Prevalence of nasal colonization of methicillin-resistant *Staphylococcus aureus* among schoolchildren of Barabanki district, Uttar Pradesh, India

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**Abstract**

**Introduction:** The study aimed to determine the prevalence of nasal colonization of methicillin-resistant *Staphylococcus aureus* (MRSA), the minimum inhibitory concentration (MIC) of oxacillin and vancomycin, inducible clindamycin resistance, and antimicrobial resistance pattern of *S. aureus* among children of Barabanki district, Uttar Pradesh, India. **Materials and Methods:** School-going children of age group of 5–15 years were identified and selected according to the inclusion and exclusion criteria. Two nasal swabs were collected from each child as per the Centers for Disease Control and Prevention guidelines and transported to laboratory. Swabs were cultured on mannitol salt agar and 5% blood agar and incubated for 18–24 h at 37°C. Identification was done as per routine laboratory protocol. Detection of MRSA was done through cefoxitin 30 µg discs and D-zone test. Antibiotic susceptibility pattern of *S. aureus* by Kirby–Bauer disc diffusion method along with MIC for oxacillin and vancomycin was performed simultaneously according to Clinical Laboratory Standards Institute guidelines. **Results:** Out of 300 children, 140 (46.67%) were found to be nasal carriage for *S. aureus*, among which MRSA was found to be 23 (7.67%). All *S. aureus* and MRSA isolates were sensitive to vancomycin with MIC <2 µg/ml, whereas 23 *S. aureus* were found resistant to oxacillin with MIC value >4 µg/ml. Resistance to penicillin and co-trimoxazole was highest, whereas all were sensitive to linezolid. MRSA showed 100% susceptibility to linezolid, followed by gentamicin (91.4%) and tetracycline (87%). **Conclusion:** With the risk involved in transmission of infection, steps for identifying the carriers and its eradication should be carried out. Rational use of antibiotics should be given preference too.

**Keywords:** Methicillin-resistance *Staphylococcus aureus*, nasal colonization, *Staphylococcus aureus*
CA-MRSA is usually associated with skin and soft tissue infections such as cellulitis, furunculosis, and abscesses; however, life-threatening infections including necrotizing pneumonia, bacteremia, osteomyelitis, and septic shock have also been reported. CA-MRSA strains have higher epidemicity and are more virulent in comparison to HA-MRSA and thus are more likely to cause outbreaks and spread in community. Studies have suggested that healthy children may act as an asymptomatic reservoir of CA-MRSA and are responsible for transmission of MRSA infection in community. The Indian study shows prevalence of *S. aureus* among children aged 5–15 years are 16%–52%, of which 4%–19% were MRSA.

Skin and soft tissue infections are more predominant followed by bloodstream infections in both CA-MRSA and methicillin-sensitive *S. aureus* (MSSA) infections. Leukocytosis is most significantly associated with MRSA infection than MSSA infections and is good predictor for staphylococcal bone and joint infections. CA-MRSA nasal carriage has been associated with invasive staphylococcal infections as suggested by genetic evidence. Children have a higher rate of colonization of MRSA as compared to adults and contact living with those children are associated with subsequent colonization among adults. CA-MRSA has a higher probability of infection as compared to MSSA. CA-MRSA may also spread to the hospital and is found highly virulent.

Several studies have been conducted to determine the risk factors associated with colonization of MRSA among healthy children. Some studies suggested that socioeconomic status, frequent medication with antibiotics, hospitalization, chronic disease, and previous infection with MRSA may be the risk factors associated with colonization of MRSA, but these factors have not been identified in majority of colonized children.

The enormous disease burden due to CA-MRSA infection raised the need for active surveillance of the organism. The primary care physicians may play an important role in the early detection of the colonization among the children and prevent its transmission of MRSA in community by screening and decolonizing the children. The public health importance lies in the fact that the reservoir of the CA-MRSA disease is the carrier state. Thus, detection of such carrier state prevents the development of serious infections caused by MRSA strains requiring hospitalization. Such surveillance is necessary to evaluate the prevalence of nasal carriage of MRSA in community to prevent the transmission among the healthy individuals as well as the diseased one. The present study to determine the prevalence of nasal carriage among schoolchildren along with the minimum inhibitory concentration (MIC) of oxacillin and vancomycin for *S. aureus* and antimicrobial resistance pattern of *S. aureus*.

