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Fishes and smoked meat delicacies as sources of multidrug resistant bacteria and parasitic worms

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Frozen fishes, stock fishes, smoked pork and smoked beef (suya) that have become popular delicacies in eateries, road side stalls and similar places were analysed for bacteriological and parasitological burden. Samples were collected from Nsukka and Obollo-Afor districts of Enugu State, Nigeria and were screened using basic microbiological procedures. One hundred and eighty four persons from the study areas were also screened for parasitic worms and consumption of test delicacies. Results revealed the presence of some opportunistic and overt pathogenic bacteria, some of which exhibited resistance to a multiple of antibacterial agents. In addition, *Taenia solium* and *Taenia saginata* were detected in some smoked pork and beef samples respectively. Among the human respondents who acceded to suya and pork consumption (78.26% of total), 64.2% were positive for *T. saginata* while 28.0% harboured *T. solium.* Also detected among human respondents were members of the giant roundworms *Ascaris lumbricoides.* Occurrence of these isolated organisms in the test meat products raises hygiene and safety questions and the need for public health awareness and consciousness in this regard.

Key words: Fishes, meat delicacies, multidrug-resistant bacteria, parasitic worms.

INTRODUCTION

Emergence of antibiotic resistant pathogen is one of the most serious threats to public health in the 21st century (Willey et al., 2007). Multidrug resistance is emerging worldwide at an alarming rate among a variety of bacterial species, causing both community-acquired and nosocomial infections (Nordman et al., 2012). This may have arisen from extensive use of antibiotics in agriculture such as for treatment of infections, growth enhancement and prophylaxis in food animals at low concentration (Burgos et al., 2005). The prevalence of these drug and multi-drug resistant bacteria in food animals and their products may serve as a potential transfer route of antibiotic resistant bacteria and resistant genes into human food-chain and environment. This has the potentials to pose a health threat to lives (Tansuphasiri et al., 2006).

Some food products may be wholesome prior to processing. For instance, chicken is wholesome while the chicken is alive. However, the sterility can be compromised by some contaminants from the environment and in the process of cutting, packaging as well as during distribution (Ingham, 2001). Animals are known to constitute a vast reservoir of enteric bacteria with the general problem of drug resistance and environmental contamination through organic wastes and vectors (Bahrndorff et al., 2013; Adeleke and Omafuvbe, 2011; Olaitan et al., 2011; Samuel et al., 2011). Most of the contaminants of food animals and fishes originate from the alimentary tracts, respiratory tracts and or external surfaces of either life animal or the handlers. In Suya (a spicy, barbecued, smoked or roasted meat product) for instance, the possible contaminants come from the carcass itself, the handlers and or even from the spices used in the preparation. According to Edema et al.

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(2008) all suya processors prepared the suya at their wooden stalls located by the roadsides. The surroundings are considered unhygienic given that garbage and dirty wastes litter the food processing environment with open gutters nearby, all of which attracted houseflies. None of the processors in their report was observed to wash their raw meat before suya preparation. Slabs and trays used for cutting and smoking were noted to be inadequately cleaned. Utensils made of plastic, metal or enamel were washed only once and the water used repeatedly until it becomes obviously oily, cloudy and dirty. The water used contained a significantly high coliform count in the order of 10^5 cfu/ml while the processing slab and raw meat both had counts of aerobic mesophiles above 5×10^5 cfu/g.

From the standpoint of microbiology, fishes and related products are risk foodstuff group. Particularly *Clostridium botulinum* type E and *Vibrio parahaemolyticus* rank among pathogenic bacteria associated with fishes. Freezing fish and related products in the sea-water, intensive handling, long-time transport or cooking in fishing containers straight on the deck contribute to their contamination with microorganisms (Novotny et al., 2004). Suya as a source of helminthic infection is worrisome considering that cattle and sheep are known (Adams and Moss, 1999) to be intermediate hosts of these worms from where man gets infected. The concern here is that suya is prepared (Edema et al., 2008) in such a way that intramuscular survival of these worms is not only possible but likely and man gets infected through injestion of food materials containing the encysted larval stage of these parasites.

