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Methicillin-resistant staphylococci among school children in Mariental, Namibia

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ABSTRACT

Methicillin-resistant Staphylococcus aureus (MRSA) is resistant to beta-lactam antibiotics, while some strains are multi-drug resistant and may produce disease-causing toxins. Drug resistant strains are often responsible for chronic, persistent and recurrent infections, which pose a challenge for healthcare practitioners. Our study aimed to determine the prevalence of nasal MRSA and methicillin-resistant coagulase-negative staphylococci (MRCoNS) among school children in the Mariental community, southern Namibia. This was a cross-sectional study in the Mariental District. Nasal specimens (swabs) were collected from 272 randomly selected learners aged 6–14 years attending school in the area during the months of March, September and October 2016. Isolation and identification of staphylococci were performed using standard microbiological methods. Methicillin-resistant isolates were identified by their resistance towards cefoxitin (30 µg) using the Kirby-Bauer disk diffusion assay. Enterotoxin production among multi-drug resistant MRSA isolates was detected with a SET-RPLA toxin detection kit. Methicillin-resistant S. aureus was isolated from 48 (17.6%) learners and MRCoNS from only seven (2.6%). Methicillin-resistant S. aureus colonization was significantly higher ($P = 0.003$) in the age group 11–14 years than in the group 6–10 years. Among the 433 staphylococcal isolates screened for cefoxitin resistance, 51 (11.8%) were MRSA and seven (1.6%) were MRCoNS. From the 51 MRSA isolates, 22 (43.1%) were multi-drug resistant of which six were enterotoxigenic. This is the first report on MRSA and MRCoNS among school children in Namibia. The presence of multi-drug resistant and potentially virulent staphylococci among school children in Mariental, Namibia, is of concern. Self-infection by these bacteria poses various health risks for the children. It is recommended that school health programmes improve current hygiene practices. Frequent handwashing can prevent staphylococcal disease and spread of resistant strains among learners and the wider community.

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Introduction

The human nasal cavity predominantly contains Staphylococcus aureus and Staphylococcus epidermidis as normal microflora [14]. These bacteria are however opportunistic pathogens, causing infections when conditions are favorable [22], such as when colonizing bacteria enter normally sterile body sites through damaged skin or mucosal surfaces [19]. Nasal carriage of S. aureus is a major risk factor for becoming infected with this bacterium [23]. Skin infections [17], ear infections [11] and tonsillitis [27] with S. aureus as a causative agent are common among school-aged children. Enterotoxin production is an important virulence factor of such infections.

Healthy school children under 16 years are potential carriers of S. aureus, especially methicillin-resistant S. aureus (MRSA) and multi-drug resistant strains [3]. These children are asymptomatic reservoirs for community-associated MRSA (CA-MRSA) which enables the bacteria to rapidly spread within communities [2]. Methicillin-resistant S. aureus is resistant to beta-lactam antibiotics, while some strains are multi-drug resistant and may produce disease-causing toxins. Drug resistant strains are often responsible for chronic, persistent and recurrent infections, which pose a challenge for healthcare practitioners.

There is currently a lack of published evidence-based research and statistics on the prevalence of methicillin-resistant staphylococci and their associated virulence factors in Namibian school children. Our study therefore aimed to determine the prevalence of nasal MRSA and methicillin-resistant CoNS (MRCoNS) among school children in the Mariental community, southern Namibia. The ability of these bacteria to display antibiotic resistance and produce enterotoxins were also investigated. To our knowledge, this is the first report on MRSA and MRCoNS among school children in the Mariental District. Information obtained from this study can be useful in assisting school health programmes.