### Materials and Methods

#### Study design

The present study was a prospective, cross-sectional, and observational study to determine the prevalence of nasal carriage of MRSA among the schoolchildren of age group of 5–15 years of age. The present study was conducted in the Department of Microbiology of the institution.

#### Study population

The study participants are schoolchildren of age group of 5–15 years of Barabanki district, Uttar Pradesh, India.

#### Sample size

The calculated sample size was 300 schoolchildren on the basis of current population of approximately 510,000 school-going children of age group of 5–15 years in Barabanki district. Six schools were included in the study. A total of fifty schoolchildren were included from each of these six schools. Students were selected randomly from the class on the basis of inclusion criteria of the study.

#### Selection criteria

A. **Inclusion criteria**

a. Schoolchildren of age group of 5–15 years. Selection of the children was done randomly

B. **Exclusion criteria**

a. History of hospitalization in the past 1 year
b. Oral antibiotic use in the past 3 days and intramuscular use in the past 28 days
c. The presence of another illness requiring antibiotics.

#### Data collection

After enrollment of the children for the study on the basis of the inclusion and exclusion criteria, information was recorded on a preformed questionnaire which includes identification details, demographic variables, present medical history, physical examination, and previous medical history.

#### Sample collection

Two nasal swabs were collected from each child. Procedure was explained, and a written consent and assent was taken from parents and the child, respectively, before sampling. Swab was collected as per the Centers for Disease Control and Prevention guidelines described below. Same swab was used for sampling other nares in each subject. After collection of sample, swab was labeled properly and transported to the laboratory in trypticase soy broth for further processing.

#### Sample processing

The processing of swabs was done as follows:

a. **Inoculation of swabs:** One nasal swab from each patient was used for the preparation of Gram’s stained smear. Another swab was inoculated on 5% sheep blood agar (BA) and mannitol salt agar (MSA) media and incubated aerobically overnight at 37°C. After 24 h of incubation, BA and MSA media were observed for growth

b. **Identification of *S. aureus***: Identification of isolates were done on the basis of colony characteristics on BA and MSA,
Gram staining, and biochemical tests: catalase, slide and tube coagulase, phosphatase, and DNAase test. Colonies of *S. aureus* on BA were large (2–4 mm diameter), circular, convex, smooth, shiny, opaque, and easily emulsifiable. While, on MSA colonies, they appear yellow with yellow zones in media. On Gram-staining, it characteristically appears as spherical cocci of diameter approximately 1 µm arranged in clusters. Only isolates showing positive results for catalase test, slide and tube coagulase test, phosphatase test, and DNAase test were included in the study.

c. Detection of MRSA: All isolates confirmed as *S. aureus* were further tested for detection of methicillin resistance by Kirby–Bauer disc diffusion method using cefoxitin 30 µg discs (HiMedia Labs, India) as per Clinical Laboratory Standards Institute (CLSI) 2015 guidelines.[10]

d. Determination of antibiotic susceptibility pattern and inducible clindamycin resistance of *S. aureus*: It was performed using Kirby–Bauer disc diffusion method on Muller-Hinton agar using the zone interpretative criteria for sensitive, intermediate, and resistant as per CLSI guidelines. The following antibiotic discs procured from HiMedia, India, was used penicillin (10 units), oxacillin (1 µg), erythromycin (15 µg), clindamycin (2 µg), tetracycline (30 µg), gentamicin (10 µg), vancomycin (30 µg), linezolid (30 µg), co-trimoxazole (1.25/23.75 µg), and ciprofloxacin (5 µg). Inducible clindamycin resistance was detected by placing the clindamycin and erythromycin disc at a distance of 15–26 mm apart. Flattening of the zone of inhibition adjacent to the erythromycin disk indicates inducible clindamycin resistance.

e. MIC of oxacillin and vancomycin: MIC of oxacillin and vancomycin was determined as per CLSI guidelines.[10] Doubling dilution of the oxacillin and vancomycin was prepared in a range from 0.5 to 64 µg/ml and 0.5–128 µg/ml, respectively. The presence of >1 colony or light film of growth after incubation was considered as resistant.

**Statistical analysis**
Chi-square test was applied to test significant differences, *P* < 0.05 was considered statistically significant.

