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MICROBIAL PROFILE, ANTIBIOTIC SENSITIVITY AND HEAT RESISTANCE OF BACTERIAL ISOLATES FROM COMMERCIAL ROASTED BEEF (SUYA) IN ABUJA, NIGERIA.

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ABSTRACT

Aim: This study was aimed at determining the prevalence, antibiotic resistance and heat resistance profile of bacterial isolates obtained from ready to eat roasted beef (suya) sold in Abuja, Nigeria.
Methods and Results: Fifty samples of suya were purchased from different vendors within the Federal Capital Territory and assessed for total viable bacterial counts. The sensitivity of the identified bacterial isolates to conventional antibiotics was evaluated using the disc diffusion method. Isolates resistant to at least three antibiotics were assessed for resistance to heat at 55 ^{o}C, 60 ^{o}C, 65 ^{o}C and 70 ^{o}C for 5 mins, 10 mins and 15 mins. The isolates identified were *Staphylococcus aureus* (54%), *Escherichia coli* (4%), *Salmonella species* (26%), *Bacillus species* (16%). The total viable bacterial counts ranged from 4.0x 10^{8} - 2.2 x 10^{9}cfu/g. *Bacillus spp*. was found to be most resistant to heat and thrived at 70 ^{o}C. The other organisms isolated showed varied sensitivity to heat.
Conclusion, Significance and Impact of study: Organisms capable of endangering human lives were isolated from the suya samples and in numbers that could likely cause health problems in healthy and immuno-compromised individuals. The presence of resistant pathogenic bacterial strains in ready to eat roasted beef could be a cause of serious concern which needs to be treated with urgency.

Key words: Suya, heat resistance, total viable bacterial count.
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INTRODUCTION

Roasted beef (*suya*) is a ready- to – eat snack prepared by smoking or roasting (barbecuing) already spiced (with finely grounded roasted peanut cake, red pepper, salt, grounded ginger, grounded garlic, chunked fresh tomatoes and minced fresh onions) raw boneless meat. The meat source could either be beef, bovine or mutton [1]. The smoke from the fire has a preservative effect on the suya. Its source is Northern Nigeria among the Hausa speaking natives, where rearing of livestock is a prominent means of livelihood and a main source of earnings among the natives. Other ready - to – eat beef products common to this community include Kilishi- dried spiced meat; Balangu and Kundi. Suya is the most prevalent ready- to – eat meat product and it has an extensive distribution in Nigeria [2].

The low standard of living and unhygienic sanitary practices of most of the people involved in its preparation, poses risk of enteric infections due to microbial contamination of the meat. Sporadic cases of gastroenteritis and symptoms of other food borne infections after consumption of suya have been reported [2, 3]. In developing countries, despite the apparent dearth of sustainable disease surveillance and reporting, it is widely known that cholera, salmonellosis, campylobacteriosis, shigellosis, typhoid, brucellosis, poliomyelitis, and *Escherichia coli* infections are prevalent [4]. A lot of factors affect the growth of these microorganisms on meat. These factors include temperature, pH, water availability, presence of nutrients, moisture, gaseous requirement, and atmosphere of storage [5]. These organisms may have public health importance due to resistance to only one key antimicrobial agent, but they also often demonstrate cross or co-resistance to multiple classes of antimicrobials, which makes them multidrug resistant (MDR).

There are documentations [2, 6] on the bacterial contamination of suya processed in Abeokuta (South western Nigeria) and Makurdi (North Central), however there are no reports on the quality assessment of suya processed in Abuja arguably, the most important city in Nigeria today. The town is largely populated and suya can be seen hawked on the roads, government offices and even at the airport. Travelers including foreigners are often seen buying this delicacy. Abuja is a densely populated town in the Federal Capital Territory, North Central Nigeria and is home to people with varied earnings and standard of living. The aim of this study was to assess the quality of roasted beef (*suya*) sold in Abuja, Nigeria

MATERIALS AND METHODS

Materials: Antibiotic discs were obtained from Oxoid, UK, they include: amoxicillin (10µg), nalidixic acid (30µg), tetracycline (30µg), vancomycin (30µg), gentamycin (10µg), streptomycin (10µg), chloramphenicol (30µg), erythromycin (15µg), cefuroxime (30µg), nitrofurantoin (300µg), fluconazole (10µg) and ciprofloxacin (5µg), tryptic soy agar (Oxoid Ltd., England), tryptic soy broth (Oxoid Ltd., England), Sabouraud dextrose agar (SDA, Oxoid, UK), nutrient agar.

