RESEARCH PAPER

Carriage rate of *Staphylococcus aureus* and its susceptibility towards mupirocin among healthcare workers at a tertiary care hospital in North India

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ABSTRACT

Introduction : Methicillin resistance in staphylococcal isolates, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase-negative staphylococcus (MRCoNS), have become increasingly prevalent and constitute a serious threat to public health globally. In hospital settings, methicillin resistant Staphylococcal colonization among healthcare workers (HCWs) is a major concern as they may transmit bacteria to patients as a result of inadequate infection control practices. Thus, surveillance of HCWs' nasal colonization with MRSA and/or MRCoNS and mupirocin nasal decolonization have been used to prevent Staphylococcal infections and reduce transmission in healthcare settings. However, mupirocin-resistant Staphylococci have emerged as a result of prolonged and extensive usage of this antibiotic.

Result: Nasal swabs from 400 HCWs, including 224 males (56%) and 176 females (44%) were included in the study. A total of 265 (66.3%) samples yielded Staphylococcal isolates, comprising of 37 (14%) *Staphylococcus aureus* and 228 (86%) coagulase negative Staphylococci (CoNS). Methicillin resistance was identified in 11% (29/265) *S. aureus* and most of the CoNS isolates (46.4%, 123/265). Among the MRSA nasal carriers, there were two doctors, six nurses, and other HCWs. Twenty-two of them were persistent nasal carrier whereas seven were transient carriers. Four MRSA isolates exhibited low mupirocin resistance, while three MRCoNS showed high-level mupirocin resistance. Antibiogram of the MRSA isolates revealed higher resistance in penicillin (86.5%), followed by levofloxacin (78.4%) and erythromycin (37.6%).

Conclusion: In our study, 10.9% of the healthcare workers were identified as MRSA nasal carriers. We also differentiated short term MRSA carriers (transient carriers) and chronic carriers of MRSA (persistent carriers) by taking repeated swabs after five days. Treatment was given to the persistent carriers of MRSA i.e., intranasal 2% mupirocin ointment twice daily for five days, which is according to Infectious Diseases Society of America guidelines. Therefore, there is an urgent need to give priority for the safety of HCWs so that they do not become a risk for patients rather become a protector for them as they remain in contact with them for longer time. Regular screening of MRSA among HCWs should be done by following standard procedures.

Keywords: Staphylococcus aureus, MRSA, Mupirocin resistance, Healthcare workers, Nasal carriage.
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INTRODUCTION

Staphylococcus species are gram-positive, occasionally capsulated, spherical cocci arranged in clusters and have a diameter of around one micrometer.[1] Based on coagulase enzyme production, they have been grouped into coagulase positive (*Staphylococcus aureus*) and coagulase negative Staphylococci (CoNS).[2] The coagulase-negative Staphylococci is classified on the basis of standard bacteriological procedures to distinguish between *S. aureus* which are pathogenic and other class of Staphylococci which are non-pathogenic.[3] Staphylococci is the second-most common cause of nosocomial bloodstream infections and a pathogen affecting humans. *Staphylococcus* bacterium has a potential to cause infections, ranging from moderate to severe skin infections to critical conditions like osteomyelitis, pneumonia, endocarditis etc.[4] The anterior nares are the main site for *S. aureus* carriage as there is relative absence of human defences in this area, the staphylococcal cells flourish here and are capable of withstanding the local antibacterial defences.[4,5] This can be either transient (lasting hours or days) or persistent. This carrier status acts as a risk factor for nosocomial infection between patients in general hospitals.[6] Using a variety of proteins and cell surface elements, this bacterium can create strong bonds with nasal epithelial

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cells, changing into persistent carriers.[6,7] However, other body sites are also frequently colonised such as hands, skin, axillae, and intestinal tracts.[8]Methicillin-resistant Staphylococci, such as methicillin-resistant coagulase-negative Staphylococcus (MRCoNS) and methicillin-resistant *S. aureus* (MRSA), have become increasingly common and constitute a serious threat to global public health.[2] MRSA strains can be categorized into two categories based on the source of infection: community (CA-MRSA) followed by hospital (HA-MRSA) infection. Patients with older age have a higher incidence of pneumonia, bacteremia, and persistent infections from these strains of CA-MRSA but HA-MRSA mostly affect healthy young patients through soft tissue infections.[4] Previous studies suggest possibilities that HCWs play a substantial role in MRSA transmission, highlighting the importance of rapid and accurate identification of MRSA carrier HCWs.[9] The knowledge of frequency of nasal carriage of *S. aureus* and MRSA among HCWs along with their current antimicrobial profile becomes necessary in the selection of appropriate treatment options for these carriers.[9 ,10]

