DETECTION OF STAPHYLOCOCCI IN STREET-VENDED READY-TO-EAT MEAT IN NSUKKA AND ITS ENVIRONS

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ABSTRACT

Ready-to-eat (RTE) street-vended meat in Nsukka and its environs were investigated for the presence of staphylococci. The samples were pre-enriched in peptone water for 24 hours at 37°C and subsequently streaked on mannitol salt agar plates and incubated at 37° C for 24 hours. The staphylococcal isolates were identified to species level by sequencing of sodA and 16S rDNA genes. Genes coding for TSST-1 (tst), ETA (eta), ETB (etb), and ETD (etd) were investigated by PCR. Phenotypic determination of resistance to 17 antimicrobial agents was carried out using the disc diffusion method, while genes coding for resistance to methicillin (mecA), aminoglycosides (aph(2)-aac(6), ant(4), aph(3)-III), erythromycin (ermA, ermB, ermC, ermT, mphC, msrA, and msrB) and tetracycline (tet(M), tet(O), tet(K), and tet(L) were determined by PCR amplification using their specific primers. Twenty-eight (11.0%) of the R-T-E meat samples contained staphylococci. Twenty-four of the Staphylococcus strains were identified to species level and they belonged to 6 species, namely S. scuiri (54.1%), S. lentus (16.6%), S. saprophyticus (12.5%), S. carnosus (8.4%), S. piscifermentans (4.2%) and S. epidermidis (4.2%). Four (16.7%) of the Staphylococcus species harboured the eta gene. Resistance genes detected in the Staphylococcus species were: mecA (25%), tetK (25%), mphC (12.5%), ermT (8.3%), and ermC (4.2%).

Keywords: Amplification, Food poisoning, Prevalence, Primers, Identification, Isolates, Ready-to-eat meat, Resistance

INTRODUCTION

Meat is defined as the flesh of animals which is suitable for use as food. Meat is valued as a complete protein food containing all the amino acids necessary for the human body. (Britannica, 2024). Meat is considered a good medium for microbial growth and survival due to its high content of blood, water, and suitable pH (Nester *et al*., 2009). Food poisoning organisms or their toxins are consumed in contaminated meat, causing foodborne infections and intoxication (Nyenje *et al.,* 2012). The increasing rate of meat contamination has generated a lot of health concerns and great attention has been paid to the zoonotic implications (Nester *et al*., 2009). Most of the disease-causing agents have the

potential to be transmitted through food including meat (Elmali *et al.*, 2005).

Members of the genus *Staphylococcus* have been associated with staphylococcal food poisoning. The onset of symptoms in staphylococcal food poisoning is usually rapid and in many cases acute, depending on individual susceptibility to the toxin, the amount of contaminated food eaten, the amount of toxin in the food ingested, and the general health of the victim (Pepe *et al.*, 2006). Street vended ready-to-eat (RTE) meat is a common business in many parts of Nigeria. In major cities and small towns in Nigeria, RTE meats are very popular street foods. Vendors of the RTE are patronized at their stands from about mid-day until late at night. In Nigeria, RTE meats are often prepared under questionable sanitary conditions and this has raised public health concerns about street vending operations (Mbata, 2005). Therefore, the present study was carried out to assess the prevalence, toxigenic potential and antibiogram of zoonotic bacterial isolates from RTE meat vended in Nsukka, Enugu State, Nigeria.

MATERIAL AND METHODS

Sampling Procedure: The study was conducted in Nsukka town and its environs in Enugu State, Nigeria between June 2010 and January 2011 (eight months). A cross-sectional survey experimental design was used. 255 RTE meat samples were purchased and transported separately in a sterile polythene bag to the Public Health and Preventive Medicine Laboratory, Faculty of Veterinary Medicine University of Nigeria, Nsukka for assay. The samples consisted of 105 roasted suya meat, 61 cooked chicken meat, 17 cooked goat meat, and 72 cooked pork meat. A purposive sampling technique was used to select meat sampling spots based on the availability of the vended markets. The specific locations sampled were collected were Nsukka Motor Park (NMP), Obollo Afor Market (OAM), Nsukka Mechanic Village (NMV) and Nkwo Ibagwa Market (NIM).

Isolation and Characterization of Staphylococci from Ready-to-Eat Meat Samples: One gram of each meat sample was aseptically transferred into 9 ml of 0.1% sterile peptone water, shaken thoroughly, and incubated overnight at 37^{o}C (Cheesbrough, 2006). A loopful of the pre-enriched suspension was streaked on mannitol salt agar plates and inoculated plates were incubated at 37^{o}C for 18 – 24 hours for *Staphylococcus* isolation. Suspected colonies of *Staphylococcus* were purified on nutrient agar. Colonies from the pure cultures were Gram stained and those that produced Gram-positive cocci in bunches were tested for catalase production (Cheesbrough, 2006).

