ANTIBIOTIC SUSCEPTIBILITY PATTERN OF BACTERIAL ISOLATES FROM COMMERCIALLY PREPARED SLICED, SAUCED ROASTED BEEF (SUYA) IN ABRAKA, DELTA STATE, NIGERIA

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Abstract
Abraka is a University Town and students in the area eat sliced roasted and sauced beef (Suya) as a delicacy. Recently, there was a wave of bacterial food poisoning in the area. This study was to determine the involvement of Suya in the food poisoning. A total of 150 samples were randomly obtained and analyzed by standard microbiological techniques: The total bacterial counts ranged from 5.7x105 CFU/ML- to 7.4×105 CFU/ML. A total of six pathogenic microbes were analyzed: Staphylococcus aureus, Enteroococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, and Proteus vulgaris. Staphylococcus aureus was the most prevalent with percentage distribution of 30(28.80%), while Salmonella typhi was the least with percentage distribution of 5(4.80%). All the isolates demonstrated resistance to spafloxacin and ampicillin. However, multidrug resistance to additional antibiotics was observed randomly amongst gentamycin (10µg), ciprofloxacin (10µg), augmentin (30µg), ofloxacin(10µg) pefloxacin (10µg), erythromycin(10µg), ceftaroline (30µg), zinnacef(20µg), amoxicillin-clavulanic acid (30µg), ceftazidime (30µg), cefuroxime (CXM) and tarivid (10µg). Multiple antibiotic resistance index (MRI) of 0.35-0.42.

Key words: Suya, Antibiotic resistance, Pathogenic bacteria.

INTRODUCTION

Meat remains a main source of protein as well as vitamins for most people in many parts of the world, thus it is essential to the maintenance of body cells and prevention of diseases (Fowotade et al., 2017). In Nigeria, "suya" is a much-cherished dietetic meat which cuts across socio-economic, age, religious and educational barriers (Inyang, *et al.*, 2005; Adebayo-Tayo *et al.*, 2008). The finely sliced meat is marinated in a range of spices and afterwards barbecued (Egbebi and Seidu, 2014). The slaughtering practice affords far-reaching contamination of hygienic tissue with Gram-positive and Gram-negative enteric bacteria and helminthes linked to humans, animals and the environment (Igyor and Uma, 2005; Avwioro et al., 2010; Ajayi et al., 2013). Periodic cases of gastroenteritis after consumption of suya points to the fact out that the product may constitute food safety risk if it not prepared hygenically (Odusote and Akinyanju, 2003; Inyang, *et. al*., 2005). The incidence of *Staphylococcus spp, Salmonella spp, Streptococcus spp, Enterobacter spp, Proteus spp, Bacillus spp and Pseudomonas spp and fecal E. coli* have been reported from both "suya" and smoked fish. Many of which have demonstrated multidrug resistance to tested

antibiotics (Adams and Moss 1999; Eze, *et al.*, 2013). Traditional approaches to the safety and quality of meat have relied on regulatory inspection and sampling regime. However, these measures cannot guarantee total consumer protection unless 100% inspection and sampling are employed. In the meat industry, this height of scrutiny is unworkable for various economic and logistic reasons (Dickson and Anderson, 2012). Fresh beef is usually healthy, but becomes contaminated during processing if is not done hygienically (Anderson, 2012). Fortunately, a large amount of bacterial colonies which have been isolated from beef have been non-pathogenic, nevertheless, human pathogens such as *Salmonella, Campylobacter* and *Listeria and helminthes* have been recovered (Ajayi *et al.*, 2013; Gilbert and Harrison, 2011). The indications of food diseases such as gastroenteritis point out that the products constitute a food safety risk (Uzeh, *et al*., 2006; Oduola *et al.*, 2010). Animals are well-known to constitute a huge reservoir of drug resistant enteric pathogens as well as infections/diseases that arise from the consumption of these MDR bacteria-laden animals, which can bring about failure of conventional treatments, longer treatments and death (Adeleke, Omafuvbe, 2011; 2013; Olaitan, *et al*., 2011; Samuel, *et al.*, 2011; Ilegbedion 2013). Even worse still, they may serve as a potential transfer route of the antibiotic resistant bacteria and resistant genes into human food-chain and environment (Nordman, *et al*., 2012; Willey, *et al*., 2007). Therefore, this study intended to evaluate total bacterial count and coliform counts in different suya samples, identify and characterize isolated microbes as well as establishes the antibiogram profiles of MDR isolated bacteria from "suya" meat from Abraka Delta State, Nigeria.

