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NASAL CARRIAGE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN HEALTH CARE WORKERS AND HEALTHY INDIVIDUALS IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Staphylococcus aureus is one of the pathogenic microorganisms widely present in human. Approximately, 25-30% of normal individuals are carrying S. aureus in their anterior nares and skin. It, commonly, causes both nosocomial and community-acquired infection. It can be treated with antibiotics, but with time, some strains of S. aureus have become resistant to antibiotics such as penicillin, methicillin, oxacillin. S. aureus having resistance against methicillin, is known as methicilin resistant Staphylococcus aureus (MRSA), was first discovered in 1961. Longer stay in hospital and prolonged antibiotic administration to the patients resulted in methicillin resistance. Presently, the prevalence rate of methicilin resistant Staphylococcus aureus in nasal swabs among health care workers (HCWs) and healthy individuals in a tertiary care hospital was studied. 60 nasal swab samples were collected and cultured on the blood agar plates. S. aureus was identified and confirmed using gram staining and biochemical tests. By Kirby-Bauer disc diffusion method, antibiotic susceptibility test was performed for the detection of MRSA using cefoxitin disc. In result, 41.66% S. aureus and 31.66% MRSA strains were isolated. MRSA carriage rate in females was higher than males. High prevalence rate of MRSA was detected in 21-40 years of age group. MRSA isolated in health care workers was 32.65%, among which, carriage rate was found highest in nurses. The present study concluded that percentage of MRSA prevalence was quite high among hospital care workers particularly in nurses. Therefore, periodical surveillance of MRSA should be done in all the health care workers in the hospital.

KEY WORDS

Antibiotic Resistance, Cefoxitin, Gram positive microbes, Health care workers, MRSA, Nasal carriage.

INTRODUCTION

Staphylococcus aureus is an opportunistic and commensal pathogen in healthy individuals and many homoeothermic species. In 30-50% healthy people, it commonly found on the skin and in anterior nares [1]. Worldwide, it is one of the leading causes of human bacterial infection [2].

S. aureus is gram-positive cocci, approximately 1 µm in diameter, and form grape-like (Greek *staphyle*) clusters indicative of the ability to divide in more than one plane. They grow both aerobically and anaerobically and most strains ferment mannitol anaerobically. They are catalase and coagulase positive. On blood agar, they form characteristic golden (Latin *aureum*) or white colonies [3].

S. aureus causes majorly skin infections and life-threatening diseases such as urinary tract infection, respiratory tract infection, intestinal tract infection, sepsis and soft tissue infection, blood-stream infections. It is generally non-pathogenic where it is present as commensal except when it gets access to deep tissues through broken skin, resulting in surgical site or wound infection, through the bloodstream leading to bloodstream infection or bacteraemia [4].

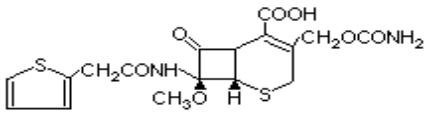
S. aureus has shown resistance to many classes of antibiotics. To treat staphylococcal infection, the first choice of antibiotics was Penicilin. In 1944, more than 90% *S. aureus* strains become resistant by destroying the penicillin by penicillinase [5]. To treat penicillin resistant *S. aureus*, methicillin (semi synthetic penicillin) was

used, but in 1962, *S. aureus* has also developed resistance against methicilin. Methicillin resistant *S. aureus* (MRSA) is mediated by the presence of PBP-2a which is expressed by an exogenous gene, *mec*A [6]. Methicillin-resistant *Staphylococcus aureus* (MRSA) had become a progressively more important human pathogen [7].

