Nasal carriage of methicillin resistant *Staphylococcus aureus* amongst meat sellers in Abakaliki Metropolis, Ebonyi State, Nigeria

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**ABSTRACT**

Methicillin resistant *Staphylococcus aureus* (MRSA) remains a global threat to human race due to its multidrug resistance propensity and avalanche of diseases associated with it. Though nasal carriage of MRSA has been reported amongst meat sellers and animal handlers elsewhere, carriage of this organism amongst this population in Ebonyi State has not been documented. In this study, eighty seven nasal swab samples were randomly collected from meat sellers in Abakaliki ‘meat market’ following informed consent. The samples were subjected to standard microbiological techniques to identify *S. aureus*. Resistance to methicillin was obtained using cefoxitin. A total of 20 isolates of *S. aureus* were recovered, representing an overall nasal carriage of 23% (20/87) while MRSA carriage of 15% (3/20). The antibiotic susceptibility profiles of the isolates to commonly used drugs show high resistance to tetracycline (80%), cotrimoxazole (70%) and erythromycin (65%). All isolates were susceptible to imipenem and vancomycin. The presence of MRSA in the nostrils of meat sellers screened in this study portends potential danger to this group, their families and the general public due to the versatility and intransigence of this organism in human infections. It is therefore recommended that more studies involving larger population be carried out to establish the prevalence of this organism in the wider Ebonyi society in order to enable healthcare providers to design preventive measures.

**Keywords:** Nasal carriage, methicillin resistant *Staphylococcus aureus*, antibiotics, susceptibility, cefoxitin, vancomycin.

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**INTRODUCTION**

*Staphylococcus aureus* is a known pathogen and has been implicated in a wide range of diseases, ranging from minor skin infections, such as furunclosis and carbunclosis to severe and highly debilitating diseases such as pneumonia, endocarditis and bacteraemia (Zetola et al., 2005; Jensen and Lyon, 2009). The anterior nares has remained a major site of staphylococcal colonization in humans (Williams, 1946; Chambers and Deleo, 2009; Bien et al., 2011) and carriage of Methicillin Resistant *Staphylococcus aureus* (MRSA) is a major risk factor for subsequent infection (Kluymans et al., 1997).

Nasal carriage of *S. aureus* has been reported across various settings in Nigeria (Lamikanra et al., 1985; Onanuga et al., 2005; Abdulhadi et al., 2008; Nwankwo and Nasiru, 2011; Onanuga and Tremedie, 2011), and infections associated with *S. aureus* has also been reported in various parts of the country (Shittu et al., 2006; Ghebremedhin et al., 2009; Nworie and Umeh, 2010; Nworie and Eze, 2010; Shittu et al., 2011).

Immediately after the introduction of methicillin in the 1960s, methicillin resistance emerged in some strains of *Staphylococcus aureus* (Grundmann et al., 2006; Sakoulas and Moellering, 2008). MRSA exhibits high-
level resistance to methicillin, and other beta-lactam antibiotics through the acquisition of the mecA gene (Lambert, 2005). This gene encodes penicillin-binding protein 2a which belongs to a group of membrane-bound enzymes that catalyse the trans-peptidation reaction that is necessary for cross-linkage of peptidoglycan layer (Grundmann et al., 2006). The mecA gene complex also allows cross resistance to non-beta-lactam antibiotics such as erythromycin, clindamycin, gentamicin, cotrimoxazole and ciprofloxacin due to the presence of insertion sites for plasmids and transposons (Chambers, 2001). Involvement of MRSA in diseases of animals has been reported worldwide and its zoonotic potentials have also been documented (Elchos et al., 2008; Nemati et al., 2008; Smith et al., 2009). Colonisation with live-stock associated MRSA sequence type (ST) 398 among animals and humans have been reported (Witte et al., 2007; Smith et al., 2009; Golding et al., 2010). In addition, MRSA clonal complex 398 (CC398) has also been isolated from animal species and has become an emerging cause of human infections, often associated with livestock exposure (Price et al., 2012). Higher prevalence of MRSA has been reported amongst pig and veal calf workers in the Netherland (Van Cleef et al., 2011) and consumption of foods containing S. aureus can cause severe gastro-intestinal illness (Crago et al., 2012).

