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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 deep-seated 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^{o}C for 24hrs. Sub-cultures from the identified and confirmed gram-positive cocci were plated on MacConkey agar and incubated at 37^{o}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 to gentamicin, erythromycin, trimethoprim-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^{o}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^{o}C and examined for the growth of at-least 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 yielded 40 (34.5%) gram-positive, catalase positive and tube coagulase test positive *S. aureus*.

Table 1: Demographic Profile of Study Population

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**

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*.

Antibiotic Susceptibility Pattern 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.

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

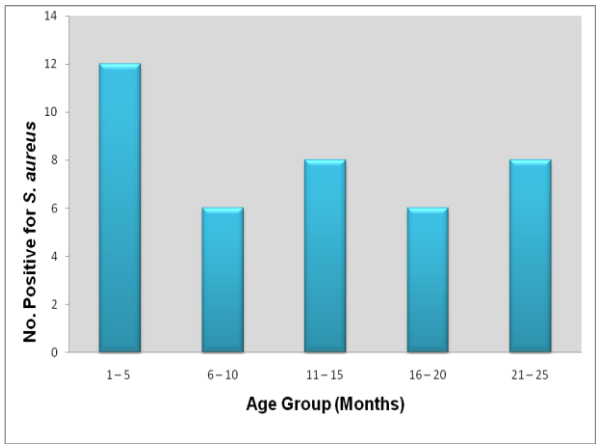
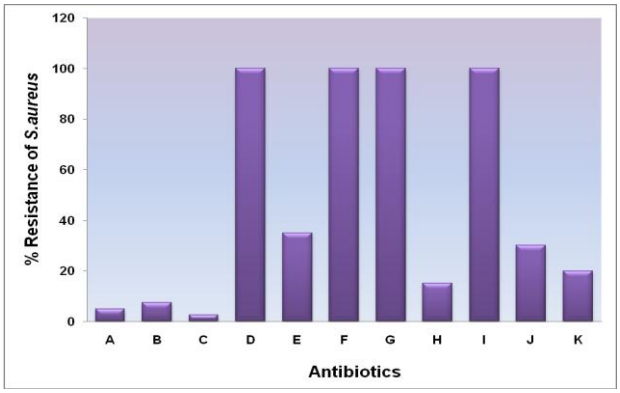


Figure 1: Prevalence of *Staphylococcus aureus* amongst Study Population



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.

Figure 2: Resistance Profile of *Staphylococcus aureus* with respect to Antibiotic 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 a 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 are easily available in the open market, inexpensive and can be administered by the mothers. Further studies are required to determine the susceptibility of *S.aureus* to β-lactam 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.

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