



Isolation, Characterisation and Antibiotic Susceptibility of Staphylococcal Isolates with Special Reference to Methicillin-Resistant *Staphylococcus aureus* From the Anterior Nares of Healthcare Workers in a Tertiary Healthcare Centre

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Abstract

Background: *Staphylococcus aureus* (*S. aureus*) and its resistant form methicillin-resistant *S. aureus* (MRSA) is one of the most common nosocomial pathogens causing a wide range of infections in humans. The anterior nares are the main ecological niche for *S. aureus*. Nasal carriage of *S. aureus* acts as an important reservoir of infection among the colonised healthcare workers and they transmit the infection to the community. The aim of the present study was to estimate the nasal colonisation of *S. aureus* (with special reference to MRSA) in healthcare workers (doctors and nursing staff) and its antibiotic susceptibility pattern.

Methods: A descriptive study was planned in the Department of Microbiology, JLN Medical College, Ajmer (Rajasthan, India) after due approval from the institutional ethics committee. A total of 170 healthcare workers of either sex aged between 18 to 60 years were screened for *S. aureus*. Identification was done using standard microbiological techniques, by studying their morphology, colony and biochemical characteristics. MRSA was detected by cefoxitin disc diffusion test, oxacillin disc diffusion test, minimum inhibitory concentration (MIC) of oxacillin by E-test and oxacillin screen agar test. The observations were described in proportions and Chi-squared test was used to find independence. Statistical significance was considered at 5 %.

Results: Among 170 samples, 159 (93.53 %) samples (50 doctors and 109 nursing staff) had staphylococci colonisation. Among these 159 isolates, 34 (21.38 %) were *S. aureus*. Further, 8 (5.03 %) *S. aureus* isolates were resistant to both cefoxitin and oxacillin and had oxacillin MIC values ≥ 4 $\mu\text{g/mL}$ and were considered MRSA. All the MRSA were detected in the nursing staff (males: 5.50 %, females: 1.83 %). All *S. aureus* and MRSA isolates were found sensitive to linezolid, vancomycin and mupirocin (minimum inhibitory concentration ≤ 4 $\mu\text{g/mL}$).

Conclusion: Screening and treatment of healthcare workers colonised with MRSA should be an important component of hospital infection control policy. These measures will prevent spread of infection to patients and the community and thereby reduce the morbidity, mortality and healthcare costs associated with nosocomial infections.

Key words: Antibiotic susceptibility pattern; Healthcare workers; Methicillin-resistant *Staphylococcus aureus*; Minimum inhibitory concentration; Nosocomial infections.

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Introduction

Staphylococci are ubiquitous colonisers of skin and mucosa and highly successful opportunistic pathogens. *S. aureus* is one of the most harmful species of staphylococci encountered.¹ It is one of the most pathogenic bacterial species in humans causing a wide variety of infections ranging from mild skin and soft tissue infections (furuncles, carbuncles etc) to severe life-threatening infections like chronic bone infections, necrotising pneumonia, bacteraemia, septicaemia, acute endocarditis, myocarditis, pericarditis, osteomyelitis, encephalitis, meningitis, chorioamnionitis, mastitis, toxic-shock syndrome, scalded skin syndrome²⁻⁵ and intravenous infections or at other sites where tubes enter the body (indwelling medical devices).⁶ It is distinct from coagulase-negative staphylococci (CoNS) eg, *S. epidermidis*, and is more virulent despite phylogenetic similarities between them.^{7,8}

The key characteristics of *S. aureus* are colony pigmentation, production of free coagulase, clumping factor, protein-A, heat-stable nuclease, lipase and acid production from mannitol.³ The species *aureus*, refers to those colonies that often have a golden colour when grown on solid media, while CoNS form pale, translucent, white colonies.

Staphylococcal infections occur frequently in hospitalised patients and have severe consequences, despite antibiotic therapy.⁹ *S. aureus* are generally susceptible to β -lactam antibiotics, but extensive use of this class of drugs has led to increasing emergence of resistant strains.¹⁰ The most notable example is the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA), which was reported just one year after the introduction of methicillin.¹¹ Also known as “a superbug”, MRSA has become a major problem in most medical institutions because it is creating life-threatening situations.¹¹ MRSA is a major healthcare-associated (HA-MRSA) as well as a community-associated (CA-MRSA) infection.⁶

Healthcare workers (HCWs) constitute an important reservoir of *S. aureus*. Nasal carriage of *S. aureus* acts as an important reservoir of infection among those colonised, who may then transmit the infection to co-workers and others in the community.¹² Approximately 20 % of individuals are persistent carriers, about 60 % are intermittent carriers and 20 % almost never carry *S. aureus*.¹³ Several studies have reported that the rate of the

nasal carriage of *S. aureus* among the HCWs ranges from 16.8 % to 56.1 %.¹⁴⁻¹⁷ Studies conducted in different hospital settings worldwide including India, have reported the prevalence of MRSA in HCWs in the range of 5.8 % to 17.8 %.^{18-22, 9, 12} The growing problem in India is that MRSA prevalence has increased from 12 % to 80.83 %.²³ The healthcare workers who are found to be colonised with *S. aureus* are advised to apply mupirocin ointment in their anterior nares and they should be retested for the nasal carriage of *S. aureus* after 3 months of treatment.⁹

The aim of the present study was to estimate the nasal carriage and antimicrobial susceptibility pattern of *S. aureus* and MRSA isolates among the HCWs in a tertiary healthcare centre. The prevalence of *S. aureus* carriers and its resistance to methicillin will help the institution develop a better MRSA infection control policy.