In hospitals, methicillin resistant *Staphylococcus aureus* (MRSA) strains became a major problem due to its rapid emergence. MRSA strains were restricted to hospitals only as Health care associated MRSA (HA-MRSA). But now, these strains were also emerging in the community, known as Community acquired MRSA (CA-MRSA) [8]. It has become a major pathogen in the community, hospitals, long term care facilities and tertiary care hospitals. The reservoir of MRSA is infected and colonized patients, and its major mode of transmission from patient to patient is through contaminated hands of healthcare workers and also its transmission is related with a dermatological condition or sharing of closed livings [9]. On admission approximately half of the MRSA infections diagnosed in the hospital and patients were not recognized as being colonized and attained colonization or infection directly or indirectly from patients.

Staphylococcus aureus is an adaptable bacterium that has an ability to attain antibiotic resistance. In 1960's, methicillin was recognized as the most recommendable agent for routine susceptibility testing, but now it is not used in treatment of MRSA [8]. Therefore, resistant strains are termed as 'methicillin-resistant *S. aureus*' (MRSA). Later oxacillin as an alternative to methicillin was used in susceptibility tests for detection of MRSA.

Recently, Cefoxitin has been investigated as an alternative agent for detection of MRSA by disc diffusion method and all studies indicated that tests with Cefoxitin are more reliable than those with oxacillin as cefoxitin is an inducer of *mec*A gene [10]. Cefoxitin is a bactericidal agent that acts by inhibition of bacterial cell wall synthesis. It has activity in the presence of some beta-lactamases, both penicillinases and cephalosporinases, of Gram-negative and Gram-positive bacteria [11].



$C_{16}H_{16}N_3NaO_7S_2$

Structure of Cefoxitin

Mechanisms of antibiotic resistance in Staphylococcus aureus[12] are enzymatic inactivation of the antibiotic (penicillinase and aminoglycoside-modification enzymes), alteration of the target with decreased affinity for the antibiotic (notable examples being penicillin-binding protein 2a of methicillin-resistant S. aureus), trapping of the antibiotic (for vancomycin and possibly daptomycin), efflux pumps (fluoroquinolones and tetracycline).

MRSA isolates in the hospitals and communities have been recognized as a major challenge. Therefore, the knowledge of prevalence of MRSA and their current antimicrobial profile become important in the selection of appropriate treatment of such infections. The present study is to determine the prevalence of MRSA in nasal swab samples from HCWs and healthy individuals in a tertiary care hospital.

MATERIALS AND METHODS

Study Area

The study was carried out in the microbiology laboratory of Tertiary care hospital, New Delhi, India. The nasal swabs were collected from healthy individual of both sexes and all age groups working in the hospital and people accompanying the patients in the outpatient department. The duration of the study was for three months from April 2014 to June 2014.

Sample Collection

Sterile cotton wool swabs moistened with sterile normal saline were used to collect the specimen from the anterior nares. The swabs were rubbed very well by rotating 5 times over the inner wall of the nasal septum. The swabs were kept in sterile test tubes, labeled it and transported to laboratory within an hour of its collection.

Culture and identification

Swabs were inoculated on to blood agar within one hour of collection and were incubated in 5% CO_2 at 37°C for 24hrs. After 24 hrs incubation, identification of cultured isolates was done according to the standard protocol. Colony morphology, hemolytic pattern, gram reaction and microscopic features were used as primary identification criteria for Gram positive coccus (Picture 1-2). Then, catalase and coagulase test was performed using slide test method for further identification [13].

Catalase test:

This is performed to differentiate between genus Staphylococcus and Streptococcus: Place a drop of H_2O_2 on a clean glass slide.

Take a small portion of test organism using a sterile wooden stick or plastic loop and mix with the drop of H_2O_2. Observe for immediate bubbling (gas production). If active bubbling is seen it is catalase +ve and confirms that the test organism is staphylococcus. While Streptococcus is catalase –ve.

Slide Coagulase test:

This test is done to detect bound coagulase: Place a drop of normal saline on a clean slide. Take few isolated colony from the plate and mix it. Add one drop of plasma onto the slide. Mix well and look for clumping within 10-15 seconds.