**Ethical considerations**
Ethical clearance was taken from the Institutional Ethical Committee for the study. Informed consent and assent form was duly filled and signed by the guardian and the child, respectively.

**Results**

In the present study, 300 school-going children of age 5–15 years were included in the study and nasal swab was collected from each child. Of these children, nasal carriage of *S. aureus* was found in 140 (46.67%) children, whereas MRSA colonization was found in 23 (7.67%) children. Nasal colonization of MRSA was found almost equal in both the age groups, i.e., 5–10 years and 11–15 years, but not *S. aureus* which is higher in 11–15-year age group. Male child were found to have a slightly higher nasal colonization with both *S. aureus* and MRSA as compared to female [Table 1].

MICs of oxacillin and vancomycin for *S. aureus* were determined by agar dilution method. Table 2 documented that all *S. aureus* and MRSA isolates were sensitive to vancomycin with MIC value <2 µg/ml, whereas 23 *S. aureus* were found resistant to oxacillin with MIC value >4 µg/ml.

Inducible clindamycin resistance was determined while performing antimicrobial susceptibility testing by D-zone test showed that none of the *S. aureus* isolates had positive D-zone test. Antimicrobial susceptibility profile of *S. aureus* is documented in Table 3. Resistance to penicillin and co-trimoxazole was highest and found in 83.6% and 67.1% of *S. aureus* isolates. Drugs with higher susceptibility were gentamicin (97.1%), clindamycin (86.4%), erythromycin (82.9%), tetracycline (78.6%), and ciprofloxacin (75.7%). All *S. aureus* were uniformly sensitive to linezolid (100%).

Antimicrobial susceptibility profile of MRSA is documented in Table 4. Penicillin was found resistant in all the MRSA isolates followed by co-trimoxazole (60.9%). MRSA showed 100% susceptibility to linezolid followed by gentamicin (91.4%) and tetracycline (87%). Moderate susceptibility was found with erythromycin (69.6%), fluoroquinolones (65.3%), and clindamycin (60.9%).

**Discussion**

Nasal colonization of *S. aureus* and MRSA among asymptomatic children may act as a reservoir of infections, which may be transmitted to the other healthy children or unhealthy children and to the adults living in close contact.[11] The infections caused by *S. aureus* may range from mild local to life-threatening infections.[12] The colonization of *S. aureus* was not a problem till the development of resistance. Methicillin resistance among *S. aureus* is a great concern as nasal colonization with these resistant isolates is difficult to eradicate as well as these isolates have a high rate of transmission.

**Table 1: Distribution of methicillin-resistant Staphylococcus aureus and Staphylococcus aureus on the basis of age and sex**

| Variables | MRSA (%) | Staphylococcus aureus (%) | *P* |
|-----------|----------|--------------------------|-----|
| Age (years) |          |                          |     |
| 5-10      | 12 (4)   | 62 (20.67)               | 0.48|
| 11-15     | 11 (3.67)| 78 (26)                  |     |
| Total     | 23 (7.67)| 140 (46.67)              |     |
| Sex       |          |                          |     |
| Male      | 16 (5.33)| 76 (25.33)               | 0.17|
| Female    | 7 (2.33) | 64 (21.33)               |     |
| Total     | 23 (7.67)| 140 (46.67)              |     |

*Percentage in the parentheses is calculated out of a total number of children, MRSA: Methicillin-resistant Staphylococcus aureus.*
In the present study, 140 (46.67%) school-going children found to have nasal colonization with *S. aureus*. This finding is consistent with a study conducted by Reta et al., which have found a slightly higher rate of 52.3%. However, a lower rate of 41%, whereas Chatterjee et al. have found a similar result in a study done in children <5 years of age with 90% resistance to ampicillin and 49% to ciprofloxacin.

Inducible clindamycin resistance was not found in any isolates of MRSA or *S. aureus*. However, several studies have found inducible clindamycin resistance among the isolates obtained as a nasal colonizer from school-going children. Chatterjee et al. have found 0.7% of inducible clindamycin resistance, whereas Pathak et al. and Shetty et al. have found 35% and 55% of resistance.

