
	Total	74.418	66			
Level of education	Between Groups	0.276	1	0.276	1.403	0.241
	Within Groups	12.769	65	0.196		
	Total	13.045	66			
Types of occupations	Between Groups	4.191	1	4.191	4.729	0.033*
	Within Groups	57.600	65	0.886		
	Total	61.791	66			

(*p-value ≤ 0.05)

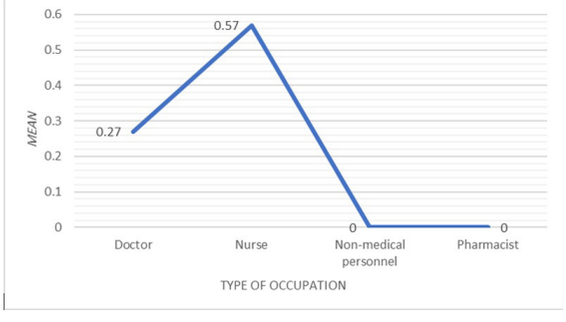

Fig. 5. Incidence of MRSE in the skin among healthcare workers.

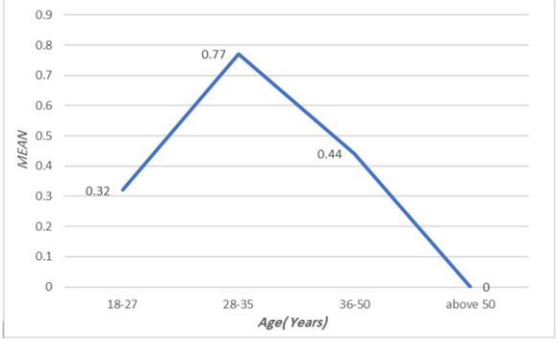

Fig. 6. Incidence of MRSE in the skin among healthcare workers according to age group.

nitol fermenting and mannitol non-fermenting (MF and MNF) (ASM-Microbes 2022). In addition, the detection of catalase enzymes is essential to ensure whether Gram-positive cocci belong to catalase-positive or catalase-negative Streptococci (20). In many instances, more than one type of colonies was found in the same sample, i.e., MF & MNF on the same MSA plate therefore, the number of Staphylococci exceeded the number of samples.

Coagulase enzyme is considered as the most important and reliable criteria for the identification of pathogenic *Staphylococcus* (21). All catalase-positive colonies were examined for their ability to produce coagulase by tube coagulase test (22). Production of coagulase enzyme is a confirmatory test for differentiation between Coagulase-positive Staphylococci (CoPS) and Coagulase-negative Staphylococci (CoNS) isolates.

The selective MSA with 6 µg/ml oxacillin was used to isolate MRS from clinical samples as an initial step of the diagnostic process which inhibits commensal microbial that mask MRS colonies. Even if MRS are present in lower number, the use of MSA with 6 µg/ml of oxacillin improves their recovery (23). Out of one hundred and seventy-five *Staphylococcus* isolates ninety-eight (54.4%) were identified

Table 7. Incidence of MRSE in skin among healthcare workers according to the demographic data

Variables		Sum of Squares	DF	Mean Square	F	p-value
Gender	Between Groups	0.088	1	0.088	0.352	0.555
	Within Groups	16.544	66	.251		
	Total	16.632	67			
Age	Between Groups	2.272	1	2.272	4.165	0.045*
	Within Groups	36.007	66	0.546		
	Total	38.279	67			
Ward	Between Groups	18.401	1	18.401	1.884	0.175
	Within Groups	644.717	66	9.768		
	Total	663.118	67			
Duration of work	Between Groups	1.288	1	1.288	1.281	0.262
	Within Groups	66.403	66	1.006		
	Total	67.691	67			
Level of education	Between Groups	0.004	1	0.004	0.029	0.865
	Within Groups	9.466	66	.143		
	Total	9.471	67			
Types of occupations	Between Groups	2.772	1	2.772	4.073	0.048*
	Within Groups	44.919	66	0.681		
	Total	47.691	67			

(*p-value ≤ 0.05)