MATERIAL AND METHODS

Sample collection: A total of 150 samples were obtained at random from Abraka, Delta State, Nigeria. The samples were kept in an ice box at 0°C and transferred to the laboratory for analysis.

Microbiological analyses: 10gram of the mashed samples was weighed and aseptically introduced into 9ml of sterile distilled water, well shaken and a five-fold dilution was carried out in different test tubes. One milliliter of each of dilution factor 10^1 and 10^3 was pipetted and plated on nutrient agar, MacConkey agar, M. endo agar, M.enterococcus agar, *Salmonella Shigella* agar, eosin methylene blue agar and was incubated at 37°C for 24 hours. Morphological and biochemical characterization and identification was done on the bases of the following; Gram reaction, urease test, indole test, methyl-red, vogues Proskauer test, citrate test, coagulase, oxidation/ fermentation test, decarboxylase test, lactose, manitol , fructose, glucose, sucrose for biochemical characterization, to uniquely classify the isolates (Olutiola *et al.*, 2009).

RESULTS

Table 1 shows total bacterial counts (TBC) of the suya samples analyzed. Suya samples randomly collected were carefully analyzed for their microbial profile. The bacterial counts vary according to each of the samples, the total bacterial count ranged from 5.7x105 CFU/ML to - 7.4×105 CFU/ML. The study reveals that among the "suya" samples collected from the various locations, samples in sample J (Umono street) had the highest total bacterial count of (7.4×105 CFU/ML). The least bacteria count was recorded in sample G (FSP road) with 5.7x105 CFU/ML. Samples from locations A had a count of 6.10x105 CFU/ML, B with count of 6.80x105 CFU/ML, C with count of 6.5x104 CFU/ML, D with count of 6.2x106 CFU/ML, E with count of 7.1x105 CFU/ML, F with count of 6.4x105 CFU/ML, H with count of 6.7x105 CFU/ML and I with count of 5.7x105 CFU/ML.

Table 1: Total viable counts of bacteria on suya samples

Sample location	Mean number of colonies	Total bacterial counts (10^5 CFU/ml)
A	61.00±8.18[a]	$6.10x10^5$
B	68.00±4.00[a]	$6.80x10^5$
C	65.00±18.02[a]	$6.5x10^4$
D	62.33±20.00[a]	$6.2x10^6$
E	71.00±3.60a	$7.1x10^5$
F	64.66±9.29[a]	$6.4x10^5$
G	5.03±5.03[a]	$5.0x10^5$
H	67.33±4.04[a]	$6.7x10^5$
I	57.00±7.54[b]	$5.7x10^5$
J	74.66±12.50[b]	$7.4x10^5$

Key: CFU/ml-Colony Forming Unit/Milliliter. Values were represented as mean +standard deviation of three replicates in all groups. Values with dissimilar superscript were significantly different at ($p<0.05$).

Incidence and percentage distribution of bacterial isolates are presented in table 2. A total of 6 microbes were isolated from the suya samples. The bacteria isolated were *Staphylococcus aureus, Escherichia coli, Enteroococcus faecalis, Proteus vulgaris Salmonella typhi,* and *Pseudomonas aeruginosa. Staphylococcus aureus,* the most occurring microbes with percentage distribution of 30(28.80%), as *Salmonella typhi* was the least occurring microbe with percentage distribution of 5(4.80%). *Pseudomonas aeruginosa* with percentage distribution of 17(16.30%) followed by *Proteus vulgaris* with percentage distribution of 15(14.40%), *Enteroococcus faecalis*, with percentage distribution of 14 (13.40%) and *Escherichia coli* with percentage distribution of 10(9.60%) respectively.

