

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

# Amit Kumar Singh<sup>1</sup>, Loveleena Agarwal<sup>1</sup>, Akash Kumar<sup>1</sup>, Chandrim Sengupta<sup>1</sup>, Ravinder Pal Singh<sup>1</sup>

<sup>1</sup>Department of Microbiology, Mayo Institute of Medical Sciences, Barabanki, Uttar Pradesh, India

#### 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

#### Introduction

*Staphylococcus aureus* has been a major cause for community as well as nosocomial infections in different countries including India.<sup>[1,2]</sup> *S. aureus* asymptomatically colonizes different regions of healthy individual most commonly being the anterior nares where it can survive for months.<sup>[3]</sup> Although the infections were easy to treat earlier even after the development of resistance to penicillin, the problem arises with the emergence

Address for correspondence: Dr. Amit Kumar Singh, Department of Microbiology, Mayo Institute of Medical Sciences, Barabanki, Uttar Pradesh, India. E-mail: amit.singh.dr@gmail.com

Access this article online		
Quick Response Code:	Website: www.jfmpc.com	
	DOI: 10.4103/jfmpc.jfmpc_345_16	

of methicillin-resistance *S. aureus* (MRSA). Methicillin is semi-synthetic penicillin used for treatment of infections caused due to *S. aureus* strains resistant to penicillin. With the increasing pressure of infections due to *S. aureus*, there has been an increasing prevalence of MRSA infections in India.<sup>[4]</sup> Earlier MRSA infections were only associated with hospitalized patients known as hospital-associated MRSA (HA-MRSA). However, nowadays, it is also spreading among the healthy individuals without any contact with health-care workers, especially in children known as community-associated MRSA (CA-MRSA).<sup>[5]</sup>

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Singh AK, Agarwal L, Kumar A, Sengupta C, Singh RP. Prevalence of nasal colonization of methicillin-resistant *Staphylococcus aureus* among schoolchildren of Barabanki district, Uttar Pradesh, India. J Family Med Prim Care 2018;7:162-6.

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.<sup>[5,6]</sup> 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.<sup>[6]</sup> 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.<sup>[7]</sup> The Indian study shows prevalence of *S. aureus* among children aged 5–15 years are 16%–52%, of which 4%–19% were MRSA.<sup>[8,9]</sup>

Skin and soft tissue infections are more predominant followed by bloodstream infections in both CA-MRSA and methicillin-sensitive *S. aureus* (MSSA) infections.<sup>[4]</sup> Leukocytosis is most significantly associated with MRSA infection than MSSA infections and is good predictor for staphylococcal bone and joint infections.<sup>[10,11]</sup> CA-MRSA nasal carriage has been associated with invasive staphylococcal infections as suggested by genetic evidence.<sup>[12]</sup> 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.<sup>[13]</sup> Colonization with MRSA has a higher probability of infection as compared to MSSA.<sup>[14]</sup> CA-MRSA may also spread to the hospital and is found highly virulent.<sup>[15]</sup>

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.<sup>[16-18]</sup>

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  $\mu$ m 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<sup>[19]</sup>
- 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.<sup>[19]</sup> Doubling dilution of the oxacillin and vancomycin was prepared in a range from 0.5to  $64 \,\mu\text{g/ml}$  and  $0.5-128 \,\mu\text{g/ml}$ , 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  $\mu$ g/ml, whereas 23 *S. aureus* were found resistant to oxacillin with MIC value >4  $\mu$ g/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.<sup>[7]</sup> The infections caused by *S. aureus* may range from mild local to life-threatening infections.<sup>[5,6]</sup> 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 (%)	Р
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)	

Staphylococcus aureus

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

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

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

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 colonization rate of 41%, whereas Chatterjee et al. have found a slightly higher rate of 52.3%.[9,20] However, a lower rate of colonization was found in the study done by Shetty et al.(25%) and Ramana et al. (16%).[8,21] Nasal carriage of MRSA was found in 23 (7.67%) schoolchildren in the present study. Colonization with MRSA is high as compared to study conducted by Shetty et al., Ramana et al., and Chatterjee et al., who have found carriage rate of 3%, 3.06%, and 3.89%, respectively.[8,9,21] However, Reta et al. have found a much higher rate (13.8%) of MRSA colonization.<sup>[20]</sup> This higher prevalence of nasal colonization may be attributed to the difference in geographical distribution and characteristics of population in the study as well as other factors such as sampling technique, its transportation, culture media, and methods of isolation and identification of S. aureus and MRSA.

