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Isolation and Antimicrobial Resistance Phenotype of *Salmonella* species from Ready-to-Eat Roasted Meat (Suya) in Ilorin, Kwara State, Nigeria

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ABSTRACT

Salmonella are pathogenic bacteria that can contaminate food products during or after processing. They are among the most important food borne pathogens worldwide contaminating a wide range of animal products including meat products. Human illnesses due to this pathogen are attributed to poor biosecurity in production and improper processing/handling of meat and meat products. This is more likely where surveillance and regulatory control are weak. Ready-to-eat (RTE) foods, like roasted meat (Suya) in Ilorin, do not undergo any treatment to ensure its safety before consumption, therefore the risk of food borne disease occurrence must be considered if these pathogens are present in the food. This study aimed to evaluate the prevalence of *Salmonella* in Suya in Ilorin, Nigeria. A total of 102 Suya samples were collected and analysed for the presence of *Salmonella*. Samples were obtained from 5 major markets, consisting of a total of 15 Suya retail outlets. Organism isolation and identification were done using conventional methods. Confirmation of suspected isolates was done using Microbact GNB 24E. Five samples (4.9%) were positive for *Salmonella*. Confirmed *Salmonella* isolates were evaluated in vitro for susceptibilities to 12 commonly used antimicrobial agents. All (100%) of the confirmed *Salmonella* isolates exhibited multiple resistance to at least 3 antibiotics and exhibited five antimicrobial resistance phenotypic patterns. The classes of antibiotics they were resistant to are: aminoglycosides, cephalosporin, penicillin and tetracycline. The isolation of Multiple Drug Resistant (MDR) *Salmonella* species in this study makes Suya potential vehicle for the transmission of *Salmonella*. Effort should be made to sensitize producers of Suya on the potential risk of the product being vehicles for the transmission of food borne pathogens and the need to maintain high level hygiene during production. The public should be educated to only patronize reliable Suya joints. Constant research work should be carried out to evaluate the level of contamination of ready to eat foods like Suya in order to be able to take informed decisions.

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INTRODUCTION

Salmonellae are zoonotic enteric bacteria found worldwide **(Hassanin *et al.*, 2014)**. They basically inhabit animal and human intestines and are shed through faeces. When ingested, they produce toxins in host cells and can cause salmonellosis which may present as gastroenteritis. The zoonotic potential of these pathogens in the food supply and their abilities to produce toxins causing diseases and death underscore the importance of the organism **(Jantsch *et al.*, 2011; Norma and Santos, 2018).** Non-typhoidal *Salmonella* can be transmitted from animal – to - human and from human – to - human. They usually invade the gastrointestinal tract and cause salmonellosis generally resulting in food poisoning **(Feasey *et al.*, 2012; Oludairo *et al.*, 2022a).** Typhoidal *Salmonella* are human - restricted pathogens that cause systemic illness progressing to an asymptomatic chronic carrier state in some individuals. Typhoidal *Salmonella* can cause food-borne infection, typhoid fever and paratyphoid fever **(Malick *et al.*, 2018).**

Typhoid fever is caused by *Salmonella* invading the blood stream, the organism can in addition spread throughout the body, invade organs and secretes endotoxins (the septic form). This can lead to

life-threatening hypovolemic shock and septic shock that require intensive care including antibiotics for control.

Ready-to-eat (RTE) foods are food products produced for direct human consumption without the need for further cooking or other processing effective to eliminate or reduce to an acceptable level the microbial load **(Elviss and Jorgensen, 2012).** Suya is a spicy, barbecued, smoked or roasted RTE beef product commonly produced by the Hausas of Northern Nigeria **(Egbebi and Muhammad, 2016).** It is the most popular RTE beef product in Nigeria, served or sold at road sides, restaurants, parties, bars and other recreational centres. It is produced from boneless meat, hung on stick and spiced with peanut cake, salt, vegetable oil and other flavourings. They are thereafter roasted on a glowing charcoal fire **(Abdullahi *et al.*, 2014).** The sources of ingredients, process of production and method of handling may predispose Suya to contamination by organisms such as *Salmonella.*

The production and consumption of RTE roasted meat products cut across religion, social status and gender **(Moshood *et al.*, 2012).** Suya is the most popular, easily assessable meat product within Ilorin metropolis. One of the sole problems in the delivery of Suya is the poor product sanitation and retail which significantly influence its bacteria quality. Majority of those involved in the processing and sale of Suya are usually illiterates without formal training in food preparation, which is necessary in the hygienic handling of foods **(Falegan *et al.*, 2017).**

