Incidence of nasal colonization of methicillin resistant *Staphylococcus aureus* (MRSA) among school children

Tika Bahadur Thapa[1*] , Angela Rai[2]
Xavier International College, Boudha,, Nepal
*Correspondence: tika.thapa@xavier.edu.np

Date of Received: 22nd December ,2021 Date of acceptance: 10th February,2022

Abstract

Staphylococcus aureus resistant to several antibiotics especially methicillin resistant or MRSA, is a global public health problem. This study investigated the susceptibility pattern of *S. aureus* isolates as well as MRSA isolates from school children of age group 5-15 years from two different schools, one being private and other government school. Characterization and identification of *S. aureus* and MRSA was confirmed by microbiological methods and antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method. 100 different specimens were processed; 50 specimens from each private school and government school. A total of 9 (9%) isolates of *S. aureus* were isolated from the nasal samples. Among the isolates, 7 (77.78%) were found to be MRSA. The *S. aureus* and MRSA were not obtained from the nasal samples of school children from private school. Incidence of *S. aureus* and MRSA was higher in the age group 11-15 years (11.43% and 10%). Similarly, female children showed higher prevalence of *S. aureus* and MRSA as well (10.52% and 8.77%). Ofloxacin and ciprofloxacin were found to be most effective against both *S. aureus* and MRSA. All MRSA strains showed sensitivity towards vancomycin. All the isolates were resistant against penicillin, amoxycillin and cloxacillin. Results of this study show the growing urgency to promote activities which aids to improve the hygienic behavior of school children.

Keywords: *S. aureus*; MRSA; nasal carriage; school children; antibiotic resistance

Introduction

Staphylococcus aureus is a major bacterial human pathogen that causes a wide variety of clinical manifestations (Lowy 1998). *S. aureus* is a normal flora of nasal cavity and studies have shown that about 20% of individuals are persistent nasal carriers of *S. aureus* and around 30% are intermittent carriers, whereas about 50% are non-carriers (Wertheim et al 2005).

Originally, when penicillin-G was initially introduced in the early 1940s, over 85% of the *S. aureus* isolates were susceptible to penicillin; but penicillin resistant *St aureus* (PRSA) appeared within 3 years. *S. aureus* had acquired the ability to inactivate the β-lactam ring of penicillin with the help of plasmid-encoded β-lactamase enzyme (De Lencastre et al 2007). Methicillin, the first of the semi-synthetic penicillinase-resistant penicillin, was introduced in 1961 to target strains of penicillinase-producing *S. aureus* (Lowy 2003). However, resistance to methicillin was reported very quickly after its introduction (Jevons 1961). *S. aureus* resistant to oxacillin, methicillin and a few other related antibiotics are all known under the generic term methicillin resistant *S. aureus* or MRSA (Chambers 2001). MRSA strains harbor mecA gene, which is carried by a unique mobile genetic element, Staphylococcal Cassette Chromosome mec (SCCmec) integrated into the *S. aureus* chromosome (Hiramatsu et al 2002). The methicillin resistance gene (*mecA*) encodes an altered penicillin - binding protein PBP2a, which decreases the affinity of the *S. aureus* to β-lactam antibiotics (Hiramatsu et al 2001).

Since the first identification of MRSA in 1960s, it has become the most common cause of nosocomial and community infections worldwide. It has spread beyond the confines of health care facilities, emerging anew in the community, where it is rapidly becoming a dominant pathogen (Deresinski 2005). Today CA-MRSA has become the most frequent cause of skin and soft-tissue infections acquired in the community. Group with high-intensity physical contact is especially affected which includes school going children (Noorbakhsh et al 2007). Methicillin-resistant *S. aureus* (MRSA) carriage in healthy children is a major asymptomatic reservoir with an ability to quickly spread of MRSA within the community (Davoodabadi et al 2016). So this study primarily focuses on the distribution and incidence of *S. aureus* and MRSA which may be present in the school children. Antibiotic susceptibility test helps in understanding the present pattern of *S. aureus* as well as MRSA towards the tested antibiotics. This study also explores susceptibility pattern of the isolated MRSA strains towards vancomycin, which is the only drug of choice for treatment with MRSA infections.

