Original Paper

Nasal Carriage of *Staphylococcus aureus* and Antibiotic Susceptibility Pattern among Children in Freetown, Sierra Leone

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ABSTRACT

Nasal carriage of *Staphylococcus aureus* has been demonstrated to be a major risk factor for invasive *S. aureus* infections in various population including children. The extent of *S. aureus* carriage in Sierra Leonean children is largely unknown. To determine the prevalence and pattern of antibiotic susceptibility of nasal *S. aureus* among children in Freetown, Sierra Leone, samples were collected from anterior nares of children less than two years at the Ola During Children's Hospital between October 2008 and April 2009. Of the 116 children screened during the study period, *S. aureus* isolates were found in the nasal specimens of 40 (34.5%) of the children. Antimicrobial susceptibility testing to norfloxacin, gentamycin, erythromycin, trimethoprim-sulfamethazole, doxycycline, tetracycline and amoxycillin-clavulanic acid were observed to be 95, 35, 30, 20, 15, 7.5 and 2.5% respectively. All the isolates were susceptible to oxacillin and resistant to chloramphenicol, penicillin G, amoxycillin and ampiclox. Regular monitoring of antimicrobial susceptibility pattern may be useful.

Keywords: Antibiotic Susceptibility, Children, Nasal carriage, Staphylococcus aureus

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INTRODUCTION

Staphylococcus aureus is associated with a variety of clinical infections including septicaemia, pneumonia, osteomyelitis and toxic shock syndrome with substantial rates of morbity and mortality (CDC 1999; Shopsin and Kreiswirth 2001). In paediatric age group, staphylococcal infections account for most superficial and deepseated soft tissue infections. Besides being found in many environments, the anterior nares are the primary reservoir of the bacteria in both adults and children with approximately one third of the population colonized at any given time (Kluytmans et al., 1997; Milles et al., 2008). Nasal carriage of S. aureus has been identified as a major risk factor for both community acquired and nosocomial infections (Oguzkaya~Artan et al., 2008).

Interest in the epidemiology of staphylococcal infection has grown in recent decades as increasing number of the organisms are becoming resistant to a wide range of antimicrobial agents. Treatment of infections caused by S.aureus has become problematic as it is being observed to be multi-drug resistant in many parts of the world (Kolawole et al., 2005; Oguzkaya~Artan et al., 2008). Hence, an understanding of the prevalence and antimicrobial susceptibility pattern of the organism is important in suggesting strategies for management of the infections caused the bacteria. The aim of the study therefore is to determine the prevalence of S. aureus isolated from the nares of children less than 2 years attending the Ola During Childrens Hospital in Freetown and their pattern of antimicrobial susceptibility.

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MATERIALS AND METHODS Study Site

The study was carried out at the Ola During children's hospital which is the main referral hospital in the country. It is a 300-bed hospital located in the East end of Freetown. The hospital contains a neonatal intensive care unit that cares for premature neonates.

Study Population and Specimen Collection

The institutional research mandate as stipulated by the ethical committee was obtained and subjects participated after informed consent was obtained from the mothers/guardians. Nasal swabs were collected from both anterior nares of children between 1 and 25 months, starting in October 2008 through April 2009. The samples were collected using pre-moistened rayon tipped swabs (culture swab BBL, Dickinson Inc. Cockeysville, MD). Each swab was introduced about two (2) centimetres into the nasal cavity and gently rubbed through both nostrils as described by Rijal et al. (2008).

Laboratory Methods

After collection, specimens were transported to the laboratory and processed within 30 minutes of collection. The swabs were first plated on blood agar plates and incubated at 37°C for 24hrs. Sub-cultures from the identified and confirmed gram-positive cocci were plated on MacConkey agar and incubated at 37°C for 24hrs. Colony morphology suggestive of S. aureus was identified by gram-stain, catalase test, and confirmed by tube coagulase test (Cowan and

Steel, 1993). Disc diffusion susceptibility testing gentamicin, erythromycin, trimethoprimto sulfamethazole, doxycycline, tetracycline, amoxicillin. chloramphenicol. penicillin G. amoxicillin/clavulanic acid and ampiclox was performed according to the Clinical and Laboratory Standards Institute (CLSI, 2006) guidelines. Turbidity was set to 0.5 McFarland standard and plates were incubated at 35°C for 18 - 24 hrs. Zone of inhibition around each of antimicrobial disc was measured with a meter rule in millimeters and interpreted accordingly (CLSI, 2006). S.aureus strain ATCC 25923 was used as a control. Methicillin resistance was done by means of the agar screening method on Mueller Hinton agar (Difco) containing 6mg/l of oxacillin and 4% chloride. The plates were incubated for 24 h at 35°C and examined for the growth of atleast a colony on the agar surface.

RESULTS

Demographic Profile of Study Population

A total of 116 children, age range 1-25 months (mean =11.96) were screened for S. aureus. Forty-nine (42.24%) were males and sixty-seven (57.78%) were females. 1-5 months age group constituted the majority of subjects screened (Table 1).