Mupirocin (pseudomonic acid A or bactroban) is a tropical antibiotic synthesised from Pseudomonas fluorescens, has been available for many years. It inhibits the synthesis of proteins by particularly binds to bacterial isoleucyl -tRNA synthetase (IRS).It is approved for use as ointment to treat subsequent wound infections and impetigo caused by *S. aureus*.[11]Mupirocin-resistant strains are divided into two different groups: low-level resistance (MuL) strains, that exhibit a minimum inhibitory concentration (MIC) between 8 and 256 µg/ml as well as high-level resistance(MuH) strains, which have a MIC of > 512 µg/ml.[2] The mutation in the ileS-2 mupA gene coded chromosomally leads to low-level resistance. The mupA gene, which generates an additional enzyme, isoleucyl tRNA synthetase provides high-level resistance. Determining the minimum inhibitory concentration levels helps to differentiate MupRL and MupRH.[12]

MATERIALS AND METHODS

Study design

Our study was conducted for a period of 6 months from May to October 2024 in the Department of Microbiology of SGT hospital. A total of 400 nasal swabs were collected on a random basis. 70 swabs were from clinical staff working in departments like General medicine, Orthopedics (15), Obstetrics and gynaecology (37), Diagnostic laboratory (62),CSSD (23) while 50 swabs collected from staff working in hospital mess.

Inclusion criteria

Healthcare workers working in SGT hospital and who will be consenting to give samples will be eligible to give samples.

Exclusion criteria

Healthcare workers who are on antibiotics for last two weeks.

Sample collection

Nasal swabs were taken from both the nostrils with the help of sterile cotton swab moistened with normal saline from anterior nares of Health Care Providers (HCP). The swab was introduced 1-2 cm in the nasal cavity and rotated 3 times both clockwise and anticlockwise. and transported immediately to tryptic soy broth to provide appropriate environment to bacteria and followed to the microbiology laboratory for further processing.

Culture and identification of bacteria

The samples were inoculated immediately on blood agar and smear was prepared for Direct examination by Gram staining. Inoculated blood agar plates were incubated for 18-24 Hrs. at 37ºC. The beta hemolytic colonies on blood agar were further identified as Staphylococci by Gram staining which shows Gram positive cocci in clusters. All these colonies were subjected to Slide coagulase test & Tube Coagulase test to confirm it to be *Staphylococcus aureus.*

Detection of MRSA

All isolated *S. aureus* were tested with 30µg cefoxitin on Muller Hinton Agar (MHA) for MRSA screening. E-zone size was interpreted according to CLSI guidelines. An inhibition zone diameter of ≤21mm was reported as MRSA and ≥22mm was reported as methicillin-sensitive *Staphylococcus aureus* (MSSA).

Antibiotic Susceptibility Testing

All Staphylococcus isolates will be routinely subjected to antimicrobial susceptibility testing (AST) on Mueller-Hinton agar using the disc diffusion (Kirby-Bauer) method. Following antibiotics will be subjected for the AST- penicillin (10 µg); erythromycin (15µg); gentamicin (10µg); ciprofloxacin (5µg); cotrimoxazole (1.25/23.75µg); linezolid (30µg); clindamycin (2 µg); tetracycline (30 µg); chloramphenicol (30µg); cefoxitin (30 µg), Minocycline (30 µg), Nitrofuratoin (300 µg). AST will be done according to latest CLSI guidelines.

Minimum inhibitory concentration of mupirocin detection by Epsilomer (E) test

Screening for high level and low-level mupirocin susceptibility will be done for all staphylococcal isolates. The MIC for mupirocin will be determined using E-strips. The plates were also observed for zone of inhibition intersecting the graduated strip of Ezy-MIC and readings was noted and was divided into[13] –

1. Mupirocin Sensitive (S): MIC of < 4 µg/ml.
2.Low Level Mupirocin Resistance (MupL): MIC of 8–256 µg/ml,
3.High Level Mupirocin Resistance (MupH): MIC of > 512 µg/ml.

