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Original Research Article

Bacterial status and antibacterial susceptibility profiles of selected pathogens associated with suya meat samples purchased in Bori metropolis, Rivers State, Nigeria

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Ready-to-eat Suya meats were randomly sampled and assessed for bacterial status and antibacterial susceptibility profiles of selected pathogens. Bacteriology of Suya samples and antibacterial susceptibility test were determined using standard microbiological and disc diffusion methods respectively. Aerobic plate counts (APCs) of roasted and vended Suya ranged from $2.4x10^4$-$1.39x10^5$ CFU/g whereas total coliform counts (TCCs) ranged from $1.5x10^4$-$6.2x10^4$ CFU/g. A total of ten (10) bacterial species were identified including six (6) Gram-positive and four (4) Gram-negative bacteria. The most frequently isolated bacteria were *Staphylococcus aureus* (20%), *Escherichia coli* and *Micrococcus luteus* (16%) respectively. Antibacterial susceptibility test results indicated that Ciprofloxacin (29-41mm) and Levofloxacin (29-38mm) demonstrated high level sensitivity and broad spectrum activity on selected pathogens respectively whereas *Klebsiella pneumoniae* showed high resistance to Ampiclox (0.0mm) and Erythromycin (0.0mm) respectively. The high incidence of bacterial contamination in Suya suggests that they are potential reservoir for possible outbreak of food-borne diseases. Furthermore, proper antibiotic therapy and patient management in cases of consumption of contaminated Suya is hereby seriously advocated.

Key words: Suya, bacteria, antibacterial, susceptibility, pathogens

INTRODUCTION

"Suya" is a traditional barbecue, smoked or roasted obtained from thinly sliced boneless meat and marinated with various spices such as clove, ginger, pepper, salt, peanut cake, vegetable oil as well as food additives and flavorings (Akpamu 2011; Egbebi and Seidu, 2011), enjoyed as a delicacy in West Africa (Eke et al., 2013). It is a street-vended food which provides a source of inexpensive, convenient and often nutritious menu for cities, urban and rural areas; a major source of income for vast number of persons and creates opportunity for self-employment.

However, the preparations of food usually result in their inadvertent contamination (Mead, 2004) especially if improperly handled or abused. Reports by several workers indicate that a wide spectrum of microbes such as *Bacillus* sp, *Clostridium* sp, *Enterobacter* sp, *Escherichia* sp, *Klebsiella* sp, *Micrococcus* sp, *Proteus* sp, *Pseudomonas* sp, *Salmonella* sp, *Shigella* sp, *Staphylococcus* sp and *Streptococcus* species have been isolated from ready-to-eat Suya in various parts of Nigeria (Uzeh et al., 2006; Moshood et al., 2012; Salihu et al., 2013). Most of these genera are known to be of public health concern and have been associated with cases of gastroenteritis and other foodborne diseases (Moshood et al., 2012). Cases of haemolytic anaemia and cancer from non-microbial sources have also been reported after ingestion of suya due to adulteration of food additives (Williams et al., 1998) and production of carcinogenic chemicals from smoke (Edema et al., 2008).

The sources of these contaminations have been linked to poor hygienic conditions of the handlers and environment, raw meat, spices and packaging materials as well as cross-

Figure 1: Roasted ready-to-eat suya meat samples displayed on tray and newspaper with spices exposed to the environment

Figure 2: Typical scenes of ready-to-eat Suya meats displayed on grilled stand, tray and on newspaper with spices exposed at (1) Market road and (2) Poly road environments respectively.

processing contaminations (FAO, 1999; Odey et al., 2013; Hassan et al., 2014). The sporadic cases of food infection after consumption of Suya suggests that the product indeed constitute a food hazard risk (Inyang et al., 2005). Part of these food safety measures would be treatment with appropriate antibiotics. Although irrational antibiotic use has led society to antibiotic resistance – a serious health problem world-wide which is now trying to be solved by many approaches (Pavyde et al., 2015).

The purpose of this study therefore are/was to determine bacterial status and antibacterial susceptibility profiles of selected pathogenic species associated with Suya purchased in Bori metropolis.

