



Short Communication

Nasal carriage of methicillin-resistant *Staphylococcus aureus* by primary school pupils in a university staff school, Zaria, Nigeria.

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ABSTRACT

Strains of *Staphylococcus aureus* were isolated from the anterior nares of healthy pupils and their antibiotic susceptibility patterns were determined. 116 isolates of *Staphylococcus aureus* (100%) were biochemically characterized as coagulase positive *S. aureus*. Susceptibility profile of the isolates revealed that 15(14.85%) methicillin-resistant *Staphylococcus aureus* (MRSA) and 84(83.16%) methicillin-susceptible *Staphylococcus aureus* (MSSA) were recovered. Lower percentages of resistant strains of *Staphylococcus aureus* to mupirocin (11.88%) and ciprofloxacin (0.99%) were observed. Vancomycin-intermediate *Staphylococcus aureus* (VISA) (2.97%) with MIC values between 8-16 µg/ml were recorded. The public health significance of community-acquired methicillin-resistant *Staphylococcus aureus* (CAMRSA) in healthy human subjects is highlighted.

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Keywords: methicillin-resistant *Staphylococcus aureus*, anterior nares, vancomycin-intermediate *Staphylococcus aureus*, mupirocin susceptibility.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first reported in the United Kingdom. It later became the leading cause of nosocomial infections during the last decade and has since then generated considerable concern among medical and public health professionals (Li et al., 2005, Desjardins et al., 2006).

Community-acquired methicillin-resistant *S. aureus* (CA - MRSA) infections are increasingly caused by MRSA strains outside the hospital setting (Ayliffe, 1997; Shopsin et al., 2001; Shukla et al., 2004a, 2004b). The documented risk factors for MRSA infections include residence in a long-term care facility (LTCF), dialysis, recent hospitalization or surgery, the presence of an indwelling catheter and the use of injectable

drugs, and contact with colonized health care providers (Christol et al., 2001).

Outbreaks of MRSA infections in healthy persons have been reported recently, and these human subjects were without previously recognized risk factors (Li et al., 2005).

It is, therefore, important to investigate the incidence of community-acquired *S. aureus* infections amongst healthy primary school pupils. Hence, the aims and objectives of the present investigation were to: isolate *S. aureus* strains from apparently healthy children, characterize the isolates biochemically and determine the susceptibility profile of the isolates to antibiotics in common use.

MATERIALS AND METHODS

Study population and period of study

Healthy children attending the Ahmadu

Bello University Staff School, Zaria, Nigeria were the study population. Questionnaires were administered to the parents of the pupils. Data such as pupil's name, date of birth, sex, date of last visit to hospitals and the use of antibiotics in the past 12 weeks at the time of the study were obtained. The study was undertaken from May to September, 2006.

Specimen collection and primary isolation of *S. aureus*

Sterile cotton swabs were used to obtain specimens from the anterior nares of the subjects. The swabs were immediately used to inoculate Mannitol Salt Agar (MSA) plates. These plates were incubated at 35 °C for 24 hours. Ten golden colonies of presumptive *S. aureus* were picked and purified on nutrient agar plates. The pure cultures of *S. aureus* were streaked aseptically on Nutrient Agar (NA) slants and incubated at 37 °C for 24 hours. The cultures were kept at refrigeration temperature until needed for other tests.

Biochemical identification of isolates

Catalase and tube coagulase tests were performed.

Preparation of 0.5 McFarland Standard

One percent (1%) v/v of sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99ml of water and mixed well. Similarly, 91% w/v solution of Barium chloride was prepared by dissolving 0.5g of dihydrate Barium chloride ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) in 50ml of distilled water. 0.6ml volume of barium chloride solution was added to 99.4ml of sulphuric acid solution and mixed.

Antimicrobial susceptibility testing

The technique of Bauer et al. (1966) for antimicrobial susceptibility testing was used. Mueller–Hinton Agar (Oxoid) was prepared according to manufacturer's instructions. These plates were inoculated by streaking with standardized isolates (0.5 McFarland). Antibiotic discs were placed on the inoculated plates. After 30 minutes of pre-incubation, plates were incubated at 37 °C for 18–24 hours. Zones of inhibition were observed and their diameters measured with a metric rule. Using the interpretative chart, the zone size of each antimicrobial inhibition was determined

as resistant, intermediate or susceptible (CLSI, 2005).

Minimum Inhibitory Concentration (MIC) of presumptive vancomycin-resistant *Staphylococcus aureus*

The E test for determining the MIC of the isolates was performed according to the recommendation of CLSI (2005).

RESULTS

The antibiotic susceptibility profile of *S. aureus* isolates is shown in Table 1. There were 15(14.85%) methicillin-resistant *Staphylococcus aureus*. The isolates with intermediate susceptibility and high susceptibility were 2(1.98%) and 84(83.16%) respectively. Using the interpretative chart recommended by CLSI (2005), the susceptibility of the isolates was determined. Resistance to mupirocin and ciprofloxacin was low (11.88%). Susceptibility to vancomycin was 97.03%. The data obtained from the questionnaire administered to the pupils showed that 36 (36%) of the healthy subjects have not been to the hospital 12 weeks prior to the study, but harbored MRSA strains.

Table 2 shows the relationship between age and visit to the hospital 12 weeks before study commenced. The relationship between sex and visit to the hospital 12 weeks before study commenced is shown in Table 3. Table 4 shows the MIC values for the vancomycin-intermediate *Staphylococcus aureus* (VISA) isolates.

