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Bacteriological Status and Antibiotics Susceptibility Pattern of Isolates from 'Suya' (Roasted Beef) Sold in Calabar, Nigeria

Emmanuel Effiong Bassey*[1], Maurice Mbah[1], Nneka Ndifon Agbiji[2]
Department of Medical Parasitology and Entomology, Faculty of Medical Laboratory Science, University of Calabar, Calabar, Nigeria [1], Department of Microbiology, Faculty of Biological Sciences, Cross River University of Technology, Calabar [2].
Author for Correspondence*: emmanueleffiong2046@gmail.com/+234-703-647-2628/
ORCID ID: https://orcid.org/0000-0003-2029-5611/https://dx.doi.org/10.4314/sokjmls.v9i3.16

Abstract

Ready-to-eat Suya meats were randomly sampled and assessed for bacterial status and antibacterial susceptibility profiles of the isolated bacterial pathogens. Bacteriology of Suya samples and antibacterial susceptibility test were determined using standard microbiological and disc diffusion methods respectively. The mean aerobic bacterial count (ABC) from point A, B, C and D ranged from 5.9×10^7 to 2.04×10^8 CFU/g, 6.4×10^6 to 2.44×10^8 CFU/g, 5.8×10^6 to 2.60×10^8 CFU/g and 2.9×10^6 to 1.76×10^7 CFU/g respectively. A total of six (6) bacterial genera were identified including twenty-six (26) Gram-positive and sixteen (16) Gram-negative bacteria. The most frequently isolated bacteria were *Bacillus* sp (35.7%), *Staphylococcus 'sp'*(26.2%), *Klebsiella* sp (19%), *Escherichia coli (11.9%), Enterobacter 'sp' (4.8%)* and *Proteus 'sp'* (2.4%) respectively. Antibacterial susceptibility test results indicated that Ciprofloxacin, Gentamycin and Sparfloxacin demonstrated high level sensitivity and broad-spectrum activity on test isolates whereas Amoxicillin, Augmentin and Septrin showed little or no effectiveness on the test organisms. 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.

Keywords: Antibiotic, Bacteriological, Calabar, Susceptibility, Suya.

Introduction

Suya is a traditional sliced meat barbecue spiced with seasoning and vegetables (e.g. grounded peanut cake, red pepper, grounded ginger, grounded garlic, salt, chunked cabbage, tomatoes and onions). Its origin can be traced to the Hausa speaking people in Northern Nigeria, where livestock rearing is a major occupation and a means of livelihood. In Nigeria and other African countries, Suya vendors are found in both villages and cities, making it one of the most patronized street- vended food (Amadi *et al*., 2016; Bello and Bello, 2020).

Safety of street- vended food is a matter of great concern, especially in developing nations. Unsafe water used in preparation, poor personal hygiene of vendors, inadequate storage infrastructure, and poor handling practices pose a high risk of foodborne illnesses to consumers. Pathogenic organisms such as bacteria, viruses, fungi or parasites can be transmitted via contaminated food (Amaeze *et al*., 2016; Tarh *et al*., 2023). Although the global impact of foodborne diseases is unknown, in 2015, more than 600 million cases of foodborne diseases was reported worldwide, of which 419,000 resulted in death, with African countries recording the highest burden (WHO, 2015; Oduori *et al*., 2022). Although Nigeria has no official foodborne disease surveillance system, more than 200,000 deaths recorded annually are linked to foodborne pathogens (Onyeneho and Hedberg, 2013).

Although ready-to-eat foods are not completely devoid of microorganisms. However, the numbers and types of organisms present in food products is of public health importance. In Nigeria, different spectra of bacteria have been isolated from ready-to-eat Suya. These include

Staphylococcus sp, *Micrococcus* sp, *Escherichia coli*, *Klebsiella* sp, *Salmonella* sp, *Bacillus* sp, *Pseudomonas* sp, *Clostridium* sp and *Proteus* sp. These genera are known pathogens, some of which have been implicated in foodborne outbreak (Amaeze *et al*., 2016; Agade *et al*., 2019; Amadi *et al*., 2020; Okunromade *et al*., 2020). Most importantly, isolates from ready-to-eat Suya demonstrated resistance to most recommended antibiotics. This is worrisome in the management of foodborne related diseases (Bello and Bello, 2020). Sources of microbial contamination of ready-to-eat food can be attributed to contaminated water use in washing, cross contamination via the handlers, packaging materials, unhygienic environment and exposure to insect vectors (Amadi *et al*., 2020).

