Nasal carriage of MRSA and its antimicrobial susceptibility pattern in healthy individuals and hospitalized patients

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Abstract

Staphylococcus aureus is one of the most common and important pathogen, responsible for the majority of nosocomial infections. Nasal carriage is a major risk factor for S.aureus infection that differs from person to person. 200 nasal swabs (100 from hospitalized patients and 100 from healthy individuals) have been collected as per standard policy protocols. Detailed history was collected and only those personnel were included in the study who had not taken any antibiotic 7 days before sample collection. S.aureus isolates were confirmed by various biochemical tests as per latest CLSI guidelines. Cefoxitin Disk Diffusion test was performed for the detection of methicillin resistance and antibiotic susceptibility was performed against different antibiotics as per CLSI guidelines. Out of the 100 healthy subjects 49% S.aureus and 8.16% MRSA isolates were obtained while as out of 100 hospitalized subjects 71% S.aureus and 39.4% MRSA isolates have been detected from the nasal swabs. Penicillin was found to be the most resistant drug in both groups of isolates where as Vancomycin and Linezolid have been the drugs of choice. A significant difference was analyzed when the resistant rate of HA-MRSA against HA-MSSA was compared (p<0.0001). Significant difference was noticed while comparing resistant patterns of CA-MRSA against CA-MSSA (p<0.0001). A significant difference was verified when we compared the resistant rate HA-MRSA against CA-MRSA (p<0.0001). Prevalence of nasal carriage MRSA was higher in hospitalized patients as compared to healthy subjects.

Keywords: CA-MRSA, HA-MRSA, nasal carriage, soft tissue infection

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Introduction

Staphylococcus aureus is a dynamic and adaptable bacterium that has an incredible talent to attain antibiotic resistance. Introduction of penicillin had a dramatic impact during the ancient times when mortality from S.aureus disease was high but this glorious moment was short lived due to the emergence of penicillinase producing S.aureus (De Lencastre et al., 2007). Meticillin resistant Staphylococcus aureus (MRSA) strains had rapidly emerged during 1960 and became a major problem in hospitals immediately after the meticillin was introduced in 1959. Till recent times these MRSA strains were restricted to hospitals only as Health care associated MRSA (HA-MRSA). But now these strains have also emerged in community, called as Community associated MRSA (CA-MRSA). The ecological niches of S.aureus are anterior nares (Gilmour, 2008). Colonization of Staphylococcus aureus strains may serve as endogenous reservoirs for subsequent clinical infections (Safdar et al., 2008) and the risk was even higher for MRSA. The incidence of
community-acquired and hospital acquired \textit{S.aureus} infections has been rising with increasing emergence of drug-resistant strains called methicillin resistant \textit{S. aureus} (MRSA) (Deresinski, 2005). Nasal carriage rates of MRSA have been reported to be 0.8 to 3.0% among adults in the community elsewhere in the world (Jernigan et al., 2003). Among healthcare workers in hospital setting, it ranges from 6.0 to 17.8% (Cesur et al., 2004). The aim of the current research was to (a) study the nasal carriage rate of \textit{Staphylococcus aureus}, in community and in hospital settings and their antimicrobial susceptibility (b) to compare the nasal carriage rate of HA-MRSA with that of CA-MRSA and their antimicrobial susceptibility.

\textbf{Materials and Methods}

\textit{Study area}

The study was conducted in healthy Community and a Tertiary Care Hospital of Kashmir valley located in north India. A total of 200 nasal swabs (100 from hospitalized patients and 100 from healthy individuals) have been collected as per standard policy protocols.

\textit{Ethical consideration}

Before samples were collected, information regarding the study was explained to the Ethical Committee of the Institute, Community individuals and Hospitalized patients, after getting approval oral consent for participation in the study was obtained.

\textit{Questionnaire}

Relevant and detailed history was collected and only those personnel were included in the study who had not taken any antibiotic 7 days before sample collection.

\textit{Specimen collection: Nasal swab}

Sterile cotton tipped swab was moistened in a culture tube containing 2 ml of 0.1% buffered Tween-80 (Hi-media, Mumbai, India). 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 (Dar et al., 2006).

