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Screening for detection of methicillin-resistant *Staphylococcus aureus* in Doon Valley Hospitals, Uttarakhand

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Abstract

Present study was conducted to assess the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) among patients at various hospitals in Doon Valley, Uttrakhand. A total of 300 nasal swabs (male patients: 177, female patients: 123) were subjected to bacteriological investigation following established protocol. Isolates were verified by mannitol fermentation, Gram staining, DNAse test and coagulase positivity. *S .aureus* was isolated in 111 (37%) participants (M: 37%, F: 36.5%). Out of 111 *S. aureus* isolates, 38 (34.2%) were methicillin resistant (MRSA). Among them, 25 (38%) were male and 13 (29%) were from female. Highest MRSA colonization rate was found among dialysis ward patients (55.5%), followed by burn ward (32.5%) and general medical ward (22.7%) patients. The study also revealed that administration of recent antibiotic was chief predisposing factor for MRSA colonization. High MRSA carriage rate found in this study indicates demand for standard infection control to curb transmission.

Key words

Hospitals, Methicillin-resistant, *Staphylococcus aureus*

Introduction

Staphylococcus aureus is a facultative anaerobic, Gram-positive coccus, arranged in grape-like clusters when viewed under a microscope and has large, round, golden-yellow colonies. *S. aureus* often lives harmlessly on skin and even in nasal passages. In this setting, usually no manifestation of bacterial illness appears. However, damaged skin with minor injuries like scratch, cuts, grazes, bruising etc., can facilitate *S. aureus* to cause a wide range of infections.

Methicillin, an antibiotic closely related to penicillin, was introduced in 1959 to treat *Staphylococcal* and other bacterial infections. After introduction within one to two years, resistance to methicillin was observed in the strains. These *S. aureus* bacteria were then termed as methicillin-resistant *Staphylococcus aureus i.e.*, MRSA. Since then it has overcome most of the therapeutic agents that have been developed in recent years, and hence chemotherapy for this species has always been empirical. Methicillin resistance in *S. aureus* is primarily mediated by *mec*A gene, which directs synthesis of altered penicillin-binding protein 2a (PBP 2a or PBP 2') (Chambers, 1997). MRSA is one of the most important pathogens in health care facility-associated infections and community-acquired infections leading to serious illnesses with high rate of morbidity and mortality, which makes this microbe a longstanding subject of epidemiological investigation. These strains are resistant to macro-lides, lincosides, aminoglycosides and beta-lactams which include penicillin and cephalosporins (Katayama *et al*., 2003). Many of these isolates have found to be susceptible only to glycopeptide antibiotics such as vancomycin (Assadullah *et al.,* 2003). But low level resistance even to vancomycin is emerging at present.

Serious endemic and epidemic MRSA infections occur globally as infected and colonized patients in hospitals

mediate dissemination of these isolates and hospital staff assists further transmission. Evolution of resistance to multiple antibiotics and control of disease transmission in hospitals or communities have been recognized as major challenges as bacterial population expresses resistance phenotype varies according to environmental conditions (Qureshi *et al.,* 2004).

Depending on the strain, *S. aureus* is capable of secreting several toxins. Many of these toxins are associated with wide range of clinical illnesses, ranging from common skin infections such asimpetigo and cellulitis to more serious manifestations of osteomyelitis, necrotizing fasciitis, pneumonia,abscess and sepsis. Anterior nares and wounds are the main reservoir of *S. aureus*. Wound infection can result in associated tissue damage (White *et al.,* 2001).It is assumed that most of the infections are derived from nasal carriage (Nguyen et al., 1999; Kluytmans *et al.,* 1997; Von Eiff *et al.,* 2001) with nose acting as a primary ecological reservoir of *S. aureus* in humans. Factors determining colonization may include the host, the environment and the nasal microbial ecology (Verbrugh, 2009). Association between the nasal carriage and subsequent infection has been comprehensively established in a variety of clinical environments. Accordingly, awareness of MRSA prevalence becomes necessary in selection of appropriate empirical treatment of these infections. In view of the above, the present study aimed to determine the prevalence of MRSA among the patients of different wards, and corresponding risk factors for acquisition of MRSA.

Materials and Methods

Study subject : The study was conducted on 300 inpatients of small (36-85 beds) to large sized (226-725 beds) hospitals in Doon Valley, North India, during the period 2010-2011. *S. aureus* strains were isolated from nasal specimens and wounds collected from these patients after 48 hr of admission to different wards such as general, burns and dialysis ward.