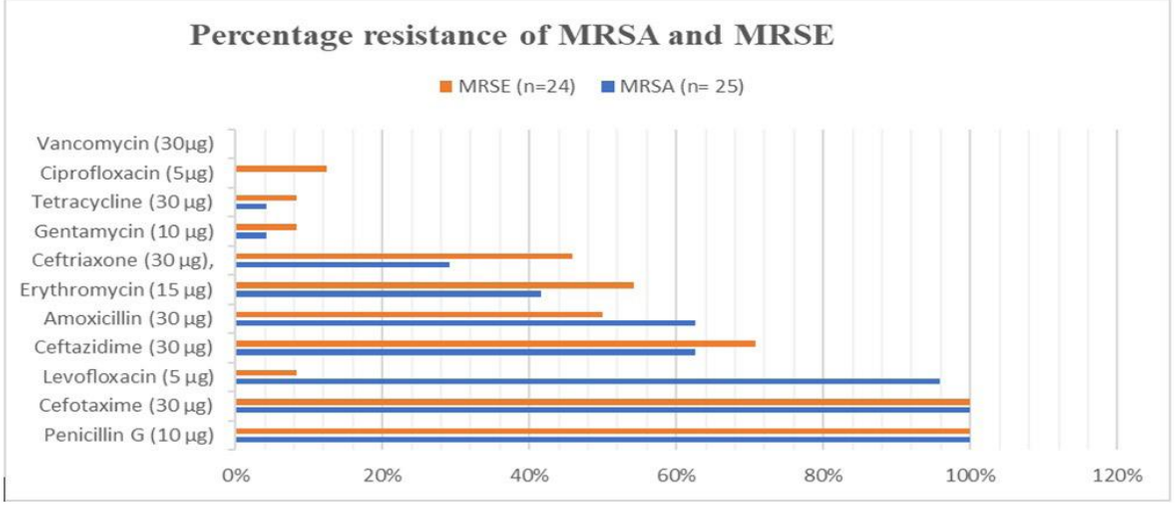

Fig. 7. Percentage resistance of MRSA and MRSE against eleven antibiotics.

as being MRS. Surprisingly, MRCoNS constituted the highest percentage 75.3%. CoNS-related infections are difficult to treat because they have a higher chance to be resistant to methicillin or that they are less susceptible to glycopeptide. The epidemiology of CoNS in healthcare settings is substantially less studied than it is for MRCoPS (24).

Frequency of MRCoPS and MRCoNS was related to demographics. Out of 23 isolates of CoPS from HCW, 92.3% of isolates were MRCoPS and higher percentages were represented in doctors. In contrast, nurses showed incidence of higher percentage of MRCoNS in both nasal cavities (75%) and skin (67.7%) compared to doctors. The reason for these results could be the lengthier period that both doctors and nurses spend in the hospital and, the frequent ex-

posure to patients. Regarding recovery of MRCoNS, our findings are comparable with other reports. Al-Tamimi et al., (2020) (14) reported that nasal MR-CoNS accounted for 73.3% of total isolates, whereas Kumar et al., (2011) (25) investigated nasal screening of HCW, and reported that 45% of CoPS were resistant to methicillin and 55% CoPS isolates were methicillin-susceptible.

In our study, from environment isolates, twenty-two MRCoNS were detected, patients' rooms revealed the highest percentage of recovery (63.7%) followed by the reception table (22.7%). The reason for highest recovery of MRCoNS in patient rooms could be overcrowding as each room accommodated at least three patients. Additionally, when they leave the hospital, other patients go to the same rooms without proper sterilization. Moreover, the focus on caring for COVID 19 patients has occasionally disrupted the cleaning and disinfection practices, which in turn increases the risk. Interestingly, while we implement measures to prevent the spread of infections through surface contact precautions, they can unintentionally create an environment for MRCoNS highlighting the intricate balance needed for effective infection control, in healthcare settings during such crises.

Isolates of MRCoPS were confirmed as being *S. aureus* by targeting *coa* gene using PCR. All the 25 isolates of MRCoPS from various sources were characterized as *S. aureus* which MRSA makes 26% out of ninety-eight isolates. The *coa* gene was present in all *S. aureus* isolates. Therefore, the *coa* gene can be used as a genetic marker to distinguish *S. aureus* from other isolates. This observation agrees with report from Effendi et al., (2019) (26), stating that the *coa* gene is a readily used epidemiological tool for detecting *S. aureus*. They found that out of 160 samples, by using a coagulase test 20 (12.5%) isolates were confirmed as *S. aureus* and 19 (95%) isolates carried *coa* gene. Javid et al., (2018) (27) screened 192 isolates to identify *S. aureus* by targeting *nuc* gene and *coa* gene. They found that 39 (20.31%) isolates of *S. aureus* were confirmed by targeting *nuc* gene. Out of these 39 *S. aureus* isolates, 25 (64.10%) isolates carried *coa* gene.

Furthermore, we found that twenty-four isolates of MRCoNS were confirmed as *S. epidermidis* by targeting *SesC* gene using PCR. MRSE makes 24% out of ninety-eight isolates. All twenty-four isolates of MRCoNS tested had the *SesC* gene. As a result, the *SesC* gene can be utilized as a genetic marker to differentiate *S. epidermidis* from other isolates. Similarly, Behshood et al., (2020) (28) stated that *S. epidermidis* can easily be identified by *SesC* gene since all *S. epidermidis* isolates contained *SesC*.