The fact that there are sporadic cases of gastroenteritis and symptoms of food infection after consumption of Suya indicate the products may constitute food safety risk **(Inyang *et al.*, 2005).** The presence of *Salmonella* in Suya can pose health threat to consumers as it could lead to *Salmonella* infections which could be invasive and life threatening. *Salmonella* may be transmitted from raw meat to cooked meat by hands, surfaces or utensils **(Jay, 2000).** *Salmonella* strains that are widely resistant to commonly prescribed antibiotics have been reportedly isolated from Suya **(Tsaku *et al.*, 2019).**

Foodborne diseases (FBD) pose a detrimental public health challenge globally as they cause alarming morbidity and mortality, marked economic loss as well as reduction in life quality and productivity **(Ameme *et al.*, 2016**). It is estimated that food borne hazards cause morbidity in 600 million people with 420,000 mortalities, globally **(Akinyemi *et al.*, 2012, WHO, 2015).** The aim of the study is to determine the presence of *Salmonella* spp. from RTE Suya within Ilorin, Kwara State, Nigeria by isolating and confirming *Salmonella* spp. using conventional methods and Microbact GNB 24E and to determine the antimicrobial resistance pattern of isolated *Salmonella* spp. using Kirby Bauer disc diffusion method.

MATERIALS AND METHODS

Sample Collection

Samples were collected in Ilorin, the capital of Kwara state, North-Central Nigeria. The city is located on latitude 8.500^{o} N and longitude 4.550^{o} E, it is $765km^2$ in area and situated about 320m above sea level. Ilorin is strategically a gateway between the Northern and Southern parts of the country which makes it accessible to all parts of the country by air, road and rail **(Aiyedun *et al.*, 2017**). It has an estimated population of 935,000, making it the biggest city in Kwara State and the 12th largest built-up urban area in Nigeria **(Demographia, 2018).**

Samples were collected for the study in 15 different randomly selected Suya retail outlets out of the 50 outlets identified to be located within Ilorin metropolis. One hundred and two (102) Suya samples were purchased at 15 randomly selected retail outlets around Ilorin metropolis into clean, dry and sterile polythene bags and transported in insulated containers with ice packs to the Food Safety Laboratory, Department of Veterinary Public Health and Preventive Medicine University of Ilorin for analysis. Analyses were carried out within 1 - 4 hours of sample collection.

Sample analysis

The sample analyses were done in accordance with the procedures by **ISO, 2007** and **Oludairo *et al.*, 2013a;** About 1 gram of each sample were inoculated into 9 ml of buffered peptone water and incubated at 37 ºC for 24 hours. One milliliter of aliquot from the buffered solution were inoculated into 9 ml of Selenite F broth and incubated at 37 ºC for 24 hours. One loopful of inoculum from the Selenite F broth were streaked in sterile Petri dishes containing xylose lysine deoxycholate (XLD) and incubated at 37 ºC for 24 hours. After incubation, suspected *Salmonella* growth which appeared as smooth, moist and pinkish colonies with dark centers were selected and distinct colonies were streaked into SSA (modified) and incubated for 37 ºC for 18-24 hours. Presumptive positive *Salmonella* isolates were inoculated into nutrient agar slants and incubated at 37 ºC for 24 hours, after which they were removed and stored in the refrigerator at 4ºC for further analysis (**Oludairo *et al.*, 2022a**).

Growths that were characteristic of *Salmonella* – smooth, moist, pink colonies with dark centres, were inoculated into Urea agar, Triple Sugar Iron (TSI) agar, Simmons Citrate Agar, Methyl Red Voges - Proskauer (MRVP) medium and Motility Indole Lysine (MIL) medium after 24 hours subculture on XLD. On urea agar slant, presumptive *Salmonella* species were negative, this was indicated by the colour of the slant not changing from its initial light brown colour after

incubation at 37 ºC for 24 hours. For TSI test, presumptive *Salmonella* species produced yellowish acidic butt and reddish alkaline slant. Some species produced gas with hydrogen sulphide evident as blackening of the slant/butt of the test tube (**Quinn *et al.*, 1994**).

Indole, Methyl Red, Voges - Proskauer and Citrate Utilization (IMViC) tests were carried out to confirm presumptive *Salmonella* isolates. *Salmonella* species were negative for indole as they did not change the colour of the media from the initial yellow colour after the addition of Kovac's reagent. They were positive for methyl red test which was evident in the change of the colour of the media from orange to red after the addition of methyl red reagent. The isolates were negative to Voges - Proskauer as they do not change the colour of the media from its initial orange colour after the addition of Barritt's A and Barritt's B reagents. The *Salmonella* isolates were also positive to Simmons citrate as was indicated in the change of the media from its initial green colour to light blue.