Admittedly, the sources of contamination of suya and fishes as well as frozen meat are well known. However, little is known about the incidences of the bacteria and parasites in these food samples and the sensitivity pattern of these bacterial isolates. Edema et al. (2008) had earlier reported on the bacterial and fungal contaminant of suya in a different study area but no study has hitherto reported on this in the study area covered by this work and none has evaluated the heliminthic contamination and antimicrobial sensitivity profile of bacteria isolated there from. Importantly, there is an observable increase in the incidence of worm infection in Nsukka metropolis. The source of this infection is still obscure and the possibility of suya as a contributor is worth evaluating. This work was therefore carried out to determine the possibility of suya acting as a source of helminthic infections since these have become endemic in the study area. It is also aimed at assessing frozen and dried fishes for multidrug resistant bacteria considering the obvious public health implications.

MATERIALS AND METHODS

Sampling procedure

The samples of frozen fishes and stock fishes were randomly obtained from different grocery stores and markets in Obollo-Afor and Nsukka, Enugu State, Nigeria. Suya and smoked beef samples were randomly obtained from various suya spots within these areas. These samples were taken in sterile containers to the laboratory for analysis within six hours of collection. For each specimen (pork, frozen fishes, stock fishes and suya) a total of two samples per week were collected for 8 weeks (n = 16) and analyzed.

Parasitological analysis

One gram each of the samples (suya) and smoked pork was weighed into test tubes containing 4 ml of normal-saline for emulsification and dissolution. The suspension was made up to 7 ml by addition of 3 ml of normal-saline. The emulsified samples were sieved and 3 ml of diethyl ether was added to the resulting suspension. The suspension was shaken vigorously and centrifuged at 3,000 rpm for 5 min. The supernatant was decanted and a drop of the sediments was placed on a clean glass slide containing drops of iodine solution to stain sample. The identification of cysts in the sample was made according to the methods of worm isolation and identification (Ochei and Kolhatkar, 2007; Eggleston et al., 2008).

Examination of individuals for intestinal helminths

A total of 184 adult individuals (100 women and 84 men) from Nsukka and Obollo-Afor, both in Enugu state were examined for intestinal helminths using the methods of Adeoye et al. (2007) and Eggleston et al. (2008). Informed consent was obtained from participants who were also given questionnaires. Clean and sterilized plastic containers, appropriately labelled, were given to them for collection of fecal samples. Using an applicator, each stool sample was examined for its consistency, colour and presence of blood, mucous, adult worms and proglottides of tapeworms. Subsequently, further examinations were done on stool specimens using saline (0.85%) preparation, iodine stain, formol (10% formol saline) ether concentration for ova and cysts and the cellophane (Kato-katz) thick faecal smear techniques (Adeoye et al., 2007; Manson-Bahr and Bell, 1987). Eggs and cysts were recognized by their perculiar characteristics.

Determination of suya and smoked pork consumption pattern of participants

Questionnaires aimed at obtaining personal data and information on the period and frequency of suya consumption vis-a-vis noticeable signs and symptoms of worm infection were administered to the participants. Emphasis was placed on individuals who have consumed suya within 5-12 weeks (the usual incubation period of most cestodes) before examination while not neglecting others.

Microbiological analysis

All the samples (meat and fish) were prepared for bacteriological analysis following the methods of Stiles and Ng (1980) by weighing 10 g of each sample, blending and dispersing in 90 ml of sterile 0.1% peptone water. Appropriate dilutions were made, also with 0.1% peptone water blanks and subsequently 0.1ml of each was separately inoculated onto nutrient and MacConkey agar plates. Two sets of plates were prepared for each sample and incubated one set at 35°C and the other at 45°C for 24 h. Bacteria growths were isolated and identified by assessing colony characteristics and Gram reaction and by conduction coagulase and catalase tests,

Table 1. Genera and number of bacteria isolated from test samples.

