Multiple Antibiotic Resistant Surveillance of Oxacillin-resistant *Staphylococcus aureus* Isolated from Beef and Frozen Poultry Meat (Chicken)

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Authors' contributions

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ABSTRACT

Introduction: Oxacillin resistant *Staphylococcus aureus* (ORSA) associated with beef and frozen chicken is a threat to public health because of the potential ability of them being multiple antibiotics resistant.
Aim: The aim of this study is therefore to isolate, determine the susceptibility of *S. aureus* to oxacillin and subsequently other antibiotics so as to establish their antibiotic profiles.
Methodology: Beef meat (20) and frozen chicken (20) samples were collected from different markets and restaurants in Oyo town, Oyo state, Nigeria. *S. aureus* was isolated from these samples using Mannitol Salt Agar and pure culture obtained. Pure culture of isolates was characterized and identified based on morphological, biochemical properties and were further subjected to susceptibility to oxacillin and other antibiotics.
Results: Eighty *S. aureus* were isolated from beef meat and frozen chicken with occurrence of 34

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and 46 respectively. Twenty five out of the 80 isolates were susceptible to oxacillin and termed as oxacillin susceptible *S. aureus* (OSSA) while 55 were resistant to oxacillin and termed as oxacillin resistant *S. aureus* (ORSA). *S. aureus* (ORSA and OSSA) from beef are susceptible (> 80%) to augmentin, gentamicin, ceftriaxone, ofloxacin, cefuroxime and erythromycin. However, rate of resistance of ORSA to ceftazidime and cloxacillin was high (> 70%). The susceptibility rate among the ORSA and OSSA isolates from chicken is also slight high with minimum of 71% for ofloxacin.
Conclusion: The overall antibiotic profiling revealed that cefuroxime, gentamicin, ceftriaxone, erythromycin, ofloxacin and augmentin are viable antibiotics. Food products remain a potential interface in the transmission of *Staphylococcus* species and therefore there is constant need for the surveillance and subsequently recommendation on the good hygiene practice.

1. INTRODUCTION

Food is the good material for the proliferation and colonization of disease-causing microorganisms due to the easy provision of the essential nutrients required by microbes to grow [1-6]. Among the food-borne pathogens, *Staphylococcus aureus* is one of the common causative agents of foodborne diseases [7,8] and have successfully acquired resistant genes that enable them to be resistant to different antibiotics especially β-lactams [9]. *S. aureus* remains an important pathogen responsible for food-borne illness in human medicine, being globally disseminated. The main sources of the pathogen could be the inadequate personal hygiene of food handlers [10] and poor storage conditions of food products. However, staphylococci are frequently associated with food products like meat, dairy products, cream-filled bakery items and salads [11].

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first identified in the United Kingdom in 1961 [12]. Since that time, MRSA has also been referred to as oxacillin-resistant *S. aureus* (ORSA). Methicillin (oxacillin)-resistant *S. aureus* has gained importance internationally as the cause of both nosocomial and community-acquired infections [13]. Methicillin-resistant *S. aureus* (MRSA) has been recognized as major cause of healthcare-associated infection worldwide. MRSA strains appear to have been transferred from health-care (nosocomial) setting into the community and have emerged in particularly associated with community associated infections in humans [14,15]. It has also been reported that isolates of *S. aureus* that are resistance to the penicillins are generally known to exhibit resistance to methicillin and oxacillin [16]. Generally, there is relative high level of virulence observed in ORSA compared to

that of oxacillin-susceptible *S. aureus* (OSSA) strains. In a study reported in United States, it was found that primary nosocomial bacteremia that occurred due to ORSA was approximately threefold higher in direct hospitalization costs than case obtained from OSSA [17].

The occurrence of ORSA in poultry products and beef constitute a serious risk to human health. In fact, the major risk is envisaged with the possibility of production of staphylococcal enterotoxins (SEA, SEC, SED and SEE) by MRSA strains, which can cause staphylococcal foodborne illness. Raw meat handling, cross contamination, and undercooked meat consumption are the major critical control points in the meat processing to prevent the spreading of ORSA which consequently result in staphylococcal infections. This study aimed at isolating, determining the susceptibility of *S. aureus* to oxacillin and subsequently other antibiotics so as to establish their antibiotic profiles.

