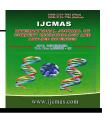
International Journal of Current Microbiology and Applied Sciences ISSN: 2319-7706 Volume 2 Number 12 (2013) pp. 569-574 http://www.ijcmas.com



Original Research Article

Screening For Detection of MRSA in Patients and Hospital Staff of a Tertiary Institutional Hospital

Fatima Khan^{1*}, Indu Shukla¹, Meher Rizvi¹, Asfia Sultan¹, Praveen Kumar², Tariq Mansoor³ and Satish Chandra Sharma⁴

¹Department of Microbiology, JNMCH, AMU, Aligarh; ²IIMT Aligarh, India ³Department of Surgery, JNMCH, AMU, Aligarh, India ⁴Department of Otorhinolaryngology, JNMCH, AMU, Aligarh, India *Corresponding author

ABSTRACT

Keywords

S.aureus, MRSA; colonization; health care workers; PCR. Methicillin resistant Staphylococcus aureus (MRSA) is one of the most significant pathogens responsible for nosocomial infections, with a differential ability to spread and cause outbreaks in hospitals. Once introduced into a hospital, MRSA can spread until a large silent reservoir of colonised patients develops who become a continuous source of transmission of infection to other patients and the hospital staff. The study was conducted in the Department of Microbiology, Jawaharlal Nehru medical College and Hospital, AMU Aligarh, to screen patients and their attendants and the hospital staff of the intensive care unit, the surgery (including the burn ward) and the orthopaedics ward for the presence of colonisation by MRSA. Nasal swabs and swabs from the finger webs were collected from all the subjects. In all the samples showing S. aureus, detection of methicillin resistance was done phenotypicaly by oxacillin disc diffusion test and genotypicaly by multiplex PCR for mecA and fem- B genes. Of the subjects, 85(63.4%) were found to be colonizers of S. aureus, while the carriage rate for MRSA was 47%. Anterior nares were found to be the more common site of colonization 63(74.1%) and colonization was highest amongst the nursing staff (76.5% - S. aureus; 61.8% -MRSA). These reservoirs of MRSA represent a serious threat to the health of hospitalised patients. Screening for MRSA colonisation should be done regularly in hospitals along with awareness programmes among the hospital staff for the prevention of spreading of MRSA infection. Emphasis should be laid on hand washing as it is the single most efficient and cheap method of prevention of transmission of infection between patients.

Introduction

Methicillin resistant *Staphylococcus* aureus (MRSA) is one of the most

significant pathogens responsible for nosocomial infections, with a differential

ability to spread and cause outbreaks in hospitals(Text-Williams *et al.*,1979). Mortality and morbidity rates in MRSA infections is roughly twice the level of those seen in cases of methicillin sensitive staphylococcal infections.

MRSA is usually introduced into an institution by a colonised or infected patient or health care worker(Mulligan *et al.*,1993) Several modes of transmission exist, including transient colonisation of hospital staff and contact with heavily contaminated fomites and environmental surfaces around infected patients (Crossly *et al.*, 1979; Muder *et al.*,1991).

However, the most important mode of transmission of MRSA within institutions appears to be poor hand hygiene (Boyce, 2001). Once introduced into a hospital, MRSA can spread until a large silent reservoir of colonised patients develops who become a continuous source of transmission of infection to other patients and the hospital staff.

Currently, the treatment options MRSA are limited to very few and expensive drugs, like vancomycin and teicoplanin. The emergence vancomycin resistant S. aureus (VRSA) and vancomycin intermediate strains of S. aureus (VISA) (Asadullah et al., 2003; Himartshu et al.,1997) shrink the treatment options even further. Because of the scarcity of the treatment options and the morbidity and mortality associated with MRSA infection, concerted efforts should be made to control the spread of this lethal pathogen. This study was designed to screen the health care workers (HCWs) and the patients in the intensive care unit, the surgery(including the burn ward) and the orthopaedics ward for colonisation with S.aureus and MRSA

and to eradicate the same by advising 1% mupirocin ointment and 2% chlorhexidine gel applied thrice daily for 15 days.