Table 2: Occurrence and percentage detection of isolated bacteria from the suya samples

Sample location	*S. aureus*	*S. faecalis*	*E. coli*	*P. aeroginosa*	*S. typhi*	*P. vulgaris*	Total	**%**
	3	1	1	1	1	2	9	8.60%
B	3	1	1	3	0	2	10	9.60%
C	3	1	1	2	1	1	9	8.60%
D	3	1	1	1	1	2	9	8.60%
E	3	2	1	1	1	1	9	8.60%
F	3	1	1	3	0	1	9	8.60%
G	2	1	1	1	0	1	6	5.70%
H	3	1	0	1	0	2	7	6.70%
I	3	2	1	1	1	1	9	8.60%
J	3	3	2	3	0	2	11	10.50%
Total	39	14	10	17	5	15		
%	28.80%	13.40%	9.60%	16.30%	4.80%	14.40%	89	

Table 3: Morphological and biochemical identification of isolates

Morphological and Biochemical Tests	*Staphylococcus aureus*	*Enteroococcus faecalis*	*E. coli*	*Pseudomonas aeruginosa*	*Salmonella typhi*	*Proteus* vulgari
Gram reaction	+	+	_	_	_	_
Shape	Cocci	Cocci	Rod	Rod	Rod	Rod
Indole	_	_	+	_	_	_
Citrate	+	+	_	+	_	_
Voges-Proskauer	+	+	_	_	_	_
Methyl red	_	+	+	_	+	_
Oxidase	_	_	_	+	_	_
Urease	+	_	_	_	_	+
Catalase	+	_	+	+	+	+
Mannitol	+	+	+	_	+	_
Fructose	_	_	+	+	_	_
Glucose	+	+	A/G	_	_	_
Lactose	+	+	+	_	_	+
Sucrose	A/G	A/G	+	_	_	_
Coagulase	+	_	_	_	_	_
Decarboxylase	_	_	Lysine +	Arginine +	Lysine +	_

Key: + Positive, - negative, A = acid production, G =gas production.

Table 3 showed morphological and biochemical classification of isolates obtained from the suya samples. The distinction in bacterial culture, morphological, and biochemical characteristics of six isolates were enumerated and identified which include *Staphylococcus aureus, Proteus vulgaris Pseudomonas aeruginosa, Escherichia coli Enterococcus faecalis* and *Salmonella typhi.* These bacteria were characterized on the basis of some key reactions which include a range of temperature for growth, production of catalase, fermentation and utilization of sucrose, glucose, maltose and lactose, ability to swarm on agar, containing the enzyme cytochrome oxidase, possess the enzyme coagulase, decarboxylase production, the ability to use citrate as its sole source of carbon, production of the enzyme tryptophanase which can convert the amino acid and tryptophan to indole, the production of urease and methyl red. Specific biochemical reactions such as indole, citrate, Voges-Proskauer, methyl red, oxidase, urease, catalase, mannitol, fructose, glucose, lactose, sucrose, coagulase and decarboxylase were also used for the identification of the organisms.

Table 4 shows the antibiotic susceptibility profiling of isolated bacteria, the susceptibility profile revealed that all the isolated bacteria demonstrated resistance to spafloxacin and ampicillin. However, resistance to three additional antibiotics was observed among Staphylococcus aureus which include pefloxacin 10µg, erythromycin 10µg and zinnacef 20µg. Resistance to three additional antibiotics which include ofloxacin 10µg, erythromycin 10µg and zinnacef 20µg was observed amongst *Enteroococcus faecalis*. Resistance to four additional antibiotics which include gentamycin 10µg, ciprofloxacin 10µg, Augmentin 30µg, pefloxacin 10µg was also observed amongst *Escherichia.coli. Pseudomonas aeruginosa* demonstrated resistance to three additional antibiotics which include pefloxacin 10µg, erythromycin 10µg and zinnacef 20µg. *Salmonella typhi* demonstrated resistance to four additional

antibiotics which include ciprofloxacin 10µg, ofloxacin 10µg and erythromycin 10µg. *Proteus vulgaris* demonstrated resistance to four additional antibiotics was observed which include gentamycin 10µg, ofloxacin 10µg, pefloxacin 10µg and zinnacef 20µg. Multiple antibiotics resistance of 0.35 was observed among *Staphylococcus aureus*, *Enteroococcus faecalis, Pseudomonas aeruginosa* and *Salmonella typhi* while multiple antibiotics resistance index of 0.42 was seen among *E. coli* and *Proteus vulgaris*.