Methods

This descriptive study was carried out in the Department of Microbiology, Jawahar Lal Nehru (JLN) Medical College and Hospital, Ajmer, Rajasthan, India from November 2016 to December 2017. The study was approved by the Ethics Committee of JLN Medical College, Ajmer and written informed consent was obtained from all the participants.

A total of 170 HCWs aged 18 to 60 years, actively involved in healthcare provision in different departments of JLN Medical College were enrolled for the study. Each participant was interviewed using a questionnaire on general socio-demographic information, personal details and clinical symptoms. Exclusion criteria included healthcare workers not actively involved in patient care or those suffering from underlying chronic disease or respiratory tract infections, with a history of recent hospitalisation, intake of broad-spectrum antibiotics, fever or those who did not consent.

Sample collection

Nasal swabs from the anterior nares of both nostrils were collected using sterile cotton swabs with transport tubes. A swab pre-moistened with sterile saline was inserted approximately 1-2 cm

into the anterior nares and slowly rotated against the nasal mucosa five times.²⁴ Both nostrils were sampled using the same swab. After collection, the swabs were re-inserted in the transport tubes, labeled properly and transported to the laboratory within 30 minutes of collection for further processing.

Sample processing

All the specimens were inoculated on 5 % sheep blood agar, nutrient agar and MacConkey agar (HiMedia Laboratories Pvt Ltd Mumbai, Maharashtra, India) and incubated at 37 °C for 24 hours. After incubation, identification of genus *Staphylococcus* was done using standard microbiological techniques, by studying their morphology, colony characteristics and biochemical properties. Staphylococci were identified as Gram positive, catalase positive, furazolidone susceptible and bacitracin-resistant. *S. aureus* colonies were further identified as slide and tube coagulase positive, polymyxin B-resistant and mannitol fermenting giving yellow pigmentation on mannitol salt agar.

Antimicrobial susceptibility testing (AST)

Antibiotic susceptibility was studied by modified Kirby–Bauer disc diffusion method²⁵ on Mueller Hinton Agar plates (120 mm diameter) using commercially available antibiotic discs (HiMedia Laboratories Pvt. Ltd. Mumbai, Maharashtra, India): penicillin G (10 units), cephalixin (30 µg), cefoxitin (30 µg), oxacillin (1µg), gentamicin (10 µg), netilmicin (30 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), levofloxacin (5 µg), erythromycin (15 µg), clindamycin (10 µg), tetracycline (30 µg), cotrimoxazole (25 µg), quinupristin dalfopristin (15 µg), vancomycin (30 µg), linezolid (30 µg), cephalothin (30 µg), amoxicillin/clavulanic acid (co-amoxiclav, 30 µg) and ampicillin (10 µg). Zone diameter interpretation for determining sensitive, intermediate or resistant isolates was done as per CLSI 2016 guidelines.²⁶

Detection of methicillin-resistant *Staphylococcus aureus* (MRSA)

All confirmed *S. aureus* isolates were tested for detection of methicillin resistance by four different methods. Kirby–Bauer disc diffusion method using oxacillin 1 µg and cefoxitin 30 µg discs (HiMedia Laboratories, Mumbai, Maharashtra, India),²⁷ minimum inhibitory concentration (MIC) testing of oxacillin by E-test and growth on Oxacillin Resistance Screening Agar (ORSA) plates²⁸

as per CLSI 2016 guidelines.²⁶ Zone of inhibition of size ≤ 10 mm was taken as resistant, 11-12 mm as intermediate and ≥ 13 mm as sensitive for oxacillin. Zone of inhibition of size ≤ 21 mm was taken as resistant, and ≥ 22 mm as sensitive for cefoxitin. On oxacillin E-test, an MIC of ≤ 2 µg/mL was considered susceptible and ≥ 4 µg/mL as resistant. Any growth on oxacillin screen agar was considered as methicillin (oxacillin) resistant.

Detection of mupirocin-resistant *Staphylococcus aureus*

The MIC of mupirocin for isolation of *S. aureus* (Mupirocin resistance) was determined by Epsilon-meter test (E-test) using HiMedia, mupirocin strip (range 0.064-1024 µg/mL) and interpreted as per CLSI 2016 guidelines.²⁶ Isolates with mupirocin MICs ≥ 512 µg/mL were considered as high-level resistant (MuH), those with MICs 8-256 µg/mL were considered as low-level resistant (MuL), and with ≤ 4 µg/mL were considered as mupirocin sensitive.