Materials and Methods

The study was conducted in Department of Microbiology, Jawaharlal Nehru medical College and Hospital, AMU Aligarh from July 2006 to July 2007. Patients and their attendants and the hospital staff (including nurses and doctors) of the intensive care unit, the surgery (including the burn ward) and the orthopaedics ward were screened for the presence of colonisation by MRSA. Informed consent was obtained prior to the screening. From each subject three swabs, one each from both the anterior nares and from the finger webs were collected. Nasal culture were performed by swabbing a sterile saline solution moistened swab for five seconds along the interior walls of DH nares(Kim etal.,2010). Similarly, sample from the finger webs were collected using a moistened swab. The samples were cultured on 5-10% sheep blood agar, MacConkey agar, Mannitol salt agar and Robertson's cooked meat broth. Only those specimens from staphylococci which isolated included in the study. All the isolates suggestive of S. aureus were identified by the standard biochemical procedures (Baird D, 2006). The methicillin susceptible strain ATCC 25923 was used as a control for the diagnostic procedures.

Oxacillin disc diffusion test

All the isolates were subjected to oxacillin disc diffusion test using oxacillin 1 µg disc. A 0.5 McFarland turbidity standard suspension of the isolate was made and lawn culture was done on Mueller-Hinton agar (MHA) plates containing 4% NaCl.

Plates were incubated at 37 °C for 18 h and zone diameters were measured. An inhibition zone diameter of ≤ 10 mm was reported as methicillin resistant and ≥ 13 mm was taken as methicillin sensitive (CLSI, 2004).

MIC determination of oxacillin

MIC was determined by agar dilution test. 10 different dilutions of oxacillin were selected such that the concentrations that allowed determination of MIC breakpoints defining susceptible ($\leq 2\mu g/ml$) and resistant ($\geq 4\mu g/ml$) (CLSI, 2004) values were included. Lowest concentration at which the growth was inhibited by 80% or more was recorded as MIC.

PCR amplification for mecA and femB genes

Duplex PCR (Jonas, 1999) was carried out on all the *S. aureus* strains found methicillin resistant on MIC determination as described by Geha *et al.*,1994. Amplicons of 310 bp were consistent with *mecA* and of 651 bp with *femB* gene amplification.

Result and Discussion

Staphylococcus aureus was isolated in a total of 85(63.4%) subjects included in the study. The total number of S aureus isolates from these 85 cases was found to be 104 as 19(22.3%) carried *S aureus* at multiple sites. Colonisation was found to be highest in the anterior nares 63(74.1%), followed by the finger webs 41(48.2%) (Table 1). Carriage was higher among the nurses 26(76.5%) than the doctors 24(70.6%). Among the patients 20(60.6%) and 15(45.5%) of their attendants were carriers (Table 2). 65(62.5%) isolates were found to be methicillin resistant by oxacillin disc diffusion test while

63(60.6%) were confirmed as MRSA by the detection of *mec-A* gene.

The carriage rate for MRSA was 47%. Similar to S. aureus colonisation, MRSA carriage was highest among the nursing staff (61.8%) followed by the doctors (47%). Overall, 15(45.4%) patients and 11(33.3%) persons accompanying them carried MRSA. Out of the 15 patients with MRSA colonisation 6(40%) had risk factors for MRSA acquisition. Two had previous history of antibiotic intake and two had history of previous history of hospitalisation. One patient was diabetic with decubitus ulcers and one had undergone some intervention in the hospital. Among the 11 attendants with MRSA colonisation, 2 had previous history of hospitalisation and two had history of antibiotic intake. Carriers (attendants) of MRSA were removed from active care of patients and were treated with 1% mupirocin and 2% chlorhexidine gel for 15 days. They were screened for the absence of MRSA carriage before resuming their normal duties.

Out of a total of 134 subjects included in the study, 63.4% yielded S. aureus from one or more sites on culture making a total of 104 isolates. Other authors have reported a similar prevalence colonisation by S. aureus (Aravind P. 2000). Out of the 85 subjects classified as carriers, 22.3% tested positive at multiple sites and 77.7% carried S. aureus only at one site. However, other authors have reported colonisation at multiple sites to be more frequent (Aravind P, 2000). Anterior nares were found to be the commoner site of colonisation (74.1%) than the finger webs (48.2%) (Table 1). Nasal carriage has been identified as one of the most common sites in other studies (Locksley et al., 1982).

