BETA-LACTAMASE PRODUCING STAPHYLOCOCCUS AUREUS ISOLATED FROM SOME MEAT AND MEAT-BASED FOODS

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ABSTRACT

Food poisoning due to *Staphylococcus aureus* isolated from meat and its products has been reported as a leading cause of morbidity, hospitalization and even death. This study was therefore aimed at evaluating the prevalence of β-lactamase producing *Staphylococcus aureus* in meat and meat-based foods using agar plate and tube method of iodometric methods by Devapriya *et al.* (2013). Results obtained connotes that 32 of the 60 analyzed samples harboured *Staphylococcus aureus* while 20 and 22 of the isolated organisms were found to be positive for both agar plate and tube methods respectively. The 32 identified *Staphylococcus aureus* however were found showing the following resistance of 97%, 94%, 88%, 81%, 75% to nitrofurantoin and amoxycillin, tetracycline, teicoplanin and ceftriazone, cotrimozazole, and vancomycin respectively. It can be concluded that the analyzed foods were variously contaminated with beta lactamase producing *Staphylococcus aureus*. It is therefore suggested that strict hygiene practices should be encouraged in every stages of animal and food processing.

Key words: Antibiotic sensitivity, Beta-lactamase, Meat, Meat-based foods, *Staphylococcus aureus*, Two iodometric methods

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INTRODUCTION

Staphylococcus aureus is a Gram-positive bacterium and an opportunistic bacterial pathogen commonly associated with asymptomatic colonization of the skin and mucosal surfaces of humans (Olowe *et al*., 2007) and animals. It is one of the leading causes of food-borne illness in humans, and has been implicated in a number of diseases ranging from minor, uncomplicated to more serious infections such as bacteraemia and pneumonia (Charlene *et al.*, 2013; Lekshmi *et al.*, 2016). *S. aureus* is responsible for various community and hospital acquired infections and has been reported as an indicator of contamination of processed foods (Kluytmans, 2010). It is also responsible for skin infections, soft tissues infections, surgical site infections and bone and joint infections. Food poisonings as a result of *S. aureus* occur when food that contain preformed staphylococcal enterotoxins are consumed. *S. aureus* is a virulent and important human pathogen which has been attributed to its intrinsic virulence and ability to adapt to different environmental conditions. Also, by reason of its ability to develop or acquire resistance to almost any new antimicrobials (Lekshmi *et al.*, 2016). In developing countries, especially Nigeria, food-borne organisms including *S. aureus* are major cause of food poisoning (Bello *et al*., 2013). In developed countries, *S. aureus* food poisoning due to consumption of fish and other street-vended foods were reported (Iwamoto *et al*., 2010; CDC, 2011). β-lactam antibiotics are among the most frequently prescribed antibiotics worldwide in the control of *S. aureus* infections and the efficacy of the antibiotic for therapy have suffered a set-back due to the growing trend of multiple resistant strains observed in the organism to β-lactam and other antibiotics (Deurenberg and Stobbenringh, 2008; Jensen and Lyon, 2009). β-lactamase production is one of the best known mechanism of antibiotic

resistance which may be chromosomally borne or plasmid-mediated (Devapriya *et al*., 2013, Prescott *et al*., 2013). Reports regarding the prevalence of *S. aureus* on different meat stuff such as pork, chicken, beef, fried fishes and meat and production of β-lactamase by *S. aureus* from clinical documents are well documented (Bello *et al*., 2013; Torimiro *et al*., 2013). But information concerning the prevalence of β-lactamase production by *S. aureus* from meat and meat products in the studied environment is scarce. The objective of the present study were to determine the prevalence of *S. aureus* from different meat and its products. Also to determine its ability to produce β-lactamase and antibiotic profile.

MATERIALS AND METHODS

Study Area
The survey to evaluate the status of meats and meat-based food products within Ago-Iwoye, Ijebu-Ode and Sagamu were carried out between April and August, 2016. The samples were purchased from both the street vendors and big fast food joints situated in the studied areas.

Study Site
The study was carried out in the microbiology laboratory of the department of Microbiology, Faculty of Science, Olabisi Onabanjo University.

Materials
Materials used in this study were of analytical grade and were obtained from Microbiology laboratory, Department of Microbiology, Faculty of Science, Olabisi Onabanjo University in Ago-Iwoye, Ogun State.

Collection of Samples
Samples were meat and meat-based foods. Sixty meat and meat-based food (28 meat and 32 meat-based), were purchased in a pre-sterilized container. The containers were not opened until they got to the laboratory for analysis.