Calabar, being the tourist center in Nigeria attract visitors within and outside of the city, especially during festive season. Most vendors involved in preparation and sales of Suya are often seen in an unhygienic condition. Hence, this study was conducted to determine the bacterial load and antibiotics susceptibility profile of isolates associated with ready-to-eat Suya sold in Calabar.

Materials and methods

Collection of samples

Different samples of *Suya* were purchased randomly from different locations in Calabar, Nigeria. The collected samples were immediately wrapped in aluminum foil. The samples were transported to the laboratory for bacteriological analysis. Analysis was carried out within 2 hours of collection. For the purpose of this study, the samples collection points were designated as A (Bogobiri), B (Ekpo-Abasi), C (Mbukpa), D (Afokang).

Bacteriological evaluation

The total viable counts were carried out using nutrient agar by the spread plate technique according to standard procedures (Chessbrough, 2006). Briefly, 10.0 g of each sample were mashed using laboratory mortar and pestle. About 1.0 g of the crushed was serially diluted in sterile distilled water up to 10^{-6} dilution. An aliquot from dilution 10^{-5} was plated on dried sterile nutrient agar and incubated for 18-24 hours. Samples with colonies numbers between 30-300 were enumerated for colony forming units.

Isolation of bacterial isolates

Discrete colonies from the spread plates were picked and sub-cultured onto freshly prepared agar used in primary culture by streaking technique to obtain pure cultures of the isolates. The representative colonies were carefully examined microscopically to ascertain the cell morphology. The purified isolates were cultured in nutrient agar slant and kept as stock culture in bijou bottles for further analysis.

Biochemical characterization of isolates

Identification of bacterial isolates was carried out based on their cultural, morphological and biochemical characteristics (Chessbrough, 2006; Bassey *et al*., 2022).

Antibiotics Susceptibility Profile of Isolates

Preparation of 0.5 McFarland

McFarland standard was prepared by mixing 1% solution (0.5 ml) of Barium chloride ($BaCl_2$) and 1% solution of (99.5 ml) of Sulphuric acid (H_2SO_4). The mixture was placed in a screw-capped bottle and stored at room temperature.

Standardization of the inoculums

The test isolates were first grown in nutrient broth for 18-24 hours. An appropriate quantity for each test isolate was mixed into 4-5 ml of physiological saline. The suspension was diluted until it became slightly turbid to match the already prepared 0.5 McFarland standard.

Antibiotic susceptibility testing (AST)

Antibiotic susceptibility patterns of the isolated bacteria were determined by Kirby-Bauer method (Bassey *et al*., 2022), using 10 commercial antibiotics, according to the Clinical Laboratory Standards Institute guideline (CLSI, 2017): Septrin (SXT, 30μg), Chloramphenicol (CH, 30μg), Sparfloxacin (SP, 10μg), Ciprofloxacin (CPX, 30μg), Amoxicillin (AM, 30μg), Augmentin (AU, 10μg), Gentamycin (CN, 30μg), Pefloxacin (PEP, 30μg), Tarivid (OFX, 10μg) and Streptomycin (S, 30μg).

The 24 hours' broth culture of each test organism was suspended in saline solution (0.85% NaCl) and adjusted to match a turbidity of 0.5 McFarland Standard. The standardized inoculums were seeded on to the surfaces of already prepared Mueller Hinton agar plates using sterile cotton swabs. The seeded plates were left to stand for about 10 minutes then the antibiotic disks were aseptically placed on the surfaces of the seeded plates with the aid of a sterile forceps. They were then inverted and incubated at 37°C for 24 hours. After incubation, any clear circular zones of growth inhibition around the immediate vicinity of any disk indicated susceptibility to that antibiotic agent. These inhibition zone diameters were measured, and the results interpreted based on the CLSI recommendation (CLSI, 2017). All of the tests were performed in duplicates and the resulting values of the IZDs recorded.