Identification of the staphylococcal isolates to species level was done by sequencing of *sodA* and 16S rDNA genes (Poyart *et al.,* 2001). The presence of *mecA* gene in the staphylococcal isolates was investigated by PCR (Gómez-Sanz *et al.*, 2010; Lozano *et al.*, 2011). PCR amplification of genes coding for TSST-1 (*tst*), ETA (*eta*), ETB (*ETB*), and ETD (*etd*) were investigated (Kim *et al.,* 2011). Phenotypic determination of resistance to 16 antimicrobial agents was done using the disc diffusion method (CLSI, 2019), while genes coding for resistance to aminoglycosides (*aph(2)-aac(6), ant(4), aph(3)-III*), erythromycin (*ermA, ermB, ermC, ermT, mphC, msrA* and *msrB*) and tetracycline (*tet(M), tet(O), tet(K)* and *tet(L*) were investigated by PCR amplification (Gómez-Sanz *et al.*, 2010; Lozano *et al.*, 2011).

Statistical Analysis: The significance of the association between the prevalence of staphylococcal contamination and RTE meat type as well as the site of samples was analyzed using the Chi-square statistic.

RESULTS

Staphylococcus species were isolated from 28(11%) out of the 255 samples processed. The prevalence of *Staphylococcus* species was highest in suya 20(19%) and least in boiled goat meat 1(0.9%) (Table 1). There was a significant association ($p<0.05$) between staphylococcal contamination and the type of RTE meat.

Twenty-four (24) of the isolates were identified to species level, and they belonged to 6 different species namely: *Staphylococcus scuiri* – 13(54.1%), *S. lentus* – 4(16.6%), *S.*

saprophyticus – 3(12.5%), *S. carnosus* – 2(8.4%), *S. piscifermentans* – 1(4.2%) and *S. epidermidis* – 1(4.2%) (Figure 1).

Table 1: Prevalence of staphylococci in different types of ready-to-eat meat in Nsukka and its environs, Nigeria

Ready-to-eat meat type	Number analyzed	Number of samples contaminated by *Staphylococcus*
Suya	105	20(19)
Boiled chicken meat	61	4(6.6)
Boiled goat meat	17	1(0.9)
Boiled pork meat	72	3(2.9)
Total	255	28(11.0)

Number in parenthesis = percentage

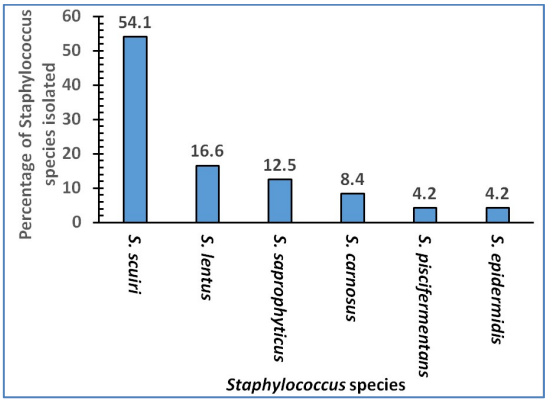

Figure 1: Percentage distribution of *Staphylococcus* species isolated from ready-to-eat meat in Nsukka and its environs

The rate of *Staphylococcus* isolation was highest in RTE meat samples obtained from NMP (39.2%), while samples from NMV had the lowest percentage (3.6%) (Table 2). There was a significant association ($p<0.05$) between the rate of contamination by *Staphylococcus* and the site of sample collection.

Table 2: Prevalence of Staphylococci in ready-to-eat meat from different locations in Nsukka and its environs, Nigeria

Location	Number analyzed	Number of samples contaminated by *Staphylococcus*
Obollo Afor (OAM)	64	10(35.7)
Nsukka Motor Park (NMP)	60	11(39.2)
Nkwo Ibagwa Market (NIM)	89	6(21.4)
Nsukka Mechanic Village (NMV)	42	1(3.6)
Total	255	28 (11.0)