In accordance with latest CLSI guidelines, Isolates with zone size 31-33 mm mupirocin disc were considered as high-level mupirocin sensitive but less than 31 mm mupirocin disc were considered as resistant strain.

Isolates with zone size 18-24 mm mupirocin disc were considered as low-level mupirocin sensitive but less than 18 mm mupirocin disc were considered as resistant strain.

3.RESULT

A total of 400 nasal swabs were collected from HCWs of which 37 (14%) were *S. aureus* carriers and among them 29(11%) were MRSA carriers.

3.1 Characteristics of Study Population.

Half of them belonged to age-group of 18-30 years (n= 200), followed by age-group 31-45 years 34.25%, (n=137), 46-60 years 11.8%, (n= 47) and above 60 years of age 4%, (n= 16).

3.2. Distribution of Bacterial Isolates.

Out of the total 400 nasal swabs, nine samples collected from nurses yielded *S. aureus* 9% (n=99), eight isolates each from GD staff 17.3% (n=8) and housekeeping staff 17.7% (n=45), five isolates from technicians 6.7% (n=74), three isolates from mess staff 6% (n=50) and two each from doctors 3.3% (n=59) and CSSD staff 8.6% (n=23) were carriers of *S. aureus.*

Distribution of MSSA was found to be highest in laboratory staffs with 19.2% (5/26), while MRSA was found to be the highest in postoperative ward staffs with 18.2% (2/11)

3.3 Antibiotic susceptibility pattern of *S. aureus*.

Antibiotic susceptibility test was done by disc diffusion method on Muller – Hinton agar medium (MHA) a/c latest CLSI guidelines to all the species of *S. aureus* isolated from antibiogram of isolates showed 97.2% sensitive to minocycline. Others member of tetracycline group also showed higher susceptibility rate i.e. 83.7% for tetracycline. Staphylococcal isolates showed higher susceptibility rates to linezolid(86.4%), chloramphenicol(78.3%), cotrimoxazole(78.3%),Clindamycin(75.6%). From the group of aminoglycosides, gentamicin showed the sensitivity of 78.3%. The susceptibility were seen moderate in vancomycin(48.6%),ciprofloxacin(37.8%) and 32.4% for erythromycin.24.3% levofloxacin were susceptible to the following staphylococcus isolates. Among the antibiotics used, least susceptibility was shown by penicillin that is 13.5%

Table no 1. Distribution of isolated bacteria

Total specimen isolated	Growth	*S. aureus*, n (%)	MRSA, n (%)	MSSA, n (%)
400	265(66.3%)	37(13.9%)	29(10.9%)	8(3.1%)

Table no 2. Age wise distribution of isolated bacteria

Age (Years)	Total isolates n (%)	*S. aureus*, n (%)	MSSA, n (%)	MRSA, n (%)	Non carrier, n (%)
18-30	200 (50)	26(13.5)	6(23)	20(77)	174(87)
31-45	137 (34.25)	7(4.3)	1(14.2)	6(85.7)	130(95)
46-60	47(11.75)	3(6.3)	1(33.3)	2(66.6)	44(93.6)
Above 60	16(4)	1(6.2)	0(0)	1(100)	15(93.7)
Total	400(100)	37(9.2)	8(21.6)	29(78.3)	363(90.7)

