Molecular Epidemiology of CA-MRSA in a Healthy Community

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ABSTRACT

BACKGROUND: Community associated MRSA infection appears to be on the rise and has been described in several well-defined populations. The aim of the present study was to evaluate the epidemiology of CA-MRSA in the healthy community of Kashmir.

METHODS: Nasal swabs, wrist swabs and ocular specimens were collected randomly from the healthy subjects. The samples were processed on selective media and identification of the *S.aureus* was performed by using biochemical tests and by the amplification of nucA gene. Methicillin-resistance was confirmed by 30µg Cefoxitin disk diffusion method and by amplification of mecA gene. Pulsed field gel electrophoresis was used for the typing of CA-MRSA.

RESULTS: Of 1000 healthy subjects that were screened in the present study, 100 (males 33, females 67) were positive for *S.aureus*. The highest prevalence of *S.aureus* (30%) was observed in the age group 50-59 years while the lowest 1% was reported in the age group 80-89 years. Of the 100 S. aureus colonization, 9 were MRSA and 91 were MSSA as determined by Cefoxitin 30µg disk diffusion test and PCR. DNA was extracted from all 9 CA-MRSA isolates and was used for amplification of mecA and nucA genes. A product size of 533bp was amplified for mecA gene and 275bp for nucA. PCR was performed for the amplification of PVL gene in all 100 isolates and only two were identified as PVL positive. The pulsed field gel electrophoresis results of CA-MRSA isolates were striking and expressed a similar banding pattern among all the isolates from the Kashmiri community.

CONCLUSION: We found that the prevalence of CA-MRSA in the healthy population of Kashmir valley is quite high.

Key Words: PFGE; PCR; Pyoderma; Soft Tissue Infection; CA-MRSA

INTRODUCTION

Staphylococcus aureus is associated with serious skin and soft tissue infections in the community. A mortality rate of 20-25% still occurs with these pathogenic bacteria despite the use of appropriate antibiotics [1]. Various particular sites like nares, axillae, vagina and damaged skin surfaces are colonized with S.aureus. About 30-50% healthy people are colonized with this particular bacterium, with 10-20% persistently colonized [2]. Methicillin-resistant Staphylococcus aureus (MRSA) emerged as a widespread cause of hospital-associated infection in the late 1990's [3]. Infections that are not acquired at a health care setting or institutions are considered community acquired methicillin resistant Staphylococcus aureus (CA-MRSA). According to the Centre for Disease Control and Prevention (CDC), the definition a CA-MRSA infection is community acquired when it is seen in an outpatient or within 48 hrs of hospitalization, when the patient lacks the following risk factors: receipt of hemodialysis, surgery, residence in long term care facility, or hospitalization during the previous year, and the presence of an indwelling catheter at the time of sample collection [5, 6]. These organisms have recently emerged as an important cause of community associated staphylococcal infections [4]. Molecular typing techniques have been widely used throughout the world for determining the evolutionary relationships of different clones and epidemiological studies of MRSA isolates [9]. MecA gene had been considered as a molecular marker for MRSA strains and is a useful tool for

its identification. Polymerase chain reaction is a technique that enables the amplification of the specific sequences of a particular gene. Among different techniques, pulsed-field gel electrophoresis of SmaI had been considered as a standard for molecular typing [11]. PFGE had been suggested to be a useful tool for epidemiological investigations of various MRSA infections [12]. Many reports from India and Asia have highlighted the prevalence of MRSA in the community [13, 14, 15, 16]. MRSA, once considered primarily a nosocomial pathogen, is being increasingly reported from India as a colonizer in healthy individuals without risk factors and a cause of community-acquired infections, including pyodermas [13, 14]. The implications of these reports for the current prescription practices for cutaneous bacterial infections are obvious. It is therefore essential to determine the susceptibility pattern of clinical isolates of S. aureus in different communities across our diverse country. The present study was undertaken to determine the prevalence of MRSA in the Kashmiri community. In this study multiplex PCR and PFGE were used genotypically for the molecular typing of CA-MRSA isolates obtained from the healthy individuals of Kashmir Valley.

METHODS AND MATERIALS

***Study population**:* From each of the 10 administrative districts of the Kashmir valley, two blocks were randomly selected and from each block two villages were chosen for sample collection to determine the prevalence of *S.aureus,* particularly MRSA. Before samples were collected, information about the study was explained to the community individuals after approval was sought from the head of each community. Oral consent for participation in the study was obtained. Detailed history was obtained and only those personnel were included in the study who had not taken any antibiotics seven days before the time of specimen collection. Questionnaires to obtain relevant information for the study were distributed to the community individuals. Ethical clearance for the study was obtained from the Institute Ethics Committee of Sher-i-Kashmir Institute of Medical Sciences Srinagar.