Preparation of Inoculum
The samples were swabbed with sterile cotton swabs dabbed in distilled water at room temperature and cultured on Nutrient agar (Oxoid, UK). The isolates from the preparation was sub-cultured on Mannitol Salt agar (Oxoid, UK).

Isolation and Identification
The isolates were identified based on their ability to grow on Mannitol Salt agar (Oxoid, UK) after overnight incubation at 37°C, Gram staining reaction, slide coagulase and catalase tests.

Antibiotic Susceptibility Test.
In vitro susceptibility of *S. aureus* to antibiotics was determined as explained by the Kirby-Bauer disk diffusion method (Bauer *et al*., 1996) on Mueller Hinton agar using the following antibiotic discs; Augmentin (30µg), Ceftriazone (30µg), Nitrofurantoin (200µg), Gentamycin (10µg), Cotrimozazole (25µg), Ofloxacin (5µg), Amoxycillin (25µg), Ciprofloxacin (10µg), Tetracycline (30µg), Pefloxacin (5µg), Teicoplanin (30µg) and Vancomycin (30µg).

Determination of β-lactamase
Agar Plate Method: The determination of the β-lactamase producing *Staphylococcus aureus* was done using the Iodometric agar and tube method (Devapriya *et al*., 2013). Briefly, pure culture of *Staphylococcus aureus* was inoculated onto nutrient agar containing 0.2% starch. After overnight incubation at 37°C, Penicillin solution was added onto the *S. aureus* grown culture plate. The excess Penicillin solution was poured off after 15 minutes. Iodine solution in 1:5 dilution was added and spread over the growth and later the excess solution was removed by inverting the plate. Reading was taken after 30 minutes at room temperature.

Tube Method: In this method, 0.5ml of Penicillin solution was dispensed into test tubes that contained the test bacteria and adjusted using normal saline to 0.5 McFarland standard to give a density of at least 10^4CFU/ml. After one hour at room temperature, two drops of starch indicator was added to the suspension, followed by one drop of iodine reagent.

RESULTS
Out of the sixty samples examined, forty-one bacterial organisms were recovered and identified. Thirty-three of these were Gram positive cocci whereas two, five and one were Gram positive rods, Gram negative rods and Gram negative cocci respectively. (Table 2). Thirty-two of the isolates were able to grow on Mannitol Salt agar and were catalase and coagulase positives. (Table 2).

Total number of isolates recovered according to location is shown in table 3. It was observed that Ago-Iwoye and Sagamu had the highest number of isolates (16 and 14) respectively. Also, highest number of *S. aureus* was isolated from barbeque meat purchased from the three locations. (Table 3). Out of the thirty-two isolates of *S. aureus* recovered, 20 and 22 had the ability to produce β-lactamase in the agar plate and tube methods respectively. (Figs 1 and 2) This was shown by the characteristic decolouration around the colonies and the disappearance of blue colour in both methods respectively. The susceptibility testing showed that two of the antibiotics (Ofloxacin and Pefloxacin) had the ability to inhibit the growth of each of the *S. aureus* isolates but to varied zones of inhibition which were not significant (4mm-12mm) and (4mm-10mm) for Ofloxacin and Pefloxacin respectively. However, high degree of resistance was observed in all the antibiotics tested against the 32 isolates of *S. aureus* especially in Nitrofurantoin (97%), Tetracycline (94%), Ceftriazone and Teicoplanin (88%). (Table 4)

Table 1. Types and number of samples with respect to location.

Samples	Ijebu-Ode	Ago-Iwoye	Sagamu	Total
Meat Pie	6	-	5	11
Barbeque meat	6	5	6	17
Barbeque chicken	-	-	2	2
Chicken Pie	2	-	2	4
Beef roll	2	-	1	3
Sausage roll	4	5	4	13
Pork	-	2	-	2
Fried meat	-	3	-	3
Cooked meat	-	1	-	1
Raw meat	-	1	-	1
Fried chicken	-	1	-	1
Cooked chicken	-	1	-	1
Smoked bush meat	-	1	-	1
TOTAL	20	20	20	60

Table 2. Morphologic features of the isolates

Sample Number	Nutrient agar		Mueller Hinton agar		Mannitol Salt agar		Gram Reaction				Catalase	Coagulase
	G	N	G	N	G	N	G+		G-			
							C	R	C	R		
60	21	09	20	10	32	09	33	2	1	5	32	32

KEY:

G = Growth
N = No growth
G+ = Gram positive
G- = Gram negative
C = Cocci
R = Rods

Table 3. Number of isolates based on food samples and locations.

