

ORIGINAL ARTICLE**The Prevalence and Risk Factors Associated with Nasal Methicillin-Resistant *Staphylococcus Aureus* Colonization among Children in a Tertiary Hospital in Nigeria****Kemi Elizabeth Tuta^{1,2*}, Abiola Olukemi Okesola¹, Chukwuma David Umeokonkwo^{2,3}****OPEN ACCESS**

Citation: Kemi Elizabeth Tuta, Abiola Olukemi Okesola, Chukwuma David Umeokonkwo. The Prevalence and Risk Factors Associated with Nasal Methicillin-Resistant *Staphylococcus Aureus* Colonization among Children in a Tertiary Hospital in Nigeria. *Ethiop J Health*

Sci.2017;29(4):487.doi:http://dx.doi.org/10.4314/ejhs.v29i4.10

Received: May 19, 2019

Accepted: May 28, 2019

Published: July 1, 2019

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Funding: Nil

Competing Interests: The authors declare that this manuscript was approved by all authors in its form and that no competing interest exists.

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ABSTRACT

BACKGROUND: Nasal methicillin-resistant *Staphylococcus aureus* (MRSA) colonization is of public health concern due to increased risk of developing invasive infections and the therapeutic challenges. This concern is more among the vulnerable group. We determined the prevalence and associated risk factors of MRSA nasal carriage among children in a tertiary hospital in Nigeria.

METHOD: We conducted a hospital-based, cross-sectional study among 300 children attending the outpatient clinic of a tertiary hospital recruited through systematic sampling technique. An interviewer-administered, structured questionnaire was used to obtain sociodemographic characteristics and exposure factors. Nasal swabs samples were collected and inoculated on mannitol salt agar and subcultured on nutrient agar to isolate *Staphylococcus aureus*. We used the conventional Polymerase Chain Reaction (PCR) technique to detect the presence of *mecA* gene for MRSA. We calculated the prevalence, prevalence odds ratio to determine risk factors for MRSA acquisition at 5% level of significance.

RESULTS: The median age was 1.7 years (6 months-16 years). Males accounted for 60.7%, and 75% of the participants were under 5 years. *Staphylococcus aureus* colonization was found in 36.3% of the participants while 5.3% of the participants had MRSA identified by detecting the *mecA* gene. History of recent surgery in the last six months was the only independent predictor of nasal MRSA colonization among the participants (aOR=12.5; 95%CI: 2.7-50.0.)

CONCLUSION: The high prevalence of MRSA colonization observed among the children in this study suggests the need to consider screening children with history of previous surgery as infection control and prevention intervention for MRSA.

KEYWORD: MRSA, Children, *Staphylococcus aureus*

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a Gram-positive bacterium that is resistant to methicillin (a member of the penicillin family), and many other β -lactam antimicrobials such as penicillin, cephalosporins, and macrolides. Methicillin-resistant *Staphylococcus aureus* strains are usually resistant to several groups of broad-spectrum antibiotics that are used on a large scale in the hospital (1). It is a significant cause of both healthcare and community associated infections globally with great economic and clinical impacts (2–4).

Methicillin-resistant *Staphylococcus aureus* infection and colonization have been reported in humans both inside and outside hospital environments in Nigeria. Several reports of human MRSA infections have been documented with prevalence ranging from 34.7% to 76.7% (5–9). A lower MRSA prevalence rate of 20% has also been reported in Zaria, Nigeria, among non-hospital sources (10). Isolating MRSA among patients has a serious implication because they pose high risk of systemic infection, wound infection with resistant antibiotics, etc. A study has, however, posited that patients going for clean elective surgeries need not be screened for MRSA (11).

The mechanism of increased spreading under antibiotic pressure may have contributed to the worldwide increase in the prevalence of MRSA in hospitals (1). Resistance in MRSA is mediated by a gene (*mec A*) that encodes the production of an altered penicillin-binding protein (PBP2a), which does not allow for the binding of β -lactams to the bacterial cell wall (12). Since β -lactams exert antibacterial activity by binding and inhibiting enzymes necessary for bacterial cell wall synthesis, these antimicrobials are not therefore effective against MRSA.

The isolation of MRSA nasal colonization were initially among hospitalized patients. This has rapidly spread to include healthy individuals in the communities. The worrisome dimension is isolation in vulnerable groups like children, with potential risk of systemic infections (13). This necessitated the need to examine children in our

environment and identify any associated risk factors to MRSA colonization. We conducted this study to assess the prevalence and risk factors of nasal MRSA colonization among children attending the outpatient clinic of a tertiary hospital in Southwest Nigeria and enable health policy makers to develop and implement an effective MRSA control policy in the hospital.