Tube Coagulase test:

This test was done to confirm the organism as *S. aureus* which gave a positive result on slide coagulase test.

Firstly, prepare a one-in-six dilution of the plasma in normal saline (0.85% NaCl) Place 1 ml of 1:6 dilutions in 3 small test tubes (positive control, negative control and test sample). Also, a tube of unseeded diluted plasma was kept to confirm that plasma did not clot spontaneously. Inoculate *S. aureus* strain in positive control, sample in test sample tube and only diluted plasma as negative control. The tubes are incubated at 37 ^{0}C in a water bath for 4 hours. The tubes are examined after 1, 2 and 4 hours for clot formation by tilting them through 90^0. The strains positive for tube coagulase test is observed and negative is left for overnight at room temperature and re-examined. The tubes which give positive result after overnight incubation is also labeled as coagulase positive. Those isolates tested coagulase positive both by slide and tube were taken as *S. aureus.*

Detection of MRSA by disc diffusion method

Colonies confirmed to be *S. aureus* were suspended in peptone water at 37^{o}C until matching with a standard turbidity (0.5 MacFarland). The suspension was used to inoculate a Mueller-Hinton agar (MHA). A Cefoxitin discs (30 µg) was placed on the plates and the plates were incubated for 24 hours at 37°C. Colonies with an inhibition zone of under 22mm for cefoxitin were read as “methicillin” resistant (Picture 3-4). In this study *Staphylococcus aureus* ATCC 25923 was used as control. This test was performed as per CLSI guidelines [14].

RESULTS

Out of 60 nasal swab samples, 25/60 (41.66%) were *S. aureus* and among which 19/60 (31.66%) MRSA strains were isolated.

In males and females, 33 and 27 nasal swabs were collected, respectively. Out of which in males 13/33(39.39%) were found to be *S. aureus* and 10/33(30.30%) isolates were MRSA and in females 12/27(44.44%) were found to be *S. aureus* and out of which 9/27(33.33%) isolates were MRSA. (Table 1) (Figure 1).

On the basis of age groups, out of 25 *S. aureus* isolates, 1 MRSA (14.28%) was isolated under the age group of (1-20 yrs), from (21-40 yrs) age group 17 MRSA(40.47%) were isolated, and from (41-60 yrs) age group 1 MRSA was isolated.(Table 2)(Figure 2)

The nasal carriage of MRSA in health care workers were 32.65% (16/49) and in persons accompanying OPD patients 27.27% (3/11).(Table 3)(Figure 3)

MRSA carriage was high among nurses (3/4; 75%) followed by house man (2/4; 50%), trainee (3/10; 30%), lab technicians (2/7; 28.57%), attendants (5/19; 26.31%), and doctors (1/5; 20%). As shown in Table 4, Figure 4.

Figure1-2: Bacterial culture (*S. aureus*) on blood agar plate and its microscopic view.

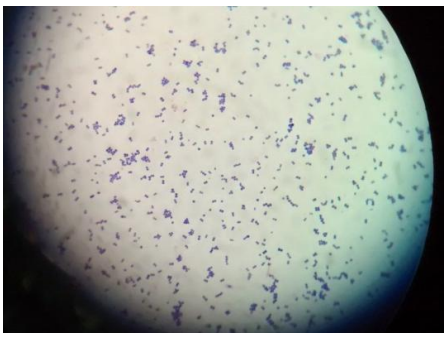
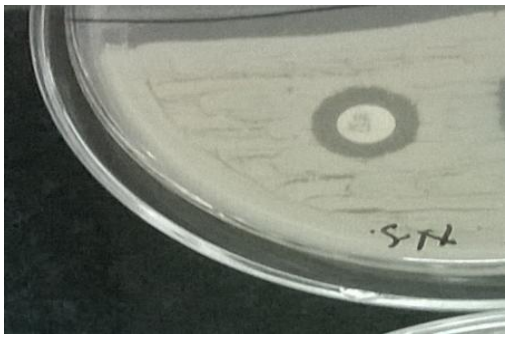