Samples	Number of Isolates				Number of *S. aureus* Isolates				Sum of Total
	I	A	S	Total	I	A	S	Total	
Meat pie	1	-	2	**3**	-	-	2	**2**	**5**
Barbeque meat	8	5	6	**19**	6	5	6	**17**	**36**
Barbeque chicken	-	-	2	**2**	-	-	2	**2**	**4**
Chicken pie	-	-	-	**0**	-	-	-	**0**	**0**
Beef roll	1	-	-	**1**	-	-	-	**-**	**1**
Sausage roll	1	-	4	**5**	-	-	2	**2**	**7**
Pork	-	2	-	**2**	-	2	-	**2**	**4**
Fried meat	-	3	-	**3**	-	2	-	**2**	**5**
Cooked meat	-	1	-	**1**	-	1	-	**1**	**2**
Raw meat	-	1	-	**1**	-	1	-	**1**	**2**
Fried chicken	-	1	-	**1**	-	1	-	**1**	**2**
Cooked chicken	-	1	-	**1**	-	1	-	**1**	**2**
Smoked bush meat	-	2	-	**2**	-	1	-	**1**	**3**
Total	**11**	**16**	**14**	**41**	**06**	**14**	**12**	**32**	**73**

KEY

I = Ijebu-Ode

A = Ago-Iwoye

S = Sagamu

Table 4. Antibiotic resistance profile of isolated *S. aureus*

Antibiotics	Number of Resistant Isolates	Percentage (%) of Resistance Isolates
AUG	14	43.75(%)
CRO	28	87.5(%)
NIT	31	96.875(%)
GEN	14	43.75(%)
COT	26	81.25(%)
OFL	7	21.875(%)
AMX	31	96.875(%)
CPX	13	40.625(%)
TET	30	93.75(%)
PFX	7	21.875(%)
TEC	28	87.5(%)
VA	24	75(%)

KEY

AUG=Augmentin (30µg) NIT=Nitrofurantoin (200µg) CRO=Ceftriazone(30µg)
GEN=Gentamycin (10µ) COT=Cotrimozazole (25µg) OFL=Ofloxacin(5µg)
AMX=Amoxycillin (25µg) CPX=Ciprofloxacin (10µg) TET=Tetracycline(30µg)
PFX=Pefloxacin (5µg) TEC=Teicoplanin (30µg) VA=Vancomycin (30µg)

Table 5. Antibiotic susceptibility profile of isolated *S. aureus*

Antibiotics	Number of Susceptible Isolates	Percentage (%) of Susceptible Isolates
AUG	4	12.5(%)
CRO	0	0.00(%)
NIT	1	3.125(%)
GEN	2	6.25(%)
COT	1	3.125(%)
OFL	11	34.375(%)
AMX	0	0.00(%)
CPX	6	18.75(%)
TET	1	3.125(%)
PFX	13	40.625 (%)
TEC	0	0.00(%)
VA	2	6.25 (%)

KEY

AUG=Augmentin (30µg) NIT=Nitrofurantoin (200µg) CRO=Ceftriazone (30µg)
GEN=Gentamycin (10µ) COT=Cotrimozazole (25µg) OFL=Ofloxacin (5µg)
AMX=Amoxycillin (25µg) CPX=Ciprofloxacin (10µg) TET=Tetracycline (30µg)
PFX=Pefloxacin (5µg) TEC=Teicoplanin (30µg) VA=Vancomycin (30µg)

Table 6. Percentage production of β-lactamase by the isolated *S. aureus* using the two methods

Method	Number (n)		Percentage (%)	
	Positive	Negative	Positive	Negative
Agar	20	12	62.5	37.5
Tube	22	10	68.75	31.25

KEY

Percentage = (n/N) x (100/1)
N=32

Table 7. Prevalence of β-lactamase producing *S. aureus* based on location

Location	Number (n)		Percentage (%)	
	Agar	Tube	Agar	Tube
Ago-Iwove	12	11	37.5	34.375
Iiebu-Ode	2	3	6.25	9.375
Sagamu	6	8	18.75	25