Materials and methods

Study area, population and sample collection

This was a cross-sectional study in the town of Mariental, located southeast of Namibia's capital city Windhoek on the B1 national highway. Approval to conduct the study was obtained from the Director, Hardap Regional Council Directorate of Education, Arts and Culture, Mariental, and the Permanent Secretary at the Windhoek Head Office, Ministry of Education, Arts and Culture. With written consent from their parents/guardians, the population that was screened for nasal staphylococci consisted of randomly chosen community individuals without any signs and symptoms of disease attending five schools in the Mariental District. Assuming that half of the study participants would carry staphylococci in their noses, sample size was calculated using the formula for sample size calculation for cross-sectional studies by Charan and Biswas [6]:

$$Sample\,size = {Z_{1-\alpha 1/2}}^2\, p(1-p)/d^2$$
$$= 1.96^2 x\, 0.5\,(1-0.5)/0.05^2$$
$$= 384\, learners$$

Four hundred and eleven parents/guardians were approached, of which only 272 (66.2%) consented. Children were divided into two age-groups: 6–10 years and 11–14 years, and consisted of 126 boys and 146 girls. Sample collection was done during March, September and October 2016. One nasal specimen was obtained from each child by gently rotating a sterile Amies transport medium swab (Labocare^{TM}, Johannesburg, South Africa) thoroughly around the perimeter of both nostrils [15]. Specimens were kept frozen at −20 °C until transporting them to the laboratory for processing.

Isolation and identification of staphylococci

To enrich for staphylococcal growth, specimen-containing swabs were cut shorter with flamed scissors and placed into test tubes each containing 2 ml sterile brain heart infusion broth (Merck, Darmstadt, Germany). These were incubated at 37°C for 24–48 h, until sufficient growth was observed. Enriched cultures were then inoculated onto selective medium Staphylococcus medium no. 110 (Oxoid, Basingstoke, England) and tryptone soy agar (Scharlau Microbiology, Spain) and incubated at 37°C for 24 h. Pure cultures were obtained by streaking different colonies from mixed culture plates onto Staphylococcus medium no.110 and tryptone soy agar. These were incubated at 37°C for 24 h. Pure culture plates were parafilmed and refrigerated at 4 °C for further assays.

Identification of S. aureus (coagulase-positive) and CoNS was done by standard microbiological procedures [15], including observation of distinctive characteristics of isolates on agar plates, Gram-positive stains, and production of the enzymes catalase and coagulase.

Detection of MRSA, MRCoNS and multi-drug resistance

Detection of MRSA and MRCoNS was done by Kirby-Bauer disk diffusion assay [15] on Mueller-Hinton agar (Mast Diagnostics, Merseyside, UK). In addition, all methicillin-resistant isolates were tested for multi-drug resistance using the antibiotics in Table 1. Altogether 352 nasal S. aureus isolates and 81 nasal CoNS were screened. By direct colony suspension, three to five well-isolated colonies from overnight tryptone soy agar (Scharlau Microbiology, Spain) plate cultures were inoculated

Table 1
Antibiotics used in this study and interpretation of inhibition zones of test cultures. Adapted from CLSI (2017) [[7]] and EUCAST (2017) [[13]].

Chemical class	Antibiotic	Disk symbol	Disk content	Resistant	Intermediate	Susceptible
Aminoglycosides	Gentamicin	GM	10µg	<18 mm S.aureus <22 mm CoNS	–	≥18 mm S.aureus ≥22 mm CoNS
β-lactams	Ampicillin	AP	25μg	<18mm	–	≥18mm
Cephalosporins (also a β-lactam)	Cefoxitin	FOX	30µg	<22 mm	-	≥22 mm
Fluoroquinolones	Ciprofloxacin	CIP	5µg	<20mm	–	≥20mm
Macrolides	Erythromycin	E	15µg	<18mm	18–20mm	≥21mm
Tetracyclines	Tetracycline	T	30µg	<19mm	19–21mm	≥22mm
Other	Rifampicin	RP	5µg	<23mm	23–25mm	≥26mm

into 10 ml sterile phosphate buffered saline pH 6.8–7.4 (Skylabs, Johannesburg, SA) and adjusted to 0.5 McFarland standard (1.5×10^8 CFU/ml, absorbance reading 0.08–0.13 at 625 nm). Adjusted cultures were then swabbed onto Mueller-Hinton (Mast Diagnostics, Merseyside, UK) agar and left to dry for 5–10 min at room temperature before dispensing the antibiotic disks (Mast Diagnostics, Merseyside, UK) onto the plates. Plates were incubated at 35°C for 18–20 h and diameters (zones of inhibition) were measured using a ruler. Clinical and Laboratory Standards Institute (CLSI) guidelines (2017) [[7]] and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint tables version 7.1 (2017) [[13]] were used to interpret results. Resistance towards cefoxitin (30 µg), in other words an inhibition zone diameter < 22 mm, indicated MRSA or MRCoNS, while resistance towards three or more different classes of antibiotics was an indication of multi-drug resistance in the bacteria.