Table 4. Antibiotic susceptibility profile of Gram +ve and Gram -ve bacterial isolates from the suya samples

Isolates	Antibiotics														
	CN 10µg	CPX 10µg	AU 30µg	OFX 10µg	PEF 10µg	E RY 10µg	CPT 30µg	SP 10µg	AMP 10µg	Z 20µg	AMC 30µg	CAZ 30µg	CXM 30µg	OFY 10µg	MAR INDEX
Gram +ve															
S. *aureus*	S	S	S	S	R	R	S	R	R	R	S	S	S	S	0.35
Enteroococcus faecalis	S	S	S	R	S	R	S	R	R	R	S	S	S	S	0.35
Gram-ve															
E. coli	R	R	S	R	R	S	S	R	R	S	S	S	S	S	0.42
Pseudomonas aeruginosa	S	S	S	S	R	R	S	R	R	R	S	S	S	S	0.35
Salmonella typhi	S	R	S	R	S	R	S	R	R	S	S	S	S	S	0.35
Proteus vulgaris	R	S	S	R	R	S	S	R	R	R	S	S	S	S	0.42

Key; CN;Gentamycin, CPX;Ciprofloxacin, AU;Augmentin, OFX: Ofloxacin, PEF;Pefloxacin, E RY:Erythromycin, CPT:Ceftaroline, SP;Sparfloxacin, AMP: Ampicillin, Z;Zinnacef, AMC :Amoxicillin-clavulanic acid, CAZ : ceftazidime, CXM

DISCUSSION

Suya which is a traditional animal protein gotten from beef hung on stick and spiced with peanut cake, salt, vegetable oil and other flavours, and roasted on a glowing charcoal fire was analyzed microbiologically. The microorganisms isolated were *Staphylococcus aureus, Pseudomonas aeruginosa Enteroococcus faecalis, Escherichia.coli, Salmonella typhi* and *Proteus vulgaris*. The result conformed to the report by Chukwura, *et al*., (2002), which declared that microbial analyses of meat in Enugu State, revealed that contamination of animal protein with diverse bacterial species. The highest bacterial count was of 7.4×105 CFU/ML, while the least bacterial count was 5.7x105 CFU/ML. Eke *et al.,* (2013) earlier reported total viable counts ranged from 1.0x103 to 4.8x103. Edema *et al.,* (2008) and Ologhobo *et al*., (2010) also reported the presence of various pathogenic bacteria in poorly processed suya Uzeh *et a*l., (2006) had earlier stated that bacterial prevalence in "Suya" products in Nigeria was of public health concern. A total of six different bacterial species were isolated, *Staphylococcus aureus* the most occurring bacterial isolate with percentage distribution of 30(28.80%), followed by *Pseudomonas aeruginosa* with 17(16.30%), *Proteus vulgaris* 15(14.40%), *Enteroococcus faecalis*, 14(13.40%), *Escherichia coli* 10(9.60%) and *Salmonella spp* had the lowest incidence with 5(4.80%) as shown in table 2. A high percentage occurrence *of Staphylococcus in* suya had been reported by Gilbert *et al*., (2001) that being normal flora of the skin, cross contamination from meat handlers during processing is a major route of transmission. The level of the presence of these organisms in food has been described as index of food hygiene

(Adesokan *et al.*, 2008). Antibiotic susceptibility profile reveals that all the isolated bacteria demonstrated resistance to spafloxacin and ampicillin. However, multidrug resistance to additional antibiotics was observed among *Staphylococcus aureus, Enteroococcus faecalis, Pseudomonas aeruginosa Salmonella* spp, *Proteus vulgaris*. Multiple antibiotics resistance index of 0.35 was observed among *Staphylococcus aureus, Enteroococcus spp, Pseudomonas aeruginosa and Salmonella spp* while multiple antibiotics resistance index of 0.42 for *E. coli and Proteus vulgaris* was observed.

Conclusion

This investigation has revealed that the preparation and sales of suya would have been carried out under grossly unhygienic and unsafe conditions, thus constituting a food safety risk.

Recommendation

Adequate monitoring of these nutrition products by educating processors, vendors and consumers on good sanitary practices, possible risk of contaminated products should be encouraged.

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