Specimen collection : A swab from both anterior nares was obtained from the patients. A pre-moistened sterile swab was cautiously pushed into each nostril so that the tip was entirely at the nasal osteum level (about 2.5 cm from the edge of the nare) and rubbing the swab 4 times around the inside of nostril by applying an even pressure and rotating the swab without interruption. Wound samples (including cuts, scrapes, scratches, punctured skin, thermal injuries etc.) were also obtained, especially from burns patients. The swabs were immediately placed in peptone water (Hi-Media, India) and kept at 4°C until inoculation.The above sample collection procedures were conducted under the supervision of a senior clinician for infusing good practices in this pre-test stage.

Media and culture conditions: Specimens were inoculated onto mannitol salt agar (MSA), a selective medium for isolation of *S. aureus* and incubated at 37^{0}C for 48 hrs (Mackie*et al.,* 1996). After mannitol fermentation, yellow or gold coloured colonies were maintained on nutrient agar (NA) and colonies on NA were subjected to additional biochemical tests.

Coagulase test: Slide coagulase test was performed by emulsifying few pure colonies of Staphylococci from blood agar on diluted plasma. However, tube coagulase test was carried out by diluting the plasma in freshly prepared normal saline (1:6). Three to four pure colonies were emulsified in 1 ml of diluted plasma and tubes were incubated at 37°C. Readings were taken at 1, 2, 3 and 4 hr and further incubated overnight at room temperature if no clot formation was observed (Mackie *et al.,* 1996).

Catalase test: Catalase test was done by transferring a small portion of the culture with clean rod onto a side with 3% (V/V) hydrogen peroxide which was kept under cover of a petri plate to avoid aerosols. If bacteria forms catalase, hydrogen peroxide is cleaved and oxygen will be evolved. Production of gas bubbles indicated positive test (Mackie and McCartney, 1998).

DNase test : Organisms were streaked on to the surface of DNase Test agar (Hi- Media) medium and incubated. 1N HCl was then poured over the agar surface. HCl precipitated and polymerised DNA which made the medium opaque. If microrganisms synthesized sufficient amount of DNase enzymes, DNA got hydrolysed and colonies were surrounded by clear zones. (Streitfeld *et al.,* 1962).

Oxacillin screen agar: This method was used for detection of methicillin resistance in *Staphylococci*. Oxacillin is more stable to other penicillinase stable penicillins. Mueller Hinton Agar supplemented with 4% NaCl and 6µg of oxacillin per ml was utilized for agar screen method (NCCLS, 2000). Inoculum suspension was prepared by selecting well characterized colonies from overnight growth on the agar surface. The colonies were added to saline to produce a suspension that matched the turbidity of 0.5 Mc Farland standard. This suspension was used to inoculate oxacillin agar screen plate by dipping a cotton swab into suspension, squeezing excess liquid from the swab and inoculating an area 10-15 mm in diameter. Test plates were incubated overnight at 35^{0}C and examined for growth of more than one colony, which indicated resistance. Recently developed chromogenic agars with various selective agents were more sensitive and specific in screening MRSA were utilized. *S. aureus* ATCC 25923 (oxacillin susceptible) and *S. aureus* ATCC 43300 (oxacillin resistant) were used for quality control of all the tests.

Results and Discussion

Of the 300 clinical samples, 111 (37%) were confirmed as *S. aureus*. The carrier rate of *S. aureus* among male and female were 37% and 36.5%, respectively (Table 1). Isolation rate of *S. aureus* from general medical wards, burns wards and dialysis wards were 44/111 (39.6%), 40/111(36%) and 27/111 (24.3%), respectively (Fig.1).

Colonization rate of *S. aureus* in the present study was relatively lower than the results of another study from Turkey (37%; vs. 56.7%) (Duran *et al*., 2006), and was compatible to the study carried out in Saudi Arabia with an overall nasal carriage of 38.0% and various carriage rates in different age groups (Saxena and Panhotra, 2003). Out of 111 *S. aureus* isolates, 38 (34.2%) were detected as methicillin resistant (MRSA) and were confirmed through different biochemical tests (Table 1).

