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Microbial Quality of Spicy Roasted Meat (Suya) Retailed in Ogbete Main Market and Oye Emene Market, in Enugu Metropolis, Nigeria

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Abstract Microbiological quality, targeting contamination with *Escherichia coli*, of ready-to-eat spicy meat product, "*Suya*" retailed in Ogbete Main Market (Location 1) and Oye Emene Market (Location 2), in Enugu, Nigeria was evaluated. Forty-eight samples of the "suya," in forms of beef, liver, intestine, and chicken, were homogenized and serially diluted with sterile distilled water and plated into Eosin Methylene Blue agar using Pour Plate Technique. Identification of isolates were based on cultural characteristics, Gram stain reaction and Biochemical (IMViC) test, and confirmed by molecular test using 16S rRNA gene. Result showed that E *coli* were isolated from all the samples. Total colony counts (TCC) of all E.*coli* isolates were at inoculums much greater than the known infective dose for the Enteropathogenic strains. Least TCC in the April-August 2015 test samples from Locations 1/2 were: 5.6 x10^2/6.0 x 10^2, respectively; and 4.4 ×10^2/3.2 x 10^2, respectively in Locations1/2 in the September- November 2016 test. The four highest TCC were from the cow intestine suya. In conclusion, "*suya*" sold to the public are contaminated, probably of faecal origin; therefore not fit for human consumption. This study also underscores the need for improved surveillance system on *suya* products, to enforce good food hygiene practices.

Keywords: *meat vendors, roasted meat, microbial contamination, food borne diseases, food hygiene, Escherichia coli*

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1. Introduction

Meats are edible animal fleshes which principally comprise of the muscular tissues, as well as internal organs such as: heart, liver, kidney, intestine and bladder generally referred to as viscera [1]. Bulk of such meat are derived from goat, cattle, pig, sheep, and poultry [2]. All such animals are used in preparing a very delicious roasted, ready-to-eat meat product commonly referred to as "*Suya*" all over Nigeria. And, its production is a special and a great art of the Hausa and Fulani around the whole savannah regions. It is a spicy, barbecued, smoked or roasted meat product, which originated from the Hausa people of northern Nigeria, where rearing of cattle is an important pre-occupation and a major source of livelihood for the people. They are also produced in other form such as "*Kilishi*" (dry ready-to-eat meat), '*Balangu*" and "*Kundi*" all which are popular street foods. *Suya*, however, is the most popular as its consumption has extended to other parts of the country [3]. Any part of animal viscera can be used, but the most commonly used part is the muscular region. Likewise, for its delicacy, this art has also been locally exported into the western worlds such that it is very common for Africans visiting Europe or United States to eagerly sort where they can be located.

Besides this juicy delicacy, meat has enormous value in the diet; they are huge sources of rich protein, so there exist large markets for them and their products worldwide at varying money value, hence their demands increase day by day across the globe. Likewise, there exist different types of other meat products all over the world, ranging from the industrially processed corned beef, ham, bacon, sausage, "hotdog," "hamburger," "beef burger," "Chicken burger," etc, to the just described indigenous Nigeria traditionally processed ready-to-eat meat ones ["*Suya*" (roasted meat), "*Kilishi*,"(dry ready-to-eat meat) and many more. Other analogous world meat varieties includes "Beef *kebab*" in Europe, "*Kyiskiyma*" in Central Africa, and "*Sogodjemine*" in Mali [4].

Generally, these varieties of meat products, including "*Suya*," are subjected to combination of several basic processing steps before reaching their final form, hence get contaminated along the line of production. Micro-organisms that occur in meat and meat products most

times are responsible for food borne illness. These micro-organisms *include Bacillus sp, Clostridium* sp, *Escherichia coli, Salmonella* sp, *Shigella sp, Staphylococcus aureus, Streptococcus pyogenes, Proteus* sp, *Pseudomonas aeruginosa, Leuconostoc* sp, *Lactobacillus* sp, *Micrococcus, Mycobacterium* sp, *Vibrio* sp, etc [4,5,6]. Other studies have pointed out that food cross-contamination during preparation contributes remarkably to the occurrence of food-borne diseases [6]. In particular, *Salmonella* may be transferred from raw meat to cooked meat by hands, surfaces or utensils [7]. In other food products, outbreaks of salmonellosis have been associated with the consumption of cut watermelon and cantaloupe in the United States of America [8,9,10]. In general, they all indicate evidences of faecal contamination. Further, this local meat product is often neglected by agencies responsible for food services as having a role in the introduction of potential food borne bacteria or parasites into populations' diet; they concentrate on officially produced licensed products [11].