Figure 3-4: Detection of MRSA and MSSA by Kirby- Bauer Disc Diffusion method



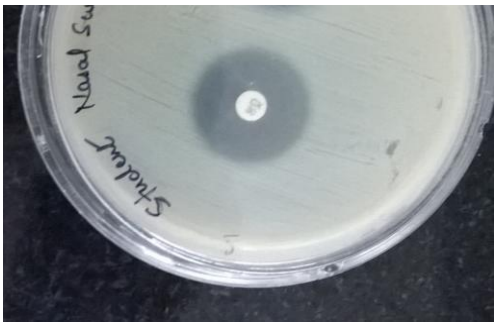


Table 1- Number and Percentage of MRSA and MSSA isolates present in Males and Females

Sex	No. of nasal swab samples	No. of *S.aureus* isolates	No. of MRSA isolates	% MRSA isolates	No. of MSSA isolates	% MSSA isolates
Male	33	13	10	30.30	03	9.09
Female	27	12	09	33.33	03	11.11

Table 2- Number and Percentage of MRSA and MSSA isolates in different age groups

AGE GROUPS (in yrs)	MRSA isolates		MSSA isolates	
	No.	%	No.	%
1-20	01/07	14.28	02/07	28.57
21-40	17/42	40.47	02/42	4.76
41-60	01/11	9.09	02/11	18.18

Table 3: Number and Percentage of MRSA and MSSA isolated from Health care workers and Persons accompanying OPD patients

	Health care workers	Persons accompanying OPD patients	Total Number
No. of Nasal Swab Samples	49(81.66%)	11(18.33%)	60
No. of MRSA	16(32.65%)	03(27.27%)	19(31.66%)
No. of MSSA	05(10.20%)	01(9.09%)	07(11.66%)

Table 4- Distribution of *S. aureus*and MRSA isolates among health care workers

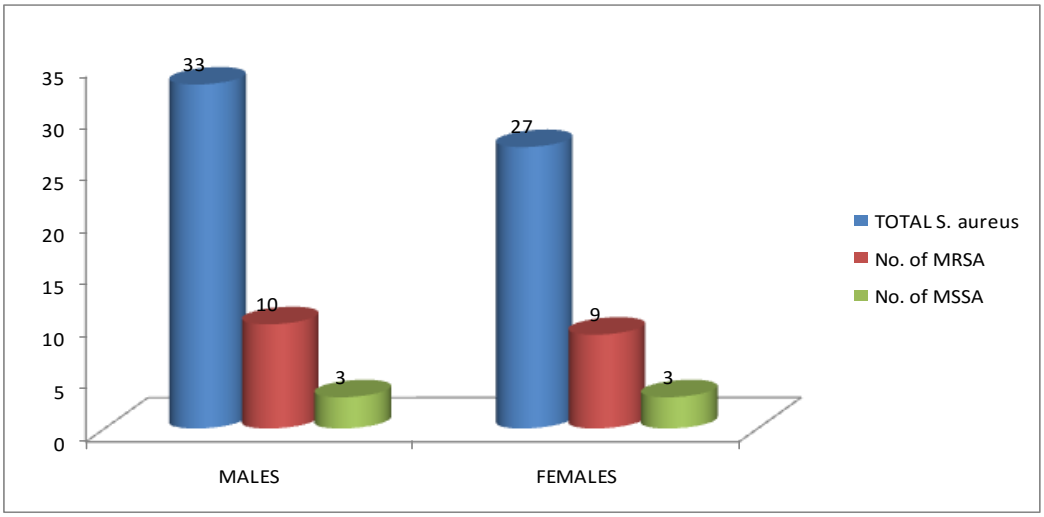
Profession	No. of nasal samples	*S. aureus* isolates	MRSA isolates	MSSA isolates
Doctors	05	02	01(20%)	01(20%)
Attendants	19	05	05(26.31%)	00
Lab. Technicians	07	03	02(28.57%)	01(14.28%)
Trainee	10	04	03(30%)	01(10%)
House man	04	03	02(50%)	01(25%)
Nurses	04	04	03(75%)	01(25%)

Table 5-Detail of OUTDOOR- Persons accompanying patients in Hospial Area; INDOOR- Working staff in Hospital or in Laboratories.

