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Original Research Article

Study of Nasal Carriage of Staphylococcus Aureus among Hospital Staff with Special Reference to Methicillin Resistance and Bacteriophage Type

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ABSTRACT

Total 150 subjects were screened for nasal carriage of Staphylococcus Aureus and for Methicillin Resistant Staphylococcus Aureus. Anterior nasal swabs were collected from medical and paramedical staff (50 Doctors, 50 Nursing staff and 50 Attendants) working in Krishna Hospital and Medical Research Centre, Karad, Maharashtra. Each specimen was processed as per standard procedure. The bacterial strains were identified by conventional method and the antibiotic resistance was carried out by disc diffusion method. A total of 74 S. Aureus strains were isolated, 63 were MSSA and 11 were MRSA (14.8%).The carriage of S. aureus in nursing staff was much higher than Doctors and attendants. All 74 S. Aureus isolates were subjected to bacteriophage typing at Department of Microbiology, Maulana Azad Medical College, New Delhi. 11 strains were typable and 63 were untypable. Among the MRSA (11 Strain) 1 was typable and phage type was 3A 71(Group II) rest are untypable. Out of 63 MSSA only 10 were typable by conventional phage typing method. Common phage group observed was Group-II in 6 Strains, and Group III in 2 strains, while another 2 strains are of mixed group. Nursing staff were found to be at more risk for colonization of S. aureus and MRSA. In females carriage rate was 20% and in males 11.3%. All MSSA and MRSA strains were 100% sensitive to Vancomycin.

Most of the MRSA strains were sensitive Ofloxacin. Mupriocin ointment advised to carriers for elimination of the strains .Continuous surveillance and improvement of hygiene standards in hospitals should be adopted.
Study was undertaken to determine the frequency of nasal carriage of S. aureus among hospital staff and to subject the isolated strains of S. aureus for antibiotic sensitivity with special reference to methicillin sensitivity and to know bacteriophage type of the isolated strains.
Key Words: MRSA- Methicillin Resistant Staphylococcus Aureus, MSSA- Methicillin Sensitive Staphylococcus Aureus.

INTRODUCTION

Nosocomial infection is a significant epidemiological problem. It can result in prolongation of hospital stay, increased mortality and morbidity and adds to the cost of health care. In the last decade gram positive bacteria became major pathogens associated with Nosocomial infections. More than 50% of these infections are caused by methicillin resistant staphylococcus aureus (MRSA).In 2007, the center for Disease control reported that MRSA causes 19000 deaths every year in US, which is more than HIV/AIDS cases. Almost 20% of people who contract MRSA are reported to die from it, and an increasing number of its victims are young. Methicillin, the first semi synthetic penicillin, was introduced into clinical use in England in 1959. European hospitals, however, observed methicillin-resistant strains of S. aureus just two year later, and by the 1980s MRSA had became widespread in hospitals throughout the world including India. [1]

In the relationship between man and S. aureus, overt infection is merely the tip of an epidemiological iceberg. Beneath the surface lurks a silent epidemic of in apparent skin and nasal carriage which plays a crucial role in persistence and spread of S. aureus and in its outstanding success as a nosocomial pathogen. This ever present reservoir of infections termed the carrier state and has an average prevalence of 25% in hospital staff. Because S. aureus primary habitat is moist squamous epithelium of the anterior nares most invasive S. aureus infections are assumed to arise from nasal carriage. [2] About 25-30 % of healthy people in community carry this organism at any given time. Almost 25% of the healthcare workers are stable nasal carriers, and 30 % 50% of them also possess the bacteria on their hand. [3] It can be disseminated by hand to nose contact by desquamation of epithelium or by droplet spread during respiratory infection. Person who have direct contact with patient including nurses, medical hose staff, clinical faculty, attending physician, paramedical personnel, nursing and medical students are more likely than other hospital personnel to be involved in disease transmission. Methicillin Resistant Staphylococcus Aureus (MRSA) is considered endemic in the UK National Health Service (NHS), and routine MRSA screening of hospital inpatients has recently been introduced in both Scotland and England. A literature review was conducted to examine whether MRSA screening in Scotland should be expanded to include the routine screening of healthcare workers (HCWs). Estimates of HCW carriage from the worldwide literature vary widely depending on the country, hospital specialty and setting (endemic, non endemic or outbreak). Recent studies conducted in endemic hospital settings report non-outbreak carriage rates of 0-15% .The role of HCW carriage in the transmission of MRSA is not well understood . Persistent carriage could act as a reservoir for infection and HCWs have

been implicated as the source in a number of published outbreak reports. [4]

Present study was undertaken to evaluate the rate of nasal carriage of S. aureus, MRSA and to know its Bacteriophage type in hospital staff, we have attempted to study nasal carriage of MRSA in hospital staff of K.H. & M.R.C, KARAD.