2. MATERIALS AND METHODS

2.1 Isolation and Identification

Twenty samples each of freshly cut small pieces of frozen chicken and beef were collected into separate sterile sample bags from different restaurants in Oyo Town, Oyo State, Nigeria. Samples were taken to the laboratory and kept at 4℃ prior to laboratory analysis. One (1) gram of the samples was mashed in 9 mL of sterilized water using stomacher machine and subsequently dilutions were carried out. One mL of the sample was inoculated into Mannitol Salt Agar (MSA) using pour plate method. It was incubated at 37℃ for 24 to 48 hours. Colonies with yellow appearance on Mannitol Salt Agar were randomly sub-cultured severally until pure

cultures were obtained. Isolates were Gram stained and subjected to catalase, coagulase and different sugars fermentation (such as mannitol, glucose, lactose and sucrose) tests [18]. Morphological and biochemical characteristics of the isolates were confirmed with the peculiar characteristics of the suspected bacteria on the Bergey's Manual Bacteriology for the purpose of identification [19].

2.2 Antibiotics Susceptibility Test

All the isolates were subjected to oxacillin screening in order to know their susceptibility to oxacillin. The susceptibility of the isolates to antibiotics were tested by Disc Diffusion method on Mueller-Hinton agar [20]. This was carried out by using Gram positive antibiotics sensitivity multi-discs (Abtek Biological Ltd. UK) that contained ceftazidime (30 µg), cefuroxime (30 µg), gentamycin (10 µg), ceftriaxone (5 µg), ofloxacin (5 µg), augmentin (30 µg), erythromycin (300 µg) and cloxacillin (30 µg). Sterile swab stick was used to inoculate isolates on set Muller Hinton agar and sterilized forceps was used to put antibiotics sensitivity multi-discs on it. It was incubated at 37°C for 24 hours. Presence of zone of inhibition was measured and recorded as sensitive or resistance based on Clinical and Laboratory Standard Institute (CLSI) standards [21].

2.3 Multiple Antibiotic Resistance (MAR) Index

Multiple antibiotic resistance index was determined for each isolate by dividing the number of antibiotics each isolate is resistance to by the total number of antibiotics used [22,23].

MAR Index =

$$\frac{Number\ of\ resisted\ antibiotics\ by\ an\ isolate}{Total\ number\ of\ antibiotics\ investigated\ against\ the\ isolate}$$

3. RESULTS

Eighty (n=80) *S. aureus* were isolated from both beef and frozen chicken out of which 34 were isolated from beef and 46 from frozen chicken. The 80 isolated *S. aureus* produced yellow colour on MSA. They were Gram-positive cocci with ability to produce catalase. They agglutinated blood serum and utilized citrate. They fermented mannitol, glucose, lactose and

sucrose. Fifty five (69%) of *S. aureus* isolates were resistant to oxacillin while the remaining twenty-five (31%) were susceptible to oxacillin (Fig. 1).

Antibiotics resistance pattern of *S. aureus* from beef and frozen chicken is shown in Table 1. The oxacillin-resistant *S. aureus* isolated from beef was observed to be highly resistant to cloxacillin (83%), followed by ceftzidine (70%) and the least was in cefuroxime (4%). Forty five percent of oxacillin-susceptible *S. aureus* isolated from beef were resistant to cloxacillin and ceftzidime. Oxacillin susceptible *Staphylococcus aureus* obtained from beef were 100% susceptible to gentamicin, erythromycin, ofloxacin and augmentin. Most of the oxacillin resistant *S. aureus* isolated from chicken were also resistant to cloxacillin (75%) and ceftzidime (72%) and the least resistance of these isolates was recorded in augmentin (3%). Seven percent of oxacillin susceptible *S. aureus* isolated from chicken were resistant to augmentin, gentamicin and ceftriaxone while the percentage of highest resistance was observed in cloxacillin (57%). Highest resistance of all the isolated *S. aureus* was observed in cloxacillin (70%), followed by ceftzidime (63%) with the least in augmentin (5%). The susceptibility of the *S. aureus* isolates to the used antibiotics is in this order: augmentin > gentamicin > ceftriaxone > ofloxacin > cefuroxime > erythromycin > ceftzidime > cloxacillin.
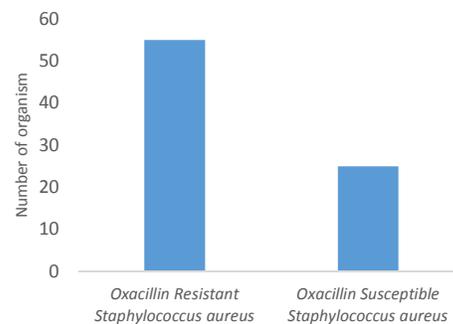