Table.1 Site of colonisation of *S. aureus* among the subjects

Site of colonisation	S. aureus isolated no. (%)
Anterior nares	44((51.8)
Finger webs	22(25.9)
Both (finger webs and anterior naris)	19(22.3)
Total	85(100)

Table.2 Pattern of colonisation among the various study groups

Subject	Total number screened	S. aureus no.(%)	MRSA no.(%)
Doctors	34	24(70.6)	16(47.0)
Nurses	34	26(76.5)	21(61.8)
Patients	33	20(60.6)	15(45.4)
Attendants	33	15(45.4)	11(33.3)
Total	134	85(63.4)	63(47.0)

Prevalence of *S. aureus* as well as MRSA colonisation was highest among the nursing staff (76.5%/61.8% respectively) followed by the doctors (70.6%/47%). Amongst the patients admitted in various wards, 20(60.6%) carried *S. aureus and* 45.4% were MRSA carriers; while 45.5% of the patients attendants accompanying them were *S aureus* colonisers and 33.3% harboured MRSA (Table 2).

By oxacillin disc diffusion test, 62.5% isolates were found to be methicillin resistant; while 60.6% isolates were confirmed as methicillin resistant by detection of *mecA* and *femB* genes on duplex PCR.

Among the patients with MRSA 40% had associated risk factors, while 36.6% of attendants harbouring MRSA had risk factors. Carriers (attendants) of MRSA were removed from active care of patients and were treated with 1% mupirocin and 2% chlorhexidine gel for 15 days. They were screened for the absence of MRSA carriage before resuming their normal duties.

The original reservoir from which the patients acquire these isolates remains unclear. Hospital personnel are among those implicated as possible sources of antibiotic resistant pathogens. these Transmission of these strains to the patients is then likely to occur during routine patient care (Cespedes al.,2002). The problem is compounded in the intensive care and the burns unit and also in the orthopaedics and surgery wards because of the long duration of stay of these patients in the hospital and intake of multiple antibiotics which becomes a risk factor for decreasing the immunity of the patients. Care of these patients is often labour intensive requiring many hours of hands on contact.

MRSA represents a serious threat to the health of hospitalised patients. Attempts to reduce the spread of MRSA have largely depended on hospital hygiene and patient isolation. These measures have met with mixed success: although some countries have almost eliminated MRSA or remained largely free of the organism,

others have seen substantial increases despite rigorous control policies(Cooper *et al.*,2004). Several surveys have confirmed that the incidence of MRSA varies by region, the proportion of isolates resistant to methicillin has ranged from less than 1% in Scandinavia to more than 30% in Spain, France, Italy and India (Voss *et al.*,1994; Herwalt *et al.*,1996; Khan *et al.*,2010; Mehta *et al.*,1996)

MRSA has become a menace in the hospitals with a scarcity of treatment options. Therefore, screening for MRSA colonisation should be done regularly in with awareness hospitals along programmes among the hospital staff for the prevention of spreading of MRSA Infection control measures infection. should be strictly adhered to in dealing with colonised subjects, like patient screening for MRSA before hospital admission and isolation. Emphasis should be laid on hand washing as it is the single most efficient and cheap method of prevention of transmission of infection between patients.

References

- Aravind, P., Krishnan, P.U, Srinivasa, H and Joseph V. 200. Screening of Burns staff of a tertiary care hospital for methicillin resistant *Staphylococcus aureus* colonisation. MJM 2000; 5: 80-84
- Asadullah, S., Kakru D.K, Thokar M.A, Bhat F. A, Hussain N and Shah, A. 2003. Emergence of low level vancomycin resisitance in MRSA. Indian J. Med. Microbiol. 21: 196-198
- Baird, D.. 2006. Staphylococcus: Cluster forming Gram positive cocci. In Mackie and Mc Cartney, Practical Medical Microbiology, eds ColleeJG, Dugid JP, Fracer AG, MarimionBP.