Analysis of antimicrobial susceptibility pattern of *S. aureus* and MRSA demonstrated that both have almost similar pattern.

*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 found 100% resistance to penicillin but only 11.8% resistance to co-trimoxazole.<sup>[20]</sup> Similarly, Ramana *et al.* have also found 100% isolates were resistance to penicillin but only 14.3% resistance to co-trimoxazole.<sup>[8]</sup> In contrast, Shetty *et al.* have found a lower percentage of resistance to penicillin (46%) followed by co-trimoxazole (39%).<sup>[21]</sup> Pathak *et al.* have also found a similar result in a study done in children <5 years of age with 90% resistance to ampicillin and 49% to co-trimoxazole.<sup>[22]</sup> The high level of resistance to few antibiotics among the rural population may be attributed to the indiscriminate use of antibiotics by the unregistered medical practitioners and over-the-counter sale of the drugs.

MRSA and *S. aureus* isolates were significantly susceptible to gentamicin, clindamycin, erythromycin, tetracycline, and ciprofloxacin. This finding is consistent with the study done 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.<sup>[20]</sup> Ramana *et al.* have also found the similar findings with gentamicin being the highest susceptibility rate followed by co-trimoxazole, erythromycin, and tetracycline.<sup>[8]</sup> All the isolates were sensitive to vancomycin and linezolid. This is similar to the study done by Shetty *et al.* and Pathak *et al.*, who have found 100% isolates susceptible to vancomycin and linezolid.<sup>[21,22]</sup>

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.<sup>[9,21,22]</sup>

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* have a MIC value <2 for vancomycin, whereas isolates detected as MRSA by cefoxitin disc diffusion method showed MIC value <2 for oxacillin. Thus, cefoxitin is a good indicator for determination of MRSA isolates in routine laboratories.

Thus, in conclusion, the present study has found a high nasal rate of colonization for S. *aureus* among school-going children with the significant colonization of MRSA, hence determining the risk involved in transmission of infection. Simultaneously,

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.

#### Acknowledgment

The study was conducted as Indian Council of Medical Research short-term studentship project for the year 2015 (Reference id: 2015-04624).

#### **Financial support and sponsorship**

Nil.

## **Conflicts of interest**

There are no conflicts of interest.

#### References

- 1. Dozois A, Thomsen I, Jimenez-Truque N, Soper N, Pearson A, Mohamed-Rambaran P, *et al.* Prevalence and molecular characteristics of methicillin-resistant *Staphylococcus aureus* among skin and soft tissue infections in an emergency department in Guyana. Emerg Med J 2015;32:800-3.
- 2. Tong SY, Kearns AM. Community-associated MRSA from the Indian subcontinent. Lancet Infect Dis 2013;13:734-5.
- 3. Sharma Y, Jain S, Singh H, Govil V. *Staphylococcus aureus*: Screening for nasal carriers in a community setting with special reference to MRSA. Scientifica (Cairo) 2014;2014:479048.
- 4. Indian Network for Surveillance of Antimicrobial Resistance (INSAR) Group, India. Methicillin resistant *Staphylococcus aureus* (MRSA) in India: Prevalence and susceptibility pattern. Indian J Med Res 2013;137:363-9.
- 5. Otter JA, French GL. Molecular epidemiology of community-associated meticillin-resistant *Staphylococcus aureus* in Europe. Lancet Infect Dis 2010;10:227-39.
- 6. DeLeo FR, Otto M, Kreiswirth BN, Chambers HF. Communityassociated meticillin-resistant *Staphylococcus aureus*. Lancet 2010;375:1557-68.
- 7. Tavares DA, Sá-Leão R, Miragaia M, de Lencastre H. Large screening of CA-MRSA among *Staphylococcus aureus* colonizing healthy young children living in two areas (urban and rural) of Portugal. BMC Infect Dis 2010;10:110.
- Ramana KV, Mohanty SK, Wilson CG. *Staphylococcus aureus* colonization of anterior nares of school going children. Indian J Pediatr 2009;76:813-6.

