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# Prevalence and Antimicrobial Resistance of *Staphylococcus aureus* Isolated from Retail Ready-to-eat Foods in Nigeria

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**Abstract:** The occurrence and antibiotic resistance of *Staphylococcus aureus* in 120 samples of ready-to-eat foods obtained from retail outlets and street vendors was studied. About 17.2% of the isolates were detected in suya, 13.4% in moin-moin, 29.7% in meat sausages and 32.6% in fish sausages. Rinse and wash water from food preparation centers had counts>$10^3$ cfu $mL^{-1}$. The antibiotic resistance of the isolates was tested using eleven different antibiotics including ampicillin, streptomycin, tetracycline, chloramphenicol and ciproflox. The isolates were resistant to two or more antibiotics and seven different resistance patterns were recorded. Resistance to streptomycin and tetracycline was found for 60.0 and 59.2%, respectively for the isolates. Lower levels of resistance were found for erythromycin (0.83%) and rifampicin (1.6%). Ten strains of the isolates showed evidence of β-lactamase activity. The results indicate that ready-to-eat food samples are frequently contaminated with *S. aureus* and could be potential vehicle of resistant *Staphylococcus* foodborne intoxications.

**Key words:** *Staphylococcus aureus*, ready-to-eat foods, contamination, antibiotic resistance,

## INTRODUCTION

*Staphylococcus aureus* continues to be a major cause of community-acquired and health-care related infections throughout the world. Animals, human food and the inanimate environment can provide a favorable environment for the transmission of *S. aureus* (Bertolatti *et al*., 2003; Lateef 2004). *Staphylococcus aureus* as an indicator of contamination of processed foods could come from the skin, mouth, or nose of handlers (Acco *et al*., 2003). Colonization of humans with *S. aureus* occurs in the anterior nares and other body sites with a carriage rate of 19-40% reported for a normal population (Noble *et al*., 1967; Acco *et al*., 2003). It has been isolated from foods such as rice, spices, meat and dairy products (Owhe-Ureghe *et al*., 1993; Sokari, 1991). In developing countries, street vending of foods is common because it offers inexpensive foods at convenient locations. In contrast to their potential benefits, concerns over the safety and quality of these foods have been raised, because the vendors lack appreciation of basic food safety issues (Bryan *et al*., 1988; Ekanem 1998; Umoh and Odaba 1999). A number of data confirm the fact that *S. aureus* causes many outbreaks of food poisoning resulting from hand contact (Bryant *et al*., 1988). As reported by Sokari (1991), 86% of meat products, 13% of cowpea-based food products and 55% of fish products from part of eastern Nigeria were contaminated with *S. aureus*.

Antimicrobial resistance associated with food and water has been a global concern (Kumar *et al*., 2005). It is now widely accepted that there is an association between the use of antimicrobial agents and the occurrence of resistance. Antimicrobials exert a selective pressure on microorganisms that acts as a driving force in the development of antibiotic resistance and therefore

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their use is considered a key issue in epidemiological studies (McGeers, 1998). Moreover, there remains the possibility that resistance may be transmitted from antibiotic resistant bacteria to the susceptible ones (Kessie *et al*., 1998). Multidrug resistant pathogens travel not only locally but also globally, with newly introduced pathogens spreading rapidly in susceptible hosts.

Antimicrobial resistant bacteria in foods threaten the efficacy of human drugs if antimicrobial resistance genes become incorporated into human bacterial populations (Smith *et al*., 2002). Surveillance of antimicrobial resistance is essential for providing information on the interventions, especially because the prevalence of resistance may vary widely between and within countries and over time (WHO, 2001).

The purpose of this study was to investigate the prevalence of *Staphylococcus aureus* in some ready-to-eat foods collected from different vendors in Umuahia Nigeria and to characterize the isolates by antimicrobial susceptibility. This will help to determine the potential hazards and possible health implications to humans that may be associated with the consumption of these foods.