Further, as risks of these E *coli* contamination, pathologic syndromes of their various five strains are serious threat to consider: Enteroheamorrhagic E. *coli* (EHEC) causes haemolytic uremic syndrome and sudden kidney failure; Enteroaggregative E. *coli* (EAEC) possess fimbriae which has aggregate tissue culture cells that binds to the intestinal mucosa and produce hemolysin, a toxin that leads to serious intestinal upset; Enterotoxigenic E. *coli* (ETEC) produces heat-labile or heat stable enterotoxins in the gut which leads to unsuspected hypersecretions in small intestine that causes a sudden, very inconvenient gastroenteritis referred to as traveller's diarrhea; Enteropathogenic E. *coli* (EPEC) attaches to mucosal epithelial cells and produces cyto-skeletal changes, squamous mucosa, and it can invade cells, enter the blood to cause very serious systemic syndromes. Lastly, Enteroinvasive E. *coli* (EIEC) infection causes a syndrome that is identical to shigellosis, with profuse diarrhea and high fever. All of these five pathologies can lead to medical emergencies [10,12,13].

Therefore, the aim of this study was to determine microbiological quality of retailed "*suya*," targeting contamination with *Escherichia coli* to understudy the situation, as well as evidence of probable faecal contamination. The specific objectives were:(i) To buy various types of retailed roasted meats (cow flesh "*suya*," chicken *suya*, intestine *suya* and liver s*uya*) from two different market locations in Enugu;(ii)To determine the presence of *Escherichia coli* in the various types of retailed roasted meat; (iii) To evaluate the degree of contamination of the various roasted ready-to-eat meat products, with *E, coli*; (iv) to also determine whether the inoculums where within infective doses.

2. Materials and Methods

2.1. Survey and Sample Collection

Between April-August 2015 and September-November, 2016 a total of 48 spicy roasted meats(suya) comprising [cow flesh *suya*, liver (cow) *suya*, intestine (cow) *suya* and chicken *suya*] from two retail outlets (Ogbete Main Market and Oye Emene Market) were analyzed for presence of *Escherichia coli* bacterial pathogens. Three of each of the four types of the roasted meats [cow flesh *suya*, liver (cow) *suya*, intestine (cow) *suya* and chicken *suya*] were collected at each of the two retail outlets. This gave a total of 24 samples. Sampling sites were randomly determined, based on the places where suya were sold within the two markets Enugu metropolisby vendors. At each point of sale, samples of each suya were bought and aseptically taken into sterile polythene bags, placed in a cold box and quickly transported to the laboratory for bacteriological analysis. All samples were analyzed within 6 hours.

Location 1 = Ogbete Main Market on geographical coordinate 625'59"N 7°28'49"E on "Infinix Hotspot 7" smart phone-compass, measured off the bus-stop at the Akwata side of the market, which is closest to the Abattoir and sampling areas.

Location 2 = Oye Emene Market on geographical coordinate 6° 28'39"N 7°34'49"E on "Infinix Hotspot 7" smart phone-compass, measured along Rehabilitation Road end of the market, which is closest to the Abattoir and sampling areas.

2.2. Determination of Geographical Coordinates

The digital phone-compass App was downloaded and installed into the "Infinix Hotspot 7" smart phone from the internet. At the precisely stated location or spot, the smart phone was put on and the compass icons clicked on, and waited for the App to boot. As soon as the phone-compass App boots, it brings out the precise geographical coordinate of the spot, this was then read off and recorded.