S/N	Sample	Number of samples tested	Bacteria isolated	Number of samples harbouring bacteria species	Percentage occurrences
1	Frozen fishes	16	*Listerria* spp.	09	56.3
			Staph. aureus	12	75.0
			Aeromonas spp.	07	43.8
			Bacillus spp.	15	93.8
2	Stock fishes	16	*Bacillus* spp.	13	81.3
			Pseudomonas spp.	05	31.3
			Staph. aureus	11	68.8
			Proteus spp.	03	18.8
			Fecal *E. coli*	11	68.8
			Salmonella spp.	06	37.5
3	Suya	56	*Bacillus* spp.	37	66.1
			Fecal *E. coli*	18	32.1
			Pseudomonas spp.	22	39.3
			Staph. aureus	43	76.8
			Salmonella spp.	18	32.1
			Streptomyces spp.	12	21.4
			Enterobacter spp.	10	17.9
			Proteus spp.	06	10.7
4	Smoked pork	56	*Bacillus* spp.	26	46.4
			Fecal *E. coli*	06	10.7
			Pseudomonas spp.	13	23.2
			Staph. aureus	17	30.4
			Streptomyces spp.	09	16.1

sugar (including xylose) fermentation and other biochemical tests such as indole production, citrate utilization, urase activity, triple sugar iron (TSI) agar tests, gas and hydrogen sulphide production tests, and oxidase tests. All these were performed in accordance with the methods of Harley and Prescott (2002) and Brown (2007). Some specific tests such as low temperature (4°C) and high temperature (44.5°C) incubation, tumbling motility, colony counts, salt tolerance and Henry illumination (Adams and Moss, 1999) were performed for specific isolates showing some tell tale characteristics.

Antimicrobial sensitivity testing

This was done as earlier described (Eze et al., 2009). Briefly, overnight Mueller-Hinton (MH) broth cultures of each isolate were standardized to match 0.5 McFarland turbidity standards. Using sterile colon swabs (Evepon, Ind. Nig.) isolates were spread on dry MH agar surfaces and allowed to dry. Sensitivity discs were subsequently and carefully placed on the agar surfaces. Plates were incubated for 24 h at 37°C. After incubation and measurement of inhibition zone diameters, susceptibility ranges were scored following CLSI (NCCLS) (2006). Isolates of the same genus were numbered to reflect sources of isolation.

Statistical analysis

Analysis of variance was used to determine the level of differences among some parameters evaluated in this study.

RESULTS

As anticipated in view of the condition under which these fish and meat products are prepared and sold, the samples had high bacterial burden as shown on Table 1. The level of worm cysts contents was relatively low with a maximum of 39.3% of the tested samples containing *Taenia saginata* (Table 2). Twenty five percent of the male respondents harboured *T. saginata* while *T. solium* were detected in 20.2%. Of the female respondents screened, 31.0% carried *T. saginata* while 24.0% had *T. solium* (Table 2). Table 3 shows that 38.0% of the female and 26.2% of the male respondents that acceded to suya consumption had worms. The percentages were lower (9.0 and 19.0% respectively) for smoked pork consumers as shown on Table 4. Among the male respondents, 26.2% harboured *Ascaris lumbricoides* while the female respondents had them up to 38.0% (Table 2).

Antibiotic resistance tests showed that up to 57.1% of species of *Aeromonas* isolated from frozen fishes were resistant to trimethoprim/sulfamethoxazole and ampicillin while 53.3% of *Bacillus* spp. isolated from the same

Table 2. Genera and number of helminths detected in samples.

S/N	Sample	Number of samples tested	Helminth detection	Number of samples harbouring helminths	Percentage occurrence
1	Suya	56	*Taenia saginata*	22	39.3
2	Smoked Pork	56	*Taenia solium*	19	33.9
3	Human (stool)	184			
			Ascaris lumbricoides	22	26.2
3a	Men	84	*T. solium*	17	20.2
			T. saginata	21	25.0
			A. lumbricoides	38	38.0
3b	Women	100	*T. solium*	24	24.0
			T. saginata	31	31.0

Table 3. Suya consumption among respondents within study period.

Sex	Number tested	Number positive	Number(percentage) of positive respondents that harboured helminths
Men	84	68	22 (26.2)
Women	100	76	38 (38.0)

Table 4. Smoked pork consumption among respondents within study period.