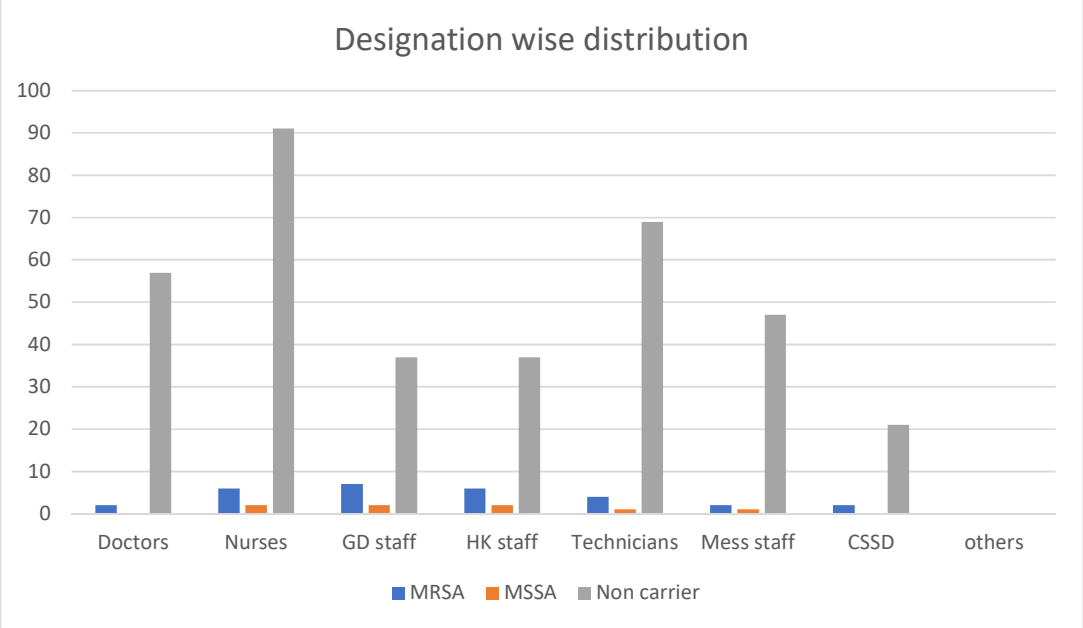

Figure.1: Designation wise distribution of Hcws carrying MRSA ,MSSA and non carrier