Materials and method

This study was carried out at two different schools, one private school and other government school inside Kathmandu valley from December 2020 to January 2021. Altogether 100 nasal

swabs, 50 from the children of private school and 50 from the children of government school were collected from participants aged between 5-15 years. Only those children willing to participate in this study were included; written consent was obtained from the students prior to collection of sample.

a) Isolation of *S. aureus*

The nasal swabs were directly inoculated into the mannitol salt agar (MSA) and incubated at 37ºC up to 48 hours. Mannitol fermenting colonies (yellow colonies) from MSA were sub-cultured on nutrient agar and incubated at 37ºC for 24 hours. Pin point-sized colony on blood agar and golden yellow colony on nutrient agar having round, convex, opaque, and smooth-glistering surface with colony diameter about 2-3 mm were indicative of *S. aureus*. Further phenotypic identification of the *S. aureus* was done by Gram staining, catalase test, oxidase test, and coagulase test (slide test).

b) Detection of MRSA

The MRSA isolates were identified by growth on Mueller-Hinton agar containing 4 µg/ml of oxacillin. All the identified *S. aureus* isolates were subjected to *in-vitro* antimicrobial susceptibility test by Modified Kirby-Bauer's Disc Diffusion test as recommended by CLSI guidelines (CLSI 2014). Cefoxitin (30 µg) was used to detect the MRSA. The inoculums were prepared by transferring 3-4 identical colonies from nutrient agar to nutrient broth and incubated for 4 hours. The turbidity of the inoculums was made equivalent to 0.5 McFarland standard. The lawn culture of the test inoculums was then prepared by swabbing Muller-Hinton agar (MHA) containing 2% NaCl with sterile cotton swab dipped into inoculums. Cefoxitin (30 µg) disc was applied to the inoculated MHA plate and incubated at 37ºC for 18 hours. After incubation, the zone of inhibition of ≤21 mm around the disc was identified as MRSA.

c) Antibiotic susceptibility test (AST)

All the identified MRSA strains were subjected to *in-vitro* antimicrobial susceptibility test by modified Kirby-Bauer disc diffusion method as recommended by CLSI guidelines (CLSI 2014). The antibiotics tested were cefoxitin (30 µg), gentamicin (10 µg), amikacin (30 µg), erythromycin (15 µg), ciprofloxacin (5 µg), tetracycline (30 µg), ofloxacin (5 µg), gentamicin (10 µg), penicillin (10 µg), amoxicillin (10 µg), cloxacillin (5 µg) and ceftriaxone (30 µg). The inoculums were prepared by transferring 3-4 identical colonies from nutrient agar to nutrient broth and incubated for 4 hours. The turbidity of the inoculums was made equivalent to 0.5 McFarland standard. The lawn culture of the test inoculums was then prepared by swabbing Muller-Hinton agar (MHA) with sterile cotton swab dipped into inoculums. Antibiotic discs were applied to the inoculated MHA plate and incubated at 37°C up to 24 hours. After incubation, the zone of inhibition around the discs was noted and the results were interpreted as sensitive, intermediate, or resistant according to CLSI guidelines (CLSI 2014).

Result

A total of 100 samples were collected from two different schools, among which 43 (43%) were males and 57 (57%) were females. The participants under investigation were in the age range 5-15 years who were categorized into two groups of 5-10 years and 11-15 years respectively. On comparison of growth of bacteria on Mannitol Salt Agar (MSA) between two schools, growth was observed in 30 (60%) out of total 50 samples from government school while none of the samples collected from private school showed growth. Similarly, out of 30 samples that showed growth, *S. aureus* was observed in only 9 (30%) of the sample. Among the *S. aureus* isolates, 7 (77.78%) were found to be MRSA. Incidence of *S. aureus* and MRSA was higher in the age group 11-15 years (11.43% and 10%) followed by age group 5-10 years (3.33% and 0). Similarly, female children showed higher prevalence of *S. aureus* and MRSA as well (10.52% and 8.77%) followed by male (6.98% and 4.65%) respectively.