Enterotoxin production

Twenty-three multi-drug resistant MRSA isolates (including the MRSA reference strain S. aureus ATCC 33,591) were selected to screen for production of one or more of the enterotoxins A-D. This was done in V-shaped 96-well microtiter plates (Thermo Fisher Scientific, Newport, UK) by reversed passive latex agglutination with a SET-RPLA toxin detection kit (Oxoid, Basingstoke, England) as per manufacturer's instructions.

A control plate was prepared as per instructions to provide references for positive tests. Each reconstituted control caused agglutination with its respective sensitized latex. Samples were prepared as follows: Each isolate was inoculated into 2-ml Eppendorf centrifuge tubes (Eppendorf, Germany) containing 1.5 ml sterile tryptone soy broth (Mast Group, Merseyside, UK) and incubated at 37 °C for 18–24 h. After incubation, broth cultures were refrigerated to 4 °C and centrifuged at 900xg for 20 min. The filtrate was retained for assay of toxin content.

The microtiter plate was arranged so that each row consisted of eight wells. Each sample needed the use of five such rows. Using a pipette, 25 µl of diluent was dispensed in each well of the five rows. Thereafter, 25 µl of test sample (filtrate) was added to the first well of each of the five rows. Starting at the first well of each row, 25 µl was picked up and double dilutions were made along each of the five rows, stopping at the seventh well (negative control well) that contained diluent only. To each well in the first row, 25 µl of latex sensitized with anti-enterotoxin A was added. To each well of the second row, 25 µl of latex sensitized with anti-enterotoxin B was added. This was repeated for the next two rows using latex sensitized with anti-enterotoxin C and D. To each well of the fifth row, 25 µl of latex control was added. The contents of each well were mixed by gentle agitation by hand. Plates were covered with lids to avoid evaporation.

After 20–24 h incubation at room temperature, each plate was examined for agglutination against a black background. If staphylococcal enterotoxins A, B, C or D were present, agglutination occurred, resulting in the formation of a lattice structure. Upon settling, this formed a diffuse layer on the base of the well. If staphylococcal enterotoxins were absent or at a concentration below the assay detection level, a lattice structure could not be formed and a tight button was observed at the bottom of each well.

Statistical analysis

A chi-square test was used using MedCalc statistical software [[20]] https://www.medcalc.org to compare percentage differences of MRSA and MRCoNS between age groups and gender. Statistical significant differences were indicated by a P-value of $\leq$ 0.05.

Table 2
Nasal carriage of MRSA and MRCoNS among learners aged 6–14 years attending five schools in the Mariental area.

School	Age group	MRSAFemales (%); Males (%)	MRCoNSFemales (%); Males (%)
A (Private)	11–14 years	2 (100.0); 2 (66.7)	0 (0); 0 (0)
B (Public)	6–10 years 11–14 years	4 (22.2); 2 (18.2) 2 (18.2); 5 (41.7)	0 (0); 0 (0) 0 (0); 0 (0)
C (Public)	6–10 years 11–14 years	2 (14.3); 1 (8.3) 3 (20.0); 2 (12.5)	2 (14.3); 1 (8.3) 0 (0); 0 (0)
D (Public)	6–10 years 11–14 years	6 (26.1); 3 (16.7) 3 (30.0); 1 (11.1)	1 (4.3); 0 (0) 1 (10.0); 1 (11.1)
E (Public)	6–10 years 11–14 years	3 (17.6); 1 (7.7) 1 (2.8); 5 (15.6)	1 (5.9); 0 (0) 0 (0); 0 (0)
Total (%) Significance level		26/146; 22/126 (17.8); (17.5) $P = 0.95$	5/146; 2/126 (3.4); (1.6) $P = 0.35$

Table 3
Multi-drug resistance among MRSA isolates.