Results and Discussion

Mean total bacterial counts from suya samples

The mean aerobic bacterial count (ABC) was evaluated following standard guideline (Table 1). Each value (1-6) in each sampling point represents mean of data obtained from four different Suya spots in same area. The mean ABC from point A, B, C and D ranged from 5.9 x 10^{7} to 2.04 x 10^{8} CFU/g, 6.4 x 10^{6} to 2.44 x 10^{8} CFU/g, 5.8 x 10^{6} to 2.60 x 10^{8} CFU/g and 2.9 x 10^{6} to 1.76 x 10^{7} CFU/g (Table 2).

Identification of bacterial isolates

Forty-two (42) bacterial isolates were obtained. Twelve (12), 11, 10 and 10 isolates were encountered from point A, B, C and D, respectively. The isolates were characterized as *E. coli*, *Enterobacter sp*, *Staphylococcus sp., Bacillus sp*, *Klebsiella sp. and Proteus sp* (Table 3).

Percentage frequency of bacterial isolates

Data showed that percentage contamination of the Suya samples from point A, B, C and D were 26.2%, 21.4%, 23.8% and 28.6%, respectively (Table 4). The most occurring bacterium from the Suya samples in zone A was *Bacillus 'sp'* and *Klebsiella 'sp'* with percentage occurrence of 36.4% each while the lowest was *Staphylococcus 'sp'* with 9.1% (Table 4). Similarly, the most encountered bacterium in point B, C and D was *Staphylococcus* sp, *Bacillus* sp and *Bacillus 'sp'* respectively. In general, the highest occurred bacterial species was *Bacillus 'sp'* (35.7%, 15/42), while the lowest was *Proteus 'sp'* (2.4%, 1/42) (Table 4).

Table 1. Microbiological guidelines for ready-to-eat meat products

Categories	ACC (Aerobic colony count)/g at 30°C	Description
1	< 10^{6}	Satisfactory
2	10^{6} - < 10^{7}	Borderline
3	$\geq$ ^{7}	Unsatisfactory

Center for Food Safety (2014).

Table 2. Mean total bacterial counts from suya samples in Nigeria

Sampling site	Sample	Colony number	Mean bacterial colony count (CFU/g)
	A1	TNTC	
	A2	59	5.9×10^7
Bogobiri	A3	204	2.04×10^8
	A4	160	1.60×10^8
	A5	TNTC	
	A6	78	7.8×10^7
	B1	244	2.44×10^8
	B2	74	7.4×10^7
Ekpo-Abasi	B3	104	1.04×10^8
	B4	TNTC	
	B5	138	1.38×10^7
	B6	64	6.4×10^6
	C1	60	6.0×10^7
	C2	126	1.26×10^8
Mbukpa	C3	260	2.60×10^8
	C4	77	7.7×10^6
	C5	94	9.4×10^6
	C6	58	5.8×10^6
	D1	54	5.4×10^6
	D2	82	8.2×10^6
Afokang	D3	29	2.9×10^6
	D4	40	4.0×10^6
	D5	176	1.76×10^7
	D6	TNTC	

Key: A = Bogobiri; B = Ekpo-Abasi; C = Mbukpa; D = Afokang; TNTC = Too numerous to count.

The antibiotic susceptibility patterns of bacterial isolates

The results of antibiotics susceptibility of isolates from Ready-to-eat Suya is presented in Table 5. The isolates showed varying degrees of sensitivity to the antibiotics and are classified based on their zones of inhibitions. Ciprofloxacin showed highest sensitivity on virtually all the test isolates (80 to 100%). Sparfloxacin was effective on all the test bacteria except *Klebsiella* sp were 62.5% resistance was recorded (Table 5). However, the majority of the isolates were resistance (100%) to amoxicillin, augmentin and septrin (Table 5).