Sampling procedure: Fifty *suya* samples were purchased from different suya selling points in Abuja, Nigeria -three replicate samples were collected from each location. The samples were transported to the laboratory in sterile bags packed in insulated containers with ice packs. Analysis was carried out within 6 hours of sampling. Where immediate microbiological evaluation was to be delayed, the samples were refrigerated at 4°C and analyzed within 24 hours [1]. All experimental determinations were made in triplicate.

Microbiological analyses: The total viable counts were carried out using tryptic soy agar (bacteria) by the spread plate technique according to standard procedures [7]. Simply, 25.0 g of sample was immersed in 225 mL of tryptic soy broth and allowed to stand for 3 h. One millilitre of the sample was aseptically transferred into 9.0 mL of TSB and subsequent serial dilutions up to10^{-5} were made. One hundred microliters of the dilutions were inoculated on the appropriate agar surface and incubated at 37°C for 24 – 48 h (bacteria). Bacterial counts were documented as cfu/g. Discrete bacterial colonies on tryptic soy agar were sub-cultured onto freshly prepared nutrient agar plate by streaking. Stock culture of the isolates were developed on slants and stored at 10 °C with transfers at intervals of 14 days. Isolates were identified by cultural and morphological characteristics as well as

biochemical tests such as the catalase, coagulase amongst others in accordance with standard methods [8].

Susceptibility Testing: The disc diffusion method was adopted for the antibiotic susceptibility testing. Eighteen to twenty-four hours culture of purified isolates from solid media was transferred into sterile normal saline until turbidity matched a 0.5 McFarland standard. The standardized culture was inoculated on the surface of Muller Hinton agar and the plates allowed standing for 30 min; with the aid of sterile forceps, the antibiotic discs were placed equidistantly from each other. The plates were incubated at 37 ^{o}C for 24 h in inverted position. Isolates resistant to at least three different classes of antibiotics were termed multi-drug resistant -MDR [9].

Thermal resistance testing: Heating menstrum (9 mL tryptic soy broth) contained in glass tubes were warmed in different water baths at temperatures of 55°C, 60°C, 65°C and 70 °C. Thereafter, 1 mL of standardized suspension of isolates (multi drug resistant) was transferred into the glass tubes containing the heating menstruum with an initial bacterial count ranging between $1.0x10^{7}$ -$1.0x10^{8}$ CFU/mL. The menstrum in the tubes was heated for 5, 10 and 15 minutes. Enumeration of organisms surviving the heating was carried out on the appropriate media, after incubation at 37 °C for 48 h. A plot of number of surviving organisms against heating time was prepared to yield a curve of rate of inactivation at four different temperatures. Based on the curve, the D values, i.e. time (minutes) at certain temperatures to reduce the number of organism by 1 log cycle was calculated from the equation D = -1/slope. A Thermal Death Time (TDT) curve was made to establish the relationship between D (minutes) with temperatures (^{o}C). The Z values, i.e. temperature intervals to reduce D value by 1 log cycle was also determined from the curve.

RESULTS

Total viable counts

The results of the total viable bacterial and fungal counts as well as the prevalent pathogenic microorganisms are represented in Table 1. From the study, it was found that the total viable bacteria count ranged from 4.0 x 10^{5} to 2.7 x 10^{6}cfu/g and the fungal count ranged from 1.0 x 10^{1} to 1.0 x 10^{3}cfu/g. The prevalent pathogenic microorganisms present in the suya samples were *Staphylococcus aureus* (27, 54 %), *Salmonella typhi* (13, 26 %), *Bacillus spp* (8, 16 %) and *Escherichia coli* (2, 4 %) as represented in Table 2.

Table 1. Mean microbial counts (counts were expressed as log 10cfu/g) and pathogenic microorganism present in samples of suya obtained from different locations in Abuja.