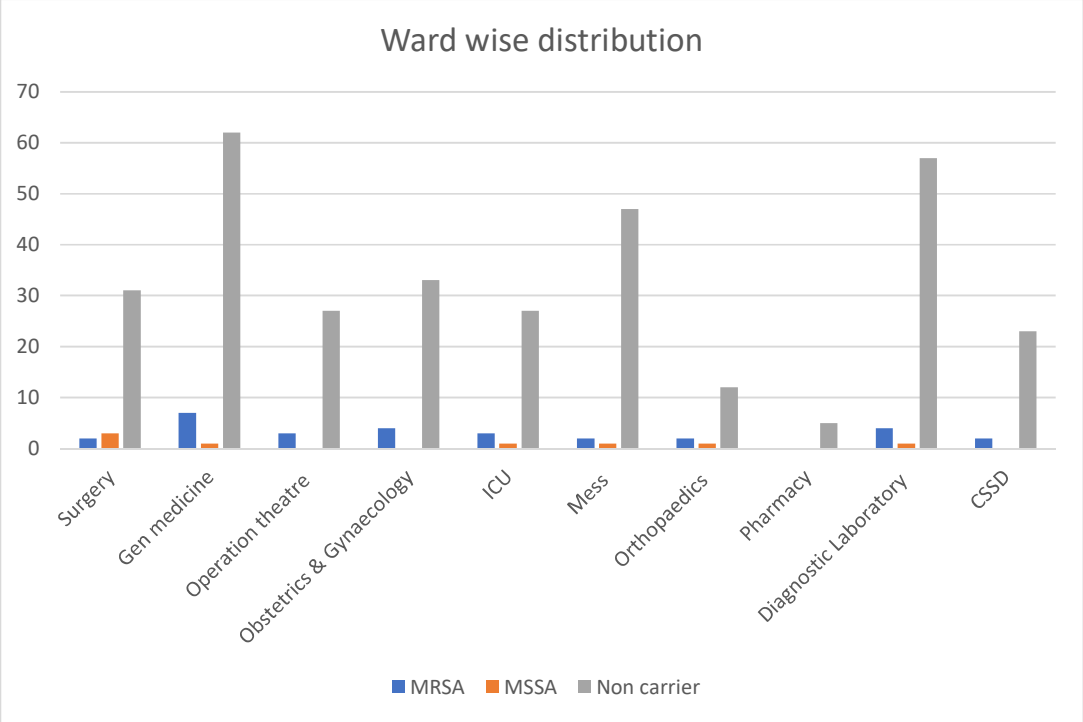

Figure.2: Ward wise distribution of Hcws carrying MRSA ,MSSA and non carrier

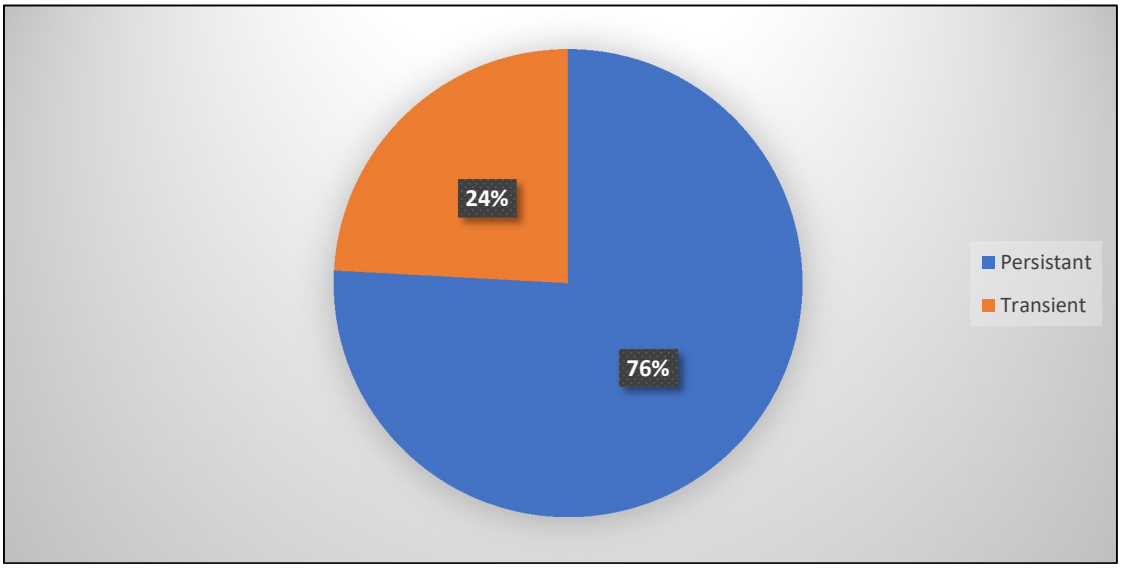

Figure 3.Pie chart showing type of methicillin-resistant *S. aureus* nasal carriers identified among HCWs

Table 3. Antibiotic susceptibility pattern of 200 mcg and 5 mcg mupirocin antibiotic

***Staphylococcus* spp.**	**Resistance to mupirocin**	
	200 µg disc No. (%)	**5 µg disc No. (%)**
MRSA (n= 29)	0 (0.0)	4 (13.8)

Table no:4 Mupirocin resistance detected using E-strip

Organism	High-level mupirocin resistance	Low-level mupirocin resistance
MRSA (n= 29)	0 (0.0)	9 (31)

DISCUSSION

Methicillin-resistant *S. aureus* nasal carriage among healthcare workers is a major concern in a healthcare setting. However, the mechanism for its carriage is complex and not entirely comprehended.[14]Since, HCWs, particularly those individuals working in critical care units, must be screened for detection of MRSA carriage. Since, such individuals can act as a potential source of disease to their patients, causing nosocomial infections.[2] *S. aureus* causes 25% of all nosocomial infections, affecting both surgical and non-surgical patients, resulting in longer hospital stays, higher drug costs, and mortality. Thus, nasal carriage of *S. aureus* is at higher risk of developing illnesses due to this bacterium. [15]

Regular screening of healthcare workers along with implementing the appropriate preventive measures are the most effective ways to control the transmission of pathogens from HCWs to patients in healthcare settings. The carriage rate of MRSA among HCWs varies between institutions and regions.[2]

In our study, 400 HCWs were included, of whom 56% (n=224) were male, surpassing the 44% (n=176) who were female. This distribution is in accordance with the findings of Aggarwal et al., which reported 54% male and 46% female participants.[16]

In our study, the prevalence rate of *S. aureus* colonization in the anterior nares is 14%, which is similar to the findings of a study conducted by Giri et al. in Kathmandu, Nepal which enrolled 238 healthcare workers and 14.7% of them were found to be *S. aureus* nasal carriers.[17]