## MATERIALS AND METHODS

### Description of Food Samples

Suya, thin slices of condiment-coated roasted beef on skewers, were purchased at random from hawkers at traditional suya spots in Umuahia. Moin-moin (cowpea pudding) prepared by placing slurries, contained in small aluminium plates or wrapped in leaves in water and boiling until the slurries set as firm gels, were purchased from retail hawkers in the market. Fish and meat sausage rolls, fried fish and meat products were purchased from retail hawkers and from supermarkets. Three independent replicate surveys of each vendor were collected. Water was scooped from dish washing and hand washing basins using sterile beakers. All the samples were transported to the laboratory of the Department of Microbiology and analysed within 1h of collection or refrigerated at 5°C before being analysed, but never longer than 2 days.

### Sample Analysis

Soaking 10 g samples separately in 90 mL of sterile distilled water and shaking them for 30 min at room temperature rehydrated the food samples. Serial dilutions of all the samples were made in sterile distilled water. The dilutions (0.1 mL) were spread on Mannitol Salt Agar (Oxoid Ltd, Basingstoke, UK) and incubated for 24-48 h at 37°C. Typical *Staph aureus* colonies on MSA were transferred to nutrient agar slants and incubated at 37°C. Only coagulase positive strains (Collins *et al*., 1990) were tested further in accordance with standard procedures (Speck, 1984).

### Characterization of Isolates

All isolates of staphylococci were inoculated on to blood agar plates, which were incubated aerobically at 37°C overnight to identify mucoid and pigmented strains. Randomly selected *Staph aureus* isolates were subjected to the following tests; catalase activity (Harrigan and McCance , 1976), growth in nutrient agar supplemented with 7.5% (w/w) and 10% (w/w) NaCl and growth in nutrient agar at 10° and 45°C (Baird-Parker, 1979). Nitrate reduction in nitrate broth and coagulase activity by the tube assay (Baird-Parker, 1979). Further characterization included production of acid from glucose, arabinose, mannitol, mannose, lactose, sucrose and xylose (Devriese *et al*., 1985),

### Antimicrobial Susceptibility Testing

The susceptibility of *Staph aureus* isolates to antimicrobial agents was tested by the disk diffusion method using Mueller-Hinton agar (Oxoid, Basingstoke England) and antibiotic disks (Abtek Biologicals Ltd.) according to the guidelines of the National Committee for Clinical Laboratory standards (NCCLS, 2001). The antibiotics used were Ciproflox (Cpx), Norfloxacin (Nfx), Gentamicin (Gen) Streptomycin (Str), Rifampicin (Rfp), Erythromycin (Ery), Chloramphenicol (Chl.), Cloxacillin (Clo), Cotrimoxazole (Cot), Amoxycillin (Amx) and Tetracycline (Tet). The commercial antibiotic disks were placed on the agar plates previously seeded with 18 h-broth cultures of the test organisms. The plates were incubated at 37°C for 48 h. The inhibition zones were measured, scored as sensitive, intermediate susceptibility and resistant according to the NCCLS recommendations. *Staphylococcus aureus* ATCC 12600 was used as a reference strain.

**Determination of Minimum Inhibitory Concentrations (MIC)**

The MIC of a commonly used antibiotic, amoxycillin was determined using the paper disc method (Oloke, 2000). Sterile paper discs were dipped into different concentrations of amoxycillin. Sterile paper discs dipped into sterile water used for the dilution of the antibiotic were used as control. Each soaked disc was then layered on Mueller-Hinton agar plates already seeded with an 18 h-broth culture of the *Staph aureus* strains in duplicate. Each plate was incubated at 37°C for 24 h and then examined for zones of inhibition. The lowest concentration of the antibiotic, which inhibited growth, was taken as the MIC.

**Assay for β -Lactamase Production**

β-lactamase production was assayed using the method of Ahmad and Yadav (1979). The cultures of the test organism was spot inoculated on to starch agar and then incubated overnight at 37°C. The plates were then flooded with freshly prepared phosphate-buffered saline containing potassium iodide, iodine and penicillin. β-lactamase converts penicillin to penicilloic acid, which reduces iodine to iodide monitored via decolorization of the starch iodine complex. The presence of clear colorless zones around the bacterial growth is an indication of β-lactamase production. All the bacterial isolates were tested for the production of β-lactamases.