Multidrug resistance in S. aureus have been associated with use of antibiotics in animal husbandry (Levy and Marshall, 2004; Erb et al., 2007) and reduction in the use of antimicrobials in veterinary medicine has been propagated as a potential control option for reducing the selective pressure of MRSA colonization in animals (Laura et al., 2012). The potential health risks of MRSA to people exposed to colonized and infected animals and livestock have been reported with higher prevalence amongst veterinarians and workers associated with animal (Farr, 2004; Anderson et al., 2008; Leonard and Markey, 2008; Van Cleef et al., 2011).

To the best of our knowledge, no population-based prevalence study has been carried out to determine MRSA carriage amongst meat sellers in Abakaliki metropolis, Southeast Nigeria. This study was therefore, aimed at determining the nasal carriage of MRSA among meat sellers in Abakaliki and their susceptibility to conventional antibiotics used in the study area.

MATERIALS AND METHODS

Study location

This study was carried out in the biggest abattoir in Abakaliki, popularly known as ‘Meat Market’. Abakaliki is the capital of Ebonyi State, Nigeria. Informed consent of the meat sellers were obtained before sample collection. Eighty seven nasal swab samples were randomly collected from the meat sellers by swabbing both anterior nares with sterile cotton-tipped moistened swabs (Transswab, Medical Wire and Equipment Co. Ltd UK). The samples were subjected to standard bacteriological techniques and isolates that showed characteristic colonies and haemolysis on blood agar, golden yellow colonies on mannitol salt agar and positive for catalase and coagulase enzymes were identified as S. aureus (Barrow and Feltham, 1993).

Determination of methicillin resistance profile of the S. aureus isolates

The cefoxitin discs (30 μg) used were procured from Oxoid, UK. The antimicrobial susceptibility profile of the isolates was determined using the Kirby-Bauer disc diffusion technique (CLSI, 2008). With a sterile wire loop, two colonies of each of the isolates were emulsified in 5 ml of sterile normal saline to a turbidity corresponding to 0.5 McFarland standard. Then 0.5 ml of each inoculum was dispensed unto the surface of dried agar plates (Muller-Hinton agar supplemented with 4% NaCl) using sterile Pasteur pipette. These were uniformly spread on the agar surface with sterile swab stick (one for each inoculum). The excess inocula were discarded into a disinfectant jar. Inoculated plates were allowed to dry for 15 min and cefoxitin discs were placed on each plate. The plates were incubated aerobically for 24 h at 35°C. Resistance to cefoxitin was interpreted according to Clinical Laboratory and Standards Institute interpretative chart (CLSI, 2008).

Susceptibility testing of MRSA isolates to other conventional antibiotics

The following antimicrobial sensitivity discs (procured from Oxoid, UK): Cefoxitin (30 μg), Vancomycin (30 μg), Tetracycline (30 μg), Gentamicin (10 μg), Ciprofloxacin (5 μg), Ampicillin (10 μg), Ofloxacin (5 μg), Ceftriaxone (30), Cotrimoxazole (25 μg), Linezolid (5 μg), Imipenem (10 μg) and Erythromycin (15 μg) were used to determine the susceptibility profile of all the MRSA strains to other conventional antibiotics used in the study area.

The antimicrobial susceptibility profile of the MRSA isolates to other conventional antibiotics was determined using the Kirby-Bauer disc diffusion technique (CLSI, 2008). With a sterile wire loop, two colonies of each of the isolates were emulsified in 5ml of sterile normal saline to a turbidity corresponding to 0.5 McFarland standard. Then 0.5ml of each inoculum was dispensed unto the surface of dried agar plates (Muller-Hinton agar supplemented with 4% NaCl) using sterile Pasteur pipette. These were uniformly spread on the agar surface with sterile swab stick (one for each inoculum). The excess inocula were discarded into a disinfectant jar. Inoculated plates were allowed to dry for 15 min and the various antibiotic discs were then placed on the inoculated plates at 25 mm away from one another and 15 mm away from the edge of the plates. The plates were incubated aerobically for 24 h at 35°C. The diameter of the zone of inhibition produced by each of the discs was measured, and recorded and interpreted based on the standard interpretative chart as described by the Clinical Laboratory Standard Institute (CLSI, 2008).