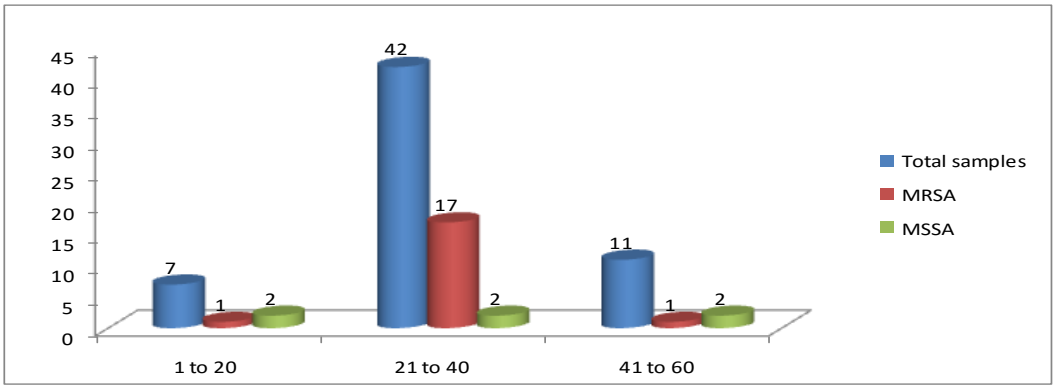
S.no.	Names of healthy persons	Age	Sex M/F	Indoor/ Outdoor	Colonies observed	Gram Staining	Catalase Test	Coagulase Test	Drug Sensitivity
									Cefoxitin (Cx) 30µg
N1.	Sushil Sharma	45	M	Outdoor	Small whitish colonies	GPC	+ve	-ve	
N2.	Ansh	17	M	Outdoor	Small colonies	GPC	+ve	+ve	24mm
N3.	Ajit	22	M	Outdoor	Small colonies	GPC	+ve	-ve	
N4.	Anubhav	22	M	Indoor Trainee)	Small colonies	GPC	+ve	-ve	
N5.	Rohit	22	M	Indoor (HCW)	Whitish colony	GPC	+ve	-ve	
N6.	Bhumi	19	F	Outdoor	Whitish colonies	GPC	+ve	-ve	
N7.	Surajbhan	21	M	Indoor (Trainee)	Small colonies	GPC	+ve	-ve	
N8.	Ruchi	30	F	Indoor (Cleaner)	Small colonies	GPC	+ve	-ve	
N9.	Shahzad	25	M	Indoor (Lab attendant)	Grey colonies	GPC	+ve	-ve	
N10.	Nitin	22	M	Indoor (Lab attendant)	Small colonies	GPC	+ve	-ve	
N11.	Malik	27	M	Indoor (Lab attendant)	Small colonies	GPC	+ve	-ve	
N12.	Chetan	21	M	Indoor (Trainee)	Whitish colonies	GPC	+ve	-ve	
N13.	Asha	34	F	Indoor (Cleaner)	Brown colony	GPC	+ve	+ve	17mm