Fig. 1. Frequency of occurrence of oxacillin-resistant and susceptible *Staphylococcus aureus*

The Multiple Antibiotics Resistance (MAR) index of *S. aureus* isolated from beef and frozen chicken is presented in Table 2. Thirteen out of 23 oxacillin resistant *Staphylococcus aureus* isolated from beef had MAR index of greater

Table 1. Antibiotics resistance pattern of *S. aureus* from beef and frozen chicken

Antibiotics	*S. aureus* from beef – number (%)				*S. aureus* from frozen chicken – number (%)				Total – number (%)	
	ORSA		OSSA		ORSA		OSSA			
	S	R	S	R	S	R	S	R	S	R
CAZ	7 (30)	16 (70)	6 (55)	5 (45)	9 (28)	23 (72)	8 (57)	6 (43)	30 (37)	50 (63)
CRX	22 (96)	1 (4)	10 (91)	1 (9)	23 (72)	9 (28)	11 (79)	3 (21)	66 (83)	14 (17)
GEN	21 (91)	2 (9)	11 (100)	0 (0)	30 (94)	2 (6)	13 (93)	1 (7)	75 (94)	5 (6)
CTR	21 (91)	2 (9)	10 (91)	1 (9)	29 (91)	3 (9)	13 (93)	1 (7)	73 (91)	7 (9)
ERY	20 (87)	3 (13)	11 (100)	0 (0)	20 (63)	12 (38)	12 (86)	2 (14)	63 (79)	17 (21)
CXC	4 (17)	19 (83)	6 (55)	5 (45)	8 (25)	24 (75)	6 (43)	8 (57)	24 (30)	56 (70)
OFL	21 (91)	2 (9)	11 (100)	0 (0)	26 (81)	6 (19)	10 (71)	4 (29)	68 (85)	12 (15)
AUG	21 (91)	2 (9)	11 (100)	0 (0)	31 (97)	1 (3)	13 (93)	1 (7)	76 (95)	4 (5)

Keys: ORSA: Oxacillin-Resistant S. aureus; OSSA: Oxacillin-Susceptible S. aureus; S: Susceptible; R: Resistant CAZ: Ceftazidime; CRX: Cefuroxime; GEN: Gentamycin; CTR: Ceftriaxone; ERY: Erythromycin; CXC: Cloxacillin; OFL: Ofloxacin; AUG: Augmentin

Table 2. Multiple Antibiotics Resistance (MAR) index of *S. aureus* isolated from beef and frozen chicken

Mar index	*S. aureus* from beef (number)		*S. aureus* from frozen chicken (number)		Total (number)		
	ORSA	OSSA	ORSA	OSSA	ORSA	OSSA	ORSA + OSSA
0.00	0	4	0	1	0	5	5
0.13	10	4	5	6	15	10	25
0.25	8	2	15	4	23	6	29
0.38	3	0	8	2	11	2	13
0.50	1	1	2	0	3	1	4
0.63	0	0	0	0	0	0	0
0.75	0	0	2	1	2	1	3
0.88	0	0	0	0	0	0	0
1.00	1	0	0	0	1	0	1
Total	23	11	32	14	55	25	80

Keys: ORSA: Oxacillin-Resistant S. aureus; OSSA: Oxacillin-Susceptible S. aureus

than 0.20 while the remaining 10 had MAR index of 0.13. Four oxacillin susceptible *S. aureus* had MAR index 0.00 to the other antibiotics used. Twenty seven out of 32 oxacillin resistant *S. aureus* isolated from frozen chicken had MAR index of more than 0.20 to other antibiotics used. MAR index of half (7) of oxacillin susceptible *S. aureus* to other antibiotics were less than 0.20 while the other half (7) were greater than 0.20. All the oxacillin resistant *S. aureus* isolated from beef and frozen chicken were resistant to at least one other antibiotics among the used antibiotics while 5 out of 25 oxacillin susceptible *S. aureus* were susceptible to all the used antibiotics with MAR index 0.00. Thirty out of the 80 *S. aureus* had MAR index of less than 0.20 whereas 50 had MAR index of greater than 0.20. One of the *S. aureus* isolates was resistant to all the antibiotics used with MAR index 1.0.