Number of isolates ($n = 22$)	Resistance pattern
1	AP-GM-RP-FOX
5	AP-RP-E-FOX
1	AP-T-E-FOX
11	AP-T-RP-FOX
3	AP-T-RP-E-FOX
1	AP-GM-T-RP-E-FOX

Abbreviations: AP – Ampicillin; E – Erythromycin; FOX – Cefoxitin; GM – Gentamicin; RP – Rifampicin; T - Tetracycline.

Results and discussion

Prevalence of MRSA and MRCoNS in school children

Nasal swabs were collected from 272 learners, randomly selected from five schools in the Mariental District. Altogether 433 isolates from the 272 swabs were morphologically and biochemically identified as Staphylococcus species by Gram staining and a positive catalase test. Of these isolates, 352 (81.3%) were identified as S. aureus by a positive coagulase test, while 81 (18.7%) were coagulase-negative (CoNS). Fifty-one (11.8%) isolates were MRSA and seven (1.6%) MRCoNS as indicated by cefoxitin resistance, in other words an inhibition zone diameter $<$ 22 mm. As indicated in Table 2, methicillin-resistant S. aureus was isolated from 48 (17.6%) learners and MRCoNS in only seven (2.6%).

The prevalence of MRSA among school children in our study (17.6%) falls in between two Ethiopian studies that found 18.8% MRSA [18] and 13.8% MRSA [24], respectively. Our MRSA prevalence is about half of the 35.9% reported by Erami et al. [12] in Iran. In comparison to another Iranian study (Abadi et al., 2014) who reported 16.7% MRCoNS among learners aged 7–19 years, our MRCoNS prevalence of 2.6% was very low. No other publications or data on the prevalence of nasal staphylococci among Namibian school children were available to date.

In the present study, gender did not affect nasal colonization with MRSA and MRCoNS, since there was no significant difference ($P > 0.05$) between males and females with regards to the prevalence of these two groups of bacteria. There was also no significant difference between age groups and colonization with MRCoNS. Methicillin-resistant S. aureus colonization was however significantly higher ($P = 0.003$) in the age group 11–14 years than in the group 6–10 years. Similar to our study, Alzoubi et al. [2] and Bharathi et al. [4] observed no significant difference in MRSA nasal colonization between males and females. Studies by Erami et al. [12] and Reta et al. [24] showed higher percentages of MRSA among nasal isolates from males, whereas Nikfar et al. [21] reported a higher incidence of MRSA isolates from females. Our MRSA isolates were significantly higher in the age-group 11–14 years, which contradicts the findings of Alzoubi et al. [2], Reta et al. [24] and Arali et al. [3] who observed no statistically significant differences in nasal MRSA isolates among age groups.

Multi-drug resistance among isolates

Of the 51 MRSA isolates in our study, 22 (43.1%) were multi-drug resistant (**Table 3**). This is of concern, but much less than the 57.6% [12] and 63.6% [10] multi-drug resistant MRSA isolated from Iranian and Brazilian children, respectively.

Methicillin-resistant CoNS did not show multi-drug resistance. The most common resistance pattern, AP-RP-FOX, was shared between seven of these isolates. Overall, our findings support the conclusion of Arali et al. [3] that healthy school children under the age of 16 years are potential carriers of MRSA and multi-drug resistant strains. This can be due to several

risk factors, such as consumption of antibiotics during the three months prior to sample collection, contact with animals or exposure to hospital environments. These factors were however not assessed in the present work.