Sex	Number tested	Number positive	Number(percentage) of positive respondents that harboured helminths
Men	84	37	16 (19.0)
Women	100	25	09 (9.0)

source were resistant to norfloxacin, erythromycin and flucloxacillin (Table 5). Strains of fecal *Escherichia coli* isolated from stock fishes were 45.5% resistant to pefloxacin, cefalexin and nalidixic acid while more than 60.0% of *Pseudomonas* spp. from the same source were resistant to ofloxacin, pefloxacin, augumentin, gentamicin, and trimethoprim/sulfamethoxazole (Table 6). Smoked pork contained in addition to other bacteria species, strains of *Staphylococcus aureus* 29.4% or more of which were resistant to 9 antibacterial agents (Table 7). The antibiograms of bacteria isolated from suya are shown on Table 8 with species of *Pseudomonas* expectedly showing the highest resistance rates of up to 77.3% against trimethoprim/sulfamethoxazole and streptomycin. In the same vein, faecal *E. coli* from the same source were at least 22.2% resistant to all the antibacterial agents with which they were challenged. Statistical analysis showed that there were no significant differences among the resistance patterns of *E. coli*, *Pseudomonas* spp, and *Salmonella* spp. isolated from stock fishes and suya. Also *S. aureus* isolated from smoked pork, stock fishes and suya showed relatively high percentage resistance values of between 46.5 and 18.2% that did not differ significantly between source groups. There was a significant difference between the occurrences of *S. aureus*, for example, in smoked pork on one hand (23.2%) and frozen fishes (75%), stock fishes (68.8%) and suya (76.8%) on the other hand.

DISCUSSION

Smoked beef (suya) and pork are increasingly becoming popular delicacies in our society especially in recreational facilities such as parks, restaurants and beer parlours. This trend cuts across social, ethnic and even religious divisions. The isolation of bacterial and parasitic agents from these delicacies and from frozen and dried fishes should therefore raise public health concern. Results of this investigation suggest that these ready-to-eat foods may be vectors in the transmission of overt or opportunistic pathogenic microorganisms as well as in the spread of multidrug resistant bacteria strains. For example, Table 6 shows that stock fishes harbour bacteria such as *S. aureus,* fecal *E. coli, Salmenella* spp. and *Pseudomonas* spp. which are known to have (opportunistic) pathogenic and public health importance (Cheesbrough, 2000). In addition, this work has shown

Table 5. Resistance pattern of bacteria isolated from frozen fishes.

Bacteria isolates	No. tested	Percentage (Number) resistant to:																
		OFX	PEF	CPX	AU	CN	S	CEP	ND	SXT	PN	NB	LC	E	APX	CH	RD	FLX
S. aureus	12	-	-	(5)41.7	-	(4)33.3	(4)33.3	-	-	-	-	(5)41.7	(4)33.3	(5)41.7	(4)33.3	(6)50.0	(5)41.7	(5)41.7
Aeromonas spp	7	(2)28.6	(1)14.3	(3)42.9	(1)14.3	(1)14.3	(1)14.3	(2)28.6	(3)42.9	(4)57.1	(4)57.1	-	-	-	-	-	-	-
Bacillus spp	15	-	-	(8)53.3	-	(7)46.7	(6)40.0	-	-	-	-	(8)53.3	(5)33.3	(8)53.3	(6)40.0	(7)46.7	(7)46.7	(8)53.3

OFX = Ofloxacin; PEF = Pefloxacin; CPX = Ciprofloxacin; AU = Augumentin; CN = Gentamicin; S = Straptomycin; CEP = Cefalexin; NA = Nalidixic acid; SXT = Trimethoprim/sulfamethoxazole; PN = Ampicillin; NB = Norfloxacin; LC = Lincomycin; E = Erythrmycin; APX = Ampicillin/cloxacillin; CH = Chloramphenicol; RD = Rifampicin; FLX = Flucloxacillin. - = Not tested (some discs were labelled as either Gram positive or negative and were used as such); Numbers in brackets are those positive for the respective tests vis-a-vis the total number tested.

Table 6. Resistance pattern of bacteria isolated from stock fishes.