\textit{Processing and identification}

Nasal specimens collected from a tertiary care hospital and healthy community was processed at the medical microbiology laboratory SKIMS as per (CLSI, 2008). Specimens have been inoculated on blood agar to look for β-hemolysis of \textit{S.aureus}, nutrient agar was used for the direct colony identification, DNase agar and mannitol salt agar (MSA) have been used as selective media for the isolation of \textit{S.aureus} and incubated at 35°C for 48 hrs. All isolates were identified routinely by Grams stain, Catalase test, Coagulate test, DNase test and Mannitol Salt agar (MSA) test. The identification of organisms was based on cellular, cultural and biochemical characteristics.

\textit{Detection of MRSA}

Resistance to methicillin was detected with the cefoxitin (30 μg) Disk Diffusion Test (Bauer et al., 1966) and interpreted according to (CLSI, 2009). A diameter of \(\geq 22\) mm was considered as susceptible and \(\leq 21\) mm as resistant as per (CLSI, 2010).

\textit{Antibiotic susceptibility}

Antibiotic Susceptibility was assessed to check out the resistance profile of both hospitalized and community \textit{S.aureus} against different antibiotics like tetracycline, oxacillin, penicillin, vancomycin, gentamycin, clindamycin, erythromycin, ciprofloxacin and Linezolid performed by Bauer et al. (1966) diffusion method.

\textbf{Results}

Out of the 100 hospitalized subjects studied, the male female ratio was 58.2:41.8, out of them only 71 subjects were positive for \textit{S.aureus}. Of these 71 patients, 45 have
been males, and 26 females and age range was 1-80 years. The highest predominance of 32% *S. aureus* was observed amongst patients in the age group of 30-39 years whereas, lowest was recorded in the age groups of 10-19. The 30µg cefoxitin disk by (disk diffusion method) was tested phenotypically for MRSA detection during the current investigation. Out of 71 hospital associated *S. aureus* and 4 CA-MRSA isolates have been detected when using by using 30µg cefoxitin disk diffusion where a zone of inhibition <21mm was recorded in all resistant strains and >22mm was computed in all susceptible strains as shown in Fig. 1.

**Fig. 1.** Susceptibility of *S. aureus* strains

Out of 71 hospital associated *S. aureus*, 28 MRSA were detected, of these, 16 and 12 have been screened in males and females respectively. Out of 100 hospitalized subjects 71% *S. aureus* and 39.4% MRSA isolates have been detected from the nasal swabs during the current study. The highest incidence (30%) hospital acquired methicillin resistant *S. aureus* (HA-MRSA) was recorded in the age group of 50-59 years while, lowest (2.5%) was recovered in the age groups of 1-9 year. In MSSA infected patients the highest frequency (33.3%) was detected in age group of 10-19 years, while lowest (1.66%) in 1-9 years respectively. A total of 100 healthy subjects have been screened in the present study, out of which 49 were positive for *S. aureus* which includes 23 males and 26 females. The highest occurrence of 30% *S. aureus* was observed in the age group of 50-59 years and lowest (1%) was found in the age group of 80-89 years. A total of 8.16% MRSA isolates were obtained. The antibacterial activity of penicillin, erythromycin, tetracycline, ciprofloxacin, clindamycin, gentamycin, vancomycin and Linezolid was recorded against methicillin sensitive *S. aureus* (MSSA) and methicillin resistant to *S. aureus* (MRSA) (Table 1).

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Resistant Percentage (HA-MRSA)</th>
<th>Resistant Percentage (HA-MSSA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>100%</td>
<td>98.4%</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>88.1%</td>
<td>78.6%</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>82.4%</td>
<td>70.7%</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>80.3%</td>
<td>65.9%</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>49.6%</td>
<td>41.6%</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>32.6%</td>
<td>25.1%</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Linezolid</td>
<td>0</td>
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</tbody>
</table>

