Research Article

Prevalence and antibiotic susceptibility profile of MRSA isolated from the Anterior Nares of school going children in Gulbarga

Sagar M Arali, Vivek Kulkarni, Manjula N. G., Subhashchandra M. Gaddad, Y. M. Jayaraj, Channappa T. Shivannavar

Department of Microbiology, Gulbarga University, Gulbarga, Karnataka, India.
Correspondence to: Channappa T. Shivannavar, E-mail: ctshiv@gmail.com

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Abstract

Background: Emergence of methicillin resistant *S. aureus* (MRSA) infections among the healthy persons in the community settings has been noted recently. MRSA infections are now classified as health-care-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) infections. Its colonization is an important risk factor for subsequent MRSA infection.

Objective: To determine the prevalence of *Staphylococcus aureus* in the school going children in Gulbarga and their antibiotic susceptibility pattern.

Methods: Nasal swabs from anterior nares were collected for the isolation of *S. aureus* from 131 healthy children aged between 5 and 15 years from university school, Gulbarga, Karnataka. The nasal swabs were inoculated in nutrient broth for enrichment of *S. aureus* then streaked on Mannitol Salt Agar (MSA) and incubated at 37 °C for 24 h. For characterization of the *S. aureus,* conventional methods such as growth characteristics on mannitol salt agar, Gram's staining, and biochemical characteristics have been performed. Antibiotic sensitivity test was carried out for the isolated *S. aureus* using different antibiotics.

Results: A total of 131 nasal swabs were screened and the incidence of *S. aureus* was observed to be 77.86%. Of the total 102 *S. aureus* isolates, 4 (3.92%) isolates were found to be MRSA and 96.10% isolates were MSSA. A total of 26 (25.4%) isolates were found to be multi-drug resistant (MDR) in this study.

Conclusion: Our results suggest that healthy school going children below 16 years of age are potential carriers of *S. aureus* and in particular MRSA and MDR strains.

KEY WORDS: Methicillin resistant *S. aureus* (MRSA), antibiotic sensitivity test (AST), anterior nares, *S. aureus,* community-associated MRSA (CA-MRSA)

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Introduction

Staphylococcus aureus is a common human pathogen found in skin infections and invasive diseases such as pneumonia, osteomyelitis, endocarditis both in the health care and community settings.[1] Since, 1960s methicillin-resistant *S. aureus* (MRSA) has been recognized as a source of health-care-associated infections.[2] Over the past decade, the traditional notion of MRSA as a pathogen is seemingly confined to the nosocomial arena that has been challenged with the emergence of community-associated MRSA (CA-MRSA) in healthy individuals without conventional risk factors for MRSA acquisition, especially in the USA.[2–4] Reports of rapidly progressive fatal disease and serious complications resulting from virulent CA-MRSA infection including sepsis, necrotizing pneumonia, and necrotizing fasciitis have alerted medical professionals and the community alike to the need to face the increasing threat from community-based MRSA infections.[5–8]

S. aureus colonizes the anterior nares and other body sites, but the anterior nares are the most consistent site of colonization.[9] Carriage of *S. aureus*, including MRSA, is common in children, and genetic evidence supports a relationship between nasal carriage of *S. aureus* and MRSA and subsequent invasive staphylococcal infection.[10,11–14] Children could act as vectors for spreading *S. aureus* and MRSA to both community and hospital environments.[15] In addition, day-care centers constitute reservoirs of MRSA where children are at increased risk of nasal colonization.[10,16,17]

The emergence of high level resistance to penicillin way back in 1948 followed by development and spread of the strains resistant to synthetic penicillin's (methicillin, oxacillin, and naficillin), macrolides, tetracycline, and aminoglycosides has made the therapy of *Staphylococcal* infections a global challenge. Widespread occurrence of MRSA eventually leads to the emergence of multidrug resistant *S. aureus*, which in turn limits the treatment options.[18]

This study is to assess trend in the overall prevalence of nasal colonization by *S. aureus* in children, to identify the potential risk factors for MRSA nasal colonization, and to describe the evolving epidemiology of nasal colonization by *S. aureus* and by MRSA specifically.

Materials and Methods

Study Design, Population, and Location

The study was conducted from November 24, 2011 to January 23, 2012 at Gulbarga University School, Gulbarga, Karnataka, India. Nasal swabs for *S. aureus* culture were obtained from healthy school going children aged between 5 and 15 years. Data of the study population such as detailed history (such as age, sex, location), risk factors (such as antibiotic intake), and previous hospitalization were recorded.