Bacteria isolates	No. tested	Percentage (Number) resistant to:																
		OFX	PEF	CPX	AU	CN	S	CEP	NA	SXT	PN	NB	LC	E	APX	CH	RD	FLX
S. aureus	11	-	-	(5)45.5	-	(5)45.5	(5)45.5	-	-	-	-	(4)36.4	(2)18.2	(3)27.3	(2)18.2	(4)36.4	(3)27.3	(4)36.4
Bacillus spp.	13	-	-	(7)53.8	-	(6)46.2	(6)46.2	-	-	-	-	(3)23.1	(3)23.1	(5)38.5	(4)30.8	(6)46.2	(7)53.8	(7)53.8
Fecal *E. coli*	11	(4)36.4	(5)45.5	(2)18.2	(4)36.4	(2)18.2	(3)27.3	(5)45.5	(5)45.5	(6)54.5	(6)54.5	-	-	-	-	-	-	-
Salmonella spp.	6	(2)33.3	(3)50.0	(1)16.7	(3)50.0	(2)33.3	(3)50.0	(2)33.3	(2)33.3	(2)33.3	(3)50.0	-	-	-	-	-	-	-
Pseudomonas spp.	5	(4)80.0	(4)80.0	(2)40.0	(4)80.0	(4)80.0	(3)60.0	(3)60.0	(2)40.0	(3)60.0	(4)80.0	-	-	-	-	-	-	-
Proteus spp.	3	A	A	(1)33.3	(1)33.3	(1)33.3	(2)66.7	(1)33.3	(1)33.3	(1)33.3	(2)66.7	-	-	-	-	-	-	-

OFX = Ofloxacin; PEF = Pefloxacin; CPX = Ciprofloxacin; AU = Augumentin; CN = Gentamicin; S = Straptomycin; CEP = Cefalexin; NA = Nalidixic acid; SXT = Trimethoprim/sulfamethoxazole; PN = Ampicillin; NB = Norfloxacin; LC = Lincomycin; E = Erythrmycin; APX = Ampicillin/cloxacillin; CH = Chloramphenicol; RD = Rifampicin; FLX = Flucloxacillin. A = All susceptible. - = Not tested (some discs were labelled as either Gram positive or negative and were used as such); Numbers in brackets are those positive for the respective tests vis-a-vis the total number tested.

Table 7. Resistance pattern of bacteria isolated from smoked pork.

Bacteria isolates	No. tested	Percentage (Number) resistant to:																
		OFX	PEF	CPX	AU	CN	S	CEP	ND	SXT	PN	NB	LC	E	APX	CH	RD	FLX
S. aureus	17	-	-	(5)29.4	-	(6)35.3	(4)23.5	-	-	-	-	(6)35.3	(5)29.4	(6)35.3	(4)23.5	(6)35.3	(6)35.3	(5)29.4
Bacillus spp.	26	-	-	(3)11.5	-	(5)19.2	(5)19.2	-	-	-	-	(5)19.2	(4)15.4	(6)23.1	(4)15.4	(6)23.1	(5)19.2	(6)23.1
Fecal *E. coli*	06	(2)33.3	(1)16.7	A	(1)16.7	A	(1)16.7	(1)16.7	(1)16.7	(2)33.3	(2)33.3	-	-	-	-	-	-	-
Pseudomonas spp.	13	(7)53.8	(7)53.8	(5)38.5	(8)61.5	(6)46.2	(8)61.5	(7)53.8	(7)53.8	(8)61.5	(9)69.2	-	-	-	-	-	-	-

OFX = Ofloxacin; PEF = Pefloxacin; CPX = Ciprofloxacin; AU = Augumentin; CN = Gentamicin; S = Straptomycin; CEP = Cefalexin; NA = Nalidixic acid; SXT = Trimethoprim/sulfamethoxazole; PN = Ampicillin; NB = Norfloxacin; LC = Lincomycin; E = Erythrmycin; APX = Ampicillin/cloxacillin; CH = Chloramphenicol; RD = Rifampicin; FLX = Flucloxacillin. A = All susceptible. - = Not tested (some discs were labelled as either Gram positive or negative and were used as such); Numbers in brackets are those positive for the respective tests vis-a-vis the total number tested.

Table 8. Resistance pattern of bacteria isolated from suya.