Prevalence of Staphylococcus aureus in the **Study Population**

Cultures obtained from the 116 nasal samples vielded 40 (34.5%) gram-positive, catalase positive and tube coagulase test positive S. aureus.

Age Group (Months)	No. Examined	No. of Males	No. of Females
1 – 5	33	17	16
6 – 10	24	7	17
11 – 15	19	9	10
16 – 20	14	6	8
21 – 25	26	10	16
Total	116	49	67

Table 1: Demographic Profile of Study Population

The age group specific prevalence of S. aureus in the study population is shown in Figure 1. The frequency of carriage differs considerably with respect to age group. Of the 13 children in the 1-5 months age group, 12 (36.4%) yielded positive cultures for Staphylococcus aureus.

Pattern Antibiotic Susceptibility of Staphylococcus aureus Isolates

All the S. aureus isolates were resistant to amoxicillin, chloramphenicol, penicillin G, and ampiclox (Figure 2). None of the isolates was resistant to oxacillin.

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Thirty-eight of the 40 (95%) *S.aureus* isolates were sensitive to norfloxacin. Isolates from fourteen patients (35%) were resistant to gentamycin, 12 (30%) to erythromycin, 8 (20%) to trimethoprim-sulfamethoxazole (6)15% to doxycycline, (3)7.5% to tetracycline and only an isolate from one patient (2.5%) was resistant to amoxycillin/clavulanic acid

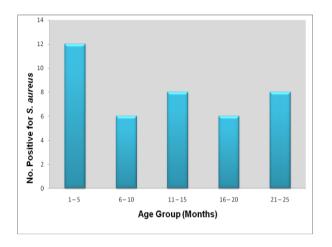
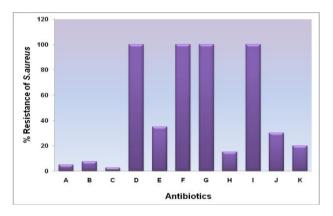


Figure 1: Prevalence of Staphylococcusaureus amongst Study Population



A= Norfloxacin, B =Tetracycline, C= Amoxycillin/clavulanic acid, D = Chloramphenicol, E= Gentamycin, F= Penicillin G, G= Amoxycillin, H = Doxycycline, I= Ampiclox, J = Erythromycin, K = Trimethoprim- sulfamethoxazole.

Figure2:ResistanceProfileofStaphylococcusaureuswithrespecttoAntibiotic Type

DISCUSSION

This study provides the first assessment of *Staphylococcus aureus* nasal carriage among children in Sierra Leone. *Staphylococcus aureus* carriage has been demonstrated to be highly variable and age dependent. The overall nasal carriage rate among the study population was found to be 34.5%, which is higher than 12.5% carriage rate among 1- to 2- year age group reported by Ko et al. (2008) in Seoul, Korea.

Unlike other studies where the prevalence of *S. aureus* colonisation increased with age in children (Bernardo and Ueno, 2008), the high occurrence of *S. aureus* nasal carriage among the children under six months of age in this study may be attributed to the fact that infants have immature immune system. Additionally, they are likely to be exposed to colonising *S. aureus* of older persons who carry them on regular basis.

However, our finding is similar to that of Lebon and colleagues recent study on the dynamics of S. aureus nasal carriage, its human and microbial determinants in the first year of life (Lebon et al., 2008). The workers documented a significant decrease in the prevalence of S. aureus carriage in children in the first year of life and suggested that it may be attributed to bacterial interference with other organisms present in the nasopharynges of children. A more extensive study will nonetheless allow а definitive assessment of this observation.

Staphylococcus aureus isolates with less than 40% susceptibility to other antimicrobials tested was documented in this study. Similar resistance level to Penicillin G. Amoxicillin and Ampicillin has been described by Bernardo and Ueno (2008) in Brazil. Furthermore, Ko et al. (2008) reported resistance of S. aureus to a wide range of antimicrobials including gentamycin, erythromycin, clindamycin and tetracycline. In Nigeria, Ako-Nai et al. (1991) documented related trend among S. aureus isolates from neonatal nasal samples with resistance to penicillin, tetracycline and chloramphenicol. In this communication, the observed resistance to a wide range of antimicrobial agents could be as a result of the exposure of children to these antimicrobial agents at a very early age, which according to Krumpermann (1983) enhances the development of resistance. Moreover, most β-lactam antibiotics in the are easily available open market. inexpensive and can be administered by the mothers. Further studies are required to determine the susceptibility of S.aureus to βlactam stable penicillins and the cephalosporins in this population.

Only two (5%) of the *S. aureus* isolates in this study were resistant to norfloxacin and none was resistant to oxacillin. Methicillin resistant *S. aureus* (MRSA) frequently causes nosocomial infections and has become one of the greatest challenges for modern antimicrobial therapy.