Sample Processing

A total of 131 samples from anterior nares of the school going children were collected using sterile cotton swabs dipped in sterile saline in propylene tubes (Hi-Media Pvt, Ltd, Mumbai). The samples were enriched further for 6–12 h in nutrient broth and after the turbidity appearance; a loopful of inoculum was streaked onto the mannitol salt agar medium, sheep blood agar, and Baird Parker agar for growth of *S. aureus.* The plates were then incubated at 37 °C for 24–48 h. Further, *S. aureus* isolates were identified by standard microbiological methods such as Gram's stain, catalase, and coagulase tests.[19] The isolates were confirmed as MRSA by disc diffusion test (1 µg oxacillin).[20] The isolated *S. aureus* were preserved in 25% glycerol tubes until further use.

Antibiotic Sensitivity Test

Antibiotic sensitivity test of isolated *S. aureus* were carried out by Kirby–Bauer disk diffusion method in accordance to CLSI guidelines.[21,22] Each isolated *S. aureus* was inoculated into nutrient broth and incubated. The growth turbidity was adjusted to 0.5 McFarland standards and used as inoculum. With the help of a sterile cotton swab, the inoculum was swabbed evenly on Mueller-Hinton agar plates and after 5 min antibiotic discs were placed on the plates. The plates were then incubated at 37 °C for 24 h. After the incubation, individual antibiotic sensitivity was measured with the help of zone measuring scale. The following antimicrobial discs were used; ciprofloxacin (5 µg), erythromycin (15 µg), cloxacillin (30 µg), vancomycin (30 µg), ceftizoxime (30 µg), ampicillin (10 µg), penicillin (10 µg), methicillin (5 µg), amikacin (30 µg), cefoxitin (30 µg), oxacillin (1 µg), and gentamicin (10 µg). All the antibiotics were obtained from Hi-Media Pvt. Ltd., Mumbai.

Results

During the study period, a total of 131 anterior samples were collected from the school going children. The overall prevalence of *S. aureus* from the nasal carriage was found to be 77.86% in our study. The incidence of *S. aureus* from the nasal samples among the different standards in school going children was found to be highest in fifth standard with 100%, followed by 2nd standard with 91.3%, and seventh standard with 83.3%. It was observed that 60% was the lowest incidence rate of *S. aureus* in fourth standard in our study (Table 1).

Incidence of *S. aureus* with relation to gender was depicted in Table 2. It was observed that 100% incidence rate was observed in females with fifth, sixth, and seventh standards whereas among the males fifth standard observed to be highest with 100%. However, lowest of *S. aureus* isolation rate was observed in both males and females of 50% (sixth standard) and 55.5% (fourth standard), respectively. A total of 73.91% of *S. aureus* were isolated from the male population and 82.25% was the overall incidence rate in female population (Table 3).

Overall prevalence rate of MRSA from the nasal carriage of the school going children was observed to be 3.92%. The highest of 20% prevalence rate of MRSA was observed in seventh standard of the school going children, followed by 10.53% in first standard. Lowest of 8.33% was observed in sixth standard students. Interestingly, none of the MRSA isolated belonged to the second, third, fourth, and fifth standard students (Table 4).

The incidence rate of multidrug–resistant (MDR) MRSA was found to be 3.92% and the carriage rate was 0.031% whereas, the MSSA incidence rate for MDR was found to be 0.27% and the overall carriage rate being 0.19%. Highest incidence for MDR in MRSA was observed in first standard with 50%, followed by sixth and seventh standard with 25%.

Table 1: Incidence of *S. aureus* among the school going children

Standard	No. of nasal samples	Incidence of *S. aureus* isolates (%)
First	24	19 (79.17)
Second	23	21 (91.30)
Third	24	15 (62.50)
Fourth	20	12 (60.00)
Fifth	18	18 (100.00)
Sixth	16	12 (75.00)
Seventh	06	05 (83.33)
Total	131	102 (77.86)

Table 2: Incidence of *S. aureus* with relation to gender

Standard	No. of samples		No. of *S. aureus* isolates	
	Male	Female	Male (%)	Female (%)
First	12	12	09 (75.00)	10 (83.33)
Second	12	11	11 (91.67)	10 (90.99)
Third	13	11	08 (61.53)	07 (63.64)
Fourth	11	09	07 (63.64)	05 (55.55)
Fifth	10	08	10 (100.00)	08 (100.00)
Sixth	08	08	04 (50.00)	08 (100.00)
Seventh	03	03	02 (66.67)	03 (100.00)
Total	69	62	51 (73.91)	51 (82.25)