***Samples and socio-demographic parameters**:* Samples from anterior nares, wrist swabs and ocular swabs were collected. Various important demographic factors like age, sex, occupation and antibiotic intake were recorded.

Specimen Collection and species identification:

Nasal swab: Sterile cotton tipped swab was moistened in a culture tube containing 2 ml of 0.1% buffered Tween-80 (Hi-media). Swab was wrung out within the tube, swirled inside the anterior nares for five clockwise and five counter clockwise rotations, re-introduced into the culture tube, and wrung out [17].

Ocular swab: Cotton swabs moistened with brain heart infusion broth have been used to culture material from the conjunctiva and lid margins of both eyes [18]. The swabs were deposited in tubes containing 2 ml of detergent fluid (0.1% buffered Tween-80), 40 µl was then drop plated on to the 5% sheep blood agar and mannitol salt agar plate, incubated for 24 hrs at 35^0 C and observed for the growth of suspected *staphylococcal* colonies. After the identification and confirmation of cultures, strains were preserved at -70^0 C in freezer vials containing 15% glycerol for further analysis.

Hand swab: Sterile cotton tipped swab was moistened in a culture tube containing 2 ml of 0.1% buffered Tween-80 (Hi-media). Swab was wrung out within the tube and rotated 5 times on both sides of the wrists and then reintroduced in to the culture tube [19]. The staphylococcal isolates were identified morphologically and biochemically by standard laboratory procedures. The coagulase plasma test was performed on organisms exhibiting typical staphylococcal colony morphology to allow for discrimination of *S.aureus* from coagulase negative staphylococci.

Resistance to methicillin was detected with the Cefoxitin (30µg) disk diffusion test [20] and interpreted as per [21]. DNA of *S.aureus* was extracted using as per Brakstad 1993 [22]. The detection of mecA and nuc genes was carried out using multiplex PCR procedure [23]. DNA of *S.aureus* was extracted using as per Brakstad 1993 [22]. The detection of mecA and nuc genes was carried out using multiplex PCR procedure [23]. The following primer sequences of mecA gene 5 AAA ATC GAT GGT AAA GGT TGC C 3 and 3 AGT TCT GCA GTA CCG GAT TTG C 5 was responsible for methicillin resistance; nucA gene 5 GCG ATT GAT GGT GAT ACG GTT 3 and 3 AGC CAA GCC TTG ACG AAC TAA AGC 5 is responsible for the production of thermo-stable nuclease and was thus included in

the multiplex PCR assay in order to confirm that the isolates are indeed *S.aureus* and not other *Staphylococcal* species, while the PVL gene 5 ACACACTATGGCAATAGTTATTT 3 and 3 AAAGCAATGCAATTGATGTA 5 encodes Panton Valentine Leukocidin toxin (PVL). A pore forming toxin was included in order to compare the CA-MRSA strains with hospital acquired methicillin resistant *Staphylococcus aureus* (HA-MRSA) strains. PCR protocol for PVL was performed [24].

DNA fingerprinting technique by pulsed field gel electrophoresis (PFGE) was used to determine and compare the PFGE patterns of MRSA strains of different sources as per Matushek *et al.,* [25] and performed in a contour clamped homogeneous electric field apparatus (CHEF DR III, Bio-Rad Laboratories, Hercules, California, USA).

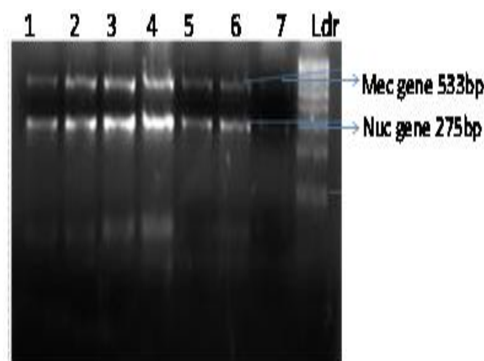
DNA fingerprints have been analyzed using the Quantity One software version 4.4.1 from Bio-Rad to construct a dendrogram of relationship among various isolates. DNA fragments on each gel were normalized using molecular weight markers to allow comparison between different gels and 1.5% band tolerance was selected for comparison of DNA profiles. Cluster analysis was performed by un-weighted paired group method with arithmetic averages (UPGMA), and DNA relatedness was calculated based on dice coefficients of similarity [26].