Isolated HA-MRSA and MSSA showed 100% and 98.4% resistance against penicillin followed by erythromycin 88.1%, 78.6%, tetracycline 82.4%, 70.7%, ciprofloxacin 80.3%, 65.9%, clindamycin 49.6%, 41.6% and gentamycin 32.6% and 25.1%, in both groups. Vancomycin and Linezolid were the most effective drugs with 100% sensitivity in both HA-MRSA and HA-MSSA respectively. A significant difference (p<0.0001) was analyzed when the resistant rate of HA-MRSA against HA-MSSA was compared. The antibiotic susceptibility of community associated methicillin sensitive *Staphylococcus aureus* (CA-MSSA) and community associated methicillin resistant *S. aureus* (CA-MRSA) to different antibiotics is shown in Table 2.
Table 2. Resistant profiles of CA-MRSA and CA-MSSA isolates

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Resistant Percentage (CA-MRSA)</th>
<th>Resistant Percentage (CA-MSSA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>99.2%</td>
<td>97.6%</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>85.3%</td>
<td>70.8%</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>20.2%</td>
<td>12.4%</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>8.7%</td>
<td>4.3%</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>7.1%</td>
<td>4.5%</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>5.8%</td>
<td>5.5%</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Linezolid</td>
<td>0</td>
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</table>

Penicillin showed resistance of 99.2%, 97.6% against CA-MRSA and CA-MSSA while erythromycin expressed 85.3%, 70.8%, tetracycline 20.2%, 12.4%, ciprofloxacin 8.7%, 4.3%, gentamycin 7.1%, 4.5% and clindamycin 5.8%, 5.5% in CA-MRSA and CA-MSSA respectively.

Again vancomycin and linezolid were the most effective drugs with 100% sensitivity and specificity in both CA-MRSA and CA-MSSA. A significant difference (p<0.0001) was verified when we compared the resistant rate of HA-MRSA against CA-MRSA.

Discussion

The distribution of methicillin resistant Staphylococcus aureus (MRSA) is worldwide but, the frequency varies among different countries. Rates of MRSA in the subcontinent countries of Pakistan and India had been observed high when compared with rates in northern Europe (Arakere et al., 2005). The highest dominance (30%) S. aureus was determined in the age group of 50-59 years and lowest in 1-9 year. Similar findings were also reported by Mahmood et al. (2010) where a prevalence of 58.5% MRSA in males and (41.5%) in females was assessed. The highest prevalence of 64.15% MRSA was analyzed in the age group of 41-80 years, while as lowest (4.91%) in 81-100 years. However, Sheng et al. (2011) noticed (53%) males and (47%) females infected with MRSA in an emergency department of a Taiwan hospital. The observation regarding the prevalence of MRSA in age group was (68.4%) in >60 years while as no MRSA was detected in the age group of 19-29 years. Similar age group patients infected by MRSA had been frequently reported in India (Mehta et al., 1998).

Staphylococcus aureus is one of the most prevalent gram positive cocci that are found as transient normal flora of human skin and mucosal surfaces in 20-90% of the population (Foster, 2004). Staphylococcus aureus is a leading cause of community acquired infections particularly in colonized humans (Kuehnert et al., 2006). Nasal carriage of S.aureus is an important risk factor for nosocomial and community acquired infection (Kluytmans et al., 1997). Staphylococcus aureus colonizes asymptotically mainly the anterior nares of humans. The present study showed an overall prevalence of 49% of S.aureus in the anterior nares of healthy subjects. The nasal carriage rate in most adult populations at a given time had been reported to be between 30-50%, but cumulative rates after several swabbing may reach 90% or more (Armstrong et al., 1976). Similar findings of 48.43% (Rajendra et al., 2011) and 49% (Oyetunji et al., 2012) were analyzed from the nasal swabs of healthy subjects. A prevalence of 8.16% CA-MRSA have been observed in the present investigation while as, Goud et al. (2011) reported 9.9% CA-MRSA rated from nasal swabs of healthy subjects. The treatment options of MRSA are limited to few antibiotics like vancomycin, linezolid and tigecycline. Reports of reduced susceptibility and resistance of S.aureus to vancomycin from Japan and USA had been reported (Klevens, 2007) but, their spread has not yet been observed...
in the present study. Penicillin expressed 100% resistance to MRSA. Similar finding was also noted by Rajadurapandi et al. (2006). Erythromycin showed (88.1%) and tetracycline gave (82.4%),

