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Research Article

Occurrence of SCCmec Type Ii Methicillin Resistant *Staphylococcus aureus* (MRSA) among Health Care Workers of a Tertiary Care Hospital of North India

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Abstract

Introduction: Methicillin Resistant *Staphylococcus aureus* (MRSA) carriage in health workers is the major cause of nosocomial infections. Typing and clonality of the isolates are essential for proper infection control.

Materials and Methods: Nasal and hand swabs in duplicates were collected from the nursing personnels of various wards and were cultured on routine bacteriological media including mannitol salt agar. MRSA isolates that were *mecA* positive were typed by Pulsed-Field Gel Electrophoresis (PFGE). SCCmec typing and PVL gene detection was also done in MRSA isolates.

Results: A total of 300 health-care workers were included in the study.15 (28%) were confirmed to be MRSA by *mecA* PCR, of which 8 (53%) were from nasal swabs and 7 (47%) were from hand swabs. Overall carriage rate was 5%. Among the Nasal swab isolates (n = 8), 7 (87%) were of SCCmec type II and 1 (13%) was of type III. Similarly, among hand swab isolates, 5 (71%) and 2 (29%) were of type II and III respectively. No PVL gene was noted in this study. Pulse field gel electrophoresis analysis showed all the MRSA isolates were of different pulsotype. In this study majority of the isolates were SCCmec type II and all were PVL negative.

Conclusions: In this Indian study, SCCmec type II was the major type of MRSA and all were hospital acquired. Multiclonality of isolates suggests multiple sources of infection and need for integrated approach for control.

Keywords: *MRSA; PFGE; SCCmec; Health Worker*

Introduction

Staphylococcus aureus can cause a variety of infections in immunocompetent as well as immunocompromised patients. Methicillin Resistant *Staphylococcus aureus* (MRSA) isolates cause a large chunk of nosocomial infections in tertiary care centers. They colonize the nasal cavities and hand surfaces of health-care workers and infections spread to patients. Typing of the isolates is very important to know the source and for controlling the infections. Epidemiologically, MRSA has been divided into Hospital Acquired MRSA (HA-MRSA) and Community acquired MRSA (CA-MRSA) [1]. The defining features of MRSA is Staphylococcal cassette chromosome *mecA* (SCCmecA) and its typing is important in characterization of MRSA. Similarly, Pantone Valentine Leucocidin gene (PVL) is important characteristic of CA-MRSA [2,3]. SCCmecA and PVL gene typing was thought to be important markers to differentiate HA-MRSA and CA-MRSA [4]. However, this distinction has become less marked now a day [5]. Pulsed Field Gel Electrophoresis (PFGE) is a molecular technique which can type the isolates. It can help to find out the common source of the isolates which in turn can lead to effective control measures.

Here, we made the study to isolate, characterize phenotypically as well as genotypically the MRSA isolates in our hospital.

Materials and Methods

Nasal and hand swabs in duplicates were collected from the nursing personnels of various wards. Proper clinical history and physical examination was carried out in each person. The swabs were cultured on routine bacteriological media including mannitol salt agar. *Staphylococcus aureus* isolates were identified by phenotypic tests and confirmed by Polymerase Chain Reaction (PCR). MRSA isolates were identified by Cefoxitin disc method and confirmed by *mecA* gene detection. The *mecA* positive isolates were typed by Pulsed-Field Gel Electrophoresis (PFGE).

Molecular Characterization

PCR amplification of *mecA* gene

Previously described primer was used to detect of *mecA* gene [6]. The reaction mixture of 25 µl contained; 50 ng of genomic DNA, 20 pmole of each primer,1U Taq DNA polymerase (Fermentas, Vilnius, Lithuania),1X buffer containing 1.5mM $MgCl_2$, and 25 mmol/l of each dNTPs (Fermentas). PCR conditions for amplification of *mecA* was at thermal temperature of 94°C for 5 minutes, followed by 34 cycles of 94°C for 1 minute, annealing at 50°C for 1.5 minutes, and extension at 72°C for 1 minute, followed by a final extension for 10 minutes at 72°C. The result was observed under UV transilluminator on 1.2% agarose gel.

SCCmec typing

SCCmec typing was defined by multiplex PCR according to the previously published protocol [6].