RESULTS

Out of a total of 87 nasal swab samples collected from the nostrils of meat sellers in Abakaliki ‘meat market’, 20 of them grew isolates identified as Staphylococcus aureus. The overall nasal carriage of Staphylococcus aureus was 23% (20/87) and that of MRSA was 15% (3/20) as shown in Figure 1. The antibiotic susceptibility
of the isolates (Table 1) to commonly used drugs showed varying susceptibilities and high resistances to tetracycline (85%), cotrimoxazole (70%) and erythromycin (65%). Other resistance values were ampicillin 55%, ciprofloxacin 35%, ofloxacin 30%, ceftriazone 25% and gentamycin 15%. No resistance was
Figure 2. Bar chart showing the antibiotic susceptibility pattern of *Staphylococcus aureus* in Abakaliki meat market.

recorded against imipenem, linezolid and vancomycin (Figure 2).

**DISCUSSION**

*S. aureus* is an important opportunistic pathogen, colonizing humans and animals. MRSA has been reported in various animals, livestock farmers and retail meat (Nemati et al., 2008; Van Cleef et al., 2011; Waters et al., 2011). The percentage prevalence of *S. aureus* and MRSA amongst meat sellers in this study was 23 and 15% respectively which was lower than results obtained elsewhere (de Boer et al., 2009; Van Cleef et al., 2011). The MRSA carriage in this study is similar to that of Cole and colleagues who reported carriage of MRSA to be 28% in California, USA (Cole et al., 2001). Higher MRSA carriage (33%) has been reported among cattle farmers in Netherlands (Graveland et al., 2010) and 45% among swine workers in US (Smith et al., 2009). However, Van Cleef et al. (2011) reported nasal MRSA carriage among workers in pig abattoirs as 5.6%. The differences in carriage can be attributed to antibiotic use in animal husbandry and poor hygiene practices among the meat sellers.

The source of acquisition of MRSA in this study was not known but might be due to contact with human or animal carriers. The nares is a natural habitat of *S. aureus* (Bien et al., 2011) and a carrier of *S. aureus* or MRSA is at potential risk of developing an infection involving the colonising pathogen (Gordon and Lowy, 2008). For instance, there is an increasing transmission of MRSA (especially Sequence type ST398) from colonised pigs to farm workers, abattoir workers and veterinarians who are in close contact with such animals in Europe (Johnson, 2011). The isolation of MRSA amongst this occupational group constitutes a serious threat to public health and healthcare system. According to Rodríguez-Noriega et al. (2010) and Smith and Pearson (2011), colonisation of man and other animals constitute a reservoir and potential source of MRSA infection.

Though MRSA has been detected in this study population, the actual mechanism of acquisition was not determined. However, according to Van Cleef et al. (2011), carriage of MRSA might arise from carrier animals and human acquisition was more likely to be contact with MRSA positive animals. Furthermore, since molecular analysis of the MRSA isolates in this study was not carried out, the circulating spa types were not identified. As a result, it was difficult to establish the origin of the colonizing type which would have given insight as to whether it was livestock associated MRSA or the human type and therefore the likely route of transmission.

The antibiotic susceptibility profile of the isolates to commonly used antibiotics show varying resistances. The
highest resistance was recorded against tetracycline (85%), cotrimoxazole (70%) and erythromycin 65%. There was no resistance recorded against imipenem and vancomycin. The high sensitivity of vancomycin (100%) reported in this study is closely related to the findings of Fridkin et al. (2005) and Anupurba et al. (2003) who reported 87 to 100% susceptibility to vancomycin. However, it is in disagreement with the findings of Onanuga et al. (2005) who reported MRSA sensitivity to vancomycin as 11%. The high sensitivity indicates that imipenem and vancomycin are good antibiotics for the treatment of MRSA infections in this environment. The high resistance recorded against tetracycline might be attributed to its excessive use in animal husbandry. Antibiotic use in animals for therapeutics, for food production and disease prevention has also promoted antibiotic resistance in humans (Ndi and Barton, 2012) which may explain the varying and multi-drug resistance observed in this study. The non-resistance recorded against vancomycin confirms it as the last remaining antibiotic to which MRSA strains were reliably susceptible (Chambers and Deleo, 2009).

This study therefore, recommends that more research should be carried out so as to determine the molecular epidemiology of the circulating MRSA in this risk population to confirm the route of transmission. Continuous monitoring of this organism is recommended due to its propensity to cause fatal infections across populations. Hand washing is also recommended as this may play a vital role in food safety and public health. To the best of our knowledge, this is the first documentation of nasal carriage of MRSA amongst meat sellers in Abakaliki metropolis, Ebonyi State, Southeast, Nigeria.

REFERENCES