Location	Sample size	TVAB (cfu/g)	Microorganisms
Gwagwalada	5	$8.0x10^{8}$ ±0.33 - $1.2x10^{9}$ ±0.0	*Staphylococcus aureus, Escherichia coli*
Asokoro	8	$5.0x10^{8}$ ±0.33 - $1.5x10^{9}$ ±0.58	*Bacillus sp., Staphylococcus aureus, S. typhi*
Wuse	8	$6.0x10^{8}$ ±0.33 - $2.2x10^{9}$ ±0.0	*Staphylococcus aureus, Salmonella typhi*
Garki	8	$6.0x10^{8}$ ±0.58 - $2.2x10^{9}$ ±0.33	*Bacillus sp., Staphylococcus aureus*
Jabi	8	$4.1x10^{8}$ ±0.0 - $1.6x10^{9}$ ±0.058	*Staphylococcus aureus, Salmonella typhi*
Karmo	8	$9.0x10^{8}$ ±0.0 - $1.5x10^{9}$ ±0.33	*Bacillus sp., Staphylococcus aureus, Salmonella typhi*
Lugbe	5	$1.0x10^{9}$ ±0.0 - $2.7x10^{9}$ ±0.33	*Staphylococcus aureus*

Table 2. Distribution and % frequency of occurrence of different microorganisms isolated from suya sample.

Location	*S. aureus*	*Bacillus* sp.	*S. typhi*	*E. coli*
Gwagwalada	2(4)	-	-	2(4)
Asokoro	5(10)	2(4)	1(2)	-
Wuse	5(10)	-	1(2)	-
Garki	3(6)	3(6)	-	-
Jabi	7(14)	-	5(10)	-
Karmo	3(6)	2(4)	5(10)	-
Lugbe	2(4)	1(2)	1(2)	-
Total (%)	**27 (54)**	**8 (16)**	**13 (26)**	**2 (4)**

Cultural characteristics and biochemical Identification of isolated strains

A total of 50 bacterial strains were isolated. Non-red colonies from MacConkey plates that grew with golden yellow on mannitol salt agar and were Gram positive, coagulase-positive and catalase-positive were taken as *Staphylococcus aureus*; non-red colonies from MacConkey plates that were Gram negative, indole-negative, methyl red–positive, Voges Proskauer-negative, citrate-positive, acidic butt and alkaline slant with blackening on TSI slant, urease negative and colourless colonies with black center on Salmonella-Shigella agar were taken as *Salmonella* spp.; Red colonies from MacConkey plates that grew with greenish metallic sheen on EMB agar and were Gram negative, indole-positive, methyl red-positive, Voges-Proskauer-negative and citrate-negative were taken as *E. coli*; Spreading colonies with flat orientation on nutrient agar, Gram positive bacilli which hydrolyses starch, and grows in the presence of 6.5% NaCl, catalase, citrate and Voges-Proskauer – positive were taken as *Bacillus spp*.[8]

Antibiotic susceptibility

Results from antibiotic susceptibility test (Table 3) showed that the organisms (Gram positive and negative) were most sensitive to gentamicin (100 %) and streptomycin (100 %), followed by chloramphenicol (60 %), nitrofurantoin (54 %), tetracycline (42 %) and cefuroxime (6 %). The Gram-positive isolates were most sensitive to amoxicillin (46%), followed by vancomycin (42 %) and erythromycin (38 %) while the Gram-negative isolates were most sensitive to ciprofloxacin (100 %), gentamycin (100 %) and streptomycin (100 %). Isolates of *S. aureus* were most resistant to amoxycillin (22.2 %) and vancomycin (29.6 %), *Salmonella typhi* to tetracycline (84.6 %), *Bacillus spp* to vancomycin (62.5 %) and *E. coli* to nalidixic acid (50 %) as represented in Table 4.

Table 3. Antibiotic susceptibility study of the isolates from suya samples

Antibiotics	Sensitive	Resistant	Intermediate
Gentamicin	50 (100%)	0	0
Vancomycin	21 (42%)	14 (28%)	0
Streptomycin	50 (100%)	0	0
Amoxicillin	23 (46%)	9 (18%)	3 (6%)
Chloramphenicol	30 (60%)	7 (14%)	13 (26%)
Tetracycline	21 (42%)	28 (56%)	1 (2%)
Erythromycin	19 (38%)	16 (32%)	0
Nitrofurantoin	27(54%)	19 (38%)	1 (2%)
Cefuroxime	3(6%)	41 (82%)	4 (8%)
Ciprofloxacin	22(44%)	0	0
Nalidixic acid	0	10 (20%)	3 (8%)

Table 4. Percentage *In-vitro* resistance pattern of each bacterial isolates from suya

S/N	Isolates	Frequency	Antibiotics									
			Ge	Vc	St	Am	Ch	Te	Er	Ni	Cf	Cp	Na
1	*S. aureus*	27	0	29.6	0	22.2	7.4	18.5	25.9	0	11.1	NA	NA
3	*Bacillus sp*	8	0	62.5	0	25	50	50	62.5	0	50	0	NA
2	*Salmonella typhi*	13	0	NA	0	NA	15.3	84.6	NA	38.4	0	0	23
4	*E. coli*	2	0	NA	0	NA	50	0	NA	50	50	0	50

Key: NA- Not applicable Amoxicillin (Am) Ciprofloxacin (Cp) Cefuroxime (Cf) Cholramphenicol (Ch) Erythromycin (Er) Gentamicin (Ge) Nalidixic acid (Na) Nitrofurantoin (Ni) Streptomycin (St) Tetracycline (Te), Vancomycin (Vc).