Number in parenthesis = percentage

Twenty-five percent of the isolates were resistant to cefoxitin, oxacillin, and tetracycline, while none of the isolates was resistant to teicoplanin, ciprofloxacin, linezolid, and chloramphenicol (Table 3). A total of 12 resistance patterns were recorded for the staphylococcal isolates (Table 4). Out of the 24 staphylococcal isolates identified, 7(29.2%) were multi-drug resistant, being resistant to three or more classes of antimicrobials. Some of the resistance genes detected in the *Staphylococcus* species were*: mecA* (25%), *tetK* (25%), *mphC* (12.5%), *ermT* (8.3%), and *ermC* (4.2%) (Table 5). Six of the strains were resistant to oxacillin and cefoxitin and they all harboured the *mec*A

Four (16.7%) of the 24 *Staphylococcus* strains haboured the *eta* gene which codes for exfoliative toxin (ET) production. The four *eta*-positive strains of *S. scuiri* were all isolated from suya. None of the species haboured genes coding for toxic shock syndrome-1 (TSST-1), Panton-Valentine leukocidin (PVL), and enterotoxin production.

DISCUSSION

Ready-to-eat (RTE) meat is an important source of bacterial agents of zoonotic importance (Clarence *et al.,* 2009). The detection of *Staphylococcus* species in this study suggests post-processing contamination of the street-vendor RTE meat. The overall *Staphylococcus* contamination rate of 11% in this study is lower than the 33.7% *S. aureus* contamination rate recorded by Ndahi *et al.* (2014) from raw meat and meat products in Zaria, Nigeria, but lower than 3.2% of S*. aureus* contamination rate recorded by Nyenje *et al.* (2012) from RTE foods from roadside cafeterias and retail outlet in Alice, Eastern Cape Province, South Africa. Salihu *et al*. (2010) also reported a prevalence of 69.9% of *S. aureus* in traditionally prepared fried ground beef in Sokoto, Nigeria. These reports indicate that *Staphylococcus* is a significant pathogen found in RTE meat and can pose human health hazards.

Table 3: Antimicrobial resistance profile of *Staphylococcus* species isolated from ready-to-eat meat from different locations in Nsukka and its environs

Antimicrobial agents (Disc potency in ug)	Number (%) of resistant isolates
Fusidic acid (10)	19(79.2)
Cefoxitin (30)	6(25)
Oxacillin (10)	6(25)
Tetracycline (30)	6(25)
Clindamycin (10)	5(20.8)
Erythromycin (30)	5(20.8)
Vancomycin (30)	3(12.5)
Mupirocin (5)	2(8.4)
Sulphamethoxazole/trimethoprim (25)	2(8.4)
Gentamicin (10)	1(4.2)
Kanamycin (30)	1(4.2)
Streptomycin (30)	1(4.2)
Tobramycin (10)	1(4.2)
Chloramphenicol (10)	0(0)
Ciprofloxacin (5)	0(0)
Linezolid (10)	0(0)
Teicoplanin (5)	0(0)

Table 4: Resistance patterns of *Staphylococcus* species isolated from ready-to-eat meat from different locations in Nsukka and its environs

S/N	Resistance patterns	Number of isolates with pattern
1	FA	7(29.2)
2	TE	2(8.3)
3	FA-OX-FOX	3(12.5)
4	FA-TE	2(8.3)
5	FA-TE-OX-FOX	1(4.1)
6	FA-MUP	1(4.1)
7	FA-VA	1(4.1)
8	TE-SXT	1(4.1)
9	FA-CC-E	3(12.5)
10	FA-CC-VA-ER-OX-FOX	1(4.1)
11	FA-CC-VA-ER-TE	1(4.1)
12	S-SXT-KA-GE-TB-MUP	1(4.1)

FA = Fusidic acid, TE = Tetracycline, OX = oxacillin, FOX = Cefoxitin, MUP = Mupirocin, VA = Vancomycin, SXT = Sulphamethoxazole/trimethoprim, CC = Clindamycin, E = Erythromycin, S = Streptomycin, KA = Kanamycin, GE = Gentamicin, TB = Tobramycin, Number in parenthesis = percentage

Staphylococcus in food indicates post-terminal processing contamination by food handlers in various RTE foods (Feglo and Sakyi, 2012). In addition, processed RTE meats are always kept exposed, while awaiting buyers, making them vulnerable to contamination with different pathogens. Besides, not all consumers are patient enough to wait for the vendor to reheat the meat before purchasing. *S. aureus* has been isolated in the nose, throat, hands, fingertips, hairs, and skin of more than 50% of apparently healthy food handlers (Acco *et al.,* 2003). The detection of *Staphylococcus* species in the RTE meat in this study revealed the likelihood of contamination due to improper handling by the vendors. Careless sneezing and coughing among vendors may result in the contamination of RTE products. Flies, nose picking, and improper washing of hands before handling RTE meat or utensils can also be sources of contamination.