DISCUSSION

Susceptibility profile of the coagulase positive *Staphylococcus aureus* to the antibiotics used revealed that there were 15(14.85%) methicillin-resistant *S. aureus* (MRSA) and 84(83.16%) methicillin-susceptible *S. aureus* (MSSA). The emergence of MRSA in the community is a major public health problem because they were initially restricted to the hospital environment.

The results of this study are worrisome as previous reports showed that MRSA infections in four apparently healthy children with no exposure to nosocomial infections caused death (CDC, 1999). These strains have been designated as community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA). MRSA strains have been

Table 1: Antibiotic susceptibility profile of *Staphylococcus aureus*.

Antibiotic	No / resistant	No / % intermediate	No / % susceptible
Methicillin(1µg)	15(14.85)	2(1.98)	84(83.16)
Mupirocin high (200µg)	12(11.88)	0(0.0)	89(88.12)
Mupirocin low (5µg)	12(11.98)	0(0.0)	89(88.12)
Ciprofloxacin (5µg)	1(0.99)	3(2.97)	97(96.04)
Penicillin (10 i.u)	45(44.55)	0(0.0)	56(55.45)
Vancomycin (30µg)	3(2.97)	0(0.0)	98(97.03)

Table 2: Relationship between age of pupil and visit to hospital 12 weeks before investigation

Age group	PNH	PH
5-8	9(25)	16(25)
9-15	27(75)	48(75)
Total	36(36)	64(64)

PNH = Pupils who had not visited the hospital;

PH = Pupils who had visited the hospital.

Table 3: Relationship between sex of pupil and visit to hospital 12 weeks before investigation.

Sex	PNH	PH
Males	16(44.4)	29(45.3)
Females	20(55.4)	35(54.7)
Total	36(36)	64(64)

PNH = Pupils who had not visited the hospital;

PH = Pupils who had visited the hospital.

Table 4: Minimum inhibitory concentration (MIC) of vancomycin-intermediate *Staphylococcus aureus* isolates.

Concentration of vancomycin (µg /ml)	No recorded as VISA
4	0
8	2
16	1

isolated from apparently healthy women in Zaria, Nigeria (Onanuga et al., 2005) and hospitalized patients.

Children between the ages of 5 and 15 were the study population in this study. Previous studies showed that the proportion of children (<15 years of age) with community-acquired *Staphylococcus aureus* (CA-MRSA) infections were higher than those with

hospital-acquired strains (Shopsin et al., 2001; Dufour et al., 2002).

Discharged patients and health care providers may transmit nosocomial MRSA strains in the community and they could be associated with the dissemination of these organisms to the pupils. Some of these colonized persons could be workers in the staff school.

The interpretative criteria for susceptibility of staphylococci to mupirocin according to Finlay et al (1997) were used. High susceptibility percentages of *Staphylococcus aureus* strains to mupirocin were observed (88.12%) in this study. This agrees with the work of Harbarth et al. (1999) who found that 100% of the MRSA infection was cleared following topical application of mupirocin aimed at preventing *Staphylococcus* infections in surgical and haemodialysis patients.

Norazoh et al. (2001) also observed high mupirocin susceptibility of MRSA strains (98.75%). Hence, mupirocin is recommended as an antibiotic of choice where methicillin resistance is observed- particularly in staphylococcal skin infections. There is, however, a need to place a strict policy on mupirocin usage in Nigeria. Elsewhere, other researchers have shown that mupirocin resistance has increased over the years. The rate of mupirocin resistance has been observed to be higher in community-acquired MRSA isolates than in hospital-acquired strains (Gupta et al., 1999; Fujimura and Watanabe, 2003; Saxena et al., 2003).

The susceptibility of *S. aureus* to ciprofloxacin (96.04%) is suggestive of the high efficacy of this fluoroquinone in treating MRSA infections in this study population and reflects the fact that fluoroquinones are less abused in this environment.

The interpretative criteria for susceptibility of staphylococci to vancomycin according to CLSI (2005) were used. Vancomycin is still very effective against typical MRSA strains in Zaria as shown by the high susceptibility of the *S. aureus* strains (97.03%). There were only 3(2.97%) presumptive vancomycin-resistant *Staphylococcus aureus* (VRSA) isolates in the present study. The Minimum Inhibitory Concentration (MIC) of the isolates were determined by E test and results showed that the values were between 8-16µg/ml and hence they were classified as vancomycin-intermediate *S. aureus* strains (VISA). Dole (2005) also observed VISA strains among hospital-acquired MRSA strains. Palazzo *et al* (2005) reported that four VRSA strains were found outside hospital environment in Brazil. Vancomycin has been used extensively for the treatment of MRSA infections but previous reports have shown that high level vancomycin-resistant *S. aureus* had emerged as at 1996 (Kluytmans *et al.*, 1997).

MRSA as a multi-drug resistant strain will continue to increase the spread of nosocomial and community-acquired *S. aureus* with reduced susceptibility to commonly used antibiotics. Prevalence rate of 10-fold increase in MRSA infections have been recorded in United States over an 8-year period (CCDR, 2005).

Conclusion

Most of the MRSA strains were acquired from outside the hospital settings. It is imperative that surveillance for MRSA infections in the community be done on a regular basis. The use of mupirocin for clearance of nasal carriage of CAMRSA infections must be closely monitored to avoid rapid resistance developments as observed by some researchers in other countries.

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