**Statistical Analysis**

Three replicates were used for all the parameters tested and the mean of three values reported. Results were subjected to a one-way analysis of variance to compare mean values.

## RESULTS AND DISCUSSION

The enumeration of *S. aureus* in food products is employed generally as a sanitation index (Adams and Moss, 1996). The presence of these organisms in food beyond certain critical limit is interpreted as indicating that the food in question has been exposed to condition that might introduce or allow proliferation of pathogenic microorganisms (Mukhopadhyay *et al*., 2002). The results obtained by the plating analysis of the different food products and related samples are shown in Table 1. Average staphylococcal counts noted were 1.8 and $2.1\times10^3$ cfu $g^{-1}$, respectively and detected in about 32.6% of fish sausages and 29.7% of meat sausages. Presence of *S. aureus* was observed in 17.2% of suya and 13.4% of moin-moin samples. The mean $\log_{10}$ (±SD) counts of this microorganism per g of the different food products were not statistically different ($p>0.05$). Mean staphylococcal counts for wash water from food preparatory centers exceeded those of food samples in all cases (Table 1). These findings agree with previous results of contamination of ready-to-eat foods, which ranged from 13 to 86% (Sokari 1991; Mosupye and von Holy, 2000). Higher prevalence and mean counts of *S. aureus* have however, been reported in other ready-to-eat products (Adesiyun and Balsbirsingh 1996; Umoh and Odaba, 1999).

*S. aureus* is an important food-poisoning organism because of its cosmopolitan distribution in nature. Its presence in ready-to-eat foods may be traced back to the environment or human sources

Table 1: Prevalence of *S. aureus* in ready-to-eat foods*

| Samples | No Examined | Percentage of product Contaminated | cfu $mL^{-1}$ or cfu $g^{-1}$ ($log_{10}$) |
|---|---|---|---|
| Fish sausages | 12 | 32.6 | 3.25±1.0 |
| Meat sausages | 12 | 29.7 | 3.32±1.15 |
| Fried fish | 15 | 8.3 | 2.5±0.78 |
| Fried meat | 15 | 6.6 | 2.34±0.91 |
| Suya | 18 | 17.2 | 3.51±1.01 |
| Moin moin | 18 | 13.4 | 3.39±1.08 |
| Wash water | 15 | 27.6 | 4.49±1.21 |
| Rinse water | 15 | 18.1 | 3.38±1.00 |
| TOTAL | 120 | | |

*Values represent the means of triplicate determinations

(Newsome 1988; Neihart *et al*., 1988; Bergdoll, 1989). The high incidence of *S. aureus* in meat and fish products may be a reflection of, repeated hand contact with these foods at the point of sale (Sokari 1991). In addition, temperatures of 26-38°C are often encountered in the retail points from which the samples were purchased; hence, growth of this organism may have been favored. Outbreaks of staphylococcal food-poisoning have been reported to occur as the result of contamination of precooked food, often through unsanitary handling and holding food at temperatures that allow the growth and toxin production (Newsome, 1988; Bergdoll, 1989; Synder and Poland, 1991). Whatever the cause, appropriate control and good hygienic display of foods in protected cabinets will minimize potential hazards. The observed high counts of *Staph aureus* in rinsing water and wash water samples from food restaurants may be attributed partly to post hand contamination from the variety of customers who patronize these eating-houses. Additionally this may result from the washing of cookery, cutlery and other utensils (Sokari, 1991). In traditional Nigeria restaurants, it is common to observe customers eating with bare hands. The sanitary conditions of the environment of these restaurants may also lead to contamination of foods. The microbial contamination of ready-to-eat foods could be closely related to the method of preparation and handling. As expected, fried fish and meat products had lower contamination levels probably due to the combined treatment of frying followed by garnishing with onions and pepper. Heat stressed microorganisms that survived frying was probably capable of growing if sample were not conserved under appropriate temperature conditions. International microbiological standards recommend limits of bacterial contamination in the range of $10\text{-}10^2$ cfu $g^{-1}$ for total aerobic plate counts (ICMSF, 1986). About 33% of the total food samples investigated was contaminated with *S. aureus* at $10^2$ cfu $g^{-1}$ levels. In comparison, although contamination of ready-to-eat foods with *S. aureus* is not common in the UK, according to studies by Elson *et al*. (2004), 75% of ready-to-eat cold sliced meats and pate from catering and retail premises were of satisfactory/acceptable microbiological quality and about 25% were unsatisfactory/unacceptable.