In the present study, *S. aureus* nasal carriage among nurses was found to be 9% and 7% among technicians while higher carriage rate (17%) was noted among housekeeping staff. This finding is in accordance with a study conducted by Khanal et al. reporting 11.8% *S. aureus* nasal carriage among nurses and 6.3% between laboratory personnel and 18% among housekeeping staff among the 204 HCWs included in the study.[18]

The maximum number of MRSA in our study were isolated from GD staff and housekeeping staff, 24% and 20% respectively, which is similar to the study conducted by Rawani et al. in Chhatisgarh.[1]

In department-wise distribution of HCWs in our study, the maximum MRSA isolates were obtained from General Medicine department (24%), Obstetrics & Gynaecology was 13.7% which is similar with the study conducted by Mangalgi et al. from Karnataka with total population of 265.[19]

In contrast, Duong et.al reported 29% MRSA carriers among ICU staff in the study.[10] Out of 10 and 7 swabs collected from Radiology and ENT respectively, none of them were found to be MRSA carriers. Comparable results were reported by Khanal et al. who enrolled 204 HCWs and none of the radiology and ENT staff were found to be MRSA carriers.[19] None of the pharmacy staff were MRSA carriers, which is similar to study conducted by Shibabaw et al.[20]A total of 10% nasal swabs collected from operation theatre staff showed MRSA growth. Giri et al. obtained 6.7% MRSA carriage in operation theatre which is comparable with our findings.[21]

Antibiotic resistance rate was analysed for all the isolates in our study, revealing higher rates of resistance among *S. aureus* compared to CoNS. Tetracycline was found to be an effective antibiotic with 83% and of *S. aureus* and CoNS isolates showing susceptible. El Aila et al. from Israel also found 85% susceptibility rate to tetracycline by *S. aureus* colonizers.[22] Linezolid susceptibility in the present study was 95%, which is similar to findings of a study conducted by Aggarwal et al. reporting 100% susceptibility to the same antibiotic by *S. aureus* isolated from HCWs.[23]

Topical nasal application of mupirocin for a short-term period of 4-7 days is the most successful treatment for eradicating MRSA nasal colonizers, with a success rate of approximately 90% after one week. After treatment, over 60% had a longer follow-up time ranging from 14 days to 365 days in various studies.

The present study detected mupirocin resistance among MRSA isolates by using mupirocin disc, revealing low-level mupirocin resistance as 13.7%, however, none of them showed high level mupirocin resistance. The same rate of mupirocin resistance among MRSA isolates were shown using mupirocin E-strip. Similar findings were reported by Abdulgader et al. from Africa, with MupL as 18% and MupH as 4%.[24]Premanand et al. reported 100% sensitivity towards mupirocin for MRSA isolates.[2]

However, with mupirocin E-strip, 20% showed MupL and 30% showed MupH. Similar study was done by K elanithi et al. in Puducherry, India with 30% MupH and 12% MupH.[25] In contrast, the mupirocin resistance was found to be 59.6% in a study conducted by Jagadeesan et al.[26] This could be due to the repeated use of mupirocin which may lead to development of its resistance. One of the MRSA showed 100% sensitivity in 5µg and 200 µg however, was low level mup resistance when subjected to E-strip.[27]

CONCLUSION

In our study, 10.9% of the healthcare workers were identified as MRSA nasal carriers. We also differentiated short term MRSA carriers (transient carriers) and chronic carriers of MRSA (persistent carriers) by taking repeated swabs after five days. We

found that 78% of our HCWs were persistent carriers which was comparatively higher from other studies. Treatment was given to the persistent carriers of MRSA i.e., intranasal 2% mupirocin ointment twice daily for five days, which is according to Infectious Diseases Society of America guidelines. In order to prevent emergence of mupirocin resistance, transient MRSA carriers were not treated by using the same. After mupirocin treatment, repeated swabs from transient carriers were collected and processed and all were found to be negative and the treatment was completed.

Given that healthcare workers can facilitate cross contamination to patients, it is imperative for them to adhere to appropriate hygiene practices, routine use of masks and gloves to avert the transmission of MRSA to both healthcare workers and patients. Therefore, there is an urgent need to give priority for the safety of HCWs so that they do not become a risk for patients rather become a protector for them as they remain in contact with them for longer time. Regular screening of MRSA among HCWs should be done by following standard procedures.

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