Table 3. Morphological and biochemical characteristics of bacteria isolated from suya

Gram	Shape	CAT	OX	IN	MOT	MR	VP	CIT	TSI				Isolates per location	Presumptive identity
									Slant	Butt	H_2S	Gas		
-ve	Rods	+	-	-	+	-	+	+	Y	Y	-	+	A = 4, B = 0, C = 3, D = 1	*Klebsiella* sp
+ve	Cocci	+	-	-	-	+/-	+	+	P	Y	-	+	A = 1, B = 5, C = 2, D = 3	*Staphylococcus* sp
-ve	Rods	+	-	-/+	+	+	-	+	P	Y	+	+	A = 0, B = 1, C = 0, D = 0	*Proteus* sp
-ve	Rods	+	-	-	+	-	+	+	Y	Y	-/+	+	A = 0, B = 1, C = 0, D = 1	*Enterobacter* sp
+ve	Rods	+	+	-	+	+	+	+	P	Y	-	+	A = 4, B = 2, C = 5, D = 4	*Bacillus* sp
-ve	Rods	+	-	+	+	+	-	-	P	Y	-	+	A = 2, B = 0, C = 0, D = 3	*Escherichia coli*

KEY: P = Alkaline; Y = Acid; H_2S = Hydrogen sulphide; + = Positive; - = Negative; A = Bogobiri; B = Ekpo-Abasi; C = Mbukpa; D = Afokang; CAT = Catalase; OX = Oxidase; MOT = Motility; MR = Methyl red; VP = Voges-Proskaure; CIT = Citrate; TSI = Triple sugar iron agar.

Table 4: Bacteria isolates from roasted 'Suya' from different location in Calabar

	Sources				
Bacteria	**Bogobiri**	**Ekpo-Abasi**	**Mbukpa**	**Afokang**	**Total (%)**
Klebsiella sp	4	0	3	1	8 (19.0)
Staphylococcus sp	1	5	2	3	11 (26.2)
Proteus sp	0	1	0	0	1 (2.4)
Enterobacter sp	0	1	0	1	2 (4.8)
Bacillus sp	4	2	5	4	15 (35.7)
E. coli	2	0	0	3	5 (11.9)
Total	**11 (26.2)**	**9 (21.4)**	**10 (23.8)**	**12 (28.6)**	**42 (100)**

Table 5: Antibiotics susceptibility of isolates from ready-to-eat Suya sold in Calabar

Antibiotic tested	Disc (µg)	*Klebsiella* sp (8)		*Staph.* sp (11)		*Proteus* sp (1)		*Enterobacter* sp (2)		*Bacillus* sp (15)		*E. coli* (5)	
		S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Augmentin (AU)	10	1 (12.5)	5 (62.5)	4 (36.4)	7 (63.6)	0 (0.0)	1 (100)	0 (0.0)	2 (100)	5 (33.3)	10 (66.7)	0 (0.0)	5 (100)
Chloramphenicol (CH)	30	6 (75)	2 (25)	10 (90.9)	1 (9.1)	1 (100)	0 (0.0)	1 (50)	1 (50)	12 (80)	3 (20)	3 (60)	2 (40)
Sparfloxacin (SP)	10	3 (37.5)	5 (62.5)	11 (100)	0 (0.0)	1 (100)	0 (0.0)	2 (100)	0 (0.0)	9 (60)	6 (40)	4 (80)	1 (20)
Tarivid (OFX)	10	0 (0.0)	7 (87.5)	11 (100)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	7 (46.7)	8 (53.3)	2 (40)	3 (60)
Pefloxacin (PEF)	30	1 (12.5)	7 (87.5)	8 (72.7)	3 (27.3)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	10 (66.7)	5 (33.3)	5 (100)	0 (0.0)
Ciprofloxacin (CPX)	30	8 (100)	0 (0.0)	10 (90.9)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	1 (50)	15 (100)	0 (0.0)	4 (80)	0 (0.0)
Septrin (SXT)	30	0 (0.0)	8 (100)	7 (63.6)	4 (36.4)	0 (0.0)	1 (100)	0 (0.0)	2 (100)	8 (53.3)	7 (46.7)	1 (20)	4 (80)
Gentamicin (CN)	30	7 (87.5)	0 (0.0)	11 (100)	0 (0.0)	1 (100)	0 (0.0)	1 (50)	0 (0.0)	13 (86.7)	2 (13.3)	3 (60)	2 (40)
Streptomycin (S)	30	2 (25)	6 (75)	8 (72.7)	3 (27.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100)	1 (6.7)	14 (93.3)	0 (0.0)	5 (100)
Amoxicillin (AM)	30	0 (0.0)	8 (100)	0 (0.0)	11 (100)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100)	5 (33.3)	10 (66.7)	0 (0.0)	5 (100)