More interestingly, MRSA colonization among paediatric population has significantly been associated with MRSA infection (Yhu-Chering *et al.*, 2006) especially in children without identifiable risk factors. Considering data from most countries worldwide (Ako-Nai *et al.*, 1991; Yhu-Chering *et al.*, 2006; Donkor and Nartey, 2008) and the indiscriminate use of antimicrobials in this African country, the detection rate of methicillin resistance is surprising. However, this could be compared with a study involving 21,000 young healthy children in Portugal (Tavares *et al.*, 2010).

Clearly, the data of this present study indicate that the *S. aureus* colonization rate among children less than six months of age and resistance to commonly used antibiotics especially the β lactams were high. Providentially, the absence of methicillin resistant isolate appears to be uncommon when compared with reports from other countries. This MRSA colonization rate is reassuring and emphasizes the need for local surveillance studies. The need to be prudent in the use of antimicrobial drugs in this country is also pertinent.

REFERENCES

Ako-Nai AK, Torimiro SE, Lamikanra A, and Ogunniyi AD (1991). A Survey of Nasal Carriage of *Staphylococcus aureus* in a Neonatal Ward in Ile-Ife, Nigeria. *Ann Trop Paediatr.* **11**:41-5.

Bernard M and Ueno M (2008). Incidence of *Staphylococcus aureus* Colonization in Children Attending Day-Care Centers. *Rev Panam Infectol*. **10**:20-23

Centers for Disease Control and Prevention (CDC) (1999). National Nosocomial Infections Surveillance System Report. Data Summary from January 1990 - May 1999. *Am J Infect Contr.* **27**: 520 - 532.

Clinical and Laboratory Standards Institute (CLSI), (2006). Performance Standards for Antimicrobial Disk Susceptibility Tests; Sixteenth International Supplement, Clinical and Laboratory Standards Institute, *Document M100-S16.* Wayne, PA, USA.

Cowan ST and Steel KJ (1993). Cowan and Steel's Manual for Identification of Medical Bacteria. Cambridge University Press, Great Britain. Pp: 225-235. Donkor ES and Nartey E (2008). Nasal Colonisation of Drug Resistant Bacteria in Ghanaian Children Less than Five Years. *Internet J Microbiol.* **5**:

www.ispub.com/ostia/index.php?xmlFilePath=jour nals/ijmb/

Kluytmans J, Van Belkum A and Verbrugh H (1997). Nasal carriage of *Staphylococcus aureus*: Epidemiology Underlying Mechanisms, and Associated Risks. *Clin Microbiol Rev.* **10**: 505-520.

Ko KS, Ji-Young L, Jin YB, Kyong RP, Ji-Young R, Ki TK, Sang TH, Kang-Mo A, and Jae-Hoon S (2008). Characterization of *Staphylococcus aureus* Nasal Carriage from Children Attending an Outpatient Clinic in Seoul, Korea. *Microbial Drug Resistance*. **14**: 37-44

Kolawole DO, Bisi-Johnson MA and Shittu AO (2005). Epidemiological Analysis of Clinical Isolates *Staphylococcus aureus* in Ile-Ife, Nigeria. *Pakistan J Bio Sci.* **8**: 1016-1020.

Krumpermann PH (1983). Multiple Antibiotics Resistance Indexing of *E. coli* to Identify High Risks Sources of Fecal Contamination of Food. *App Environ Microbiol.* **46**:165-17

Lebon A, Labout, JAM, Verbrugh AH, Jaddoe VWV, Hofman A, van Wamel W, Moll HA and van Belkum A (2008). Dynamics and Determinants of *Staphylococcus aureus* Carriage in Infancy: The Generation R Study. *J Clin Microbiol.* **46**: 3517–3521

Melles, DC, Tenover FC, Kuehnert MJ, Witsenboer II, Peeters JK, Verbrugh HA and Van Belkum A. (2008). Overlapping Population Structures of Nasal Isolates of *Staphylococcus aureus* from Healthy Dutch and American Individuals. *J Clin Microbiol.* **46**:235-41.

Oguzkaya-Artan M, Baykan Z and Artan C (2008). Nasal Carriage of *Staphylococcus aureus* in Healthy Preschool Children. *Jpn J Infect Dis.* **61**: 70-72.

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. Shopsin B and Kreiswirth BN (2001). Molecular Epidemiology of Methicillin Resistant *Staphylococcus aureus*. *Emerging Infectious Dis*. **7**: 323-326.

Tavares DA, Sa-Leao R, Miragaia M and de Lencastre H (2010). Large Screening of CA-MRSA among *Staphylococcus aureus* Colonizing Healthy Young Children Living in Two Areas (Urban and Rural) of Portugal. *BMC Infect Dis*. **10**:

http://www.biomedcentral.com/1471-2334/10/110

Yhu-Chering H, Yi-Hong C, Lin-Hui S, Rey-In L and Tzou-Yien L (2006). Methicillin-Resistant *Staphylococcus aureus* Colonization and Its Association with Infection among Infants Hospitalized in Neonatal Intensive Care Units. *Pediatrics*.**118**:469-474.