KEY

Percentage = (n/N) x (100/1)
N=32

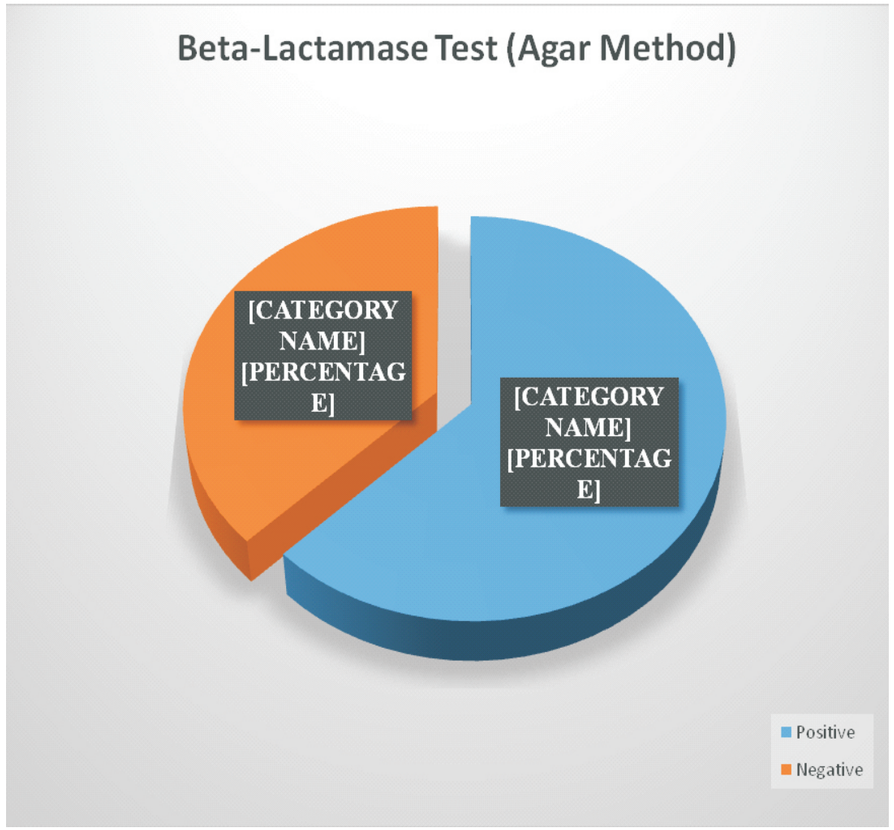

Fig. 1: Chart showing percentage result for beta -lactamase test using agar method.

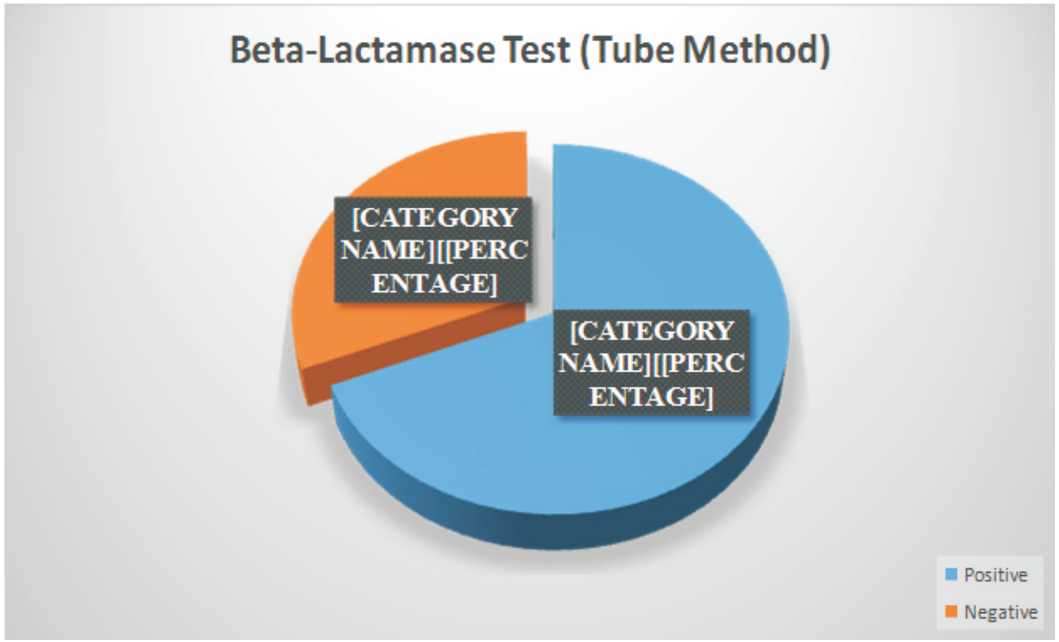

Fig. 2Chart showing percentage result for beta-lactamase test using tube method.