Table 3: Incidence of MRSA among university school going children

Standard	No. of nasal swab samples	No. of *S. aureus* isolated	No. of MRSA (%)
First	24	19	02 (10.53)
Second	23	21	00 (0.00)
Third	24	15	00 (0.00)
Fourth	20	12	00 (0.00)
Fifth	18	18	00 (0.00)
Sixth	16	12	01 (8.33)
Seventh	06	05	01 (20.00)
Total	131	102	04 (3.92)

Table 4: Prevalence of MDR-MRSA and MDR-MSSA from nasal swabs

Standard	MRSA	No. of MDR-MRSA (%) (*n* = 4)	Carriage rate of MDR-MRSA (*n* = 131)	MSSA	No. of MDR-MSSA (%) (*n* = 98)	Carriage rate of MDR-MSSA (*n* = 131)
First	02 (10.53)	02 (50)	0.015	17 (89.47)	08 (0.08)	0.061
Second	00 (0.00)	00 (0.00)	0	21 (100.00)	01 (0.01)	0.008
Third	00 (0.00)	00 (0.00)	0	15 (100.00)	02 (0.02)	0.015
Fourth	00 (0.00)	00 (0.00)	0	12 (100.00)	03 (0.03)	0.023
Fifth	00 (0.00)	00 (0.00)	0	18 (100.00)	06 (0.06)	0.046
Sixth	01 (8.33)	01 (25)	0.0076	11 (91.67)	04 (0.04)	0.031
Seventh	01 (20.00)	01 (25)	0.0076	04 (80.00)	02 (0.02)	0.015
Total	04 (3.92)	04 (3.92)	0.031	98 (96.10)	26 (0.27)	0.198

Table 5: Percent of antibiotic resistance in *S. aureus* of nasal swabs among school going children

Antibiotic	No. of sensitive *S. aureus* (%)	No. of intermediate *S. aureus* (%)	No. of resistant *S. aureus* (%)
Ciprofloxacin (CIP)	68 (66.67)	28 (27.4)	6 (5.8)
Erythromycin (E)	36 (35.2)	50 (49.0)	16 (15.6)
Cloxacillin (COX)	0 (0)	74 (72.54)	28 (27.4)
Vancomycin (VA)	77 (75.4)	20 (19.6)	5 (4.9)
Ceftizoxime (CZX)	85 (83.3)	4 (3.9)	13 (12.7)
Ampicillin (AMP)	59 (57.8)	16 (15.6)	27 (26.4)
Penicillin G (P)	6 (5.8)	0 (0)	96 (94.1)
Methicillin (MET)	97 (95.0)	1 (0.98)	4 (3.9)
Amikacin (AK)	100 (98.03)	2 (1.96)	0 (0)
Cefoxitin (CX)	94 (92.1)	6 (5.8)	2 (1.9)
Oxacillin (OX)	95 (72.5)	1 (0.98)	6 (4.5)
Gentamicin (GEN)	99 (75.5)	3 (2.2)	0 (0)

Table 6: Antibiotic resistance pattern of *S. aureus* isolates with relation to age

Antibiotic	Age group 5–10 years(*n* = 75)			Age group 11–15 years (*n* = 27)		
	No. of sensitive (%)	No. of intermediate (%)	No. of resistant (%)	No. of sensitive (%)	No. of intermediate (%)	No. of resistant (%)
Ciprofloxacin (CIP)	50 (38.1)	22 (16.7)	3 (2.2)	18 (13.7)	6 (6.8)	3 (2.2)
Erythromycin (E)	27 (20.6)	37 (28.2)	11 (8.3)	9 (6.8)	13 (9.9)	5 (3.8)
Cloxacillin (COX)	0 (0)	55 (41.9)	20 (15.2)	0 (0)	19 (14.5)	8 (6.1)
Vancomycin (VAN)	53 (40.4)	19 (14.5)	3 (2.2)	24 (18.3)	1 (0.7)	2 (1.5)
Ceftizoxime (CZX)	67 (51.1)	1 (0.7)	7 (5.3)	18 (13.7)	3 (2.2)	6 (6.8)
Ampicillin (AMP)	54 (41.2)	16 (12.2)	5 (3.8)	5 (3.8)	0 (0)	22 (16.7)
Penicillin G (P)	6 (4.5)	0 (0)	69 (52.6)	0 (0)	0 (0)	27 (20)
Methicillin (MET)	72 (54.9)	1 (0.7)	2 (1.5)	25 (19.08)	0 (0)	2 (1.5)
Amikacin (AK)	73 (55.7)	2 (1.5)	0 (0)	27 (20)	0 (0)	0 (0)
Cefoxitin (CN)	70 (53.4)	5 (3.8)	0 (0)	24 (18.3)	1 (0.7)	2 (1.5)
Oxacillin (OX)	71 (54.1)	1 (0.7)	3 (2.2)	24 (18.3)	0 (0)	3 (2.2)
Gentamicin (GEN)	73 (55.7)	2 (1.5)	0 (0)	26 (19.8)	1 (0.7)	0 (0)

