



## Antibiotic Susceptibility of *Staphylococcus aureus* Isolated from Retailed Raw Beef at Choba Market, Rivers State

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### Authors' contributions

This work was carried out in collaboration between both authors. Author EOC designed the study, wrote the protocol and draft manuscript. Author POO managed the analyses of the study. All authors contributed to the literature review and approval of the final manuscript. Both authors read and approved the final manuscript.

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### ABSTRACT

**Aims:** The aim of the study was to determine the presence of antibiotic resistant *Staphylococcus aureus* in retail raw beef in Choba market, Nigeria.

**Study design:** The study is based on a completely randomized design with two replicates and the mean being calculated.

**Place and Duration of Study:** Major's Laboratory, Department of Microbiology, University of Port Harcourt within three months.

**Methodology:** The presence, characterization and antibiotic susceptibility of *S. aureus* from 30 retail raw beef samples was done using standard microbiological method involving the use of mannitol salt agar (MSA) and Mueller Hinton agar (MHA).

**Results:** All the samples were positive for *Staphylococcus* species of which 18 (60.00%) were positive for *S. aureus*. The *S. aureus* strains were least resistant to gentamycin (22.22%) and cotrimoxazole (38.89%) with varying resistance against erythromycin (83.33%), tetracycline (88.88) and chloramphenicol (61.11%). All the *S. aureus* isolates in this study were 100% resistant to

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cloxacillin, amoxicillin and augmentin or amoxicillin-clavulanic acid combination. These results also showed the potential dissemination of multidrug-resistant *S. aureus* strains in the raw beef samples examined.

**Conclusion:** The results of this study suggested high-level contamination of meat with multi-drug resistant *S. aureus* and this highlights the public health consequences associated with consuming such unhygienic products if poorly prepared.

**Keywords:** Antibiotic sensitivity; beef; multidrug-resistant; staphylococcus aureus.

## 1. INTRODUCTION

Meat of animal origin is the major source of protein and valuable qualities of vitamins for most people in many parts of the world, thus they are essential for the growth, repair and maintenance of body cells and necessary for our everyday activities [1,2]. Due to the chemical composition and biological characteristics, meats are highly perishable foods providing excellent source of nutrients for growth of several hazardous microorganisms that can cause infection in humans and spoilage of meat and economic loss [3]. The microorganisms of meat surfaces include *Listeria monocytogenes*, *Micrococcus* spp., *Staphylococcus* spp., *Clostridium* spp., *Bacillus* spp., *Lactobacillus* spp., *Brochotrix thermophacta*, *Salmonella* spp., *Escherichia coli*, *Serratia* spp. and *Pseudomonas* spp. [4,5]. Growth of food borne pathogens such as *Salmonella*, toxin-producing strains of *E. coli*, *L. monocytogenes*, *C. perfringens* and *S. aureus* are of concern with meat and poultry products [6].

*Staphylococcus aureus* is one of the most important Gram-positive organisms that have gained attention because of its role in both hospital and community acquired infections [7]. This bacterium multiplies quickly at room temperature to produce toxins that cause food poisoning. Naturally, its distribution was ubiquitous in the world, but the most important infection source of *S. aureus* was food [8].

Staphylococcal food poisoning is one of the most economically important foodborne diseases and produces gastrointestinal illness through a wide variety of toxins, including staphylococcal enterotoxins characterized by vomiting and diarrhea within 2 to 6 h after the consumption of contaminated food in the United States [9-11]. *Staphylococcus aureus* and other pathogens in meat, as a result of improper hygienic practices at the point of handling by slaughter personnel during meat production and other faulty abattoir processes such as improper evisceration,

increase the chances of cross-contamination of gut pathogens to meat [12,13].

Treatment of *S. aureus* infections involves the use of antibiotics. However, use and misuse of antibiotics for prophylactic or sub-therapeutic treatment in livestock and the resultant residues in general, have been responsible for the development of multidrug-resistant bacterial isolates, which is a major public health issue [7,14,15]. According to Haskell et al. [14] several bacterial species have acquired resistance to a number of antibiotics and the rate of development of new antibiotics is not keeping up with the pace of development of resistance.