However, Mahmood et al. (2010) computed (73.5%) for erythromycin and Durgadas et al. (2009) reported 78% in tetracycline. Vancomycin was the effective drug with 100% vulnerability in both MRSA and MSSA isolates. These results are similar to the findings of El-Azizi et al. (2005) and Mahmood et al. (2010). Gentamycin expressed 32.6% resistance in MRSA isolates, whereas Durgadas et al. (2009) observed (32%). Groh’s et al. (2003) studied that aminoglycosides particularly gentamycin are bactericidal agents possessing rapid lethal activity on susceptible MRSA strains both in-vitro and in-vivo. Moreover, Jacqueline et al. (2004) concluded that despite aminoglycosides resistance among clinical MRSA isolates being widespread, gentamycin remains active against most MRSA strains in European countries. Ciprofloxacin revealed a resistant rate of 80.3% in MRSA whereas Mahmood et al. (2010) verified (72.3%) however, Shakya et al. (2010) expressed a low resistance of 37.5% and 37.7% in MRSA and MSSA isolates. The community acquired MRSA have typically established resistance to β-lactam group and erythromycin while retaining susceptibility to clindamycin, trimethoprim-sulfamethoxazole and floroquinolones whereas, health care associated MRSA are often multidrug resistant (Naimi et al., 2003). In Community acquired *S. aureus*, penicillin was the least effective drug with a confrontation of 99.2% against CA-MRSA whereas Wen et al. (2007) observed 100% resistance. Erythromycin expressed (85.3%) resistance whereas, (92%) was reported by Wang et al. (2004). Wen et al. (2007) studied a higher resistance of 100% while Rahul et al. (2006) exposed a much lower resistance of 42.9% for erythromycin. Vancomycin and Linezolid with 100% sensitivity were noted as the drugs of choice against CA-MRSA and CA-MSSA. Similar results were established (Rahul et al., 2006; Wen et al., 2007). Gentamycin with a resistant rate of 7.1% and 4.5% was also analyzed to be an effective drug against CA-MRSA and CA-MSSA, similar observations were made (Rahul et al., 2006; Wen et al., 2007; Shiv et al., 2009; Oyetunji et al., 2012). Clindamycin was another drug of choice with a sensitivity rate of 94.2% in CA-MRSA but, Shiv et al. (2009) and Debora et al. (2010) observed (95%) and (94.5%) sensitivity. Concerns had been raised regarding inducible clindamycin resistance that might emerge during treatment of CA-MRSA infection with this drug. From the above study it was clear that hospital acquired strains were more resistant to antimicrobials than community strains. Unless we take necessary precautions and measurements, microbes will develop 100% resistance to these antibiotics.

**Conclusion**

Prevalence of nasal carriage MRSA was higher in hospitalized patients as compared to healthy subjects. Community acquired MRSA were more susceptible to various antibiotics as compared to hospital acquired MRSA. Vancomycin and Linezolid were the drugs of choice for both groups of organisms. The following recommendations are essential in the containment of resistance to antimicrobial agents:

1. There is a need to reassess policies in antimicrobial drugs use within and outside the hospital environment.

There is also need for regular monitoring of the antimicrobial susceptibility status of important pathogens particularly MRSA so as to ensure the administration of an effective antibiotic.
2. Vancomycin, Teicoplanin, Linezolid and Teigecycline are effective against MRSA. These drugs should not be used as empirical therapy otherwise there are plenty of chances for development of resistant strains which would be resistant to almost all antibiotics.

3. High cost of treatment is one of the major causes of non compliance by patients. In a developing country like India majority of the patients are unable to afford a full course. This results in a vicious cycle of inadequate treatment leading to emergence of further resistance and spread of MRSA. Therefore, it is imperative to explore alternate effective antibiotic regimen to eradicate MRSA from the hospital and avoid spread in the community.

4. Screening should be made an essential tool in order to prevent spread of MRSA from hospital to community and community to hospital settings.

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