Antibiotic susceptibility pattern of *S. aureus*

Antibiotic susceptibility testing was performed on all 9 of the *S. aureus* isolates, out of which, 100% sensitivity was observed towards Ofloxacin and Ciprofloxacin. Similarly, 100% resistance was observed among the *S. aureus* isolates against Penicillin- G, Amoxycillin and Cloxacillin. One (11.11%) of the isolates was intermediate towards Tetracycline, Gentamicin and Amikacin while 8 (88.89%) of the isolates showed sensitivity against Tetracycline, Gentamycin and Amikacin. Seven (77.78%) of the isolates were resistant towards Cefoxitin discs thus confirmed MRSA (Table 1).

Table 1: Antibiotic susceptibility pattern of *S. aureus*

Antibiotics used	Susceptibility pattern		
	Sensitive (%)	Intermediate (%)	Resistant (%)
Cefoxitin (30µg)	2 (22.22%)	0	7 (77.78%)
Ofloxacin (5 µg)	9 (100%)	0	0
Penicillin- G (10 µg)	0	0	9 (100%)
Ciprofloxacin (30 µg)	9 (100%)	0	0
Amoxycillin (10 µg)	0	0	9 (100%)
Tetracycline (30 µg)	8 (88.89%)	1 (11.11%)	0
Gentamycin (10 µg)	8 (88.89%)	1 (11.11%)	0
Amikacin (30 µg)	8 (88.89%)	1 (11.11%)	0
Cloxacillin (5 µg)	0	0	9 (100%)
Ceftriaxone (30 µg)	5 (55.56%)	0	4 (44.44%)

Antibiotic susceptibility pattern of MRSA

Out of total 7 isolates of MRSA, all 7 (100%) of them were sensitive against Vancomycin. One (14.29%) of the isolate was sensitive, one (14.29%) was intermediate and five (71.43%) were resistant against Erythromycin. 100% sensitivity was observed towards Ofloxacin and Ciprofloxacin. Highest percentage of resistance (100%) was observed among the *S. aureus* isolates against Penicillin- G, Amoxycillin and Cloxacillin (MDR). One (14.29%) of the isolates was intermediate towards Tetracycline, Gentamicin and Amikacin while six (85.71%) of them were sensitive against Tetracycline, Gentamycin and Amikacin (Table 2).

Table 2: Antibiotic susceptibility pattern of MRSA

Antibiotics used	Susceptibility pattern		
	Sensitive (%)	Intermediate (%)	Resistant (%)
Vancomycin (30 µg)	7 (100%)	0	0
Erythromycin (15 µg)	1 (14.29%)	1 (14.29%)	5 (71.43%)
Cefoxitin (30µg)	2 (28.57%)	0	5 (71.43%)
Ofloxacin (5 µg)	7 (100%)	0	0
Penicillin- G (10 µg)	0	0	7 (100%)
Ciprofloxacin (30 µg)	7 (100%)	0	0
Amoxycillin (10 µg)	0	0	7 (100%)
Tetracycline (30 µg)	6 (85.71%)	1 (14.29%)	0
Gentamycin (10 µg)	6 (85.71%)	1 (14.29%)	0
Amikacin (30 µg)	6 (85.71%)	1 (14.29%)	0
Cloxacillin (5 µg)	0	0	7 (100%)
Ceftriaxone (30 µg)	4 (57.14%)	0	3 (42.86%)

Discussion

An important finding of this study was that the children of private school didn't show any nasal carriage of *S. aureus*. Various factors including recent exposure to antibiotics, host humoral immunity and other environmental factors including microbial interference (such as *Streptococcus pneumonia*) might be associated with the absence of colonization of *S. aureus* (Chen et al 2011). Proper personal hygiene practice among the school children is also likely to be relevant factor (Tong et al 2012).