While, highest of 0.08% was observed in MDR-MSSA in first standard, followed by 0.06% and 0.04% in fifth and sixth standards.

The antibiotic resistance pattern of the isolated *S. aureus* from the nasal samples of the school going children is shown in Table 5. A highest of 94.1% resistance was observed to penicillin, followed by 27.4% to cloxacillin, 26.4% to ampicillin, 15.6% to erythromycin, and 12.7% to ceftizoxime. Low resistance rate was observed to cefoxitin (1.9%), methicillin (3.9%), oxacillin (4.5%), vancomycin (4.9%), and ciprofloxacin (5.8%). None of the *S. aureus* isolates showed resistance to amikacin and gentamicin, respectively.

Antibiotic resistance patterns with relation to age group of 5–10 years was found to be highest with penicillin (52.6%), whereas in age group of 11–15 years was comparatively less with 20%. Ciprofloxacin and oxacillin showed similar resistance rate of 2.2% in both the variable age groups. The lowest of 1.5% resistance rate was observed in vancomycin, methicillin, and cefoxitin in the age group of 11–15 years and 1.5% for methicillin in age group of 5–10 years. However, all the isolates observed 0% resistance to amikacin and gentamicin (Table 6).

Discussion

This study revealed *S. aureus* nasal carriage rate of 77.86%, which is higher than the quoted figures of 20–40%.[9] Studies by Chatterjee et al.[23] and Ramana et al.[24] showed the less prevalence rate of 52.3% and 16%, respectively, from the nasal carriage of the school going children. Chatterjee et al. also reported the prevalence of MRSA carriage rate to be

3.98% which is similar to this study of 3.92%, whereas Ramana et al. reported a very high of 19% MRSA in nasal carriage. Chatterjee et al.[23] also reported the prevalence of *S. aureus* in the age group of 5–9 years with 47.8% and 10–15 years with 62.3% and prevalence of MRSA in these age groups were found to be 2.1% and 5.8%, respectively and the study reports the incidence of *S. aureus* in 5–15 years varying with 40–70%. Reported prevalence in the age group of 5–15 years varies from 40% to 70%.[25,26] A community-based study in the USA showed a prevalence of 42% in the age group of 5–19 years.[27] Intravenous drug addicts, patients with insulin dependent diabetes mellitus, those on haemodialysis or continuous peritoneal dialysis, HIV infected persons, and persons with skin infections are at increased risk of *S. aureus* nasal colonization.[28,9] Subjects in our study were healthy children, in whom most of these factors were negated.

Study by Fomda et al.[29] showed the overall prevalence of *S. aureus* to be 27.92% in the healthy individuals which is very less compared to this study (77.86%). The prevalence of *S. aureus* range from 20% to 40% from the nasal carriage among the healthy individuals and higher prevalence rates may be attributed in the overcrowded population.[9,26,30] The prevalence of MRSA nasal colonization was high (1.8%) in their study as compared to this study.