MATERIAL AND METHODS

The study was conducted at the department of microbiology, Krishna Institute of medical sciences and K. H. M. R. C. Karad.

Specimen Collection: Specimens were collected from the anterior nares with sterile dry cotton swabs. The swabs was circled in both nostril of medical and paramedical Staff which includes 50 Doctors, 50 Nursing staff and 50 Attendants working in hospital with consent.

Microscopy: Each specimen was smeared on clean, dry glass slide and was stained with gram stain. The stained smear was microscopically examined under oil immersion lens (100 xs) for the presence of gram positive cocci and epithelial cell.

Bacteriological culture: All specimens were inoculated on Blood agar, chocolate agar MacConkey agar, mannitol salt agar and incubated at 37^{o} for24- 48 hrs in incubator. Well isolated colonies were initially Gram-stained and then biochemical characterized according to Bergey 'manual of Determinative Bacteriology. S. aureus strains were identified on the basis of colony morphology, microscopy and confirmatory biochemical test such as catalase test, coagulase test, modified Hugh and leifson (0/F) test, mannitol sugar fermentation test, phosphatase test.

Antibiotic Susceptibility Test: It was performed by standard Kirby- Bauer disc diffusion technique against routine antibiotics (Bauer A.W. and Kirby M.M.1966).Muller –Hinton agar medium was used for the antibiotic sensitivity testing. Oxford Strains of Staphylococcus aureus NCTC 6571 was used as control throughout the study. The inoculums of the test strain to a density visually equivalent to 0.5 McFarland turbidity standards was prepared by growing the strain in peptone water. The entire surface of plate of medium was inoculated using sterile cotton swab soaked in the inoculum and allows drying before the application of antibiotic discs. To check methicillin resistance for all isolates 1 µg oxacillin discs (Hi Media Mumbai India). [5] The plates were inverted and incubated at 35-37^{0}C for 24 hrs. The diameter of the clear zone around the disc was measured using a measuring scale and the results were interpreted as susceptible, moderately susceptible or resistant as per recommendations.

Bacteriophage typing

All strains were stoked in nutrient agar bulb and send to National phage typing center at Department of Microbiology, Maulana Azad Medical College, and New Delhi. The phage typing system was originally introduced by Wilson and Atkins and later it was reclassified and used internationally with 23 standard typing phages in 5 groups. Typing can be done using a conventional set 23 phages and a set of MRSA phages which include ten phages Viz.M3,M5,MR8,MR12, MR25, 622, 30,33, 38, and 56B. National phage typing center at Department of Microbiology, Maulana Azad Medical college (M.A.M.C), New Delhi have propagated and standardized the phages that they received from Central Public Health Laboratory ,Colindale U K., and which will be used for typing of S. aureus and MRSA strains . In present study, S. aureus and Methicillin resistant Staphylococcus aureus are phage typed by conventional method. MRSA phages are

not used for MRSA strains. The strain to be typed is inoculated on plate of nutrient agar to produce a lawn culture. After drying, the phages are applied over marked squares in a fixed dose. After overnight incubation, the culture will be observed to be lyses by some phages but not by others. The phage type strains are expressed by the designations of phages that lyse it.

RESULTS

Table no 1:

Age Group	MALE				FEMALE			
	No. of male	**S. aureus**	**MSSA**	**MRSA**	**No of female**	**S. aureus**	**MSSA**	**MRSA**
0-15 Yrs	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
16-30Yrs	44	24	22	02(8.3%)	50	19	14	05(26.3%)
31-45Yrs	33	18	15	03(16.6%)	15	10	09	01 (10%)
46 onwards	06	02	02	00(0%)	02	01	01	00(0%)
Total	83	44	39	05(11.3%)	67	30	24	06(20%)

Table shows the distribution of S. aureus, both MRSA and MSSA in different age groups of male and female staff members. Out of 83 males in various age groups, 44 had nasal carriage of S. aureus. Among these isolates 5 (of 44) i.e. 11.3% showed methicillin resistance. Maximum numbers of isolates were from age group 16-30 yrs. as majority of staff members belong to these groups. Out of 67 female screened 30 subjects were nasal carriages of S. aureus. Out of these isolates, 24 were MSSA and 6 were MRSA. High carriage rate was again in age group 16-30 yrs. which is similar to male age group.

Table no .2
ISOLATION OF S. AUREUS AND MRSA IN DIFFERENT PROFESSION

Profession	S. aureus	Methicillin Sensitive	Methicillin Resistance	% of Methicillin Resistance
Attendants N=50	25	23	02	8 %
Nurses N=50	26	19	07	26.9 %
Doctors N=50	23	21	02	8.6%
Total N= 150	74	63	11	14.8 %

Table shows distribution of S. aureus and of MRSA in three different professionals. A total of 74 S. aureus isolated in 150 staffs, 63 were MSSA and 11 were MRSA. Out of these 74 S. aureus

11 (14.8%) were MRSA. The carriage of S. aureus in nursing staff was much higher than doctors and attendants. This may be due to close contact with patient or due to constant contact with hospital environment by their natural duty.