- 13th edition , Churchill livingstone Edinburg. 245-261
- Boyce, J.M., 2001. MRSA patients proven methods to treat colonization and infection. J Hosp. Infect. 48: S9-S14
- Casewell, M.W., 1995. New threats to the control of methicillin resistant *Staphylococcus aureus*. J Hosp. Infect. 30(Suppl): 465-471
- Cespedes, C., Miller, M, Quagliarello, B, Vavagiakis, P, Klein, R.S and Lowy F.D. 2002. Differences between Staphylococcus aureus isolates from medical and nonmedical personnel. J .Clin. Microbiol. 2594- 2597
- Cooper, B.S., Medley, G.F, Stone, S.P, Kibbler, C.C, Cookson, B.D, Roberts, J.A, Duckworth, G, Lai, R and Ebrahim, S. 2004. Methicillin resistant *Staphylococcus aureus* in hospitals and the community: Stealth dynamics and control catastrophes. PNAS. 101(27): 10223-10228.
- Crossly, K., Landesman B, and Zaske, D. 1979. An outbreak of infections caused by strains of *Staphylococcus aureus* resistant to methicillin and aminoglycosides. J. Infectious. Dis. 139: 280-287
- Fatima Khan, Indu Shukla and Meher Rizvi. 2010. Cefoxitin Disc Test as a Marker for Detecting Methicillin Resistance in *Staphylococcus aureus* Isolates. J .Pure. Appl .Microbiol. 4 (2): 831-835.
- Geha, J., J. R.Uhl, C. A. Gustaferro and Persing, D.H. 1994. Multiplex PCR for identification of methicillin resistant staphylococci in the clinical laboratory. J .Clin. Microbiol. 32:1768-1772.
- Herwalt, A.H., 1996. Control of methicillin resistant *Staphylococcus aureus* in the Hospital setting. American. J. Med. 106: 11S-18S.

- Himartshu, K., Aritaka N, Hanaki H, Kawasaki S, Hosoda Y, Hori S, Fukuchi Y, and Kobayashi, I . 1997. Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneously resistant to vancomycin. Lancet. 350: 1670-1673.
- Jonas, D., Grundmann, H, Hartung D, Daschner F.D, and Towner, K.J. 1999. Evaluation of mec A and fem B duplex polymerase chain reaction for detection of methicillin resistant *Staphylococcus aureus*. Eur. J. Clin.Microbiol.Infect.Dis. 18(9): 643-647.
- Kim, D.H., Spencer M, Davidson SM, Li L, Shaw J.D, Gulczynski D, Hunter D.J, Martha J.F, Miley G.B, Parazin S.J, Dejoie P and Richmondm J.C. 2010. Institutional prescreening for detection and eradication of methicillin resistant *Staphylococcus aureus* in patients undergoing elective orthopaedic surgery. JBJS. 92: 1820-1826.
- Locksley, R.M., Cohen M.L, Quinn T.C, Tompkins I.S, Coyle M.B, Kihara J.M, and Counts G.W. 1982. Multiply antibiotic resistant *Staphylococcus aureus*: introduction, transmission and evolution of nosocomial infection. Annals. Intern. Med. 97: 317-334.
- Mehta, A., Rodrigues C, Kumar R, Rattan A, Sridhar H, Mattoo V and Ginde V. 1996. A pilot programme of MRSA surveillance in India. J Postgraduate. Med . 42: 1-3.
- Menezes, G.A, Harish, B.N, Sujatha S, Vinothini K and Parija, S.C. 2008. Emergence of vancomycin resistant intermediate *Staphylococcus species* in

- Southern India. J. Med.Microbiol . 57: 911-912.
- Muder, R.R., Brennen C, and Wagner, M.M. 1991. Staphylococcal colonization and infection in a long term care facility. Annals. Intern. Med. 114: 107-112.
- Mulligan, M.E., Murray-Leisure K.A, Ribner B.S, Standiford H.C, John J.F, Korvick J.A, Kauffman C.A, and Yu V.L. 1993. Methicillin resisitant Staphylococcus aureus: a consensus review microbiology, of the pathogenesis, epidemiology with implications for prevention and management. American. J .Med. 94: 313-325.
- Text Williams, D.N., and Peterson, P.K. 1979. Endocarditis caused by coagulase negative staphylococci. Infection. 7: 5-9.
- Tiwari, H.K., and Sen, M.R. 2006. Emergence of vancomycin resistant *Staphylococcus aureus* (VRSA) from a tertiary care hospital from northern part of India. BMC Infect. Dis . 6:156
- Voss, A., Milatovic, D, Wallrauch-Schwarz C, Rosdahl V.T, and Braveny, I. 1994. Methicillin resistant Staphylococcus aureus in Europe. Eur. J. CLin. Microbiol .Infect. Dis. 13: 50-55.
- Wayne Pa. Clinical and Laboratory Standards Institute. 2004. Performance standards for antimicrobial susceptibility testing,14th information supplement.M100-S14.