In this study, 9% of the total school children were found to be nasal carriers of *S. aureus*. This result is slightly less than the study conducted in Kathmandu valley in the year 2008 which reported the nasal carriage rate of *S. aureus* as 11.8% in school children (Joshi et al 2008). However, in the same year, the study performed in Pokhara reported the nasal colonization of *S. aureus* as 31.0% (Rijal et al 2008) which showed comparatively higher prevalence of *S. aureus* in the school children. The variation in the prevalence of *S. aureus* among different studies could be due to the several factors such as difference in personal hygiene and lifestyle among different population characteristics, crowded living environment, direct skin to skin contact, sharing of individual objects, etc (Tong et al 2012).

The nasal carriage rate of MRSA among the school children, in this study, was found to be 77.78%. An estimated prevalence of nasal carriage of MRSA in this study was comparatively high compared to the reported prevalence of 5.2%, 17.1% and 56.1% in the previous studies conducted in Nepal (Rijal et al 2008; Joshi et al 2008; Adhikari et al 2018). Differences in the prevalence of nasal carriage of MRSA strains may be due to differences in the quality and size of samples and the use of different techniques and different interpretation guidelines.

The reported carriage rate of MRSA in this study was significantly high as compared to systematic review from Ethiopia and Gharyan city, where the MRSA colonization rate was 13.8% (Reta et al 2017) and 39.6% (Salem et al 2019) respectively. The methicillin resistant *S. aureus* (MRSA) carriage rate seemed to be very low among healthy school children in the Tibet Plateau in Southwest China (Deng et al 2014). Similarly, the nasal carriage rate of MRSA was 4% in the primary school children of Zakho city, Iraq (Assafi et al 2017). In compared to the report from the recent study performed in Sana's city, Yemen that reported only 1.5% of the nasal carriage rate of MRSA in the school children (Mohammed et al 2020), the estimated prevalence of MRSA in this study was significantly very high. This confirms that the carriage rate of MRSA vary widely depending upon the climatic condition and regions as well.

In the present study, the rate of isolation of *S. aureus* in male school children was 6.98% and 10.52% in female school children. In a similar study conducted in Urmia, the result showed that the rate of isolation of *S. aureus* in male was 17.0% and in female was 23.5% (Sadeghi et al 2017). Similarly, the study conducted in Nigeria showed the prevalence of *S. aureus* as 57.3% in females and 55.39% in males (Nsofor et al 2015). These results indicate that the nasal colonization of S. aureus in female is higher than the male counterparts which is similar to the findings of our study. On the other hand, in a similar study conducted by Reta et al, nasal carriage of *S. aureus* was found to be 23.7% in male children and 17.3% in female children (Reta et al 2015). Also, a study showed the prevalence of *S. aureus* as 19.07% in female children and 28.2% in male children of municipality schools in India (Bharathi et al 2014) which showed that the prevalence of *S. aureus* was more in male than in female; however, the carriage of MRSA was more or less same (7.23% Vs. 7.69%). This result is comparatively higher than the report of the present study that showed the nasal carriage rate of MRSA in males and female school children as 4.6% and 11.63% respectively.

Nasal carriage of *S. aureus* was also studied in two different age-groups: 5-9 years and 10-15 years. In the study, 3.33% of the children belonging to the age group 5-9 years and 43% belonging to the age group 10-15 years were found to harbor *S. aureus*. The present study showed a higher colonization of *S. aureus* in the age group 10-15 years. A recent study performed in Sana's city, Yemen also reported the higher prevalence of *S. aureus* in children of age group 10-15 years (Othman et al 2020). Different studies show variations in the rate of colonization of MRSA. In the present study, nasal carriage of MRSA in 5-9 years age group was found to be 0% and its carriage in the age group 10-15 was found to be 10%. This result is somewhat similar to that of the study in Turkey which reported 0.3% of the nasal carriage of MRSA among the healthy children of age group 4-6 years (Ciftci et al 2007). However, the carriage rate differs from the study conducted in Bharatpur that reported the nasal carriage of MRSA in 3-9 years age group as 2.1% and its carriage in the age group 10-15 as 3.6% (Adhikari et al 2018). In Ethiopia, Reta et al further reported colonization of MRSA was 12.7% and 15.4% among 6-9 years old children and 10-12 years old children respectively (Reta et al 2015).