RESULTS

Of the 1000 healthy screened subjects, 100 (10%) were positive for *S.aureus* (33 males and 67 females). The highest prevalence (30%) of *S.aureus* was observed in the age group 50-59 years while the lowest 1% was reported in the age group 80-89 years (**Table 1**). The high prevalence of 44.4% MRSA was recorded in females in the age group of 40-49 years, and lowest 11.1% was observed in males in the age group of 1-9, 10-19, and 70-79 years respectively (**Table 1**). The main occupation of the healthy subjects was farming and participants were residents of the far-flung areas of the Kashmir Valley. Of the 100 *S.aureus* isolates, 49% and 30% were obtained from nasal samples and wrist swabs and 21% were from ocular sources of healthy subjects. Of these 100 isolates, 9% confirmed MRSA and 91% MSSA by Cefoxitin 30µg disk diffusion test. The maximum number of MRSA were isolated from nasal specimens (5), followed by soft tissue infection sites (4). No MRSA was isolated from the ocular sources.

DNA was extracted from all nine CA-MRSA isolates and was used in multiplex PCR for the amplification of mecA, and nucA genes. All nine strains were amplified successfully for the respective genes. A product size of 533bp was amplified for mecA gene, and 275bp was amplified for nucA [Fig. 1]. PCR was performed for the amplification of PVL gene in all 100 isolates and only two isolates were identified as PVL positive and both of them were CA-MRSA assessed in pyodermal infectious persons residing in the community (these patients were enrolled because they were community-dwelling and apparently healthy). The identity of the PCR products was confirmed by running a 1000 bp ladder along the amplified products on 1.5% agarose and product size of 176 bp was amplified [Fig. 2]. Pulsed field gel electrophoresis (PFGE) was performed on all CA-MRSA isolates using *Sma*I as a restriction endonuclease. Cluster analysis was performed by using the unweighted pair group method based on Dice coefficients. Pulsed field type clusters were defined by using a

Figure 1: Fig. 1: Amplification of mecA (533bp) and nucA (275bp) genes of (CA-MRSA) isolates. Lane 1 positive control, lane 2 -6 CA-MRSA strains, 7 Negative strain and Lane 8 showed 1000 bp Ladder.



similarity coefficient of at least 80%. The PFGE results of CA-MRSA isolates were striking and expressed a similar banding pattern among all the isolates from the Kashmiri community. Two clusters (a and b) formed by all the genotypes represented 100% similarity within the groups [Fig. 3]. All the 9 isolates belong to a single clone prevailing in the community, meaning there had not been any clonal evolution among the MRSA isolates in the healthy population of Kashmir. Although the similar banding pattern was observed in all MRSA isolates, only 9 different antibiograms have been observed in the

Figure 2: Amplification of PVL (176bp) gene. Lane 1 showed negative strain, and 2-3 PVL positive strains and lane 4 indicate 1000 bp Ladder

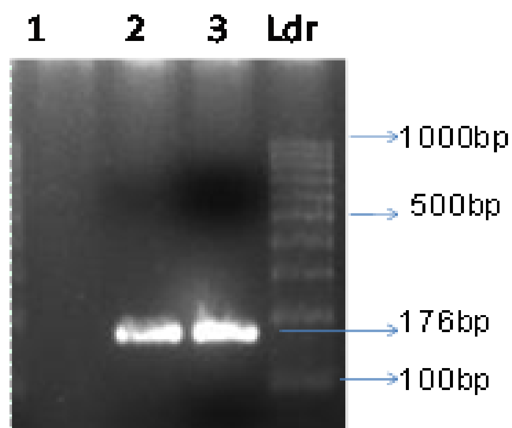


Table 1: Age wise/Sex wise distribution of CA-MSSA/CA-MRSA

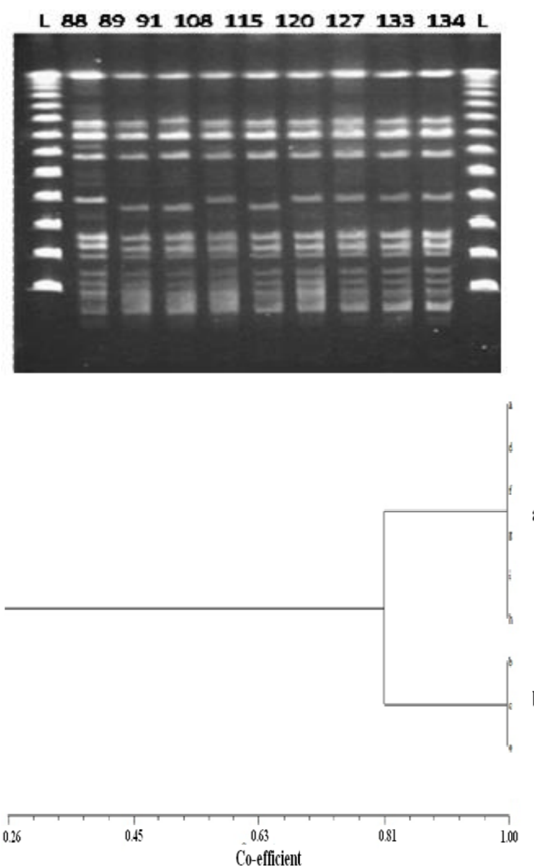
Age Group	No. of *S.aureus*	Male	Female	No. of MRSA
1-9 yr	10	3	7	1(11.1%)
10-19	5	2	3	1(11.1%)
20-29	22	4	18	2(22.2%)
30-39	11	2	9	0
40-49	10	3	7	4(44.4%)
50-59	30	10	20	0
60-69	3	2	1	0
70-79	8	6	2	1(11.1%)
80-89	1	1	0	0
TOTAL	100	33	67	

respective isolates.