METHODS AND METHODS

Sample collection

Suya meats were randomly sampled and packaged in sterile aluminum foil after purchase from eight (8) retail outlets at two major roads by 12noon and 7.00pm in Bori metropolis. These roads are Market and Poly Roads with display of Suya meats shown in Figure 1 and 2 respectively. The samples were transported to the laboratory within 30min for bacteriological analysis.

Bacteriological analysis

Serial dilutions were carried out after blending 10g of Suya meat samples in 90mL peptone water (Titan Biotech Ltd. Bhiwadi-301019, Rajasthan, India.) diluents to obtain a (10^{-1}) homogenate. Aerobic plate counts (APC) were determined on surface-dried nutrient agar (Scharlau Chemie S.A. Barcelona, Spain.) and plates were incubated in duplicates at 37°C for 24hours. Total viable colonies (30-300) were enumerated as colony forming units (CFUs). Coliforms including *Escherichia coli* colonies were determined on MacConkey agar (Oxoid. Unipath Ltd, Basingston, Hampshire, England.) using the spread plate method and duplicate plates were incubated at 37°C for 18-24hours.

Biochemical characteristic tests

Identification of bacterial isolates was carried out based on their cultural (appearance; pigmentation, consistency, margin and elevation), morphological (Gram's and endospore stain, size and shape) and biochemical characteristics (catalase, oxidase, indole, coagulase tests, citrate utilization, Methyl red and Voges- Proskauer tests, etc.) as well as sugar fermentation tests (glucose, fructose, galactose, lactose, sorbitol and arabinose) (Harrigan and McCance, 1976; Sneath et al., 1986; Forbes et al., 2007).

Disc diffusion Susceptibility Test

Susceptibility tests were performed by the disc diffusion method (Bauer et al., 1966; NCCLS, 1997; CLSI, 2011). An 18hours cultures of test organisms incubated at 37°C were standardized by diluting to 0.5 McFarland turbidity standard before spreading over the surface of Mueller Hinton agar (MHA) (Titan Biotech Ltd. Bhiwadi-301019, Rajasthan, India.) plates using sterile cotton swab/glass

Table 1. Bacterial counts (cfu/g) of roasted (R) and vended (V) Suya meat samples purchased from two locations in Bori metropolis

Purchase location	Sample type	APC	TCC
Market Road	Vended	2.4×10^4	3.9×10^4
	Roasted	1.39×10^5	6.2×10^4
Poly Road	Vended	4.5×10^4	2.7×10^4
	Roasted	7.4×10^4	1.5×10^4

Legend: APC = Aerobic plate count; TCC = Total coliform count

Table 2. Percentage occurrences of isolated bacteria from roasted and vended suya

		Market Rd		Poly Road		
		V	R	V	R	Total (%)
Bacteria	GR	(n=2)	(n=2)	(n=2)	(n=2)	(n=8)
Escherichia coli	—	0	2	0	2	4 (16)
Staphylococcus aureus	+	2	1	2	0	5 (20)
Listeria. monocytogenes	+	0	1	0	0	1 (04)
Bacillus subtilis	+	1	0	0	0	1 (04)
Klebsiella pneumoniae	—	1	0	0	0	1 (04)
Staphylococcus epidermidis	+	0	1	0	1	2 (08)
Streptococcus agalactiae	+	0	2	0	0	2 (08)
Micrococcus luteus	+	1	2	0	1	4 (16)
Shigella species	—	0	1	0	1	2 (08)
Yersinia pestis	—	1	1	1	0	3 (12)

Legend: GR = Gram's reaction; — = Not isolated; + = Isolated; Number in parenthesis is in %; V = Vended and R = Roasted ready-to-eat Suya samples.