Since large numbers, typically $>10^6$ cfu $g^{-1}$, are required for the production of enough toxin to cause illness, contamination is necessary but is not alone sufficient for an outbreak to occur (Adams and Moss, 1996). In particular, holding the product for sale at temperature and time that allow the organism to grow to hazardous levels could be risky. It is an acknowledged fact that unsold samples are usually presented for sale the next day perhaps with gentle heat treatment. Such unwholesome practices could result in higher levels of toxins due to build-up on subsequent days. *S. aureus* strains demonstrate elevated thermal resistance, which precludes inactivation by current culinary heating techniques (Synder and Poland, 1991).

The antibiotic sensitivity test and assay for the production of β-lactamase were conducted to ascertain the source of the multi drug resistance phenomenon frequently encountered among bacterial isolates in Nigeria. Notwithstanding the comparably low counts of *S. aureus* in the ready-to-eat food

Table 2: Antibiotic Resistance In S.aureus Strains Isolated from Ready- to-eat Foods

| Samples | No. of strains tested | Percentage resistance to antimicrobial agents | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Cpx | Nfx | Gen | Clo | Str | Rfp | Cot | Ery | Chl | Amx | Tet |
| Meat and Fish Sausage | 24 | 12.5 | 0.0 | 70.8 | 12.5 | 75.0 | 4.2 | 4.2 | 4.20 | 0.0 | 42.0 | 70.0 |
| Fried Meat and Fish | 30 | 0.0 | 3.3 | 6.7 | 13.3 | 73.3 | 0.0 | 0.0 | 0.00 | 0.0 | 0.0 | 33.3 |
| Suya | 18 | 5.6 | 11.1 | 83.3 | 75.0 | 94.4 | 5.6 | 16.6 | 0.00 | 0.0 | 38.9 | 56.6 |
| Moin-moin | 18 | 11.1 | 11.1 | 56.6 | 11.1 | 16.6 | 0.0 | 0.0 | 0.00 | 11.1 | 16.6 | 66.6 |
| Rinse water Andwash water | 30 | 3.3 | 6.7 | 50.0 | 10.0 | 50.0 | 0.0 | 0.0 | 0.00 | 10.0 | 20.0 | 3.3 |
| Total | 120 | 5.8 | 5.8 | 49.2 | 22.5 | 60.0 | 1.6 | 3.3 | 0.83 | 5.8 | 2.9 | 59.2 |

Table 3. Resistance of S.aureus strains of different origins to a variety of antibiotics

| Food sample | No. of strains Tested | Number resistant to 2 or more antibiotics | Occurrence (%) |
|---|---|---|---|
| Meat and fish sausages | 24 | 18 | 75.0 |
| Fried meat and fish Products | 30 | 10 | 33.3 |
| Suya | 18 | 17 | 94.4 |
| Moin-moin | 18 | 12 | 75.0 |
| Water | 30 | 22 | 73.3 |
| Total | 120 | | |