The aim of the study was to determine the presence and characterize antibiotic resistance profiles of *S. aureus* strains isolated from raw beef vended in Choba market, Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Source of samples

The raw beef samples were randomly purchased from meat vendors at Choba market and transferred in flask coolers and immediately transported to the Department of Microbiology Laboratory for analysis.

### 2.2 Microbiological Analysis

Twenty-five grams of composite beef samples were aseptically weighed into a stomacher bag containing 225 mL of sterile normal saline. This was followed by maceration in a stomacher blender for 1-2 min to obtain the homogenate. After a ten-fold serial dilution, 0.1 mL of appropriate dilutions of the homogenate were spread plated in duplicates on mannitol salt agar (MSA) and incubated at 35°C for 24 h. Discrete colonies were further purified on freshly prepared MSA before storing in nutrient agar slant for confirmation. Identification of bacterial isolates was carried out based on cultural, physiological and biochemical characteristics of the isolates. Identification was confirmed using Cowan [16]

and Bergey's manual of determinative bacteriology [17].

### 2.3 Antibiotic Susceptibility Testing

Antibiotic sensitivity patterns of all the confirmed *S. aureus* was performed by standard disk diffusion method according to Kirby-Bauer on Mueller-Hinton agar (Titan, Biotech Ltd, Indian) following the procedures recommended by CLSI [18]. Ten commonly used antibiotics ( $\mu\text{g}/\text{disc}$ ) viz. amoxicillin-clavulanate or augmentin (AUG) 30, amoxicillin (AMX) 25, erythromycin (ERY) 5, tetracycline (TET) 10, cloxacillin (CXC) 5, gentamycin (GEN) 10, cotrimoxazole (COT) 25, chloramphenicol (CHL) 30 (Abtek, UK) were tested. From an overnight culture in brain heart infusion broth, a  $10^8$  cell/mL (0.5 MacFarland turbidity standards) bacterial culture was prepared in sterile saline, from which 0.1mL was inoculated onto Mueller Hinton agar, after which antibiotic discs were carefully and aseptically placed on the surface of the agar. The plates were incubated at 37°C for 24h. Zone of inhibition was measured in millimeter. Multidrug resistance was reported as a single isolate resistant to three or more unique antibiotics.

### 3. RESULTS AND DISCUSSION

The results of the study showed that all the 30 samples of raw beef analyzed were positive for *Staphylococcus* species of which 60% of the confirmed isolates were *S. aureus*. All the isolates fermented mannitol salt agar and appeared golden yellow showing the biochemical characteristics previously reported by Konuku et al. [19] for *Staphylococcus* spp. The result of the occurrence of *Staphylococcus* spp. in the beef samples examined is in conformity with previous report by Schlegelová et al. [20] and Crago et al. [21] who described *S. aureus* as a common pathogen of raw meats. The 60% occurrence of *S. aureus* is in agreement with reports by Waters et al. [22] that 37 to 77% of all meat and poultry types examined were contaminated by *S. aureus*. On their part Rahimi et al. [23] reported a 60.3% of *S. aureus* in raw beef samples examined.

The presence of antimicrobial resistant bacteria in meat has been widely reported from different parts of the world [24,25]. The use of antibiotics in livestock and the resultant residue contributes to high levels of antibiotic resistance in *S. aureus* found in meat products [7,14]. All the *S. aureus* isolates in this study were 100% resistant to cloxacillin, amoxicillin and amoxicillin-clavulanic

acid combination (Table 1). This agrees with previous report by Brinda et al. [26] of a 100% resistance of *S. aureus* strains isolated from raw beef. In contrast to the findings of this study, Waters et al. [22] reported a 0.00% resistance of *S. aureus* in US meat and poultry to cloxacillin. The 88.88% resistance to tetracycline is not within the range (25.00 to 82.1%) reported by previous authors [2,8,10,22,26,27,28] though comparable to the 82.10% reported by Jaja et al. [2]. This could be due to varied usage of various antibiotic products in each country, as different countries have different products available and registered for use in different species. Varying resistance of *S. aureus* from raw meat have been reported also by a number of authors, ranging from 25.00% to 73.30% [2,7,26,27,28].