Statistical analysis

The descriptive statistics for quantitative data was expressed as mean and standard deviation and qualitative data was expressed as proportions. Chi-squared test was used to find independence of attributes at 5 % level of significance ($\alpha = 0.5$). The JASP 0.11.1.0 statistical package was used for statistical analysis.

Results

In the present study, nasal swabs were randomly collected from a total of 170 HCWs from various clinical departments and screened for the study of *Staphylococcus* colonisation. Out of a total of 170 samples, 159 (93.53 %) had staphylococci colonisation. Of these 159 HCWs, with age group ranging between 18 to 60 years, 99 (62.26 %) were males and 60 (37.74 %) were females. The colonisation rate was 31.45 %, 34.59 %, 18.24 % and 15.72 % in the age groups '18-30', '31-40', '41-50' and '51-60' years, respectively (Figure 1).

From these 159 subjects, 50 were doctors and 109 were nursing staff. Of the 50 doctors, 37 (74 %) were males and 13 (26 %) were females. Among the 109 nursing staff, 62 (56.88 %) were males

and 47 (43.12 %) were females. The maximum carriage rate in doctors was observed in the age group 31-40 years ie, 60 %, where 50 % were males and 10 % were females. In the nursing staff group, maximum carriage was seen in 18-30 years age group where 20.18 % were males and 18.35 % were females accounting for a total of 38.53 % carriage rate in their group (Figure 1).

In the present study *Staphylococcus* colonisation

was detected in 159 (93.53 %) healthcare workers which comprised 34 (21.38 %) *S. aureus* and 125 (78.61 %) CoNS isolates. Dual colonisation with *S. aureus* and CoNS was observed in 10 samples. The carriage rate of *S. aureus* was significantly higher in nursing staff (26.60 %) as compared to doctors (10 %) ($\chi^2 = 5.62, p = 0.018$). Professors/associate professors/assistant professors and resident doctors were found to have *S. aureus* nasal carriage rate 16.67 % and 7.89 %, respectively (Figure 1).

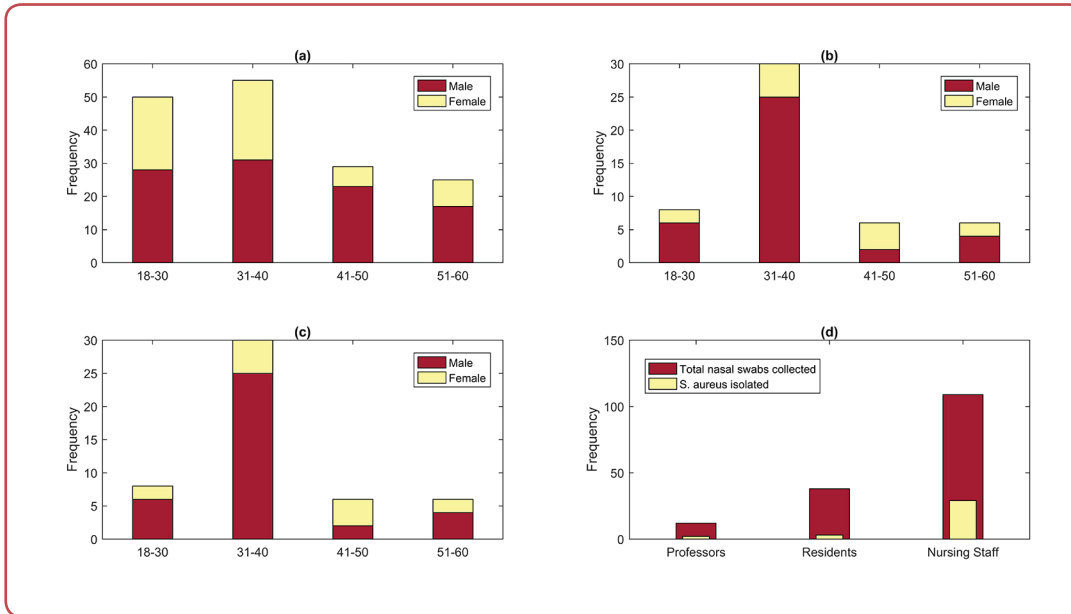


Figure 1: Stacked bar plots showing demographic profile (a) Age wise gender distribution of all healthcare workers ($n = 159$), (b) age-wise gender distribution of doctors ($n = 50$), (c) age-wise gender distribution of nursing staff ($n = 109$), (d) Total number of nasal swabs collected and *S. aureus* isolated in healthcare workers ($n = 159$)