Bacteria isolates	No. tested	Percentage (Number) resistant to:																
		OFX	PEF	CPX	AU	CN	S	CEP	NA	SXT	PN	NB	LC	E	APX	CH	RD	FLX
S. aureus	43	-	-	(18)41.9	-	(20)46.5	(18)41.9	-	-	-	-	(17)39.5	(15)34.9	(16)37.2	(20)46.5	(18)41.9	(20)46.5	(19)44.2
Bacillus spp.	37	-	-	(20)54.1	-	(21)56.8	(21)56.8	-	-	-	-	(18)48.6	(13)35.1	(17)45.9	(19)51.4	(15)40.5	(21)56.8	(21)56.8
Fecal *E. coli*	18	(6)33.3	(6)33.3	(4)22.2	(7)38.9	(4)22.2	(6)33.3	(6)33.3	(7)38.9	(7)38.9	(7)38.9	-	-	-	-	-	-	-
Enterobacter spp.	10	(4)40.0	(4)40.0	(3)30.0	(5)50.0	(3)30.0	(5)50.0	(4)40.0	(4)40.0	(5)50.0	(5)50.0	-	-	-	-	-	-	-
Salmonella spp.	18	(7)38.9	(8)44.4	(5)27.8	(9)50.0	(4)22.2	(7)38.9	(8)44.4	(8)44.4	(8)44.4	(9)50.0	-	-	-	-	-	-	-
Pseudomonas spp.	22	(11)50.0	(15)68.2	(9)40.9	(16)72.7	(10)45.5	(17)77.3	(15)68.2	(11)50.0	(17)77.3	(17)77.3	-	-	-	-	-	-	-
Proteus spp.	06	(1)16.7	(1)16.7	A	(2)33.3	A	(1)16.7	(1)16.7	(1)16.7	(1)16.7	(2)33.3	-	-	-	-	-	-	-

OFX = Ofloxacin; PEF = Pefloxacin; CPX = Ciprofloxacin; AU = Augumentin; CN = Gentamicin; S = Straptomycin; CEP = Cefalexin; NA = Nalidixic acid; SXT = Trimethoprim/sulfamethoxazole; PN = Ampicillin; NB = Norfloxacin; LC = Lincomycin; E = Erythrmycin; APX = Ampicillin/cloxacillin; CH = Chloramphenicol; RD = Rifampicin; FLX = Flucloxacillin. A = All susceptible. - = Not tested (some discs were labelled as either Gram positive or negative and were used as such); Numbers in brackets are those positive for the respective tests vis-a-vis the total number tested.

these organisms to be multidrug resistant with strains of *Salmonella* isolates for example, exhibiting more than 33% resistance to nine antibacterial agents. In a similar vein, strains of *E. coli* showed more than 36% resistance to 7 antibacterial agents while more than 60% of the *Pseudomonas* isolates were resistant to 8 antibiotics. While the medical implication of these is obvious, the public healthimport is more glaring in the light of the transferability of these traits among both pathogenic and potentially pathogenic bacteria (Eze et al., 2010). Bacteria isolated from smoked pork (Table 7) and those isolated from suya (Table 8) have shown similar attributes, thus raising the same serious concern. Bacteria isolated from frozen fishes are the least worrisome except for the drug resistant patterns and presence of *Aeromenas* spp. which have been implicated (Adams and Moss, 1999) in gastroenteritis and extraintestinal infections associated with immunocompromised hosts.

This study has also shown that the highly cherished smoked beef (suya) and pork often contain cysts of *T. saginata* and *T. solium* (Table 2). While taeniasis caused *T. saginata* can result in allergic reactions, chronic indigestion, constipation and inflammation of the appendix, *T. solium* is acknowledged as the most harmful tapeworm in humans (because of its ability to cause cysticercosis especially neurocysticercosis). Their presence in the study samples is therefore undesirable. The risks this may portend is corroborated by the more than 26% presence of helminths among suya consumers (Table 3) and 19% presence of the same worms among smoked pork male consumers (Table 4). Although a statistical correlation has not been established, these results suggest that suya and smoked pork may be “vectors” (at least in part) of the increasing cases of worm including *Ascaris lumbricodies* (Table 1) infestation in the study areas. Overall, this study has portrayed the analyte delicacies as potential vehicles for the transmission of parasitic worms and multidrug resistant bacteria. Further (correlation) research and public awareness programmes are recommended.

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