Staphylococcus may produce enterotoxins in the contaminated RTE meat which could cause gastroenteritis (Argudín *et al.*, 2010). *Staphylococcus* can also infect other tissues when immune barriers have been breached (e.g. skin and mucosal lining) resulting in the development of furuncles and carbuncles (Taylor and Unakal, 2023). In infants, the organism can cause a severe disease called staphylococcal scalded skin syndrome (SSSS) (Ross *et al.*, 2024). Some strains of *Staphylococcus* which produce exotoxin and TSST-1 have been reported to be the causative agents of Toxic Shock Syndrome (Sila *et al.,* 2009).

In this study, the high prevalence of *Staphylococcus* recorded in OAM and NMP compared to other locations may be an indication of poor hygienic practices carried out in the two vending areas. The two locations are situated around motor parks and busy daily markets, thus increasing the chances of the RTE meat being contaminated. USFDA (2000) stated that exposure of meat to contaminated air or dust at the points of sale is likely to increase the bacterial load as most of the bacteria are carried by contaminated dust and air. The surroundings of the vending sites were considered unhygienic given that garbage and waste littered around the processing environment.

The result of antimicrobial resistance recorded in this study showed that the *Staphylococcus* isolates were multiple drug resistant.

Table 5: Antimicrobial resistance phenotype and some resistance genes detected among the staphylococcal isolates recovered from ready-to-eat meat from different locations in Nsukka and its environs

Strain ID	Staphylococcus species	Resistance phenotype	Resistance genes detected
S_1	*S. sciuri*	FA-OX-FOX	*mecA*
S_6	*S. sciuri*	FA-OX-FOX	*mecA*
S_{11}	*S. sciuri*	FA	*ermC*
S_{12}	*S. sciuri*	FA-OX-FOX	*mecA*
S_{15}	*S. sciuri*	FA	
S_{61}	*S. piscifermentans*	TE	*tetK*
S_{67}	*S. lentus*	FA-E-CC	*mphC*
S_{68}	*S. sciuri*	FA	
S_{69}	*S. epidermidis*	TE-SXT	*tetK*
S_{70}	*S. sciuri*	TE-FA-OX-FOX	*tetK, mecA*
S_{71}	*S. sciuri*	TE-FA	*tetK*
S_{72}	*S. sciuri*	FA	
S_{73}	*S. sciuri*	FA-OX-FOX-MUP	*mecA*
S_{74}	*S. lentus*	E-CC-FA	*mphC, ermT*
S_{75}	*S. lentus*	E-CC-FA	*mphC, ermT*
S_{77}	*S. sciuri*	VA-FA	
S_{80}	*S. saprophyticus*	FA	
S_{81}	*S. carnosus*		
C_4	*S. sciuri*	S-SXT-KA-GEN-TOB-MUP	
C_{23}	*S. saprophyticus*	FA	
C_{55}	*S. carnosus*	TE	*tetK*
C_{56}	*S. saprophyticus*	ER-CC-VA-FA-OX-FOX	*mecA*
G_8	*S. lentus*	ER-CC-VA-TE-FA	*tetK*
P_{57}	*S. sciuri*	FA	

S = suya, C = chicken meat, G = Goat meat, P = pork meat

In addition to being resistant to fusidic acid and cefoxitin, some isolates were also resistant to oxacillin, tetracycline, clindamycin, erythromycin, vancomycin, mupirocin, sulphamethoxazole/trimethoprim, gentamicin, kanamycin, streptomycin, and tobramycin. This observation agreed with the study of Van *et al*. (2007) who recorded 83.3% resistance to at least one antibiotic in food-borne bacterial contaminants in Vietnam. This is of public health concern as these drugs are still in use for the treatment of food-borne infections in humans. Antimicrobial resistance associated with food has been a global concern (Kumar *et al.,* 2005). There is an association between the use of antimicrobial agents and the occurrence of resistance.

Conclusions: In this study, six different species of *Staphylococcus* were isolated from the RTE meat products in Nsukka and its environs. 54.1% of the species isolated were *S. scuiri*. An overall prevalence of 11% was recorded for *Staphylococcus* from all the samples in this study with a specific prevalence of 19, 6.6, 0.9, and 2.9% for suya, cooked chicken, cooked goat, and cooked pork respectively. There was a significant association ($p<0.05$) between staphylococcal contamination and types of RTE meat while there was no significant association ($p>0.05$) between meat contamination and location of sample collection.

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