Confirmation of suspected *Salmonella* isolates was done using Microbact GNB 24E. Presumptive *Salmonella* isolates were sub cultured on SS agar at 37 ºC for 24 hours. One to two colonies were picked using a sterile loop and suspended into 5 ml sterile normal saline in test tubes. The Microbact GNB 24E wells were labelled and filled to ¾ levels (about 4 drops / 100 µl) with the corresponding isolate suspension. The filled plates were then incubated for 24 hours at 37 ^{o} C. Two drops of Kovac's reagent, one drop each of VPI and VPII and one drop of TDA were added to wells 8, 10 and 12 respectively before taking the readings. Colour changes were compared with the standard colour chart provided by the manufacturer and number grades assigned to each well. The grades were summed up to three numbers and inputted to the Microbact 2000 identification software provided by the manufacturer, which gave the identification of test organisms as percent probabilities **(FAO, 1992, Oludairo *et al.*, 2022b).**

Antimicrobial Sensitivity Test

Antimicrobial sensitivity test was carried out on confirmed *Salmonella* isolates to detect the resistance/susceptibility of isolated *Salmonella* to 12 antibiotics using disc diffusion method **(CLSI, 2016)**. The twelve (12) antimicrobial agents tested with their concentrations are: Amoxicillin (AML) – 10 µg, Ampicillin (AMP) – 10 µg, Cefotaxime (CTX) – 30 µg, Chloramphenicol (C) – 30 µg, Ciprofloxacin (CIP) –5 µg, Colistin (CT) – 10 µg, Gentamicin (CN)– 30 µg, Neomycin (N) – 30 µg, Oxytetracycline (OT)– 30 µg, Penicillin G (P)–10 µg, Sulfamethoxazole/ Trimethoprim (SXT)–25 µg, Tetracycline (TE)– 30 µg.

Confirmed *Salmonella* isolates were subcultured on nutrient agar and incubated for 24 hours at 37 ºC. One distinct colony was inoculated into 5 ml nutrient broth and incubated for 24 hours at 37 ºC. One drop (approx. 0.03 ml) was inoculated into 10 ml sterile normal saline to get turbidity comparable to 0.5 McFarland **(Cruickshank *et al.*, 1975)**. The mixture was inoculated into Mueller Hinton agar using pour plate method, the excesses were discarded and the plates were placed in the hot air oven o allow to dry. Antimicrobial discs were then placed on the surface of the agar using a multiple disc dispenser (Oxoid, UK), after which the plates were incubated for 18 hours at 37 ºC and the zones of inhibition read according to the CLSI Standards **(CLSI, 2020).**

RESULTS

Prevalence of *Salmonella* in Suya in Ilorin

Out of 102 samples of Suya that were collected from the fifteen (15) locations in Ilorin, Kwara state, Nigeria, 5 (4.9%) were positive for *Salmonella*. The organism was isolated from four Suya stands and their prevalence were 1 (20%), 1 (20%), 1 (20%), and 2 (40%) for Tipper Garage/Unilorin PS, Zamfara Hostel, Sabo-Oke and Sabo-Oke/Maraba Junction respectively (Table 1)

Table1: Prevalence of *Salmonella spp.* isolated from Suya in Ilorin, Kwara state, Nigeria.

Serial Number	Location	Number of samples collected	Number (%) of *Salmonella* isolates
1	F-Division Road	6	0 (0)
2	Gaa-Akanbi junction	8	0 (0)
3	K-Dorms	6	0 (0)
4	Maraba Park	6	0 (0)
5	Oke-Odo Junction	10	0 (0)
6	Oke-Odo/Bekandims	8	0 (0)
7	Sabo-Oke	7	1 (0.98)
8	Sabo-Oke/Maraba Junction	7	2 (1.96)
9	Tanke Primary School Junction	6	0 (0)
10	Tipper Garage	6	0 (0)
11	Tipper Garage Market	7	0 (0)
12	Tipper Garage/Pipeline Road	6	0 (0)
13	Tipper Garage/Unilorin PS	6	1 (0.98)
14	Unilorin PS	7	0 (0)
15	Zamfara Hostel	6	1 (0.98)
	Total	102	5 (4.9)