Heat resistance and thermal death time

Results on the heat resistance nature of the isolates is shown in Table 5. A total of six isolates were killed after exposure for 5 minutes at 60 ^{o}C; while 16 and 20 isolates were killed after exposure at temperature of 60 ^{o}C for 10 and 15 minutes respectively. At a temperature of 65 ^{o}C only 9 isolates were resistant after 5 minutes of exposure, comprising mainly *Bacillus spp.* After 15 minutes of exposure at temperature of 70 ^{o}C, 8 *Bacillus species* were shown to be resistant. The thermal death time of the isolates are represented in Table 6. Isolates M41- M47 survived beyond 70 ^{o}C.

Table 5. Heat resistance (D-values in seconds) of multi-drug resistant bacterial isolates from *Suya* after 5 mins exposure time

Multi- resistant isolates	Temperatures (^{o}C)			
	55	60	65	70
M_8	100.00^{a}	-	-	-
M_{10}	107.14^{a}	103.40^{b}	-	-
M_{11}	111.01^{a}	90.90^{b}	75.00^{c}	-
M_{16}	124.48^{a}	-	-	-
M_{17}	109.89^{a}	99.00^{b}	94.63^{c}	-
M_{18}	150.00^{a}	-	-	-

M_{20}	134.50 [a]	125.00 [b]	-	-
M_{21}	92.87 [a]	116.27 [b]	-	-
M_{23}	127.11 [a]	62.50 [b]	-	-
M_{24}	128.70 [a]	128.00 [b]	-	-
M_{26}	182.92 [a]	87.97 [b]	-	-
M_{27}	105.26 [a]	101.69 [a]	-	-
M_{29}	106.00 [a]	-	-	-
M_{30}	109.89 [a]	101.69 [b]	-	-
M_{32}	126.05 [a]	117.64 [b]	-	-
M_{33}	123.45 [a]	115.38 [b]	-	-
M_{34}	127.65 [a]	71.59 [b]	-	-
M_{35}	114.06 [a]	110.70 [a]	-	-
M_{36}	100.00 [a]	90.90 [b]	-	-
M_{37}	109.89 [a]	100.00 [b]	-	-
M_{38}	106.00 [a]	-	-	-
M_{39}	106.00 [a]	65.07 [b]	-	-
M_{40}	115.38 [a]	103.40 [b]	-	-
M_{41}	130.43 [a]	122.00 [b]	117.18 [c]	107.91 [c]
M_{42}	139.53 [a]	131.00 [b]	124.48 [c]	114.06 [c]
M_{43}	126.58 [a]	119.52 [b]	114.06 [c]	105.26 [c]
M_{44}	127.65 [a]	120.48 [b]	114.94 [c]	106.00 [c]
M_{45}	133.30 [a]	125.52 [b]	119.52 [c]	109.89 [c]
M_{46}	126.58 [a]	119.52 [b]	114.94 [c]	108.69 [c]
M_{47}	138.88 [a]	130.43 [b]	123.96 [c]	113.63 [c]
M_{49}	105.26	-	-	-
M_{50}	103.44 [a]	103.44 [a]	-	-

Values with different superscripts on the same row are significantly different (P≤0.05)

Table 6. Thermal death time of of multi-drug resistant bacterial isolates from suya

S/N	Isolates	Thermal death time	
		Temperature (^oC)	Time(minutes)
1	M_8	60	5
2	M_{10}	60	10
3	M_{11}	65	10
4	M_{16}	60	5
5	M_{17}	65	10
6	M_{18}	60	5
7	M_{20}	60	10
8	M_{21}	55	15
9	M_{23}	60	15
10	M_{24}	60	10
11	M_{26}	60	10
12	M_{27}	60	10
13	M_{29}	60	5
14	M_{30}	65	5
15	M_{32}	60	15
16	M_{33}	60	15
17	M_{34}	60	10
18	M_{35}	60	10
19	M_{36}	60	15