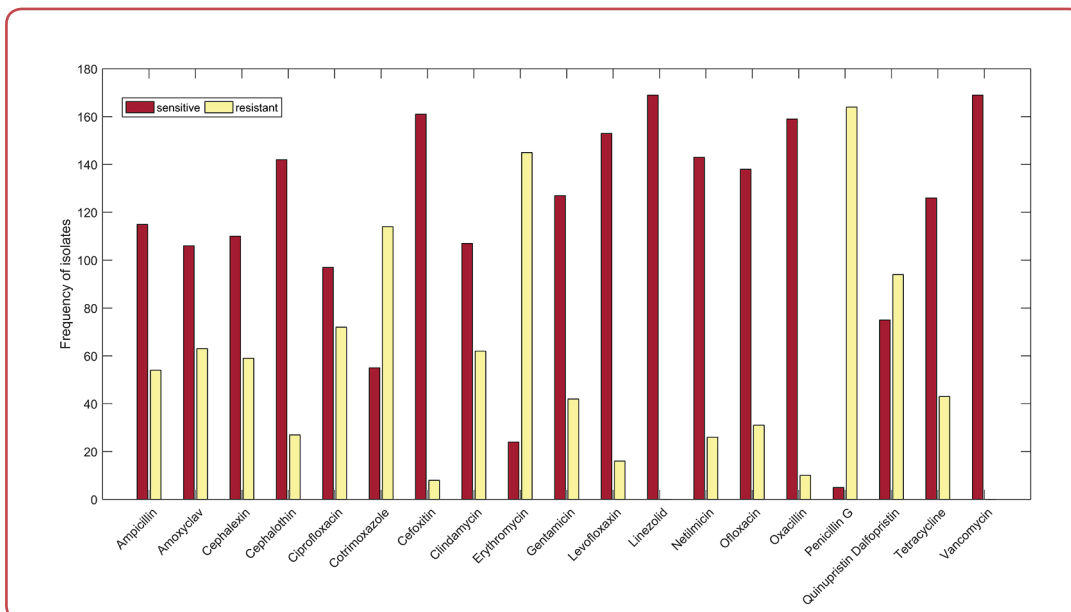


Figure 2: Bar plots showing antimicrobial sensitivity pattern of *Staphylococcus* isolates by modified Kirby-Bauer disc diffusion method ($n = 169$)

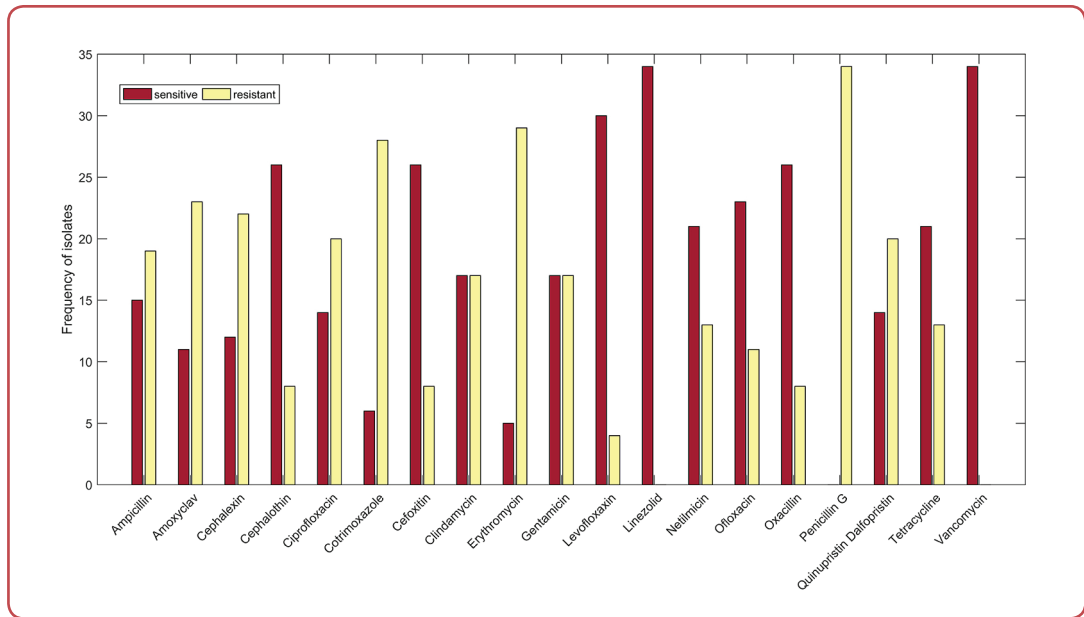


Figure 3: Bar plots showing antimicrobial sensitivity pattern of *S. aureus* isolates by modified Kirby-Bauer disc diffusion method (n = 34)

Antimicrobial sensitivity pattern of *Staphylococcus* isolates by disc diffusion method is shown in Figure 2. Ten subjects had concomitant colonisation of *S. aureus* and CoNS. Therefore, antimicrobial susceptibility testing was done for 169 *Staphylococcus* isolates.

Among the antibiotics tested, all the staphylococcal isolates were susceptible only to linezolid and vancomycin (100 %). Maximum resistance was shown to penicillin G (97.49 %). Resistance to cefoxitin and oxacillin was 4.73 % and 5.92 %, respectively.