Analysis of antimicrobial susceptibility pattern of *S. aureus* and MRSA demonstrated that both have similar pattern. *S. aureus* and MRSA isolates were found to be most resistant to penicillin,

amoxicillin and cloxacillin. No isolates were found resistant to vancomycin, ofloxacin and ciprofloxacin. This finding was similar to the findings reported in Omdurman city where all the isolates were sensitive to vancomycin and resistant to penicillin (Suliman 2014). Similar was the case in Barabanki district, UP, India, where the highest sensitiveness was against vancomycin and highest resistance was against penicillin and also cotrimoxazole (Singh et al 2018).

Acknowledgement

I would like to express my deep sense of indebtedness and immense gratitude to my respected supervisor **Mr. Rajesh Kumar Nepal,** lecturer and Head of Department of Microbiology, Xavier International College for providing an opportunity to work under his supervision and continuous guidance, without which this work would not have come into shape. I am extremely grateful to **Mr. Biraj Lohani,** lecturer of Department of Microbiology, Xavier International College for his valuable suggestions and help during my research work. I am also thankful to **Xavier International College** for providing me the laboratory facilities throughout my research work. I would also like to express my deepest gratitude and sincere thanks to my friends and families who directly or indirectly helped me in the completion of this work.

REFERENCES

Adhikari S, Khadka S, Parajuli A, KC A, Mishra R, Kandel P and Tiwari A (2018). Nasal Colonization of Staphylococcus aureus and their Antibiograms among School Children in Bharatpur, Nepal. *J of Col of Med Sci-Nepal*, ***14***: 172-177.

Assafi MS, Polse RF, Hussein NR, Haji AH and Issa AR (2017). The prevalence of S. aureus nasal colonisation and its antibiotic sensitivity pattern amongst primary school pupils. Sci J of Uni of Zakho **5**: 7-10.

Bharathi M, Lakshmi N, Kalyani C and Padmaja I (2014). Nasal carriage of multidrug-resistant MSSA and MRSA in children of municipality schools. Indian j of med microbiol **32**: 200.

Chambers HF (2001). The changing epidemiology of Staphylococcus aureus. Emerg infect dis **7**: 178.

Chen CJ, Hsu KH, Lin TY, Hwang KP, Chen PY and Huang YC (2011). Factors associated with nasal colonization of methicillin-resistant Staphylococcus aureus among healthy children in Taiwan. J of Clinic Microbiol **49**: 131-137.

Ciftci IH, Koken R, Bukulmez, A, Ozdemir M, Safak B and Cetinkaya Z (2007). Nasal carriage of Staphylococcus aureus in 4–6 age groups in healthy children in Afyonkarahisar, Turkey. Acta Paed **96**: 1043-1046.

Clinical and Laboratory Standards Institute (CLSI) (2014). M_{100}-S_{24} performance standards for antimicrobial susceptibility testing; twenty-fourth information supplement. 950 West Valley Road, Suite 2500.

Davoodabadi F, Mobasherizadeh S, Mostafavizadeh K, Shojaei H, Havaei SA, Koushki AM and Shirani K (2016). Nasal colonization in children with community acquired methicillin-resistant Staphylococcus aureus. Adv biomed res **5.**

De Lencastre H, Oliveira D and Tomasz A (2007). Antibiotic resistant Staphylococcus aureus: a paradigm of adaptive power. Cur op in microbiol **10**: 428-435.

Deng JJ, Xiao GG, Zhu Y, Zhou W and Wan C (2014). Staphylococcus aureus nasal carriage and its antibiotic resistance profiles in Tibetan school children in Southwest China. HK J Paediatr **19**:75-8.

Deresinski S (2005). Methicillin-resistant Staphylococcus aureus: an evolutionary, epidemiologic, and therapeutic odyssey. Clinic infect dis **40**: 562-573.