N14.	Nikki	20	F	Indoor (Trainee)	Small colonies	GPC	+ve	-ve	
N15.	Sunil kumar	42	M	Indoor (HCW)	Small colonies	GPC	+ve	-ve	
N16.	Vipin	30	M	Indoor (HCW)	Small colonies	GPC	+ve	+ve	16mm
N17.	Lokesh	35	M	Indoor (HCW)	Small colonies	GPC	+ve	-ve	
N18.	Rekha	20	F	Indoor (Trainee)	Small whitish colonies	GPC	+ve	-ve	
N19.	Sintheles	45	F	Outdoor	Small colony	GPC	+ve	-ve	
N20.	Khurshid	35	F	Outdoor	Grey colony	GPC	+ve	-ve	
N21.	Santosh	27	M	Indoor (HCW)	Small colonies	GPC	+ve	-ve	
N22.	Geeta	42	F	Indoor (HCW)	Small colonies	GPC	+ve	-ve	
N23.	Dr. Sangeeta	49	F	Indoor (Doctor)	Whitish colonies	GPC	+ve	-ve	
N24.	Manojsingal	38	M	Indoor (HCW)	Small whitish colony	GPC	+ve	-ve	
N25.	Snehlata	23	F	Indoor (Trainee)	Small whitish colony	GPC	+ve	-ve	
N26.	Yogesh	30	M	Indoor (HCW)	Small colonies	GPC	+ve	-ve	
N27.	Krishnavtar	34	M	Indoor (HCW)	Small brown colonies	GPC	+ve	+ve	14mm
N28.	Satish	30	M	Indoor (Lab technician)	Small colonies	GPC	+ve	+ve	12mm
N29.	Kapil	29	M	Indoor (Lab technician)	Small whitish colonies	GPC	+ve	-ve	
N30.	Rajkumar	30	M	Indoor (Lab attendant)	Small brown colony	GPC	+ve	+ve	18mm
N31.	Pawan	24	M	Indoor (Lab attendant)	Small colonies	GPC	+ve	+ve	18mm
N32.	Sudha	40	F	Indoor (Cleaner)	Small colonies	GPC	+ve	+ve	16mm
N33.	Dhruv	32	M	Indoor (Cleaner)	Small whitish colonies	GPC	+ve	+ve	24mm
N34.	Lovely	21	F	Indoor (Trainee)	Brown coloured colony	GPC	+ve	+ve	12mm
N35.	Dr. Renu	40	F	Indoor (Doctor)	Small sized colonies	GPC	+ve	+ve	10mm
N36.	Dr. Mausami	35	F	Indoor (Doctor)	Small colonies	GPC	+ve	+ve	30mm
N37.	Dhirender	23	M	Indoor (Trainee)	Small colony	GPC	+ve	+ve	12mm
N38.	Urmilla	26	F	Indoor (Nurse)	Grey colonies	GPC	+ve	+ve	15mm
N39.	Rajni	25	F	Indoor (Nurse)	Small colonies	GPC	+ve	+ve	14mm