Antimicrobial sensitivity test results

All (100%) the *Salmonella* isolates were sensitive to amoxicillin, chloramphenicol, colistin sulphate, sulfamethoxazole/trimethoprim and tetracycline. Four (80%) of the isolates were sensitive to ampicillin and neomycin while three (60%) isolates were susceptible to ciprofloxacin and gentamicin. Out of the isolates tested for sensitivity to oxytetracycline, two (40%) were sensitive while three (60%) were resistant to the antibacterial agent. On the other hand, all (100%) of the isolates were resistant to cefotaxime and penicillin G. Two (40%) *Salmonella* isolates were resistant to ciprofloxacin and gentamicin while one (20%) isolate each was resistant to ampicillin and neomycin (Table 2). The phenotypic antimicrobial resistance patterns recorded in this study were CIP–CTX–P, AMP–CTX–OT–P, CN–CTX–P, CTX–OT–P and CIP–CN–CTX–N–P (Table 3).

Table 2: Results of Antimicrobial sensitivity test carried out on isolated *Salmonella spp*. from Suya in Ilorin, Kwara state, Nigeria:

Serial Number	Sample Number	Source	Antimicrobials, Inhibition Zone and Susceptibility										
			AML	AMP	C	CIP	CN	CT	CTX	N	OT	P	SXT	TE
1	62	Sabo-oke/ Maraba Junction	32 (S)	23 (S)	33 (S)	28 (R)	15 (S)	20 (S)	0 (R)	23 (S)	29 (S)	0 (R)	38 (S)	33 (S)
2	64	Sabo-oke/ Maraba Junction	19 (S)	15 (R)	40 (S)	37 (S)	37 (S)	17 (S)	0 (R)	30 (S)	0 (R)	11 (R)	40 (S)	26 (S)
3	69	Sabo-oke	29 (S)	23 (S)	30 (S)	31 (S)	12 (R)	18 (S)	0 (R)	30 (S)	26 (S)	9 (R)	32 (S)	31 (S)
4	88	Tipper Garage / Unilorin PS	29 (S)	21 (S)	35 (S)	31 (S)	18 (S)	20 (S)	0 (R)	25 (S)	0 (R)	8 (R)	38 (S)	27 (S)
5	101	Zamfara Hostel	25 (S)	20 (S)	36 (S)	27 (R)	14 (R)	22 (S)	0 (R)	13 (R)	0 (R)	9 (R)	31 (S)	28 (S)

Key: AML - Amoxicillin, AMP - Ampicillin, C - Chloramphenicol, CIP - Ciprofloxacin, CN - Gentamicin, CT – Colistin Sulphate, CTX - Cefotaxime, N - Neomycin, OT – Oxytetracycline, P – Penicillin G, SXT – Sulfamethoxazole/Trimethoprim, TE – Tetracycline. (CLSI, 2016).

Table 3: Isolated *Salmonella spp.* from Suya in Ilorin, Kwara state, Nigeria and their antimicrobial resistance phenotypic pattern.

Serial number	Sample number	Source	Antibiogram
1	62	Sabo-Oke/Maraba Junction	CIP–CTX–P
2	64	Sabo-Oke/Maraba Junction	AMP–CTX–OT–P
3	69	Sabo-Oke	CN–CTX–P
4	88	Tipper Garage/Unilorin PS	CTX–OT–P
5	101	Zamfara Hostel	CIP–CN–CTX–OT-N–P

DISCUSSION

Salmonella is a zoonotic bacterium that could cause high economic loss and is of public health importance worldwide **(Oludairo *et al*., 2013b).** In this study, the prevalence of *Salmonella* was obtained from various Suya samples from different parts of Ilorin with overall prevalence of 4.9%. The result gotten in this study could be due to climate of the geographical location of the study area, having climatic conditions that could encourage the growth and survival of the microorganism.

The presence of *Salmonella* in Suya samples could be as a result of poor processing methods, poor hygiene practices, improper and unhygienic handling of the meat products, poor sanitation operations and the use of unclean equipment and utensils by Suya vendors. An important factor which could have significantly contributed to the presence of *Salmonella* in Suya is the environment of the retail outlets which were motor parks, market places or roadsides where suya could be easily contaminated by microorganisms carried in the air and several other insects which

usually abound at such sites. Insects such as flies can cause contamination through continuous contact with the products in addition to dust particles from heavily contaminated atmospheres around market places and motor parks. Contamination of meat product could arise from the spice used in the processing of Suya and the use of contaminated equipment/ utensils. The spice could contain lots of microorganisms which could serve as a source of contamination of the meat products. Another source of contamination could be the retailers if they do not practice good personal hygiene. Other likely sources of contamination are packaging materials like magazines, cement papers, used sheets of paper, which may have been sourced from unhygienic places.