All the *S. aureus* isolates were found to be susceptible to linezolid and vancomycin (100 %). All *S. aureus* isolates showed complete resistance to penicillin G (100 %). Extremely low susceptibility was shown for erythromycin (17.71 %) and cotrimoxazole (17.65 %). Resistance to cefoxitin and oxacillin was 23.53 % (Figure 3).

Detection of MRSA was done by four different phenotypic methods. Among the 34 *S. aureus* isolates studied, 8 isolates (23.53 %) were found to be MRSA. While in oxacillin screen agar testing, 6 (17.65 %) isolates were found to be MRSA. No isolate showed intermediate resistance. Thus, out of 34 *S. aureus* isolates, 8 (23.53 %) were MRSA and 26 (76.47 %) were methicillin sensitive *S. aureus* (MSSA) (Table 1). One MRSA isolate showed resistance to vancomycin disc on AST. However due to limited resources, further testing of this isolate

Table 1: Comparison of different phenotypic methods for detection of methicillin resistance in *S. aureus* isolates (n = 34)

<i>S. aureus</i>	Tests used for detection of MRSA			
	Oxacillin (1 µg) disc diffusion	Cefoxitin (30 µg) disc diffusion	Oxacillin screen agar	Oxacillin MIC by E-test
Methicillin-resistant <i>S. aureus</i> (MRSA)	8 (23.53)	8 (23.53)	6 (17.65)	8 (23.53)
Methicillin-sensitive <i>S. aureus</i> (MSSA)	26 (76.47)	26 (76.47)	28 (82.35)	26 (76.47)

Note: percentage is shown in parenthesis; *S. aureus*: *Staphylococcus aureus*

by MIC testing of vancomycin using agar dilution method (recommended by CLSI) to determine it as vancomycin-resistant, intermediate or sensitive could not be carried out.

In the present study, the 8 *S. aureus* isolates that were resistant to both cefoxitin and oxacillin had oxacillin MIC values ≥ 4 µg/mL (Table 2). There were no isolates found resistant to cefoxitin and intermediate resistance to oxacillin at the same

Table 2: Comparison of cefoxitin and oxacillin disc diffusion tests with minimum inhibitory concentration (MIC) of oxacillin by E-test method (n = 34)

Results of oxacillin and cefoxitin disc diffusion	MIC of oxacillin (µg/mL)	No. of isolates (%)
Resistant to both cefoxitin and oxacillin	≥ 4	8 (23.53)
Resistant to cefoxitin and intermediate resistant to oxacillin	≥ 4	0 (0.0)
Resistance to cefoxitin and sensitive to oxacillin	≥ 4	0 (0.0)
Sensitive to cefoxitin and oxacillin	< 4	26 (76.47)

Note: percentage is shown in parenthesis

Table 3: Nasal carriage of *S. aureus* and MRSA among various healthcare workers (n = 34)

Healthcare workers	No. of nasal swabs collected	No. of <i>S. aureus</i> isolated (%)	No. of MRSA isolated (%)
Professors, Associate Professors, Assistant professors	12	02 (16.67)	0 (0.0)
Resident doctors	38	03 (7.89)	0 (0.0)
Nursing staff	109	29 (26.60)	08 (7.34)
Total	159	34 (21.38)	08 (5.03)

Note: percentage is shown in parenthesis; *S. aureus*: *Staphylococcus aureus*; MRSA: methicillin-resistant *Staphylococcus aureus*

time. No isolate was found resistant to cefoxitin while being sensitive to oxacillin. 26 *S. aureus* isolates that were sensitive to both oxacillin and cefoxitin by disc diffusion method had MICs ≤ 2 $\mu\text{g}/\text{mL}$, indicating their susceptibility to oxacillin. Thus, out of 34 *S. aureus* isolates, 8 (23.53 %) were MRSA and the remaining 26 (76.47 %) were oxacillin and methicillin susceptible (MSSA).

Out of 159 isolates, 21.38 % subjects had *S. aureus* colonisation out of which 5.03 % had MRSA colonisation (Table 3). All of these MRSA carriers were detected in the nursing staff. The carriage

Table 4: Sex wise distribution of nasal carriage of *S. aureus* and MRSA among various healthcare workers (n = 34)

Healthcare workers	No. of nasal swabs collected	No of <i>S. aureus</i> isolated (%)		No. of MRSA isolated (%)	
		Male	Female	Male	Female
Doctors	50	4 (8)	1 (2)	0	0
Nursing staff	109	17 (15.60)	12 (11.01)	6 (5.50)	2 (1.83)
Total	159	21 (13.21)	13 (8.18)	6 (3.77)	2 (1.26)
		34		8	

Note: percentage is shown in parenthesis; *S. aureus*: *Staphylococcus aureus*; MRSA: methicillin-resistant *Staphylococcus aureus*

Table 5: Mupirocin minimum inhibitory concentration (MIC) of nasal isolates of *S. aureus* (n = 34)

S. No.	Mupirocin MIC ($\mu\text{g}/\text{ml}$)	No. of <i>S. aureus</i> isolates	No. of MRSA isolates
1	≤ 0.125	13	01
2	0.25	11	03
3	0.5	06	02
4	1	03	01
5	2	01	01
6	4	00	00
7	8	00	00
Total		34	08

Note: *S. aureus*: *Staphylococcus aureus*; MRSA: methicillin-resistant *Staphylococcus aureus*; MIC: minimum inhibitory concentration

rate of *S. aureus* too was significantly higher in nursing staff ie, 26.60 % with MRSA carriage rate of 7.34 %. Professors/associate professors/assistant professors and resident doctors were found to have *S. aureus* nasal carriage rate 16.67 % and 7.89 % respectively with no MRSA carriage. The MRSA carriage rate was 5.5 % and 1.83 % in male and female nursing staff, respectively (Table 4).