Enterotoxin A-D production in selected isolates

Out of the 23 multi-drug resistant isolates screened in our study, including the reference strain S. aureus ATCC 33,591, seven were enterotoxigenic as indicated by agglutination reactions which caused the formation of lattice structures in microtiter plate wells. Enterotoxin A was the most prevalent toxin, found in five of the isolates, including the reference strain. The reference strain did not produce enterotoxins B, C and D. Only one isolate produced enterotoxin B, whereas two isolates produced enterotoxin C. One isolate produced both toxins B and C. Enterotoxin D was not detected in any of our isolates. From literature, it is evident that it is not uncommon for nasal staphylococci from healthy persons to harbor enterotoxins. In Egypt, Hassanien and Abdel-Aziz [16] detected the genes for enterotoxins A, B and C in nasal S. aureus from food handlers. Similar to our results, one of their isolates carried both enterotoxins B and C. According to Brooks et al. [5] ingestion of as little as 25 µg of enterotoxin B causes vomiting and a runny stomach. In Ireland, Collery et al. [8] amplified sea, seb and *sec* genes in nasal S. aureus from healthy students. In accordance with our results and that of Collery et al. [8], one of their isolates carried enterotoxins B and C simultaneously. According to Dağı et al. [9] S. aureus strains with a combination of different enterotoxin genes can increase the incidence and severity of S. aureus infections. These researchers performed toxin assays on 104 S. aureus isolates from nasal swabs of healthy university students and found that 95.2% of isolates tested positive for at least one enterotoxin gene. In Japan, Uemura et al. [26] also used the SET-RPLA kit to screen for enterotoxins A–D in S. aureus isolated from the noses of healthy volunteers. They detected all four toxins. The absence of enterotoxin D in our study is consistent with another African study conducted in Benin, where Sina et al. [25] also did not detect this toxin in S. aureus.

Conclusion and prospects

Our study showed the presence of both MRSA and MRCoNS among school children aged 6–14 years in Mariental, Namibia. Almost half of the MRSA isolates were multi-drug resistant, which is of concern. Some of these multi-drug resistant strains were enterotoxigenic and self-infection by these potentially virulent bacteria poses various health risks for the children in our study population. School health programmes should improve current hygiene practices. Learners must be educated on the importance of frequent handwashing to prevent staphylococcal disease and spread of resistant strains among them and the wider community. We recommend mupirocin, amikacin, tobramycin and fusidic acid to be included in the antibiotic panel for prospective Namibian studies. Vancomycin, teicoplanin, linezolid and tigecycline are alternatives to beta-lactams and considered last-resort drugs for the treatment of MRSA infections. It is therefore important to include them in future studies. Risk factors for nasal carriage of methicillin-resistant strains among school children should be investigated. Prospective studies should aim to characterize and classify methicillin-resistant staphylococci at a molecular level. A variety of molecular typing methods [[1]] can be used, including accessory gene regulator (agr) typing, staphylococcal protein A (spa) typing, staphylococcal chromosome mec (SCCmec) typing, multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE).

Declaration of Competing Interest

The authors have no competing interests to declare.

CRediT authorship contribution statement

Sunette Walter: Conceptualization, Methodology, Validation, Software, Formal analysis, Writing – original draft. **Mervyn Beukes:** Supervision, Validation, Writing – review & editing. **Davis Mumbengegwi:** Supervision, Validation, Writing – review & editing. **Ronnie Böck:** Conceptualization, Methodology, Validation, Supervision, Validation, Writing – review & editing.

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References

[1] M.I.M. Abadi, R. Moniri, A. Khorshidi, A. Piroozmand, S.G.A. Mousavi, K. Dastehgoli, H.M. Ghazikalayeh, Molecular characteristics of nasal carriage methicillin-resistant coagulase negative staphylococci in school students, Jundishapur J. Microbiol. 8 (6) (2015) [online]Available from DOI[Accessed 11 September 2017], doi:10.5812/jjm.18591v2.

[2] H.M. Alzoubi, A.A. Agel, S.A. Al-Sarayreh, E. Al-Zayadneh, Methicillin-resistant Staphylococcus aureus nasal carriage among primary school-aged children from Jordan: prevalence, antibiotic resistance and molecular characteristics, J. Egypt. Public Health Assoc. 9 (3) (2014) 114–118 [online]ppAvailable from DOI[Accessed 4 June 2016], doi:10.1097/01.EPX.0000454671.83406.e8.

[3] S.M. Arali, V. Kulkarni, N.G. Manjula, S.M. Gaddad, Y.M. Jayaraj, C.T. Shivannavar, Prevalence and antibiotic susceptibility profile of MRSA isolated from the anterior nares of school going children in Gulbarga, Int. J. Med. Sci. Public Health 5 (4) (2016) 620–626 [online]ppAvailable from DOI[Accessed 7 December 2016], doi:10.5455/jmsph.2016.0805201592.