Key: R = Resistant; S = Susceptible

Discussion

The higher incidence of microbial contaminants in Suya had been previously reported in other places (Ikechukwu *et al*., 2019; Bello and Bello, 2020). Bello and Bello (2020) also reported the mean plate counts of 1.0 x 10^5 to 3.7 x 10^5 CFU/g in roasted suya meat samples. Poor water and personal hygiene qualities, traditional processing techniques and exposure of suya to unhealthy environment could be attributed to this phenomenon. Similar findings on microbial biodiversity in suya had been earlier reported (Ribah and Manga, 2018). Six species of microorganisms were identified in Suya meat samples as shown in table 3. Similar findings in biodiversity have been earlier reported (Amadi *et al*., 2016). 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 (Mensah *et al*., 2012; Okwori *et al*., 2014). Additionally, health conditions may be exacerbated by the ability of *E. coli, S. aureus*, *Enterobacter* sp, *Proteus* sp and *K. pneumonae* to form biofilms which enhances antibiotic resistance (Chen *et al*., 2013). The existence of these organisms in the suya could be attributable to the filthy environment, poor personal hygiene of the processors and retailers, the use of contaminated utensils during processing, use of contaminated materials for packaging, activities of flies as well as the addition of spices and seasonings during processing.

The results of this study differ from a study by Onuorah *et al*. (2015) who reported *Escherichia coli* (34.3%) as the most frequent while *Streptococcus pyogenes* (8.6%) had the lowest. *Bacillus* sp is widely spread in nature especially in the air, soil, water, on plants and can easily contaminate food products. This finding agrees with the study of Odey *et al*. (2013) who reported *Bacillus* sp as the most predominant organism in their study. A higher percentage of organisms had earlier been reported (Bello and Bello, 2020; Amaeze *et al*., 2016). There may be a possible outbreak of food poisoning and/or food-borne infections due to the consumption of contaminated suya meat, if appropriate quality control measures are not put in place. This may lead to serious economic and public health problems.

Antibacterial susceptibility profiles revealed that ciprofloxacin, gentamycin and sparfloxacin were the most effective antimicrobials against all the test bacterial pathogens whereas Streptomycin showed high sensitivity to *Staphylococcus 'sp' as shown in t*able 5. The findings on the antibiotic resistance of bacteria in this study deviated from the result of Barber *et al*. (2018) who reported that all *E. coli* was resistant to chloramphenicol. Nutanbala *et al*. (2011)

reported the sensitivity of *E. coli* to ciprofloxacin which is in line with the finding of this study. Ciprofloxacin belongs to the fluoroquinolone class of antibiotics and has been known to have excellent activities against Gram-negative and Gram-positive bacteria such as *E. coli* and *S. aureus,* respectively. The report of Sani *et al.* (2012) also buttressed the sensitivity of S. aureus to the fluoroquinolones. However, amoxicillin, augmentin and septrin exerted poor antimicrobial effects on the isolates as the majority of the bacterial isolates exhibited resistance to it in the present study. The mechanism of action of the fluoroquinolones is the inhibition of bacterial DNA gyrase responsible for DNA replication and transportation (Moore, 2015).

Conclusion and Recommendation

In conclusion, the results indicate that the ready-to-eat Suya meats were contaminated with a diversity of bacterial species. Apparently, this signals for surveillance and monitoring of the microbial safety of roasted and vended foods. The high-level inhibition profiles showed by ciprofloxacin, gentamycin and sparfloxacin to the 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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