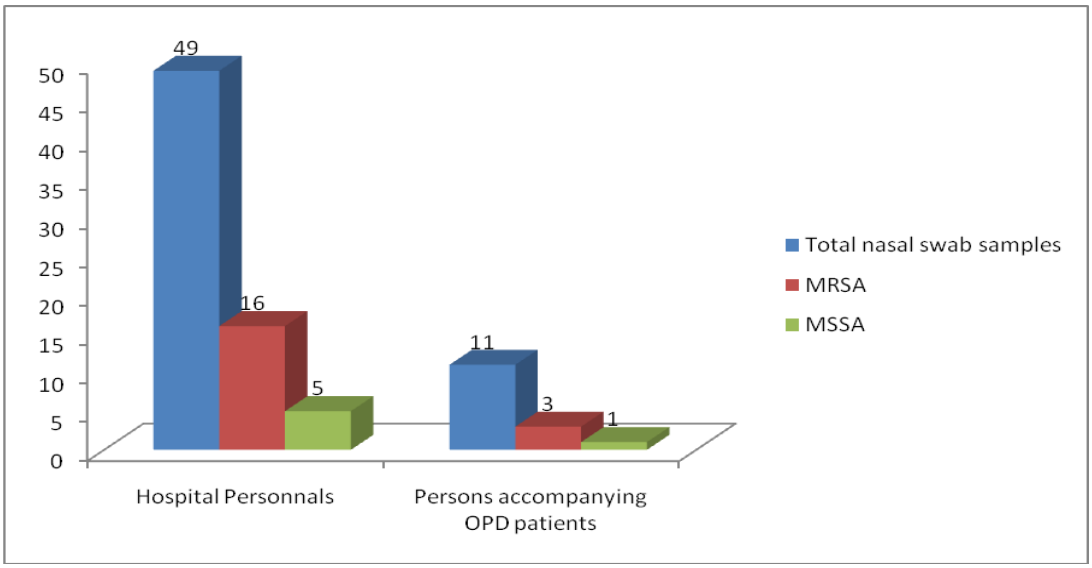
N40.	Pushparaj	50	F	Indoor (Nurse)	Small colonies	GPC	+ve	+ve	22mm
N41.	Pooja	19	F	Indoor (Trainee)	Small whitish colony	GPC	+ve	+ve	24mm
N42.	Vivek	42	M	Indoor (Lab technician)	Small colonies	GPC	+ve	+ve	22mm
N43.	Prakash	30	M	Outdoor	Small colonies	GPC	+ve	+ve	15mm
N44.	Neeraj	21	M	Indoor (HCW)	Small colonies	GPC	+ve	+ve	13mm
N45.	Himanshu	19	M	Outdoor	Small colonies	GPC	+ve	+ve	17mm
N46.	Sapna	27	F	Indoor (Lab technician)	Small colonies	GPC	+ve	+ve	10mm
N47.	Preeti	34	F	Indoor (Nurse)	Small whitish colony	GPC	+ve	+ve	No zone
N48.	Mohini	21	F	Indoor (Trainee)	Small whitish colonies	GPC	+ve	+ve	12mm
N49.	Manish	22	M	Indoor (Lab technician)	Small sized colonies	GPC	+ve	-ve	
N50.	Nupur	26	F	Indoor (Lab technician)	Small colonies	GPC	+ve	-ve	
N51.	Meena	35	F	Outdoor	Small colonies	GPC	+ve	-ve	
N52.	Sushil	56	M	Outdoor	Brown colonies	GPC	+ve	+ve	16mm
N53.	Harshgil	45	F	Outdoor	Small colonies	GPC	+ve	-ve	
N54.	Dr. Priyanka	23	F	Indoor (Doctor)	Small sized colony	GPC	+ve	-ve	
N55.	Sandhya	33	F	Indoor (HCW)	Whitish colony	GPC	+ve	-ve	
N56.	Dr. Rajeev	50	M	Indoor (Doctor)	Small colonies	GPC	+ve	-ve	
N57.	Rajkumari	42	F	Indoor (HCW)	Grey colony	GPC	+ve	-ve	
N58.	Bitoo	23	M	Indoor (HCW)	Small colonies	GPC	+ve	-ve	
N59.	Vicky	20	M	Indoor (HCW)	Small sized colony	GPC	+ve	-ve	
N60.	Aditya	24	M	Indoor (lab technician)	Small brownish colonies	GPC	+ve	-ve	