Table 4: Antibiotic resistance profiles of *S. aureus* strains isolated from ready-to-eat foods

| Resistance profile | Meat and fish sausages | Fried meat and fish | Suya | Moin-moin | Wash water | Total |
|---|---|---|---|---|---|---|
| Fully susceptible | 0 | 0 | 1 | 0 | 0 | 1 |
| Gen, Str, Tet | 10 | 12 | 14 | 12 | 8 | 54 |
| Gen, Str, Tet, Amx | 5 | 0 | 5 | 2 | 8 | 11 |
| Gen Lcn Str Tet Amx | 2 | 1 | 1 | 4 | 3 | 11 |
| Gen, Cpx, Npx, Rfp Tet, Amx. | 3 | 2 | 4 | 0 | 4 | 13 |
| Cpx Nfx Lcn Str Flx Tet Amx | 3 | 5 | 6 | 3 | 1 | 18 |
| Tet Nfx, Flx, Gen Lcn, Ery Str | 2 | 1 | 0 | 2 | 1 | 6 |
| Cpx, Nfx, Gen, Lcn Str, Flx Amx, Rfp Chl Tet | 3 | 1 | 0 | 1 | 1 | 6 |

Table 5: MIC values of amoxycillin against strains of *Staphylococcus aureus*

| MIC ($\mu g\ mL^{-1}$) | No. of strains | Percentage | $\beta$-lactamase activity |
|---|---|---|---|
| 0.25 | 1 | 5.6 | - |
| 0.5 | 0 | 0.0 | - |
| 1 | 2 | 11.1 | - |
| 2 | 5 | 11.1 | - |
| 4-64 | 8 | 44.4 | + |
| 128 | 2 | 27.8 | + |
| >256 | 0 | 0.0 | - |
| Total | 18 | | |

products examined, the fact that some of the isolated strains exhibited antibiotic resistance indicates that the products could pose a public health risk to consumers. Table 2 summarizes the resistance patterns of all the *S. aureus* strains to 11 antimicrobial agents. The percentage resistance of the isolates for the antibiotics varied from 60% for streptomycin to 0.83% for erythromycin. Most of the resistant strains for streptomycin were obtained from *moin-moin*. Fried fish and meat samples yielded low levels of antimicrobial resistant strains. High resistance was also found for tetracycline (59.2%) and gentamicin (49.2%). Lower levels of resistance were observed among the strains for chloramphenicol, norfloxacin and ciproflox. 65.8% of *S. aureus* strains were multiresistant (resistance to two or more antibiotics) (Table 3). Most of the isolates fell into one of seven major resistance groups. The predominant pattern was resistance to streptomycin, gentamicin and tetracycline (Table 4). Even though the food samples were obtained from hawkers and retail outlets at different locations, the isolates showed similar antibiotic resistance patterns. The MIC of Amoxycillin against the strains of *S. aureus* was determined (Table 5). The MIC ($\mu$g mL$^{-1}$) ranged between 0.25 to $\geq$256 $\mu$g mL$^{-1}$. Out of the 18 strains of *S. aureus* tested, 10 were found to be positive for β-lactamase production. The production of β-lactamase by bacteria with high resistance to β-lactam antibiotics has been reported (Raham Khan and Malik, 2001; Lateef, 2004).

The present data has indicated that infection of humans by transmission through food products contaminated with antibiotic-resistant strains is plausible. The antibiotic-resistant isolates may have originally come from humans, considering that the level of carriage among human *S. aureus* isolates is over 40% in Nigeria (Paul *et al*., 1982; Lamikanra *et al*., 1985). The level of resistance to antimicrobial drugs is a reflection of the indiscriminate misuse and abuse of antibiotics in the environment (Umoh *et al*., 1990; Chigbu and Ezeronye, 2003).

There is need to educate the hawkers and retailers of ready-to-eat foods on the hazards of contamination. Such control measures as displaying foods in glass cabinets, washing hands at regular intervals, not allowing customers to pick up foods with bare hands and selling off all items on the same day are necessary to prevent hazardous conditions from developing or occurring.

The antibiotic resistance patterns of ready-to-eat food strains of *Staphylococcus aureus* observed in this study suggests a greater risk in the form of transfer of resistance to other forms of bacteria since they can form commensal flora through the food chain. A comprehensive surveillance is required to determine the presence and distribution in foods, animals and agriculture in Nigeria.

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