DISCUSSION

High level of poverty, increase in population and persistent urbanization factors inherent in developing countries have led to the emergence of a novel and most dominant form of restaurants called street or vended foods. Vended / street foods are one of the most cost effective and highly patronized forms of feeding among low paid public works, students, craftsmen and high school students due to its relatively low cost and ready to eat nature. This study revealed high prevalence rate (53%) of *S. aureus* in meat and meat based foods. This is consistent with the findings of Kitai *et al*. (2005); VanLoo *et al.* (2007); Murray *et al.* (2007); Philip *et al.* (2008) and Bello *et al.* (2013). The incidence of *S. aureus* in meat and meat based foods bought from roadside vendors as against big reputable fast food centres in studied areas could be attained to differences in the level of hygienic practices and environmental conditions. Studies conducted in Thailand, Ghana and Cotonou revealed 18%, 39% and 56% street vended dishes were contaminated by *S. aureus* respectively (Mensah *et al.,* 2002; Fang *et al*., 2003 and Sina *et al*., 2011). Barbeque meat had the highest occurrence of *S. aureus* (17) (Table 3). This could be attributed to the fact that hygienic practices as related to cleaning processing, preparation and packaging of the finished products were simply ignored by most food sellers. Our results agrees with the recent study of Bello *et al*. (2013) who reported meat sausages as the second most contaminated ready to eat foods after fish sausages. Previous findings of Moushumi *et al*. (2007) and Barro *et al*. (2002) reported the contamination trends of street foods in India and Burkina-Faso respectively. This calls for crucial need to educate street food sellers about basic hygienic behaviour in order to keep the food safe for consumption. Polluted environmental conditions and the lack of hygienic practices of sellers of ready to eat foods, meat/meat-based foods easily attract flies, vectors of toxic microbial contaminants which lead to disease mediating vectors (Todd *et al*., 2007). Roberts (1982), reported that lack of keeping foods in appropriate temperature for long time represents one of the major factors of food toxic-infection. It has been observed that most food sellers used their hands to serve foods. This practice increase the contamination risks. Findings of Greig *et al*. (2007) demonstrated that 20% of collective nutritional intoxication cases resulted from food contamination or from people that have handled contaminated food. Barro *et al*., (2000) reported that in street food selling system, that the food sellers are the cashiers where there is no separate cashiers as well as the food server. It was clearly noted that the meat and meat-based foods gotten from Ago-Iwoye were the products most contaminated by *S. aureus* (16 isolates/27%), followed by those from Sagamu in which 14 isolates (23%) were recovered unlike Ijebu-Ode where only 11 (18%) isolates were observed. This could be linked to the level of development and the type of people that inhabit the place (interior or exterior of the town) Ijebu-Ode and Sagamu could have said to have experienced both population and economic growth and have more of civil servants and industries than Ago-Iwoye in which majority of the inhabitants are predominantly students, farmers and peasant traders. This study revealed that the tested *S. aureus* had the ability to produce β-lactamase and it agrees with the reports of Akindele *et al.* (2010) and Torimiro *et al.* (2013). The two iodometric methods tested for β-lactamase detection are shown in Fig. 1 and 2. The sensitivity of the agar plate method for β-lactamase production was 20/32 (63%) and tube method was 22/32 (69%). This shows that the tube method was more sensitive than the agar method in detecting the β-lactamase. This is in agreement to other studies of Mazura, (1990) and Devapriya *et al.* (2013) who reported tube method as the most sensitive after paper method. Multi-drug is of significant health importance. It was observed in this study that 97%, 94% and 88% of the isolates were resistant to nitrofurantoin, tetracycline and teicoplanin respectively among others. It has been reported that multi-resistance could be chromosomal or plasmid mediated (Anderson, 2003). Origin of multi-drug resistance in meat/meat-based foods could come from a number of ways such as human handling, fecal contamination during the production process, improperly cooked, contaminated meat products or use of contaminated cooking utensils or packaging materials etc. the multi-drug resistance *S. aureus* observed in this study is likely due in part to the use of antibiotics in feeding practices. Recent studies have also reported multi-drug resistance *S. aureus* isolated from different food products (Philip *et al*., 2008; Bello *et al*., 2013; Sina *et al*., 2011).

CONCLUSION

This study revealed the prevalence of *S. aureus* in meat/meat-based food products, ability of the organism to produce β-lactamase using agar and tube methods of iodometric and multi-drug resistance profile. This study suggests that food vendors and hawkers of ready to eat foods should be properly educated so that they will be fully aware of the dangers associated with contaminated foods especially that of *S. aureus*. This organism has been implicated in diarrheal diseases. Also, special attention should be given to the preparation, storage and service of ready to eat foods since they are the highly patronized once in order to observe good hygienic practices and food safety. Efforts should be geared towards promoting appropriate and prudent use of antibiotic in order to curtail the role of selective pressure that tends to favour the emergence of drug resistance.

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