Salmonella spp. could survive improper heating of suya meat product during processing. The presence of *Salmonella* spp. as contaminants may be attributed to inadequate heating of meat product during its preparation **(Moshood *et al.*, 2012).** The prevalence of *Salmonella* spp. recorded in this study is low when compared with 40% prevalence reported by **Oladunjoye *et al.*, (2019)**, 18.84% reported by **Ogbu *et al.*, (2016)** and 17% reported by **Konne *et al*., (2018)** but it is relatively higher than the 2.4% prevalence reported by **Tafida *et al.*, (2013)** and 0% prevalence reported by Fahim *et al.*(**Fahim *et al.*, 2016).** All the *Salmonella* spp. isolated in this study exhibited multiple drug resistance to at least 3 antimicrobial agents, it is in agreement with the report of **Fallah *et al*., (2013)** who reported multiple drug resistance to at least 3 antimicrobial agents in all the isolated *Salmonella* serovars from the study. **El Jakee *et al.*, (2014)** also reported between 12.5 - 87.5% MDR in the isolated *Salmonella* from their study.

The phenotypic resistance for routinely prescribed antimicrobial agents like penicillin G, cefotaxime, ciprofloxacin, gentamicin, ampicillin and neomycin including the 5 different microbial resistance phenotypic patterns exhibited by the 5 Salmonella isolates are of public health importance. It poses considerable health hazards to the consumers unless prudent control measures are instituted. Partly because there is the possibility of the transmission of MDR *Salmonella* from the Suya meat to consumers of the meat product. Multiple drug resistance in the organism could have been developed as a result of misuse of antimicrobial agents in the treatment of diseases in source cattle before slaughtering. Reports from different parts of Nigeria have observed temporal trends in the prevalence of resistance among enteric organisms **(Tafida *et al.*, 2013**). In veterinary medicine, antimicrobial agents are used in therapy, prophylaxis, and as growth promoters. This kind of use may be responsible for generate resistant bacteria.

During the past two decades, the emergence of antibiotic-resistant *Salmonella* has become a serious problem worldwide. Wide usage of antibiotics in the diet of domestic animals has made drug resistant bacteria which could be transferred to human beings to be on the increase. The challenge of MDR strains of *Salmonella* is increasing and most studies in different countries have reported high resistance of *Salmonella* strains to several antimicrobial agents **(Fallah *et al.*, 2013).**

The prophylactic use of many antimicrobials in animal feed can also lead to acquired antibiotic resistance **(Rajagopal and Mini, 2013).** Bacterial antimicrobial resistance is an alarming situation because it limits treatment to only antimicrobial agents to which they are sensitive **(Oludairo *et al*., 2019).** Resistance to *Salmonella* transmitted by contaminated foods of animal origin is undesirable, but it can be prevented with the rational use of antimicrobials in animal production **(Tessari *et al.*, 2013).** The detection of *Salmonella* spp. in this study is of public health importance as due to it ability to cause food borne diseases, food poisoning, diarrhoea, abdominal disorder, pains, fever and other symptoms/clinical signs.

CONCLUSION

Suya is a ready to eat meat product that is a delicacy in many parts of Africa, Asia some other parts of the world. This meat product may constitute life threatening food safety risk to both man and animals due to possible contamination of the product by microorganisms like *Salmonella*. This study isolated *Salmonella* spp. in Suya from Ilorin, Kwara State, Nigeria and determined the antimicrobial patterns of the isolates. Five out of the 102 Suya samples collected from 15 locations within Ilorin, Kwara State, Nigeria yielded *Salmonella* spp. which was recovered from 4 out of the 15 locations sampled. All the *Salmonella* isolates were multiple drug resistant to at least 3 antimicrobial agents phenotypically exhibiting 5 antimicrobial resistance patterns. The presence of *Salmonella* in Suya is of public health importance that could causes of gastro-intestinal disorders, food poisoning and food borne diseases. Adequate measures and proper hygiene should therefore be employed during the production of Suya and its spices, so that they would be devoid of potential microbial contaminants. Food Regulatory Bodies like National Agency for Food and Drugs Administration and Control (NAFDAC) should set up modalities to monitor the production/processing and packaging of Suya and other meat products in order to reduce health hazards and guarantee good health for the public. Public health education programme should be carried out to enlighten the general public about the health

implication of consuming contaminated meat products like Suya.

Conflict of interest

The authors acknowledge that there is no conflict of interest regarding the research idea and tools, actual, potential and financial, directly or indirectly.

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