The overall susceptibility test results showed penicillin G to be the least effective drug against *S. aureus* with a resistance rate of 94.1%, whereas the other antibiotics showed comparatively low resistance rate. While study by Fomda et al.[29] showed a lower resistance rate of 21.39% for penicillin. Chatterjee et al. also showed the antibiotic resistance of *S. aureus* from nasal swabs of the school going children. The resistance rates of *S. aureus* observed for various antibiotics were as follows: amoxicillin (20.9%), erythromycin (8.8%), clindamycin (5%), cefotaxime (3.7%), gentamicin (3%), ciprofloxacin (2.4%), rifampicin (1%), netilmicin, and 5 (0.3%). On the other hand, this study showed comparatively higher prevalence rates for erythromycin (15.6%) and ciprofloxacin (5.8%) and higher resistance rates for other cephalosporin groups cefoxitin (1.9%) and ceftizoxime (12.7%).[23]

Oguzkaya-Artan[31] reported the overall nasal carriage in the study population of children was 18.0% less than our reports, and the prevalence of MRSA was found to be 5.6% comparatively much higher than this study with 3.92%. Their study also reported one MRSA strain was resistant to clindamycin, and the other one was resistant to the antibiotics such as, clindamycin, erythromycin, fusidic acid, and tetracycline. The resistance rates to various antibiotics in *S. aureus* were given in their study. Resistance to erythromycin was present 16.7% isolates almost similar to our study. All the tested strains were sensitive to gentamicin, vancomycin, SXT, rifampicin, and mupirocin in their study whereas this study reported resistance rate of 4.9% to vancomycin. Resistance to two or more antibiotics was noted in 13.8% isolates whereas we observed 25% MDR-MRSA with a carriage rate of 0.0076%.

Ciftci et al.[32] from Turkey, evaluated children between the age group of 4–6 years, and found that 28.4% had *S. aureus* carriage rate and 0.3% was the prevalence rate of MRSA less than our reports. The following findings were made in other parts of the world. Creech et al.[33] and Lo et al.[34] from Taiwan reported an incidence of 36.4% and 25%, respectively, for *S. aureus* carriage rate in the nasal colonization from children, and 9.2% and 13.2% children had colonized with MRSA. However, it was observed that the resistance rate was higher compared to this study. Hussain et al.[35] from Chicago Children's Hospital evaluated both in adults and in children. The nasal carriage rates for *S. aureus* have been reported to vary from 18% to 50% in different populations.[8,9] There are studies that have evaluated the prevalence of nasal carriage of *S. aureus* in children. Harputluoglu et al.,[10,11] two studies from Turkey, evaluated from deaf and healthy children reported the prevalence of *S. aureus* as 20.7% and 35.7% in the control group, respectively. The oxacillin susceptibility in deaf children was 100% and in the control group was found to be 85% in their study.

Lo et al.[36] reported 25.8% of *S. aureus* from children and the percentage of MRSA isolates among all *S. aureus* isolates was 45%. The ratio of male and female prevalence of *S. aureus* was found to be 28.2% and 25.6%, 28% and 21.1% (2004–2006 and 2007–2009), respectively. With reference to age less than 1 year had the prevalence rates of 32.1% and 16.0%, whereas 1–5 years showed 23.2% and 24.7% and less than 5 years showed 32.0% and 24.4% (2004–2006 and 2007–2009) respectively. The incidence of MRSA was found to be 8.1% and 15.1% between the years 2004–2006 and 2007–2009. Multidrug resistance was found to be 11% and 35% in 2004–2006 and 2007–2009. Our reports, however, showed less MDR comparatively with 0.19%.

The resistance pattern of *S. aureus* for various antibiotics was reported as followed by Lo et al. Resistance to gentamicin was observed to be 9.9% and 12.9%, whereas our isolates showed 0% resistance rate. Resistance of 15.6% to erythromycin, 5.8% to ciprofloxacin, and 49% to vancomycin in this study, comparatively their study showed higher resistance rate to erythromycin with 92.4%, whereas ciprofloxacin and vancomycin isolates were found to be totally sensitive.[36]

Lee et al.[37] reported 9.3% colonized nasal carriers with MRSA. A study by Sharma et al.[20] reported *S. aureus* nasal carriage rate in general population to be 12% and MRSA was found to be 5%. The antibiotic resistance rate for gentamicin Q4 was found to be 25%, for amikacin was 16.6%, for oxacillin was 5%, for ciprofloxacin was (54.16%) whereas vancomycin showed 0% resistance rate. Comparatively, our isolates showed ciprofloxacin resistance of 5.8%), oxacillin resistance of (4.5%), gentamicin and amikacin showed 0% resistance. The resistance rate of vancomycin was found to be high with 4.9%.[37,20]

Conclusion

Our results suggest that healthy school going children below 16 years of age are potential carriers of *S. aureus* and in particular MRSA and multidrug resistant strains.

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