The *S. aureus* strains were least resistant to gentamycin (22.22%) and cotrimoxazole (38.89%). A number of authors have reported gentamicin resistance of *S. aureus* from raw meat ranging from 0.00% to 19.40% [2,8,10,15,26,27,28]. This may not be unconnected with the fact that it is in injection form and hardly abused, unlike a vast majority of antibiotics that come in capsule or tablet forms. For cotrimoxazole, contrary to the findings of this study, Effah et al. [7] reported a 57.80% resistance of Methicillin-resistant *S. aureus* isolated from raw meat. Other authors however, reported varying activities (8.00 to 34.2%) against Methicillin-resistant *S. aureus* (MRSA) from humans [29,30,31]. Bishara et al. [29] asserted that data from their study may favor the use of co-trimoxazole as a potentially cost-effective antimicrobial drug for treating MRSA infections.

*Staphylococcus aureus* is among the most prevalent causes of clinical infections globally and has garnered substantial public attention due to increasing mortality associated with multidrug resistance (MDR) [22]. According to Magiorakos et al. [32], MDR is resistance to one antimicrobial drug in three or more antimicrobial group based on the mode of action and specific to target microorganisms. The present result also shows the potential dissemination of multidrug-resistant (MDR) *S. aureus* strains in the raw beef samples examined. The *S. aureus* were multi-drug resistant, i.e., resistant to three or more antibiotic groups (Table 2). Consistent with the findings of this study, Brinda et al. [26] stated that the phenomenon of multiple resistance to antibiotics has been noticed in *S. aureus* isolates, in varying proportions; While Effah et al. [7] reported multi-

**Table1. Percentage resistance of the confirmed *S.aureus* to common antibiotics**

|                 | No | AUG    | AMX    | ERY   | TET   | CXC    | GEN   | COT   | CHL   |
|-----------------|----|--------|--------|-------|-------|--------|-------|-------|-------|
| <i>S.aureus</i> | 18 | 100.00 | 100.00 | 83.33 | 88.88 | 100.00 | 22.22 | 38.89 | 61.11 |

AUG= Augmentin or amoxicillin-clavulanate; AMX= Amoxicillin; ERY=Erythromycin; TET= Tetracycline; CXC= Cloxacillin; GEN=Gentamicin; COT= Cotrimoxazole; CHL=Chloramphenicol

**Table 2. Multidrug resistant pattern of 18 *S.aureus* isolated from raw beef**

| Antibiotics                       | No of isolates with profile (N) in % | Resistance category  |
|-----------------------------------|--------------------------------------|----------------------|
| Aug-Amx-Ery-Tet-Cxc               | 4(22.22)                             | Multi-drug-resistant |
| Aug-Amx-Ery-Tet-Cxc-Chl           | 3(16.67)                             | Multi-drug-resistant |
| Aug-Amx-Ery-Tet-Cxc-Cot-Chl       | 4(22.22)                             | Multi-drug-resistant |
| Aug-Amx-Ery-Tet-Cxc- Gen- Cot-Chl | 3(16.67)                             | Multi-drug-resistant |

Aug= Augmentin or amoxicillin-clavulanate; Amx= Amoxicillin; Ery=Erythromycin; Tet= Tetracycline; Cxc= Cloxacillin; Gen=Gentamicin; Cot= Cotrimoxazole; Chl=Chloramphenicol

drug resistance of MRSA to 16 antibiotics of which 6 of the antibiotics were among those tested in this study. The presence of multi-drug resistant strains poses serious public health risk.

#### 4. CONCLUSION

The role of food in the spread of pathogens cannot be over-emphasized in public health. Retailed raw beef remains a potential source in the transmission of pathogenic foodborne bacteria, including, *S. aureus*. Therefore, there is the need for adequate food processing especially at suitable temperatures, in order to reduce the possible microbial load in the food products. Surveillance and good hygiene practice by meat handlers is important in the face of an increasing threat of multi-drug resistant *S. aureus* both in animals and humans. The results of this study show that gentamicin and cotrimoxazole remain viable antibiotics for *S. aureus*.

#### DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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