For *S. aureus*, mupirocin MIC ≤ 4 $\mu\text{g}/\text{mL}$ is considered as susceptible. MIC 8-256 $\mu\text{g}/\text{mL}$ is considered as intermediate resistant and MIC ≥ 512 $\mu\text{g}/\text{mL}$ is considered as resistant. All *S. aureus* isolates had MIC ≤ 4 $\mu\text{g}/\text{mL}$ for mupirocin, indicating mupirocin susceptibility of all the isolates (Table 5). Further, as many as 13 *S. aureus* and 1 MRSA isolate had MIC ≤ 0.125 $\mu\text{g}/\text{mL}$ and 11 *S. aureus* and 3 MRSA isolates had MIC ≤ 0.25 $\mu\text{g}/\text{mL}$.

Discussion

S. aureus is a common component of the skin flora, and 30 % to 50 % of healthy adults are colonised with it at any given time. The primary site of colonisation of *S. aureus* in humans are the anterior nares.^{2, 29} Hospital workers have higher rates of MRSA nasal colonisation than the general population.³⁰ In the present study 21.38 % subjects had *S. aureus* colonisation. Among HCWs around the globe, the nasal carriage rates of *S. aureus* have been reported at 14 % in Nigeria, 27.5 % in Turkey, 31.1 % in Iran, 33.4 % in France and 39.3 % in Spain.³¹ The growing problem in India is that MRSA prevalence has increased from 12 % to 80.83 %.²³

Out of a total of 159 subjects (50 doctors and 109 nursing staff) *S. aureus* and CoNS appeared in 34 and 125 samples respectively. Dual colonisation of *S. aureus* and CoNS was observed in 10 samples. However, no dual isolation was observed in a study conducted by Vinodhkumaradithyaa et al.²² The prevalence of the *S. aureus* nasal carriage was higher among the male HCWs (13.21 %) than the female HCWs (8.18 %). Similar observation was reported by Rongpharpi et al.⁹ In the present study, the carriage rate of *S. aureus* was significantly higher in the nursing staff 26.60 %. Professors/associate professors/assistant professors and resident doctors were found to have *S. aureus* nasal carriage rate 16.67 % and 7.89 % respectively. Out of 34 *S. aureus*, 5.03 %

had MRSA colonisation. All of these MRSA carriers belonged to the nursing staff with MRSA carriage rate of 7.34 %. Similar studies from Barabanki, Uttar Pradesh reported 81 % *Staphylococcus*, 48 % *S. aureus* and 14 % MRSA colonization.¹² However, Bhatiani et al reported 39 % and 15 % carriage rates of *S. aureus* and MRSA in Rama Medical College, Hospital and Research Center, Kanpur which is similar to the findings presented here.³² Shobha et al found none of the healthcare workers colonised with *S. aureus*³³ while a study from south India showed 9.3 % *S. aureus* colonization.³⁴

In the present study, on MRSA detection using oxacillin disc diffusion, cefoxitin disc diffusion and MIC of oxacillin by E-test, 8 (23.53 %) isolates were found to be MRSA. In this study, 8 *S. aureus* isolates that were resistant to both cefoxitin and oxacillin had oxacillin MIC value ≥ 4 $\mu\text{g}/\text{mL}$ and 26 isolates that were sensitive to both oxacillin and cefoxitin had MIC values ≤ 2 $\mu\text{g}/\text{mL}$. Oxacillin screen agar could detect only 6 (17.65 %) isolates instead of 8 detected by the other three methods. Hence it is recommended that all four methods should be used for detection of oxacillin resistance. Pramodhini et al found oxacillin disc diffusion method to be less sensitive for the detection of MRSA.³⁵ Mohanasoundaram and Lalitha obtained 100 % concordance in disc diffusion method and oxacillin MIC using agar dilution methods.³⁶