[4] M. Bharathi, N. Lakshmi, C.S. Kalyani, I.J. Padmaja, Nasal carriage of multidrug-resistant MSSA and MRSA in children of municipality schools [Letter to the editor], Indian J. Med. Microbiol. 32 (2) (2014) [online]Available from http://www.ijmm.org/text.asp?2014/32/2/200/129840 . [Accessed 29 July 2015].

[5] G.F. Brooks, K.C. Carroll, J.S. Butel, S.A. Morse, T.A. Mietzner, Jawetz, Melnick & Adelberg's Medical Microbiology, 26th ed., s.l.: McGraw-Hill, 2013.

[6] J. Charan, T. Biswas, How to calculate sample size for different study designs in medical research? Indian J. Psychol. Med. 35 (2) (2013) 121–126 [online]ppAvailable from DOI[Accessed 22 March 2015], doi:10.4103/0253-7176.116232.

[7] Clinical and Laboratory Standards Institute (CLSI), Performance standards for antimicrobial susceptibility testingCLSI Document M100, 27th ed., Wayne: CLSI, 2017.

[8] M.M. Collery, D.S. Smyth, J.M. Twohig, A.C. Shore, D.C. Coleman, C.J. Smyth, Molecular typing of nasal carriage isolates of Staphylococcus aureus from an Irish university student population based on toxin gene PCR, agr locus types and multiple locus, variable number tandem repeat analysis, J. Med. Microbiol. 57 (2008) 348–358 [online]ppAvailable from DOI[Accessed 22 September 2017], doi:10.1099/jmm.0.47734-0.

[9] H.T. Dağı, D. Fındık, G. Demirel, U. Arslan, Detection of methicillin resistance and various virulence factors in Staphylococcus aureus strains isolated from nasal carriers, Balk. Med. J. 32 (2015) 171–175 [online]ppAvailable from DOI[Accessed 9 January 2017], doi:10.5152/balkanmedj.2015.150186.

[10] S.P. de Carvalho, J.B. de Almeida, Y.M.F.S. Andrade, L.S.C. da Silva, M.V. de Oliveira, J. Timenetsky, L.M. Marques, Community-acquired methicillin-resistant Staphylococcus aureus carrying SCCmec type IV and V isolated from healthy children attending public daycare in northeastern Brazil, Braz. J. Infect. Dis. 21 (4) (2017) 464–467 [online]Available from DOI[Accessed 22 September 2017], doi:10.1016/j.bjid.2017.04.001.

[11] A.A. Elmanama, N.E.A. Tayyem, S.A.N. Allah, The bacterial etiology of otitis media and their antibiogram among children in Gaza Strip, Palestine, Egypt. J. Ear Nose Throat Allied Sci. 15 (2014) 87–91 [online]ppAvailable from DOI[Accessed 7 November 2017], doi:10.1016/j.ejenta.2014.03.002.

[12] M. Erami, B. Soltani, A.T. Ardakani, A. Moravveji, M.H. Rezaei, S. Soltani, R. Moniri, Nasal carriage and resistance pattern of multidrug resistant Staphylococcus aureus among healthy children in Kashan, Iran, Iran. Red Crescent Med. J. (9) (2014) 16 [online]Available from DOI[Accessed 16 December 2015], doi:10.5812/ircmj.21346.

[13] European Committee on Antimicrobial Susceptibility Testing (EUCAST)Breakpoint Tables For Interpretation of MICs and Zone Diameters Version 7.1, EU (European Union)., 2017 Available from http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_7.1_Breakpoint_Tables.pdf [Accessed 9 September 2017].

[14] B.A. Forbes, D.F. Sahm, A.S. Weissfeld, Bailey and Scott's Diagnostic Microbiology, 12th ed., Mosby, St. Louis, 2007.

[15] J.P. Harley, L.M. Prescott, Laboratory Exercises in Microbiology, 5th ed., McGraw-Hill, New York, 2002.