A total of 150 subjects screened. Out of these 74 were nasal carriers, among which 11 had MRSA carriage. Out of 74 S. aureus 11 strains were typable and 63 were untypable. Among the MRSA (11 strains) 1 was typable and rest are untypable.

Table No. 3
BACTRIOPHAGE TYPING OF S. AUREUS.

Isolate No.	Phage Type	Phage group
09	3A, 71	II
16	54	III
35	3A	II
38	52, 52 A,79,80,81,96	I, Unclassified, V
41	71	II
43	29, 54	I, II
45	71	II
48	71	II
51	71	II
52	71	II
53	54	III

* Isolate no -9 is MRSA and rest are MSSA.

Out of 150 cases, 74 S. aureus were isolated from nose of hospital staff. These 74 (including 11 MRSA) were subjected to phage typing at M.A.M.C, New Delhi. Out of 11 (MRSA) only one strain was typable and phage type was 3A, 71(Group-II). Out of 63 (MSSA) only 10 were typable by conventional phage typing method. Common phage group observed was Group II in 6 strains and Group III in 2 strains, while another 2 strain are of mixed group.

Compared to MRSA resistance pattern, percentage of resistance in MSSA to various antibiotics is low. The higher percentage of resistance was seen to Penicillin (90.4%), followed by Amoxicillin (85.7%), Ceftazidime (71.4%), Gentamycin (52.3%), Co-trimoxazole (46%), Doxycyclin (41.2%), Amikacin (38%), Cephalexin (31.7%), Netimycin (30.1%), Cefotaxim (20.6%), Ciprofloxacin and Erythromycin (12.6 %) and Ofloxacin (9.5 %). All strains were uniformly sensitive to Vancomycin.

DISCUSSION

Numerous outbreaks have been reported in United States particularly within the recent years, occurring most frequently in large tertiary care referral hospitals with medical school affiliations e.g. Haley and his colleagues [6] found that among 63 hospitals participating in the National Nosocomial Infection Survey, the four medical school affiliated hospital with 600 beds reported a frequency of methicillin resistant staphylococci that increased from 2% in 1975 and 18% in 1981. They also suggested that the increase of MRSA in large teaching hospitals was due not only to large number of patient at high risk of infection, existence of special care units such as burn units and oncology units but

also interhospital spread of organism by frequent transfer of house staff and by infected patients.

Colonized patients represent major source of S. aureus infection in hospitals. Approximately 10 to 40 % of individuals cultured as outpatients or at admission will be nasal carriers of S. aureus, and act as endogenous source for already colonized patients or as a source from which organism spread to other patients. Many studies carried to find the colonized patient as a source of infection in various outbreak of MRSA. But another important reservoir that is healthcare worker from which S. aureus can be transmitted to hospital patient was ignored. In 1999 Abha, Saudi Arabia, Alghaity A.A [7] determined the prevalence of nasal carriage staphylococcus aureus, Antibiogram and MRSA prevalence in community and hospital personnel. S. aureus was isolated 26.1% of 299 adults in community and 25.4% of 279 of hospital personnel. MRSA was isolated respectively from 5.1% and 18.3% of non hospital and hospital carriers. This indicates that the carriage of MRSA in hospital environment was high and antibiogram also shows that the strain isolated from hospital personnel were more resistant than from non hospital personnel. This may be due to indiscriminate use of antibiotics in hospitalized patients and such resistant strain might colonize the hospital person and act as reservoir of infections with multidrug resistant strain. Mitsuda T, et al [8] examined two persistent MRSA carrier nurses in maternity hospital to elucidate transmission of MRSA from health care providers (HCW) to new born infants and to the nurses own families. Hence screening of HCW is necessary in patient's view and also in respect to their own family health.

Vinodkumaradithyaa, A, ET al [5] stated in surgical wards 13% of participants carried S. aureus. Out of which 13 carriers, 5 each were doctors and nursing students. One in each category was resistant to methicillin. In our study 74 (49.33%) carried S.aureus.Out of which 11 (14.8%) were MRSA.Out of these MRSA 2 were in Doctor, 2 were in attendants and 7 were in nursing staff. As compared to all above studies S. aureus carriage is high (49.33%) but MRSA carriage is nearly same (14.8%).