Hiramatsu K, Cui L, Kuroda M and Ito T (2001). The emergence and evolution of methicillin-resistant Staphylococcus aureus. Trends in microbial **9**: 486-493.

Hiramatsu K, Katayama Y, Yuzawa H and Ito T (2002). Molecular genetics of methicillin- resistant Staphylococcus aureus. Inter j of med microbiol IJMM **292**: 67–74.

Joshi, D. R., Shrestha, S. N., Bomjan, R., & Poudel, K. (2008). Nasal Carriage of Staphylococcus aureus among Healthy School Children in Kathmandu Valley. *Nepal Journal of Science and Technology*, *9*, 139-142.Jevons MP (1961). "Celbenin"-resistant staphylococci. British med j **1**: 124.

Lowy FD (1998). Staphylococcus aureus infections. New Eng j of med **339**: 520-532.

Lowy, F. D. (2003). Antimicrobial resistance: the example of Staphylococcus aureus. The J of clinic investig **111**: 1265-1273.

Noorbakhsh, S., Siadati, A., Farhadi, M., Rimaz, S., & Tabatabaei, A. (2007). Methicillin resistant Staphylococcus aureus in children. J of Comprehen Pedi **1**: 24-30.

Nsofor C, Nwokenkwo V and Nwaokpa C (2015). Nasal carriage of Staphylococcus aureus among apparently healthy school children in Owerri Metropolis, Nigeria. MOJ Cell Sci Rep **2**: 00038.

Othman AM, Al-Huraibi BS, Assayaghi RM and Al-Shami HZ (2020). Nasal Carriage and Methicillin Resistance of Staphylococcus Aureus among Schoolchildren in Sana'a City, Yemen.

Reta A, Gedefaw L, Sewunet T and Beyene G (2015). Nasal carriage, risk factors and antimicrobial susceptibility pattern of methicillin resistant Staphylococcus aureus among school children in Ethiopia. J of Med Microbiol & Diag **4**: 1.

Reta A, Wubie M and Mekuria G (2017). Nasal colonization and antimicrobial susceptibility pattern of Staphylococcus aureus among pre-school children in Ethiopia. BMC res not **10**: 1-7.

Rijal KR, Pahari N, Shrestha BK, Nepal AK, Paudel B, Mahato P, and Skalko-Basnet, N (2008). Prevalence of methicillin resistant Staphylococcus aureus in school children of Pokhara. Nepal med coll J **10**: 192-195.

Sadeghi, E., Far, A. N., Karamiyat, M., Gazzavi, A., Nikibakhsh, A. A., & Noroozi, M. (2017). Frequency of Methicillin-resistant Staphylococcus aureus Nasal Colonization among preschool and school children under 14 years old in Urmia. The J of Urmia Uni of Med Sci **27**:12.

Salem MA, At Thar AD, Belkher MS and Shaban TM (2019) Nasal and Hand Carriers and the Prevalence of Methicillin Resistant Staphylococcus aureus (MRSA) Among School Children in Gharyan City. Gastroenterol Hepatol Int **4**: 000147.

Singh A, Agarwal L, Kumar A, Sengupta C and Singh RP (2018). Prevalence of nasal colonization of methicillin-resistant Staphylococcus aureus among schoolchildren of Barabanki district, Uttar Pradesh, India. *J of Fam Med and Prim Care* ***7***: 162 - 166.

Suliman MAA (2014). Nasal Carriage of Methicillin Resistant Staphylococcus aureus (MRSA) among Healthy Primary School Children in Omdurman City (Doctoral dissertation, Sudan University of Science and Technology).

Tong SY, Chen LF and Fowler VG (2012). Colonization, pathogenicity, host susceptibility, and therapeutics for Staphylococcus aureus: what is the clinical relevance? Sem in immunopathol **34**: 185-200 Springer-Verlag.

Wertheim HF, Melles DC, Vos MC, Van Leeuwen W, Van Belkum A, Verbrugh HA and Nouwen JL (2005). The role of nasal carriage in Staphylococcus aureus infections. The Lancet infect dis **5**: 751-762.