As CLSI has recommended the determination of susceptibility of isolates to vancomycin and oxacillin by agar dilution method. The present study has determined that all the isolates of *S. aureus* and MRSA isolates were found to be most resistant to penicillin followed by co-trimoxazole. This finding is consistent with the study conducted by Reta et al., which have shown 100% sensitive to gentamicin, 97% in our study, and >88% sensitivity of other antibiotics used in our study. Ramana et al. have also found a similar findings with gentamicin being the highest susceptibility rate followed by co-trimoxazole, erythromycin, and tetracycline.

In conclusion, the present study has found a high nasal colonization rate of MRSA or *S. aureus* isolates were found to be most resistant to penicillin followed by co-trimoxazole. This finding is consistent with the study conducted by Reta et al., which have found 100% resistance to penicillin but only 11.8% resistance to co-trimoxazole. Similarly, Ramana et al. have also found 100% isolates were resistance to penicillin but only 14.3% resistance to co-trimoxazole. In contrast, Shetty et al. have found a lower percentage of resistance to penicillin (46%) followed by ciprofloxacin (39%).

**Table 2: Minimum inhibitory concentrations of oxacillin and vancomycin for *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus***

| Drugs isolates     | Oxacillin ≤2 µg/ml (sensitive) | Oxacillin ≥4 µg/ml (resistant) | Vancomycin ≤2 µg/ml (sensitive) | Vancomycin 4-8 µg/ml (intermediate) | Vancomycin ≥16 µg/ml (resistant) |
|--------------------|--------------------------------|--------------------------------|----------------------------------|-------------------------------------|-----------------------------------|
| *Staphylococcus aureus* | 117 (60.9)                  | 23 (11.4)                        | 140 (75.3)                       | -                                   | -                                 |
| MRSA               | -                             | 23 (12.6)                        | 23 (12.6)                        | -                                   | -                                 |

MRSA: Methicillin-resistant *Staphylococcus aureus*

**Table 3: Antimicrobial susceptibility pattern of *Staphylococcus aureus* (n=140)**

| Antimicrobials | *Staphylococcus aureus* susceptibility pattern |
|----------------|---------------------------------------------|
|                | Sensitive (%) | Intermediate (%) | Resistant (%) |
| Penicillin     | 23 (16.4) | - | 117 (83.6) |
| Erythromycin   | 116 (82.9) | 8 (5.7) | 16 (11.4) |
| Clindamycin    | 121 (86.4) | 5 (3.6) | 14 (10) |
| Ciprofloxacin  | 106 (75.7) | 11 (7.9) | 23 (16.4) |
| Linezolid      | 140 (100) | - | - |
| Tetracycline   | 110 (78.6) | 9 (6.4) | 21 (15) |
| Gentamicin     | 136 (97.1) | 3 (2.1) | 1 (0.8) |
| Co-trimoxazole | 41 (29.3) | 5 (3.6) | 94 (67.1) |

**Table 4: Antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* (n=23)**

| Antimicrobials | MRSA susceptibility pattern |
|----------------|----------------------------|
|                | Sensitive (%) | Intermediate (%) | Resistant (%) |
| Penicillin     | - | - | 23 (100) |
| Erythromycin   | 16 (69.6) | 2 (8.7) | 5 (21.7) |
| Clindamycin    | 14 (60.9) | 3 (13) | 6 (26.1) |
| Ciprofloxacin  | 15 (65.3) | 1 (4.3) | 7 (30.4) |
| Linezolid      | 23 (100) | - | - |
| Tetracycline   | 20 (87) | 1 (4.3) | 2 (8.7) |
| Gentamicin     | 21 (91.4) | 1 (4.3) | 1 (4.3) |
| Co-trimoxazole | 7 (30.4) | 2 (8.7) | 14 (60.9) |

MRSA: Methicillin-resistant *Staphylococcus aureus*
there is a need to identify the reservoir and emphasize in limiting the transmission. This study suggests that the primary care physicians by identifying the carrier states of MRSA among school-going children may help in reducing the disease burden in the community as well as decreases the number of hospitalization due to MRSA infections, as children come in contact with large population regularly. A resilient and stringent continued surveillance is of utmost importance. With initial resistance to primary drugs, adhering to the strict and judicious use of antimicrobials along with antibiotic stewardship and identification of reservoir may limit the spread of MRSA among the children as well as the community.

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Conflicts of interest
There are no conflicts of interest.

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