In the present study, 97.04 % staphylococcal isolates and 100 % *S. aureus* and MRSA were found to be resistant to penicillin G. Similar findings were observed by Bala et al and Bhatiani et al where penicillin was found to be 100 % resistant to all strains of *S. aureus*,^{37,32} but Rongpharpi et al reported 90 %, Duran et al reported 92.8 %, Kandle et al reported 98.9 % penicillin resistance.³⁹ All the MRSA isolates were resistant to penicillin as reported by Agarwal et al.¹² In the present study, ampicillin and co-amoxiclav showed a resistance of 31.95 % and 37.28 % for staphylococci and 55.88 % and 67.65 % for *S. aureus*. Out of 8 MRSA isolates, 6 (75 %) and 8 (100 %) isolates were found to be resistant to ampicillin and co-amoxiclav respectively. Bhatiani et al has reported a 100 % resistance to ampicillin,³² while 88.57 % and 82.00 % resistance to ampicillin by *S. aureus* isolates was observed by Rongpharpi et al and Jindal et al in studies conducted among HCWs respectively.^{9,40} Study conducted at

a tertiary care hospital in Iran reported 89.4 % resistance among MRSA.⁴¹

A total of 34.91 % *Staphylococcus* isolates, 64.71 % *S. aureus* isolates and all MRSA isolates were resistant to cephalexin in the present study while 73.7 % MRSA isolates were found to be resistant to cephalexin in a similar study.⁴² In the present study, 24.85 % and 15.38 % staphylococcal, 50 % and 38.24 % *S. aureus*, 87.50 % and 75.00 % MRSA isolates were resistant to gentamicin and netilmicin respectively. In studies by Hauschild et al and Schmitz et al, 24.4 % and 23 % resistance was shown in *S. aureus* isolates to the above aminoglycosides.^{43,44} In this study, of 34 *S. aureus* isolates, 38.24 % were resistant to at least one of the two aminoglycosides tested. Hauschild et al reported that 38.1 % *S. aureus* were resistant to one of the aminoglycosides tested.⁴³

In the present study, 42.60 % *Staphylococcus* isolates and 58.82 % *S. aureus* isolates were resistant to ciprofloxacin. Lower incidence of resistance (10.4 %) was reported by Tahnkiwale et al,⁴⁵ 41 % by Duran et al³⁸ and 90 % by a Mexican study on 211 isolates.⁴⁶ In Europe resistance by region showed a 5.6 % resistance in the northern, 6.2 % in the central and 23.6 % in the southern region.⁴⁷ Resistance to ofloxacin was shown by 18.34 % *Staphylococcus* isolates and 32.35 % *S. aureus* isolates in this study. Levofloxacin resistance stood at 9.47 % and 11.76 % for *Staphylococcus* and *S. aureus* isolates, respectively. However, 87.50 %, 75.00 % and 12.50 % MRSA isolates showed resistance to ciprofloxacin, ofloxacin and levofloxacin, respectively. In contrast, Agarwal et al reported 50 % MRSA isolates resistant to ciprofloxacin and 21.4 % for levofloxacin.¹²

Erythromycin-resistant *Staphylococcus* often exerts cross resistance to other macrolides, lincosamide and streptogramin type B (MLS_B).⁴⁸ In the present study erythromycin resistance was seen in 85.80 % and 85.29 % *Staphylococcus* and *S. aureus* isolates respectively. However, a lower resistance to erythromycin ranging between 66.66 % and 67.9 % has been observed by Bhatiani et al,³² Bala et al³⁷ and Kausalya et al.⁴⁹ Clindamycin resistance was shown in 36.69 % and 50 % *Staphylococcus* and *S. aureus* isolates, respectively, while in a study by Verma et al,²³ erythromycin and clindamycin resistance was found to be 52.8 % and 48.28 %, respectively in *S. aureus* isolates. In this study, 25.44 % *Staphylococcus* and 38.24 % *S. au-*

reus isolates respectively were tetracycline-resistant. A higher resistance was reported by Shittu and Lin and Duran et al who reported 55.9 % and 35.6 % resistance for *S. aureus* isolates, respectively.^{38, 50} In the present study, 75.00 % (6/8) MRSA isolates were found to be resistant to tetracycline which is much higher as reported by Agarwal et al.¹²

During the 17-year period of the studies by Cuevas et al there was low resistance of *S. aureus* to cotrimoxazole in all the studies (0.5 to 2.1 %).⁵¹ In this study, 82.35 % *S. aureus* isolates were resistant to cotrimoxazole while other studies conducted in India have reported a resistance of 63.84 %, ²³ 73.3 %, ³² 46.1 %, ²² 31.43 %⁹ and 57.1 %.⁴⁰ Present study showed that 87.50 % (7/8) MRSA isolates were resistant to cotrimoxazole which correlates with the study by Mohanasoundaram and Lalitha showing 82 % cotrimoxazole resistance among the MRSA isolates.³⁶ A somewhat higher resistance was reported by Pulimood et al (97.1 %)⁵² while low resistance in MRSA isolates was reported by Agarwal et al (57 %).¹²

In the present study, a total of 55.62 % (94/169) *Staphylococcus* isolates and 58.82 % *S. aureus* isolates showed resistance to quinupristin dalfopristin. All the MRSA isolates (8/8, 100 %) were found to be resistant to quinupristin dalfopristin, while in a study conducted by Kaur and Chate, only 5.56 % MRSA isolates were reported as resistant to quinupristin dalfopristin.⁵³