- 9. Chatterjee SS, Ray P, Aggarwal A, Das A, Sharma M. A community-based study on nasal carriage of *Staphylococcus aureus*. Indian J Med Res 2009;130:742-8.
- 10. Kini AR, Shetty V, Kumar AM, Shetty SM, Shetty A. Community-associated, methicillin-susceptible, and methicillin-resistant *Staphylococcus aureus* bone and joint infections in children: Experience from India. J Pediatr Orthop B 2013;22:158-66.
- 11. Ju KL, Zurakowski D, Kocher MS. Differentiating between methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* osteomyelitis in children: An evidence-based clinical prediction algorithm. J Bone Joint Surg Am 2011;93:1693-701.
- 12. Lo WT, Wang CC, Lin WJ, Wang SR, Teng CS, Huang CF, *et al.* Changes in the nasal colonization with methicillin-resistant *Staphylococcus aureus* in children: 2004-2009. PLoS One 2010;5:e15791.
- 13. Chen CJ, Hsu KH, Lin TY, Hwang KP, Chen PY, Huang YC. Factors associated with nasal colonization of methicillin-resistant *Staphylococcus aureus* among healthy children in Taiwan. J Clin Microbiol 2011;49:131-7.
- 14. Miller MB, Weber DJ, Goodrich JS, Popowitch EB, Poe MD, Nyugen V, *et al.* Prevalence and risk factor analysis for methicillin-resistant *Staphylococcus aureus* nasal colonization in children attending child care centers. J Clin Microbiol 2011;49:1041-7.
- 15. Mishaan AM, Mason EO Jr., Martinez-Aguilar G, Hammerman W, Propst JJ, Lupski JR, *et al.* Emergence of a predominant clone of community-acquired *Staphylococcus aureus* among children in Houston, Texas. Pediatr Infect Dis J 2005;24:201-6.
- Shapiro A, Raman S, Johnson M, Piehl M. Community-acquired MRSA infections in North Carolina children: Prevalence, antibiotic sensitivities, and risk factors. N C Med J 2009;70:102-7.
- 17. Woods SE, Beiter E, Drake B, Engel A. The prevalence of asymptomatic methicillin-resistant *Staphylococcus aureus* in school-age children. East J Med 2011;16:18-20.
- Rijal KR, Pahari N, Shrestha BK, Nepal AK, Paudel B, Mahato P, *et al.* Prevalence of methicillin resistant *Staphylococcus aureus* in school children of Pokhara. Nepal Med Coll J 2008;10:192-5.
- 19. Clinical and Laboratory Standard Institute. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard – Eleventh Edition and Performance Standards for Antimicrobial Susceptibility Testing; Twenty-third Informational Supplement. CLSI Document M02-A11 and M100-S24. Wayne, PA: Clinical and Laboratory Standard Institute; 2014.
- 20. Reta A, Gedefaw L, Sewunet T, Beyene G. Nasal carriage, risk factors and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* among school children in Ethiopia. J Med Microbiol Diagn 2015;4:177.
- 21. Shetty V, Trumbull K, Hegde A, Shenoy V, Prabhu R, Sumathi K, *et al.* Prevalence of community-acquired methicillin-resistant *Staphylococcus aureus* Nasal colonization among children. J Clin Diagn Res 2014;8:DC12-5.
- 22. Pathak A, Marothi Y, Iyer RV, Singh B, Sharma M, Eriksson B, *et al.* Nasal carriage and antimicrobial susceptibility of *Staphylococcus aureus* in healthy preschool children in Ujjain, India. BMC Pediatr 2010;10:100.