METHODS

Study setting and design: The study was conducted at the University College Hospital Ibadan, an 820 bedded tertiary hospital located in Oyo State, Southwest Nigeria. It runs Paediatric Outpatient Clinics daily. The clinic sees about 20-30 patients daily.

The study was a hospital-based, cross-sectional one among children of less than 16 years of age attending the Children Outpatient Clinic in University College Hospital. We included the eligible children whose mothers or guardians gave consent whereas children whose mothers or guardians did not give consent were excluded.

Sample size and sampling technique: We estimated the sample size using the formula for single proportion using the following parameters: precision of 5%, prevalence of MRSA among children in previous study of 22.1% and level of significance of 5%. We adjusted for non-response using 10% and conducted continuity correction. The estimated sample size was 300 children. We used systematic sampling technique to recruit the eligible children from the clinics. Interviewer-administered structured questionnaire was used to obtain sociodemographic characteristics and exposure factors of nasal MRSA colonization.

Specimen collection and handling: We collected nasal swab samples from each participant. The two anterior nares of each enrolled patient were swabbed with sterile cotton wool swabs moistened with normal saline. The nasal swabs were transported immediately to the laboratory and inoculated within 2 hours. The swabs were plated on mannitol salt agar (MSA) and nutrient agar and then incubated at 35-37 degree Celsius within 18-24 hours. The *Staph aureus* isolated was identified by colonial morphology, Gram stain, slide

coagulase test and confirmed by tube coagulase test. Pure colonies of *Staphylococcus aureus* isolated were sub-cultured in a 20% glycerol with tryptic soy broth at -20 degrees Celsius, for further processing. Methicillin-resistant *Staphylococcus aureus* gene (Mec A) primer, conventional PCR instrument, DNA extraction kit, Master Mix containing each DNA taq polymerase 1.25µl per reaction, buffer B of 50mM KCl, TRIS 10mM, 1.5mM, MgCl₂, 200µm dNTP, blue dye, yellow dye, Eppendorf tubes, Universal containers, Micropipette, Micropipette tips, Micro centrifuge machine, Vortex machine, Electrophoresis tank, Ultra violet box, Ethidium bromide (EtBr), DNA ladder and Jena Bioscience Bacteria DNA preparation kit were used according to the manufacturer's instruction.

About 2 distinct colonies of sub cultured *Staphylococcus aureus* suspension was harvested by centrifugation at 10000rpm for one minute, and the supernatant was discarded; then 300µl of lysis buffer and 2µl of RNase A were added to the pellet. The mixture was vortexed vigorously for 30 to 60 seconds and 8µl of proteinase K was added to it and then incubated at 60 degree Celsius for 10 min. The mixture was cooled down for 5 minutes, 300µl of binding buffer was added to it and was vortexed briefly. The solution was placed on ice for 5 minutes and centrifuged for 5 minutes at 10000rpm. A spin column was placed into a 2ml collection tube, and the mixture was discarded into it. Lysate was pipetted directly into the spin column, centrifuged for one minute at 10000rpm and the flow-through was discarded. About 500µl of washing buffer was added into the spin column, centrifuged for 30 seconds at 10000rpm and the flow-through was discarded. Another 500µl of washing buffer was added into the spin column, centrifuged for another 30 seconds at 10000rpm and the flow-through was discarded. The spin column was centrifuged again at 10000rpm for one minute to remove the residual washing buffer. The 2ml tube was discarded; the spin column was placed in the elution tube and 40 to 50µl of elution buffer was added into the center of the spin column. It was incubated at room temperature for one minute and centrifuged at 10000rpm for 2 minutes. The extracted DNA was stored at -20

degrees Celsius.

Protocol For PCR: Primer sequences used for methicillin resistance *Staphylococcus aureus* (MecA) gene primer were MecA-F5'GTGGAATTGGGCAATACACC-3' and MecA-R 5'AGTTCTGCAGTACCGGAT-3' (Biomers.net, Germany). Then, 20µl was prepared for each of the samples. The PCR set up consisted of 5µl of DNA extract, 0.40 µl of primers (forward and reverse), 10.60µl of PCR water, 4µl of Master mix (reaction buffer B, MgCl₂, DNTP), all in a tube. All tubes were sealed and briefly centrifuged then transferred into the PCR machine. Amplification was done with the following protocol: initial denaturation at 95 degree Celsius for 5 minutes, then 30 cycles of denaturation at 95 degrees Celsius for 40 seconds, annealing at 57 degree Celsius for 30 seconds, extension at 72 degree Celsius for 60 seconds and final extension at 72 degree Celsius for 7 minutes. Each amplification run contained one negative control. After amplification, electrophoretic separation of PCR products was performed on 1.5% agarose gel stained with ethidium bromide, and visualized by UV illumination.