[16] A.A. Hassanien, N.M Abdel-Aziz, Staphylococcus aureus and their enterotoxin gene in fast food and food handlers from different food premises in Sohag City, Egypt, Int. J. Agric. Sci. Vet. Med. 5 (1) (2017) [online]Available from https://www.ijasvm.com/ijasvmadmin/upload/IJASVM_58a403b503a72.pdf . [Accessed 22 September 2017].

[17] A. Jenney, D. Holt, R. Ritika, P. Southwell, S. Pravin, E. Buadromo, J. Carapetis, S. Tong, A. Steer, The clinical and molecular epidemiology of Staphylococcus aureus infections in Fiji, BMC Infect. Dis. (160) (2014) 14 [online]Available from DOI[Accessed 7 November 2017], doi:10.1186/1471-2334-14-160.

[18] T. Kejela, K. Bacha, Prevalence and antibiotic susceptibility pattern of methicillin-resistant Staphylococcus aureus (MRSA) among primary school children and prisoners in Jimma Town, Southwest Ethiopia, Ann. Clin. Microbiol. Antimicrob. (2013) [online]**12**. Available from DOI[Accessed 29 July 2015], doi:10.1186/1476-0711-12-11.

[19] Lowy, F.D. (2010) Staphylococcal infections. In: Kasper, D. and Fauci, A. (eds.) Harrison's Infectious Diseases. S.l.: McGraw-Hill, 386–399.

[20] MedCalc Statistical Software version 16.4.3. (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2016)

[21] R. Nikfar, A. Shamsizadeh, T.Z. Kajbaf, K. Panah, S. Khaghani, M. Moghddam, Frequency of methicillin-resistant Staphylococcus aureus in healthy children, Iran. J. Microbiol. 7 (2) (2015) 67–71 [online]Available from http://www.researchgate.net/publication/281999247 . [Accessed 16 December 2015].

[22] L.M. Prescott, J.P. Harley, D.A. Klein, Microbiology, 5th ed., McGraw-Hill, New York, 2002.

[23] M.J.A. Reid, R.S.B. Fischer, N. Mannathoko, C. Muthoga, E. McHugh, H. Essigmann, E.L. Brown, A.P. Steenhoff, Prevalence of Staphylococcus aureus nasal carriage in human immunodefiency virus-infected and uninfected children in Botswana: prevalence and risk factors, Am. J. Trop. Med. Hyg. 96 (4) (2017) 795–801 [online]ppAvailable from DOI[Accessed 14 July 2017], doi:10.4269/ajtmh.16-0650.

[24] A. Reta, L. Gedefaw, T. Sewunet, G. Beyene, Nasal carriage, risk factors and antimicrobial susceptibility pattern of methicillin resistant Staphylococcus aureus among school children in Ethiopia, Med. Microbiol. Diagn. 4 (1) (2015) [online]Available from DOI[Accessed 15 September 2015], doi:10.4172/21610703.1000177.

[25] H. Sina, T.A. Ahoyo, W. Moussaoui, D. Keller, H.S. Bankolé, Y. Barogui, Y. Stienstra, S.O. Kotchoni, G. Prévost, L. Baba-Moussa, Variability of antibiotic susceptibility and toxin production of Staphylococcus aureus strains isolated from skin, soft tissue and bone related infections, BMC Microbiol. 13 (2013) [online]Available from DOI[Accessed 14 January 2017], doi:10.1186/1471-2180-13-188.

[26] E. Uemura, S. Kakinohana, N. Higa, C. Toma, N. Nakasone, Comparative characterization of Staphylococcus aureus from throats and noses of healthy volunteers, Jpn. J. Infect. Dis. 57 (2004) 21–24 [online]ppAvailable from http://www0.nih.go.jp/JJID/57/21.pdf . [Accessed 22 September 2017].

[27] A.E. Zautner, M. Krause, G. Stropahl, S. Holtfreter, H. Frickmann, C. Maletzki, B. Kreikemeyer, H.W. Pau, A. Podbielski, Intracellular persisting Staphylococcus aureus is the major pathogen in recurrent tonsillitis, PLoS ONE 5 (3) (2010) [online]Available from DOI[Accessed 7 November 2017], doi:10.1371/journal.pone.0009452.