4. DISCUSSION

This study reports the occurrence of *S. aureus* in the beef and frozen chicken which could account for economic loss and public health challenge of the consumers similar to the earlier report of Hachemi et al. [8] and Wang et al. [24]. *S. aureus* was isolated from beef and chicken in which occurrence was high in chicken compare with beef in this study. All selected isolates were found to ferment mannitol, glucose, lactose and sucrose as well as coagulation of serum. The isolates' susceptibility to oxacillin was investigated which revealed their resistance to oxacillin, a derivative of penicillins and this account for the occurrence of oxacillin-resistant *S. aureus* (ORSA) in beef and chicken sold for consumption. It has earlier been reported that *S. aureus* isolates resistant to penicillins will be resistant to methicillin and oxacillin therefore ORSA isolates have been reported to be MRSA [16]. Methicillin (oxacillin)-resistant *S. aureus* has not only been implicated in nosocomial infection but also in community acquired infection through foodborne cases. Although there might be some past work in this area of study however there is need for on-going surveillance of antibiotic resistance especially pathogen that are implicated in food contamination which makes this work a relevant study.

Higher proportion (69%) of the *S. aureus* isolates were resistance to oxacillin. Of importance to notice, Abramson [17] reported ORSA isolates to be more virulence than the OSSA. Along with this study where M-ORSA isolates were isolated, Bunnoeng et al. [25] has earlier reported high prevalence of MRSA from meat in Thailand which corroborate our study. ORSA isolated from beef was resistant to cloxacillin and ceftazidime but susceptible to other antibiotics. OSSA isolates from beef were found susceptible to all antibiotics with minimum of 55% susceptibility. It is observed that there were total susceptibility of OSSA from beef to gentamicin, erythromycin, ofloxacin and augmentin however there was no 100% susceptibility among ORSA isolates. The OSSA isolates from beef were also 100% susceptible to representative of penicillins (augmentin) except cloxacillin that exhibited low activity (55%) as investigated in our study which correlated with low resistance rate exhibited by ORSA from meat (beef) as earlier reported by Pu et al. [26] and Ge et al. [27].

The resistance rate of *S. aureus* isolated from chicken was observed to be high. ORSA and OSSA isolates from chicken were observed to have high resistance rate to ceftazidime and cloxacillin as investigated in our study. However, there is high susceptibility to cefuroxime, gentamicin, ceftriaxone, erythromycin, ofloxacin and augmentin. OSSA strains from chicken had high susceptibility rate greater than 71% to ceftriaxone, gentamicin, cefuroxime, erythromycin, ofloxacin and augmentin which is similar to the report of Haydar and Erhan [28] that OSSA strains from chicken could be susceptible to other antibiotics just as reported in our findings.

The overall antibiogram of the ORSA and OSSA isolates in this work revealed that there is high level of susceptibility of the isolates to gentamicin, ceftriaxone, Erythromycin, ofloxacin and augmentin which is similar to earlier report of Haydar and Erhan [28] while high resistance was observed to the remaining antibiotics. It can be deduced that there was low resistance pressure in ORSA and OSSA isolates as found in our study. The result of this work further confirmed that there are viable antibiotics for the treatment of staphylococcal infection if it occurs however it should better emphasize that adequate care should be put in place in preventing the spread of *S. aureus* through contaminated food products like beef and chicken.

The multiple antimicrobial index as a parameter as observed in this study indicates that there are some of the isolates (ORSA and OSSA) from beef and chicken are susceptible to vast number of antibiotics in the antibiotic profile of this investigation. The isolates with MAR ranges from

0.50 – 1.00 has resistance potential to significant number of antibiotics as evidence in the findings of our study.

5. CONCLUSION

The role of food in the spread of pathogens cannot be over-emphasised in public health therefore the need to embark on the continuous surveillance is vital. ORSA and OSSA strains from beef and chicken have been investigated and their susceptibility to other groups of antibiotics are well presented in this report. The report of this study further confirmed that there are potent antibiotics especially to treat staphylococcal infection if it occurs. It could further emphasis that food products like beef and chicken are potential sources of ORSA and OSSA. This deduction create awareness that there is need for adequate food processing especially at suitable temperature so to reduce the possible microbial load in the food products. The findings of this research especially in the area of antibiotic profiling will also assist managing antibiotics for staphylococcal infection management. There is need to educate the food handlers particularly raw meat sellers to practice good hygiene so as to reduce occupational hazard in their practice.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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