In this study, 100 % *Staphylococcus* and *S. aureus* isolates showed sensitivity to vancomycin. One MRSA isolate showed resistance to vancomycin disc on AST. However, due to limited resources, further testing of this isolate by MIC testing of vancomycin using agar dilution method (recommended by CLSI) to determine it as vancomycin-resistant (VRSA), intermediate (VISA) or sensitive (VSSA) could not be carried out. In a similar study, conducted at Kasturba Medical College, Hospital, Mangalore, no vancomycin resistance was observed in MRSA isolates.⁵⁴ Complete sensitivity to vancomycin of *S. aureus* isolates was reported by Anupurba et al and Datta et al.^{55, 56} In 2003, Assadullah et al reported staphylococcal isolates with intermediate susceptibility to vancomycin in India.⁵⁷ Tiwari and Sen reported two strains of VRSA in the northern parts of India.⁵⁸ Sharma and Vishwanath studied 156 MRSA isolates which were susceptible to vancomycin by

disc diffusion method but, the MIC of 18 isolates was $\geq 4 \mu\text{g/mL}$ (VISA).⁵⁹

This study showed 100 % susceptibility to linezolid and vancomycin. Vancomycin and linezolid were found to be the most sensitive drugs against *S. aureus* in studies by Agarwal et al and Bhatiani et al.^{12, 32} Golan et al reported a significant trend in increased MRSA linezolid resistance from 2002 onwards.⁶⁰ Linezolid, a member of the new oxazolidinone class of antibiotics is highly active *in vitro* against MRSA and has excellent oral bioavailability and constitutes the drug of choice against MRSA infection, besides vancomycin. The present study supported this.

Resistance to mupirocin is being reported from across the globe with a prevalence of 0.5 % in Nigeria to 14.6 % in India.^{50, 61} Rapid resistance to mupirocin has been reported among some strains of *S. aureus* isolated from various hospitals. In the present study of 34 *S. aureus* isolates, sensitivity to mupirocin was 88 % with isolates having MIC $< 0.5 \mu\text{g/mL}$. Mohajeri et al reported 100 % sensitivity to mupirocin in the nasal carriage isolates of the patients.⁶² Though mupirocin resistance was not seen in the *S. aureus* isolates in the study by Mohajeri et al, the MIC of 9.2 % of the isolates was as high as $4 \mu\text{g/mL}$ which was very close to a low level resistance ($8 \mu\text{g/mL}$).⁶² In the study by Saderi et al, 6 strains had MIC $> 4 \mu\text{g/mL}$.⁶³ Abimanyu et al observed all MRSA isolates showed a high level mupirocin resistance and inducible clindamycin resistance.⁶⁴

Agarwal et al reported that 4 (2 %) isolates were found to be mupirocin-resistant of which three isolates were high levels resistant.¹² In the presence of mupirocin-resistant strains, treatment with mupirocin may be ineffective, especially with high-level resistance strains. Although low-level mupirocin-resistant strains can be controlled by normal dosage schedule of mupirocin, a few studies suggest that treatment failure may occur. This emphasizes the importance of identification of both high and low-level resistant strains.⁶⁵⁻⁶⁷

Simple preventive measures like hand washing, using a sterile mask, gown and avoiding touching one's nose during work, should be reinforced in all healthcare settings. This study reiterates the need for periodic surveillance, early and accurate detection and treatment of MRSA carriers. This

should be accompanied with appropriate hospital infection control measures, to prevent the nasal carriage of MRSA in hospital healthcare workers.

Conclusion

In the present study, very high carriage rate was detected in the anterior nares which are also the commonest site for *Staphylococcus* colonisation. The results obtained from the antibiogram of Staphylococci, *S. aureus* and MRSA isolates from colonised HCWs showed the increase in rates of resistance against various antibiotics. The present study confirms for the first time the presence of MRSA in HCWs working in this hospital and demonstrates the prevalence of the antibiotic resistance amongst them. Vancomycin resistance in *Staphylococcus* species is beginning to emerge as a clinical threat, yet the attention it has received is scant and serves to underscore the seriousness of the problem.

A better understanding of these issues will be a key to help in the prevention and treatment of these infections in the future and in containing the spread of these from HCWs to patients and vice versa. All the HCWs should be periodically educated and trained in the maintenance of hygiene and infection control and the effects of the use or rather, the misuse of antibiotics.

The limitations of the study

The study enrolled HCWs from a single tertiary healthcare centre, however, to generalise the results multi-centric studies are required.

Contribution of Authors

MC was involved in planning, concept design and hypothesis generation, NA did data collection, AC did data assembly, literature review and manuscript writing, MB helped in statistical analysis, GP and VR helped in data interpretation and literature review, AT helped in manuscript writing and data visualisation. All the authors collaborated and finally approved the manuscript.

Ethics Statement

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the JLN Medical College, Ajmer (No 42954-85, dated 28-10-2016).

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None.

Conflict of interest

None.

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