Electrophoresis: Twenty microliter (20 µl) of each of the PCR product was loaded into separate wells of 1.5% agarose gel stained with 0.5µg/ml ethidium bromide and assembled in a gel electrophoretic tank containing 1x Tris-borate-EDTA buffer. Then, 10 µl of 100 bp DNA ladder was loaded into the first well followed by the positive and negative controls in the next 2 wells; the amplicon was then serially loaded into the other wells. After loading, the electrophoretic tank was switched on and programmed to transmit 45 volts electric current across the gel for about 45 minutes. After electrophoresis, the agarose gel was transferred to a gel photo documentation system, UV-trans illuminator (byClinix Science Instruments Co Ltd model number 800 serial number 76526), and the result was read on a connected computer.

Interpretation of laboratory result: Samples corresponding to electrophoretic lanes with band at a level equivalent to 536 bp on the DNA molecular weight marker were documented as positive for mecA DNA. The samples that did not

meet this criterion were considered as negative.

Quality control: To confirm proper performance of the amplification process, a known MRSA DNA-positive control was run along with the samples during every PCR run and always gave a positive band at 536 bp mark. To confirm absence of contamination, a negative control using RNA/DNA free water was included in every PCR run and always showed no band.

Ethical approval: The study protocol was approved by the Institutional Review Board of the University of Ibadan and the University College Hospital both in Southwest Nigeria with approval number UI/EC/15/0156. Written informed consent was obtained from the parents or guardians of the participants and ascent obtained from the older participants.

RESULTS

The median age of the sample population was 1.7 years (6 months – 16 years). Males constituted 60.7% (182/300). Under 5 years were the majority constituting 75% (219/300) of the study population. The majority of the fathers (63.4%) and mothers (60.0%) had attained tertiary level of education (Table 1).

The majority (78.6%) of the participants were Yorubas by ethnicity, and others (21.4%) were composed of other tribes. Most of the study participants (91.7%) had no surgery in the last six months, (63.7%) were not recently hospitalized, the majority of the respondents were not residents in a long-term care facility and (77.3%) had no history of previous usage of antibiotics in the last six months (Table 2).

Staphylococcus aureus colonization was detected in 36.7% (110/300) of the participants whereas MRSA colonization was found in 5.3% (16/300) of the participants. We conducted bivariate analysis of the factors associated with nasal MRSA colonization. Age of children and history of recent surgery was found to be associated (Table 3). When these variables were modelled in logistic regression, history of recent surgery remained a significant predictor of nasal MRSA colonization (aOR 5.2; 95% CI: 1.60-16.93, Table 4).

Table 1: Socio-demographic characteristics of participants

Variable	Frequency (N=300)	Percentage
Sex		
Male	182	60.7
Female	118	39.3
Age(years)		
0-4	225	75.0
5-9	45	15.0
≥10	30	10.0
Religion		
Christianity	182	60.7
Islam	118	39.3
Father's Occupation		
Self Employed	144	48.0
Civil Servant	88	29.3
Trading	37	12.3
Farming	9	3.0
Others	22	7.4
Mother's Occupation		
Self Employed	137	45.7
Civil Servant	62	20.7
Trading	62	20.7
Farming	5	1.6
Others	34	11.3
Father's Education		
No formal	10	3.3
Primary	26	8.7
Secondary	75	25.0
Tertiary	189	63.0
Mother's Education		
No Formal	10	3.3
Primary	25	8.3
Secondary	85	28.4
Tertiary	180	60.0
Family Size		
< 6	235	78.3
≥ 6	65	21.7
Number of people sleeping in a room		
<5	275	91.7
≥ 5	25	8.3

Table 2: Clinical characteristics of the participants

Variable	Frequency	Percentage
Recent surgery		
Yes	25	8.3
No	275	91.7
Recent hospitalization		
Yes	123	41.0
No	177	59.0
Residence in long term care facility		
Yes	22	7.3
No	278	92.7
Use of antimicrobial last one year		
Yes	68	22.7
No	232	77.3
Contact with frequently hospitalized person		
Yes	15	5.0
No	285	95.0
History of chronic illness		
Yes	34	11.3
No	266	88.7
History of household contact with bedridden		
Yes	13	4.3
No	287	95.7
Current episode of URTI		
Yes	35	11.7
No	265	88.3
History of living in a hostel		
Yes	10	3.3
No	290	96.7

Table 3: Relationship between MRSA colonization and socio-demographic and clinical characteristics of the participants