Panton-Valentine leukocidin (PVL)

PVL genes were detected by co-amplification of the *lukS-PV* and *lukF-PV* genes as described by Lina., *et al* [7].

Pulsed field gel electrophoresis

Genetic relatedness of MRSA strains digested with SmaI was assessed by PFGE, as described by Singh., et al [6]. Strains were considered indistinguishable if there was no difference in bands, and related (i.e. variants of the same PFGE subtype) if they varied by 1 to 3 bands. A PFGE dendrogram was constructed using Gel Compare II 6.6 version (Applied Maths, Sint-Martens-Latem, Belgium) to calculate similarity coefficients and to perform unweighted pair group analysis using arithmetic mean clustering. Dice coefficient with 0.5% optimization and 1.0% position tolerance was used.

Results

A total of 300 health-care workers were included in the study. 212 were females and 88 were males.

A total of 70 *Staphylococcus aureus* and 50 Coagulase Negative *Staphylococcus* (CoNS) isolates were obtained. 15 (28%) were confirmed to be MRSA by *mecA* gene detection out of which 8 (53%) were from nasal swabs and 7 (47%) were from hand swabs. 13 MRSA isolates were derived from females and 2 from males. MRSA carriage rate was 6% for females and 2% for males. Overall carriage rate was 5%.

Nasal swabs yielded 57 *Staphylococcus* spp isolates out of which 18 were Coagulase Negative *Staphylococcus* (31%) and 39 were *Staphylococcus aureus* (69%). 8 were MRSA (20%). Hand swabs yielded 63 *Staphylococcus* spp isolates out of which 32 (51%) were CoNS and 31 (49%) were *Staphylococcus* aureus. 7 were MRSA (21%).

Among the Nasal swab isolates (8), 7 were of SCCmec type II (87%) and 1 (13%) was of type III. For the Hand swab isolates, 5 (71%) and 2 (29%) were of type II and III respectively.

The isolates obtained from different sites have been depicted in table 1.

	Coagulase Negative *Staphylococcus* (n = 50)	***Staphylococcus aureus (n = 70)***	**MRSA (n = 15)**	**SCCmec type II (n = 12)**	**SCCmec type III (n = 3)**
Nasal Swabs (n = 300)	18	39	8	7	1
Hand Swabs (n = 300)	32	31	7	5	2

Table 1: *Characteristics of the Staphylococcal isolates*

No PVL gene was noted in this study. Pulse field gel electrophoresis analysis showed all the MRSA isolates were of different pulsotype.

Discussion

Organisms residing on the body surface of health care workers pose a major problem in hospital acquired infections. Especially MRSA transmitted from them to patients cause variety of health problems. However, 5% of our health care workers carried MRSA which is lower than other study [8] but correlates with a study from China where the prevalence was 0.3% [9]. Our study found *Staphylococcus aureus* to be more prevalent than Coagulase negative *Staphylococcus* in nasal swabs than hand swabs. However, MRSA prevalence in both the sites was almost equal.

Panton Valentine leukocidin (*PVL*) is considered one of the important virulence factors of *S. aureus* responsible for destruction of white blood cells, necrosis and apoptosis and as a marker of community acquired MRSA. A study in Nepal found 7.1% PVL gene in hospital acquired MRSA isolates [4]. However, No PVL gene was detected in our study. It suggested all were hospital acquired isolates.

Literature suggests that Hospital acquired MRSA isolates are usually of SCCmec type I, II and III. In our study majority isolates were of type II and some were of type III. No isolates were found to be of type IV or V. Majority of nasal and hand swab isolates were of Type II than type III. Many studies found type III to be prevalent rather type II [2,10]. However, no association was found in virulence of type II and type III isolates. Our isolates were all hospital acquired unlike mentioned in other studies.

All the isolates were of different pulsotype. It is rather rare phenomenon as other studies found MRSA strains in groups of pulsotypes [9,11]. It suggests the isolates were all different and from multiple sources. So, the infection control should extensive and integrated to control the spread of MRSA isolates.

Conclusion

Around 1/4th of the health-care workers carried *S. aureus* and 5% carried MRSA which could be the major source of hospital acquired infections. Unique feature of the isolates was that majority were of SCCmec type II isolates and all were PVL negative. It suggests all health care workers harbor hospital acquired strains. All the isolates were from multiple sources, so integrated approach is required for the infection control.

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