MRSA colonization rate in hospitalized patients was compatible to the study done in other parts of India, which suggested that the average isolation rate of MRSA was between 20 to 40%. Mehta *et al*. (1998) conducted study on control of MRSA in a tertiary care center and reported an isolation rate of 33% from pus and wound swabs. Recently, Datta *et al.* (2011) also reported 35% MRSA colonization rate, but other studies has reported high proportion of hospitalized patients with MRSA carriage (Hadley *et al*., 2007; Lederer *et al*., 2007; Muñoz *et al*., 2008). The present study indicated that elderly patients, children and male patients were more colonized with MRSA (Table 2)

The MRSA isolation rate from dialysis wards general medical wards, and burns wards were 15/27 (55.5%), 10/44 (22.7%) and 13/40 (32.5%), respectively (Fig.1). The highest number of patients carrying MRSA was found to be present in dialysis ward. Studies by Kluytmans *et al*. (1997) on nasal carriage of *Staphylococcus aureus* reported patients repeatedly punctured the skin (e.g., hemodialysis or continuous ambulatory peritoneal dialysis [CAPD] patients and intravenous drug addicts) and patients with human immunodeficiency virus (HIV) infection showed increased *S. aureus* carriage rates.

Colonization and infection by *S. aureus* are found to be considerably linked with infection already prevalent among hospitalized patients. Several risk factors for acquisition of MRSA and MSSA have been identified

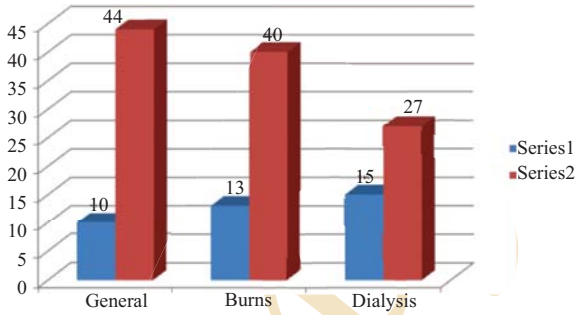

Fig. 1 : Graph showing number of patient in different wards carrying MRSA. (series 1-MRSA data, series 2-staph. aureus data)

(Table 3).

Administration of urinary catheter, antibiotics usage and hospitalization for one week or more were significantly associated with higher rate of MRSA carriage. Various studies have demonstrated that the risk factors for MRSA which include use of broad spectrum antibiotics (Miyake *et al*., 2007). Hospitalized patients particularly the elderly, the acutely ill in ICU, duration of hospitalization for more than a week, those with surgical wounds, person using intravenous catheter or in physical closeness to a patient with MRSA are at a greater risk (De San *et al*., 2007; Nijssen *et al*., 2005). Patients who have recently been discharged or transferred from another hospital are at increased risk of carrying MRSA. Different studies have claimed the transfer of MRSA between hospitals and its peripheral community. Visnuvinayagam *et al.* (2014) reported the occurrence of multiple drug resistance *S. aureus* in retail fishery outlets of India, spread of *S. aureus* through fish handlers defined as the key reason.

Measures taken to control spread of MRSA infections include laboratory-based observation, separation of colonized and infected patients, and *maintaining* proper hand hygiene. Sampling of specimen from hospitalized patients, who are at high risk of acquiring MRSA, can facilitate screening and isolation of colonized patients. Eliminating nasal colonization amidst affected patients and healthcare personnel has also been an effective control measure with variable success (Boyce, 2001).

In conclusion, the study indicates high nasal carriage rate of MRSA (34.2%) among pateints. Highest number of

Table 1 : Gender-wise prevalence of *S. aureus* isolates and the MRSA isolates

Gender	Total sample	*S. aureus* positive (%)	MRSA positive(%)
Male	177	66 (37%)	25 (38%)
Female	123	45(36.5%)	13(29%)
Total	300	111(37%)	38(34.2%)

Table 2 : Mean age and standard deviation (SD) (in years) of patients carrying MRSA

MRSA	Mean age (SD) (years)	No.
Male	44.6(22.73)	25
Female	50.5(23.71)	13

Table 3 : The risk factors for MRSA colonization during surveillance period

Risk factor	MRSA	MSSA
	38	73
Antibiotics consumption	30	09
Use of Urine catheter	17	10
Prolonged hospitalization	20	15

patients carrying MRSA were present in dialysis ward (55.6%) and lowest in general ward (22.7%). High rate of colonization was observed in males (38%). Administration of recent antibiotics was the foremost predisposing factor for MRSA colonization. Regular monitoring of MRSA in hospitals is essential with execution of effective measures to prevent transmission between healthcare facilities and the community outside it.

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