DISCUSSION

Staphylococcus aureus is a leading cause of community acquired infections, particularly in humans colonized with it [27]. During the current investigation, (10%) S.aureus carriage rate was analyzed in healthy individuals, whereas, 10-40% colonization in normal healthy individuals had been reported [28]. Staphylococcus aureus colonizes asymptomatically, mainly in the anterior nares of humans. The present study showed an overall prevalence of 49% of S.aureus in the anterior nares of healthy subjects. Similar findings were analyzed from the nasal swabs of healthy subjects [30]. Total of 9% CA-MRSA prevalence was recorded in the current study whereas, 9.9% had been observed in normal adult population [32] although, a much lower rate of 0.2% was found in another study [33]. Genotypic tests like PCR are the preferred meth od for the detection of mecA gene and nucA gene [38]. The present investigation provided an evidence for the presence of mecA gene at 533bp and nucA gene at 275bp in all CA-MRSA isolates. Similar findings had been determined [39,40]. Only two CA-MRSA isolates have been found positive for PVL gene with a product size of 176bp [41]. However, a study detected PVL gene in only one CA-MRSA strain [42]. CA-MRSA had been described to carry the loci for PVL in high frequency and to be associated with the type IV (SCCmec) [43]. In order to track the original MRSA clone and the substitute clone, the previous histories, related with hospitalization of all the subjects, including those who were PVL positive, were reviewed. The following variables have been collected: patient demographics (gender and age); ward; underlying diseases; the length of time after

Figure 3: PFGE profiles of 9 CA-MRSA strains with their dendrograms formed by UPGMA method. Lanes 88 and 134 represents the CA-MRSA isolates, lane 1 and 12 represents Ladder



Two clusters (a & b) formed by all the 9 genotypes representing 100% similarity within the groups.

admission that a sample for culture was obtained; the presence of an invasive device (e.g., a vascular catheter or a gastric feeding tube) at the time of admission; and a history of MRSA infection or colonization, surgery, hospitalization, dialysis, or residence in a long-term care facility in the 12 months preceding the culture. These two MRSA isolates have been noticed in the patients who were suffering from pyoderma as per their medical records, but lacks all risk factors, except of previous hospitalization before two months of sample collection and have been considered as carriers as per their medical records. Currently there are numerous typing techniques for the discrimination of Staphylococcus aureus isolates, which can be applied as invaluable tools by both the clinician and the epidemiologist. The greatest challenge for a clinical laboratory is to carry out an analysis that can reliably group epidemiologically related strains and discriminate them from unrelated strains. Usually, these typing systems are used for the investigation of outbreaks of nosocomial infection, and furthermore can aid in the clinical treatment of a patient, allowing for discrimination between successive and recurrent infections, and in a broader context they contribute to the understanding of the epidemiology of infections [10]. During the current investigation two clusters (a & b) were formed by all the genotypes representing 100% similarity within the groups. 100% similarity was noticed between the 5 strains isolated from nasal samples and 4 from soft tissue infection sites of wrists. Similar results have been observed [44]. However an investigation reported 4 PFGE types among 132 MRSA isolates [45]. A single MRSA clone prevailing in the community means that there had not been any clonal evolution among the MRSA isolates in the Kashmiri community. It is also possible that no new MRSA strain had been introduced in to Kashmiri community or if they have introduced they might have not persisted. Low incidence of MRSA colonization in an adult outpatient population indicated that MRSA carriers most likely acquired the organism through contact with healthcare facilities rather than in the community. Thus it is clear that care must be taken when attributing MRSA colonization to the community if detected in outpatients or during the first 24 to 48 hrs of hospitalization.

CONCLUSION

In conclusion it was found that only a single CA-MRSA clone is roaming in healthy community of Kashmir that was detected by PFGE technique. Indiscriminate use of the antibiotics has given rise to a number of CA-MRSA clones throughout the world that have spread from one region to another through different routes. Both phenotypic and genotypic methods proved to be sensitive and specific for the detection of MRSA during the current investigation. Molecular typing by PFGE should be made an essential technique in epidemiological outbreaks and has proved to be a gold standard during the present study.

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