From HCWs, twenty-three isolates (25%) were identified as MRSA, and twenty-three isolates (24%) were identified as MRSE. This study found that type of occupation and age are associated with a higher risk of having MRSA and MRSE. The highest incidence of MRSA was obtained from the doctors at a p-value (0.033), while being aged 28-35 years is associated with a higher risk of having MRSE at a p-value (0.045). Moreover, HCW who were at the paediatric ward were more likely to have MRSE. This finding agrees with the reports from Giri et al., (2021) (29). The overall percentage of nasal carriage MRSA among healthcare workers was 5.2% (12/232). The percentage of MRSA in males (8.7%), was higher than in females (4.3%). The highest recovery of MRSA was found to be at its peak among doctors (11.4%), and also healthcare workers in the postoperative ward were colonized by the highest percentage of MRSA (18.2%). Desta et al. (2022) (30) reported the percentage recovery of MRSA as 4.8% (28/580) among HCWs compared to 0.2 % (1/468) of administrative staff. Nevertheless, the present study reported higher percentages of incidence of MRSA and MRSE among HCWs. This increased incidence could be attributed to the overwhelming workload, as HCWs have faced an unprecedented surge in patient loads during the pandemic, particularly in hospitals that received high COVID-19 cases. This has led to exhaustion and lapses in infection control practices. The incidence of MRSA in doctors was the highest, this may be due to the fact that the nature of the doctors' work is different from other HCWs, as they do not remain in one ward, but rather move between wards in the hospital and from one patient to another, compared to nurses who remain in one ward and do not move to other wards within the hospital and every nurse is responsible for a certain number of patients, not like doctors who visit a larger number of patients, especially during COVID-19 where the focus on COVID-19 patient care sometimes led to reduced attention to spread MRSA than coronavirus. Also, the incidence of MRSE in nurses was the highest, which could be due to the fact that nurses are the most frequent healthcare workers in contact with

patients directly, and may be acquired as they collect sample, especially if they do not comply to safety precautions during handling samples.

Age between 28-35 years was associated with a higher risk of MRSE, and this age group is active and may have greater interaction with patients and colleagues, which may expose them to further colonization of microorganisms. The prevalence of *Staphylococcus epidermidis* that is methicillin resistant varies significantly by region as reported by Haque et al., (2011) (31). Despite being an endogenous human skin flora, it is extremely contagious both in a medical setting and in a community. Antibiotic-resistant *S. epidermidis* strains may be found on the skin of patients and healthcare professionals, on medical equipment, on personnel clothes, and on environmental surfaces as reported by Haque et al., (2011) (31). It is expected that, this opportunistic pathogen, particularly in immunocompromised people, causes various infections connected to implanted material. Therefore, the key to a successful outcome is an early and accurate diagnosis. Hence, Staphylococcus epidermidis identified in culture should not always be dismissed as a contaminant and should instead be treated appropriately along following the recommended preventative measures (31).

All isolates of MRSA and MRSE showed resistance to penicillin which is higher than that reported from Efa et al., (2019) (32) and Desta et al. (2022) (30) as they reported 51.0% and 79.0% resistance to penicillin, respectively. Resistance to cefoxitime was also 100% for both MRSA and MRSE which was higher than that reported for MRSA by Kashif Salman et al. (2018) (33). Resistance to levofloxacin was 95.8% for MRSA isolates and it was higher compared to the data reported for MRSA by Zhanel et al. (2019) (34). Whereas resistance to ceftazidime was 70.83% for MRSE similar to that reported by Mun et al. (2019) (35). But both MRSA and MRSE showed lower resistance against gentamycin (4.16% and 8.33%) and tetracycline (4.16% and 8.33%), respectively. In contrast to our findings, El Aila et al., (2017) (36) reported lower resistance of MRSA towards erythromycin, tetracycline, gentamycin, clindamycin, and ciprofloxacin as 19.6%, 9.8%, 3.9%, 3.92%, and 3.92%, respectively. Also, in a study by Chauhan et al., (2021) (37), the antibiogram of MRSA isolates showed resistance to amoxiclav (100%), erythromycin (45%), and gentamycin (40%), whereas all the MRSA isolates were sensitive to linezolid and vancomycin. By comparing the results of this study with previous work discussing the resistance of MRSA and MRSE to antibiotics, it becomes evident that incidence of resistance has increased (38). This increase in resistance could be attributed to the greater use of antibiotics during the COVID-19 pandemic, often inappropriately employed to treat suspected bacterial co-infections.

Since sampling approach depends on responses of HCWs, the generalization of the study's results is limited. Future studies are needed to target more healthcare workers in different hospitals. In addition, several logistic hurdles were experienced, mainly due to the privacy of some departments in the hospital.

CONCLUSION

This study indicated that the incidence of MRSA was mainly detected in doctors and MRCoNS in both nasal cavities and skin of nurses. The highest percentage of recovery of *Staphylococcus* was among healthcare workers in both nasal cavities and skin. Regarding the environment, patients' rooms revealed the highest percentage of MRCoNS recovery followed by the reception table. Vancomycin is still useful and effective for managing and controlling S. aureus, MRSA- and MRSE related infections. Although nasal and skin carriage of MRSA and MRSE are harmless in healthy HCWs, they can pose the risk of spreading infections to the hospital environment and subsequently transmitting to hospital patients and to the community. Therefore, it is recommended to employ proper strategies to prevent spread of these infections.

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