spreader (Selvamohan et al., 2012; Adham, 2015) and allowed to dry for 2 to 5 minutes. Ten (10) standard antibiotic discs such as Amoxacilin = AMX (25µg), Ampiclox = APX (20µg), Chloramphenicol = CH (10µg), Gentamycin = CN (10µg), Ciprofloxacin = CPX (10µg), Erythromycin = E (5µg), Levofloxacin = LEV (10µg), Norfloxacin = NB (10µg), Rifampin = RD (10µg), Streptomycin = S (10µg) (Abtek biologicals, Ltd., Liverpool, UK) were placed on pre-dried MHA using sterile forceps and incubated at 37°C for 24hours (CLSI, 2011). The diameter of inhibition zone (DIZ) were measured with a transparent ruler and expressed in millimeters (mm). The mean values of DIZ were calculated. Interpretation of results was based on the zones of inhibition, susceptible or resistant (Smith, 2004; Cheesbrough, 2006; Forbes et al., 2007).

Statistical analysis

All data were obtained from at least two replicated experiments and the mean values estimated using Microsoft Excel 2007.

RESULTS

The bacterial counts of roasted ready-to-eat and vended Suya meat samples purchased at different locations in Bori metropolis are presented in Table 1. The APC of the Suya meat samples purchased at Market road had the highest value of $1.39\text{x}10^5$cfu/g and TCC value of $6.2\text{x}10^4$cfu/g respectively. Vended samples only showed marginal differences in counts at both purchase locations.

Identified bacterial isolates from roasted and vended Suya meat samples purchased at two different locations are shown in Table 2. A total of twenty five (25) bacteria isolates were identified with *S. aureus* (20%), *E. coli* (16%) and *M. luteus* (16%) being the most frequently isolated. These identified organisms consist of nine (9) genera of which six (6) are Gram positive and four (4) Gram negative

The presence of some opportunistic and/or pathogenic organisms such as *E. coli, S. aureus, K. pneumonae*, *L. monocytogenes, B. subtilis, Shigella* species, etc., in ready-to-eat suya meat underscores the environmental and public health significance.

Table 3. shows antibacterial susceptibility pattern of four selected pathogens from Suya meat samples. Ciprofloxacin showed highest sensitivity on virtually all the test bacterial pathogens but much more against *Staphylococcus aureus* (41mm) and *K. pneumoniae* (34mm) whereas streptomycin showed highest sensitivity against *Shigella* sp (42mm). Gentamycin showed highest sensitivity against *E. coli* (36.5mm) whereas *K. pneumoniae* (0.0mm) was highly resistant to Ampiclox and Erythromycin respectively (Table 3). Percentage susceptibility shows *Shigella* sp. (60%), *S. aureus* (30%), *E. coli* and *K. pneumoniae* (10%) respectively (Table 3).

Table 3. Antibacterial susceptibility pattern of selected pathogens from Suya meat samples

Bacterial Isolate	Antibiotic standards and Zone of Inhibition (mm)										
	AMX	APX	CH	CH	CPX	E	LEV	NB	RD	S	% Sus
E. coli	21.0	23.0	18.3	17.0	25.0	23.0	36.5	17.8	22.5	10.5	1(10)
S. aureus	17.5	25.0	11.5	8.8	41.0	19.0	38.0	19.0	11.8	29.0	3(30)
K. pneumoniae	17.0	0.0	5.0	23.0	34.0	0.0	21.0	21.0	14.3	17.0	1(10)
Shigella species	11.0	21.0	15.0	23.0	33.0	30.3	29.3	29.0	32.0	42.0	6(60)

Legend: AMX = Amoxacilin; APX = Ampiclox; CH = Chloramphenicol; CN = Gentamycin; CPX = Ciprofloxacin; E = Erythromycin; LEV = Levofloxacin; NB = Norfloxacin; RD = Rifampin; S = Streptomycin; Sus = Susceptibility.