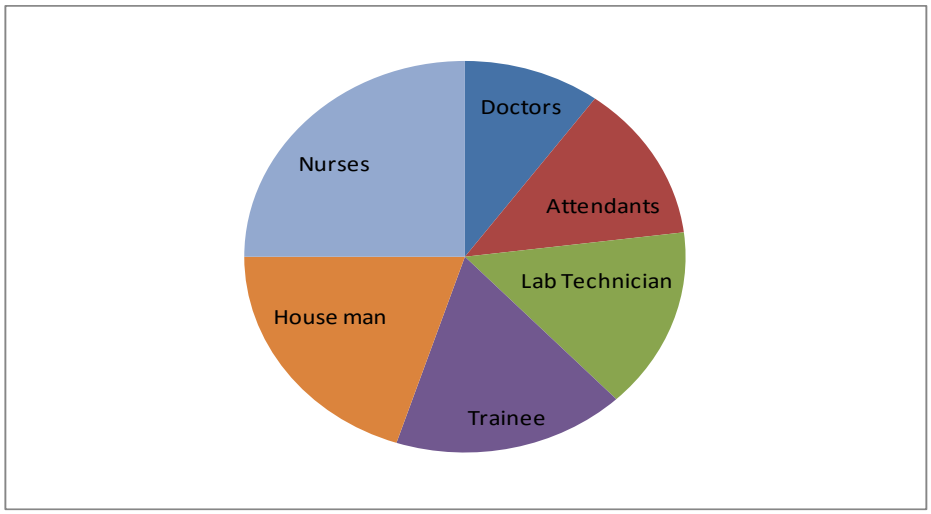
Graph1: Prevalence of MRSA and MSSA in males and females.



Graph2: Prevalence of MRSA and MSSA in Healthy persons under different age groups.



Graph3: Prevalence of MRSA and MSSA among Health care workers and Persons accompanying OPD patients



Graph4: Chart depicting the percentage of health care workers having MRSA colonization in their nasal swabs.

DISCUSSION

In healthy individuals, *Staphylococcus aureus* is a colonizer in the anterior nares. Most invasive infections are assumed to be originated from nasal carriage. Hence, it is necessary that nasal carriage of *S. aureus* strains should be prevented. People, those are in hospitals or long term care settings, have more chances of getting infected by MRSA, and now it is spreading from hospital to the community at a large scale.

According to present study, out of 60 nasal swab samples, 31.66% MRSA was detected which is showing similar result with the study done in a tertiary referral hospital in eastern Uttar Pradesh i.e., 54.8% and another study from north India reported 46% prevalence rate of MRSA[15, 16]. According to this study, prevalence rate of MRSA in males and females was found to be 30.30%and 33.33%, respectively. In contrast, another study done in South India reported 12.4% MRSA in male and 2.4% in females [17]. According to this study, prevalence rate of MRSA in health care workers was found 32.65%, whereas a study conducted in Sikkim reported 20.92% prevalence rate of MRSA in nasal samples from different categories of health care workers [18]. Another study was conducted in Moradabad, Uttar Pradesh, out of 110 nasal swabs from healthcare personnel of tertiary hospital, 36.84% were MRSA carriage in the healthcare personnel [19].

Anand *et al.* [20] have shown the comparison of cefoxitin disc diffusion test, oxacillin screen agar, and PCR for *mecA* gene for detection of MRSA. Cefoxitin was superior to oxacillin disc for detection of methicillin resistance. Rasheed *et al.* [21] also concluded that the cefoxitin disc diffusion tests can be a suitable alternative to PCR for detection of MRSA.

Some studies reported that vancomycin and mupirocin have shown 100% sensitivity against MRSA, so these drugs can be used for the treatment of MRSA infections [15, 22].

CONCLUSION

The present study found the percentage of MRSA prevalence is quite high in health care workers and healthy individuals. Prevalence of MRSA is found more in females than males. As the collected nasal samples were from a tertiary care hospital, MRSA carriage is seen more among the hospital care workers, particularly in nurses. In any medical facility, nurses are the one who are in immediate contact with many patients. It means that the health care workers should follow strict infection control practices while coming in contact with patients and also with relatives and visitors to the hospital. Therefore, healthcare workers should be provided with hand hygiene practices, contact precautions, identifying previously colonized patients. As well as, continuous monitoring of antibiotic susceptibility pattern should be done as irrational antibiotic prescription causes high prevalence of MRSA in

hospitals/community. In order to reduce the MRSA, it is necessary to formulate awareness and educational programs/policies especially in hospitals so that MRSA related infections can be controlled.

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