20	M_{37}	65	5
21	M_{38}	60	5
22	M_{39}	60	10
23	M_{40}	60	10
24	M_{41}	-	-
25	M_{42}	-	-
26	M_{43}	-	-
27	M_{44}	-	-
28	M_{45}	-	-
29	M_{46}	-	-
30	M_{47}	-	-
31	M_{49}	60	5
32	M_{50}	60	15

Key: (-) greater than 70^{o}C

DISCUSSION

The results of the survey of *suya* samples from different vendors in Abuja revealed an aerobic microbial count range of $4x10^5$ to $2.7x10^6$ cfu/g of suya. These values placed the suya samples examined at an acceptable limit according to the Public Health Laboratory Services guidelines for bacteriological quality of ready-to-eat food sampled at point of sale [10] and International Commission of Microbiological Standards for Food [11] proposed an acceptable limit for total viable count of not > 10^7cfu/g. However, the number of coliforms and *Bacillus spp* present in the *suya* samples render them unsatisfactory and unfit for human consumption. The presence of *E. coli* and *Salmonella typhi* in some of the suya samples is unacceptable and of public health concern. In a similar study, Inyang et al [2] reported a total microbial count of 10^5-10^6cfu/g and the presence of these two indicator organisms of faecal contamination in suya samples collected from Edo State, Southern part of Nigeria.

S. aureus (54 %) was most prevalent in the samples screened, this result is in agreement with the study conducted in Jordan [12, 13], followed by *Salmonella* (26%), *Bacillus spp* (16%) and *E. coli* (4%), respectively. The presence of *S. aureus*, *E. coli* and *Bacillus spp* in the sample is indicative of poor handling and sanitary conditions. *Salmonella* is an indicator organism of faecal contamination and should be absent in 25 g of sample. Its presence in suya sample renders the food unfit for human consumption [11].

From the four organisms that were exposed to heat treatment, *Bacillus spp* was shown to be most resistant. This was indicated by the continued growth of some *Bacillus spp*. after exposure for 15 minutes at temperature of 70 ^{o}C. This finding agrees with several other studies [14, 15, 16, 17, 18]. Resistance of most *Bacillus species* to heat treatment have been linked to their ability to produce spores which can survive at harsh conditions. The study by Janstova and Lukasova [14], showed that a temperature of 135 °C is adequate for the inactivation of spores even in high initial concentrations (up to 10^7 /mL) of all strains studied except for *B. licheniformis* spores, which were able to germinate even after heating at 135 °C. However some strains of *Bacillus* can survive beyond their thermostable limit.

The D-value (seconds) of *Bacillus* at D_{55}, D_{60}, D_{65} and D_{70} after 5 minutes ranged between 126.58 to 139.53, 119.52 to 131.00, 114.06 to 124.48 and 105.26 to 114.06 respectively. The D-value (seconds) of *E. coli* at D_{55} and D_{60} after 5 minutes ranged between 103.44 to 105.25 and 0 to 103.44 respectively. The D-value of any microorganism in food is an indication of the heating time required to kill 90 % of the microorganism population at the temperature of exposure. The level of heat resistance of bacteria is influenced by factors such as the inherent resistance of the organism and environmental factors such as pH, growth medium, growth stage, previous exposure to stress. The rise in temperature from 55^{o}C to 70^{o}C (in the present study) resulted in the

decrease of D-value of the isolates, this observation is similar to that reported by Janstova and Lukasova [14]. This is expected as an increase in temperature has been confirmed to reduce the D-values [19]. The D-values of *S. typhi* and *S. aureus* reported in this study was lower than that reported by Samadpour and Stopforth [19] and Ratith *et al*. [20] respectively. This could be linked to strain variation and other environmental factors.

CONCLUSION

Roasted beef (*suya*) constitutes a rich source of protein which is needed for body building and repair of worn out tissue in human. Improvement in the microbial quality of suya is very important and adequate steps must be taken to prevent contamination and spoilage by microorganisms. Organisms capable of endangering human lives were isolated from the suya samples and in numbers that could likely cause health problems in healthy individuals. It is obvious from these studies that; suya may become a potent carrier of pathogenic organism across the country. The presence of these organisms in suya could result in gastroenteritis and other more serious or fatal food infection/poisoning on consumption. The practice of preparation, handling, displaying and distribution of suya in open places where there is no emphasis on the hygiene standard should be discouraged.

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