A.P. Mehta [9] under took the Surveillance study of Methicillin-resistance Staphylococcus from 1992-1996. They evaluated the incidence in patient and carrier state of healthcare. Out of total healthcare workers 13% were carrying MRSA .This carriage was estimated during the epidemic period of study, which was nearly similar to our study. This states that carrier rate was increased in the epidemic period which is risk factor for transmission of MRSA infection. Therefore if prevalence rate of carriage of MRSA is known, the future epidemic can be controlled by decolonizing the carriers. Various other studies showed that by reducing the carriage rate of S. aureus in healthcare workers reduces the chance of epidemic in hospitals and helps to control the epidemic due to MRSA.

As various study shows MRSA isolates either from clinical specimen or from carriers (patient or healthcare workers) were resistant to most of the antibiotics and sensitive to vancomycin. Few reports of Vancomycin Intermediate Staphylococcus aureus (VISA) has been reported in Japan and France. In France Marie-Cecile Ploy, Bruno Francois, Marcelle Mounier ,Philippe Vignon and Francois Denis, [10] screened 1015 patients hospitalized in intensive care unit (ICU) and they found 15(1.5%) were colonized or infected with VISA. Accordingly, they evaluated the prevalence of carriage of VISA in the ICU staff. Out of total 98 persons 88(90%) were screened by using nasal swab (10 physician, 5 medical student, 69 nurses and 4

therapists). Seventeen(19.3%) of 88 persons screened carried a S. aureus strains susceptible to methicillin and vancomycin, 1 person (1.1%) carried an MRSA strain susceptible to vancomycin , and 3 persons(3.4%) carried a VISA strain with low level resistance to vancomycin(MIC range,4-6 ml). In our study all strains were uniformly sensitive to vancomycin.

In our study we isolated total of 74 strains from anterior nares of 150 staff including doctors (50), nurses (50), and attendants (50). Out of 74 isolates 11 were MRSA and 63 were Methicillin sensitive, all these strains are subjected to phage typing at M.A.M.C. New Delhi. Out of the 11 MRSA strains 7 strains isolated from nurses, 2 from doctors and 2 from attendants. Most of MRSA strains are non-typable by conventional phages with 100 Routine Test dilutions (RTD). Out of 74 isolates of S. aureus including MRSA, only 11 strains (14.8 %) are typable. Out of 11 MRSA only one strain was typable and phage type was 3A, 71. This strain was isolated from anterior nares of staff nurse. Out of 63 S. aureus isolates 10 isolates are typble(5 of nursing staff,2 of attendant, and 3 of doctor).Out of 5 isolates from nurses 4 were carrying same Phage type 71, one carry type 3A 71.These nurses are working in different wards. Strains isolated from attendants were 2, one carry 3A and another 29 phage type. Three doctors from different department one from surgery, one from ophthalmology and one from Anesthesia department carry S. aureus in their nose. Out of these 3 doctors, 2 doctors carry same phage type i.e. 54 and one carry 71 phage type.

Elimination Strategies for nasal carriage

It is conceivable that in populations in which S. aureus nasal carriage is identified as risk factor for infection, elimination of carriage would reduce the infection rate. Three approaches of elimination of carriage are available. The first is the local application of antibiotics, disinfectant or combination of both e.g. mupirocin antibiotic available for topical use. A second approach is administration of systemic antibiotics. To date, only rifampin has proven to be an effective agent, but side effects and the rapid emergence of resistant strain have limited its use for this purpose .The third strategy is bacterial interference, i.e., active colonization with a strain of S. aureus (type 502A) which is considered to possess minimal pathogenic properties but is able to prevent colonization by more virulent strains presumably by competition for the binding sites in the nose. In conclusion, most strategies to eliminate the carriage of S. aureus have been disappointing. Mupirocin has offered a new opportunity for this purpose and considered by far the most effective agent available. In our study we suggested mupriocin ointment and hand washing for elimination of nasal carriage in our staff. [11]

CONCLUSION

The study shows that 49.9% of hospital staff carries S. aureus in their anterior noses, out of total 74 isolates, 63(85.1%) were methicillin sensitive and 11(14.8%) were methicillin resistant. All MRSA strains were 100% resistant to Penicillin, Amoxycillin, followed by 81.8% to Cotrimazole and Cefotaxime. MSSA strains were also resistant to Penicillin but percentage was less as compared to MRSA. Both MRSA strains and MSSA strains were 100% sensitive to Vancomycin. MRSA susceptibility to ofloxacin was next to vancomycin. Nasal carriage of S. aureus was higher in nursing profession with common phage 71. Females are more carriers and age group 16-30 year in both sexes has high percentage of S. aureus and MRSA. Our study highlighted the carriage rate of S.

aureus and MRSA in our hospital settings and suggested for appropriate measure to prevent feature epidemic.

Limitations and further needs of study:

We did not confirm MRSA status by doing mecA gene due to financial constrains. We have not tested MRSA strains for teicoplanin because of non availability in our settings. Continuous surveillance is needed for further study and have to screen not only healthcare workers but also the patients attending OPD and admitted patient in hospital.

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