Variable	MRSA	No MRSA	Chi square	p value
Age group (years)				
0-4	9 (4.0)	216 (96.0)	3.17	0.075
Others	7 (8.3)	68 (90.7)		
History of recent surgery				
Yes	5 (20.0)	20 (80.0)	11.62	<0.001
No	11 (4.0)	264 (96.0)		
History of hospitalization				
Yes	5 (4.1)	118 (95.9)	0.66	0.415
No	11 (6.2)	166 (93.8)		
History of antimicrobial use				
Yes	4 (5.9)	64 (94.1)	0.53	0.819
No	12 (5.2)	220 (94.8)		
History of stay in a long-term health facility				
Yes	1 (4.6)	21 (95.4)	0.03	0.864
No	15 (5.4)	263 (94.6)		
History of bedridden household member				
Yes	1 (7.7)	12 (92.3)	0.15	0.699
No	15 (5.2)	272 (94.8)		

Table 4: Logistic regression of the factors associated with MRSA colonization

Risk factors	Adjusted OR	95% CI
Age group (years)		
0-4	1	
Others	2.0	0.70 - 5.86
Recent surgery		
Yes	5.2	1.60 - 16.93
No	1	

DISCUSSION

The prevalence of nasal carriage of *Staphylococcus aureus* varies among geographical regions, hospital settings, populations and people with different conditions. This study has shown the overall anterior nares prevalence rate of *Staphylococcus aureus* among children attending outpatient clinic. The prevalence of *Staphylococcus aureus* colonization observed was similar to previous findings in Niger Delta, Nigeria, USA and Japan among healthy adults (14,15). Our finding is also in keeping with that obtained in Iran where the prevalence of 26.6-52.3% has been reported (13). Anterior nares are the main reservoirs of *S. aureus* in children and adults. Nasal cavity colonization with *S. aureus* that is a major risk factor for different infections (16-18).

Hence, this is a worrisome situation given that these children still have poorly developed immune system and are not able to mount resistance as observed in adults. They thus need to be protected from the risks posed by these invasive organisms (19,20). In Abia State, \southeast Nigeria, higher prevalence of 50% nasal colonization in hospital and non-hospital subjects has been reported (21). However, a lower prevalence of 14% has been reported among medical students in Lagos, Southwest Nigeria (22).

There is a need to eliminate its virulent strains because of their ability to cause infections in colonized individuals both in the hospital and community settings (19; 20). Our study provided information on the level of nasal MRSA colonization among children in the hospital. The prevalence of asymptomatic nasal MRSA colonization among the study population in our study with the use of molecular technique (PCR) was 5.3%. The prevalence rate in this study may be considered low for a hospital. However, the prevalence of nasal MRSA colonization in this study was higher than those reported in Maiduguri, Nigeria of 0.01%(23), and 1.5% in USA (24), Ireland (5%) and UK (2%) (25) but lower than the 15% reported in another study in Abakaliki, Ebonyi State, Nigeria (26), the 13.6% in Taiwan (27) and the 14.85% in Zaria, Nigeria (28). These variations may be attributed to the varying populations under

study.

The highest yield of nasal MRSA colonization in this study was from the age group of 5-10 years compared to a previous study that reported the highest nasal colonization among children aged 1-6 years (29). This is believed to be due to poorly developed immune system which makes them vulnerable to bacterial invasion especially when hospitalized. It could also be due to period of peak colonization with respiratory organisms. This is a phenomenon known as bacterial interference in which one bacterial strain prevents colonization by another strain which plays an important role in establishing the pathogen (1). The risk factor for MRSA nasal colonization in our study was the history of previous surgery in the last six months. Similar observations were made in previous studies (30,31). We observed that the nasal MRSA carriage in our study is independent of previous hospitalization, antibiotic use, number of persons living in the room, household contact bedridden, daycare attendance, breastfeeding, age of 2-6 months and household contact with healthcare worker as reported in previous studies (13,32). This is similar to a study done by Adesida *et al* among medical students in a tertiary hospital in Lagos, Nigeria (22) and in contrast to studies done in Africa (33-35) plus studies done in United States (36). In this study, the temporal relationship between having surgery and MRSA colonization could not be established due to the study design. However, an earlier cohort study concluded that children going for clean elective surgery need not be screened for MRSA prior to surgery (11).

The MRSA colonization of paediatric age group is particularly of interest for a number of reasons. First, they are a very vulnerable group that need special protection from such virulent organisms. They are at risk of systemic invasion of the organism resulting in life threatening infections. Secondly, for school aged children, the risk of transmission to other children is high and that could rapidly result in epidemics if not well handled with serious effect on the health of the community. Lastly, identifying the special populations at risk of MRSA colonization has implications for its control.

ACKNOWLEDGEMENT

We acknowledge the management of Nigeria Institute Medical Research (NIMR), Lagos, for granting us access to their molecular laboratory where the PCR test was done.

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