DISCUSSION

Suya is a rich source of essential nutrient for humans and for microbial growth and survival. Its nutritional composition and characteristics makes it an excellent medium for microbial proliferation leading to rapid deterioration, loss of organoleptic property and economy. The occurrence of APC and TCC values of $1.39x10^5$cfu/g and $6.2x10^4$cfu/g in roasted Suya meat samples from Market road (Table 1) raises a food safety concerns and such high incidence of microbial contaminants have been previously reported (Salihu et al., 2010; Odey et al., 2013). This phenomenon may be attributed to poor personal hygiene and water quality, uncovered Suya, traditional processing techniques and unhealthy environment (Figure 1 and 2). Ten species of microorganisms were identified in Suya meat samples (Table 2) and similar findings in biodiversity have been earlier reported (Moshood et al., 2012; Hassan et al., 2014). This is suggestive of high level of contamination and underscores their environmental and public health significance. Several reports have implicated *S. aureus* and *Bacillus* to cause food borne diseases due to their ability to produce thermo-stable toxins and spores respectively (Mead et al., 1999; IFT, 2004; Mensah et al., 2012; Okwori et al., 2014). Additionally, health conditions may be exacerbated by the ability of *E. coli, S. aureus, S. epidermidis* and *K. pneumonae* to form biofilms which enhances antibiotic resistance (Donlan, 2001; Chen et al., 2013). Reports also reveal that an estimated 40-50% of prosthetic valve infections and 50-70% catheter biofilm infections as well as 87% of blood stream infections are attributed to *Staphylococci* (Agarwal et al., 2010; Chen et al., 2013).

Antibacterial susceptibility profiles revealed that Ciprofloxacin and Levofloxacin were the most effective antimicrobials against all the selected test bacterial pathogens whereas Streptomycin showed high sensitivity to *Shigella* sp. and *S. aureus* (Table 3). *K. pneumoniae* showed high resistance to Ampiclox, Erythromycin and Chloramphenicol but highly sensitive to Ciprofloxacin. Although, the present study precludes biofilm-formation but research has shown that they are linked with antibiotic resistance (Chen et al., 2013). Sensitivity of *S. aureus* to most of the third generation fluoroquinolones and other antibiotics has been reported (Forbes et al., 2007). Sensitivity of *E. coli* to Chloramphenicol in this study is consistent with that earlier reported (Adham, 2015) as well as to Ampiclox and Rifampicin. The high degree of sensitivity of these drugs against both Gram positive and Gram negative bacteria suggests broad spectrum activity. This study also revealed the percentage susceptibility profiles of the isolates as follows; *Shigella* sp. (60%), *S. aureus* (30%), *E. coli* and *K. pneumoniae* (10%) respectively. Thus, predicting the course of antibiotic therapy following infection with these organisms. Out of 25 isolates, four (4) were selected based on clinical significance, predictability of a bacterial isolate's susceptibility to the antimicrobials commonly used against them and availability of reliable standardized methods for testing the isolates (Forbes et al., 2007). Considering the effects of these antibiotics on the other isolates can be a subject for further research.

However, the advent of molecular/culture-independent techniques though advantageous in the identification of bacterial species than the classical culture-dependent techniques does not invalidate the accuracy/exactness of culture-dependent techniques.Where necessary or possible, by combining both isolation strategies a more complete picture of the diversity of bacterial species in any given sample can be captured (Ellis et al., 2003; Kisand and Wikner 2003; La Valley et al., 2009)

However, if the following solutions for monitoring the contamination of suya meat is put in place it will go a long way to reduce food borne diseases to the bearest minimum. There should be rural-to-city wide public enlightenment campaigns to food vendor and handlers to imbibe high level of hygienic practice. There should intensive surveillance and monitoring of roasted and vended foods to enhance microbial safety of Suya consumers. Roasted and vended foods should be adequately covered to prevent cross and post processing contaminations by microbial pathogens. Federal and State Food Regulatory Authorities/Agencies should rise up to their statutory responsibilities to ensure that the food which gets to the consumers' table are palatable, wholesome and of high quality.

Conclusions

The results indicate that the ready-to-eat Suya meats were contaminated with a variety of bacterial species thus susceptible to rapid deterioration, organoleptic changes

and economic loss. Apparently, this signals for surveillance and monitoring of the microbial safety of roasted and vended foods. The high level inhibition profiles showed by Ciprofloxacin, Levofloxacin, Rifampin and Erythromycin to selected test bacterial pathogens suggest broad spectrum activity of these antibiotics. Therefore, antimicrobial therapy and adequate patient management with these drugs following consumption of contaminated Suya is seriously advocated.

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