2.3. Bacteriological Processing of Samples

Bacteriological analysis included isolation of potential pathogens ws done [14,15]. Each of the samples were homogenized and1g of each homogenate was suspended into 9ml sterile peptone water. This was serially diluted using sterile peptone water and1ml of each of the serially diluted mixture was plated into Eosin Methylene blue Agar (L-EMB) using Pour Plate Technique.and incubated at 37°C for 24 – 48 hr.

2.4. Identification

At the end of incubation period, the cultural characteristic of the bacterial growth on Eosin Methylene Blue (EMB) agar selective medium, based on the pigmentation, elevation, consistency, surface texture and edges were observed. Positive results were those that gave the typical cultural characteristics and greenish metallic sheen on EMB. Colonies that developed typical cultural characteristics and greenish metallic sheen were counted to obtain total colony counts, and the average growth for each sample was used to determine the Most Probable Number of *Escherichia coli* growth as colony forming units (cfu) in each sample.

2.4.1. Confirmation

The suspected organisms were further identified and characterized based on their cellular morphology (Gram's

Stain), physiological and biochemical characteristics (IMViC), after Barrow *et al*. (1993) and Cheesbrough (2000), and confirmed by molecular tests using 16S rRNA gene.

2.4.2. Molecular Testing

The molecular testing was performed at Bioformatic Services, Ibadan, Nigeria. DNA was extracted using ZR Fungal/Bacterial DNA Miniprep (Zymo Research Cat Number: D6005). Then Agarose gel was prepared for electrophoresis of DNA (1gm of agarose) and PCR (2g of agarose) and allowed to completely solidify. Then, after all the necessary protocols, the eluted DNA and PCR product were loaded on wells in the gel, then the gel was run at 80-150 V for about 1-1.5 hr. Thereafter, power was turned off, electrodes disconnected from the power source and then the gel was carefully removed from the gel box, after which DNA fragments and PCR product were visualize under UV transilluminator. The PCR mix was made up of 12.5 µL of Taq 2X Master Mix from New England Biolabs (M0270), 1 µL each of 10 µM forward and reverses primer, 2 µL of DNA template and then made up with 8.5µL nuclease free water. Primer sequences were: 27F: AGAGTTTGATCMTGGCTCAG and 1525R: AAGGAGGTGWTCCARCCGCA. Cycling conditions were: Initial denaturation at 94°C for 5 min, followed by 36 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and elongation at 72°C for 45 sec, followed by a final elongation step at 72°C for 7 min and hold temperature at 10°C forever. After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. The amplified fragments were sequenced with a Genetic Analyzer 3130×L sequencer from Applied Biosystems using manufacturers' manual, while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Genomic composition of the sample was determined by mapping its sequence read against viral, bacterial and eukaryotic sequences data bank.

3. Results

Samples of *suya* investigated were contaminated with potential E *coli* pathogens.

Result showed that *E coli* were isolated from all the 48 *suya* samples (Table 1 – Table 2), except the chicken *suya* sample that swarmed with contaminants in the April-August analysis (Table 1), and so produced no determined result.

Further, Table 1, Table 2 and Table 5 showed that all the *E.coli* isolates were at inoculums much greater than the known infective dose (10 organisms) for the Enteropathogenic strains; the least total colony counts found in all the analysis was from chicken *suya* in the September-November test sample at Location 2: 3.2 x 10^2 (Table 2 and Table 5).

Two of the four least Total colony counts were from the chicken *suya* (Table 2 and Table 5), while the other two were from the beef *suya* (Table 1 and Table 5).

All the four highest total colony count in the analysis in the two periods were from the cow intestine *suya* (Table 1, Table 2 and Table 5).

The least average number of *E coli* isolated per gram of meat sample was 3.2 x 10^2cfu, and it was from the chicken sample (Table 2), and the highest was 7.3 x 10^4 and it was from the intestine sample at Location 2, both in the September-November analysis (Table 2).

Table 1. Average number of *Ecoli* colonies isolated in each serial dilution of three roasted meat (*suya*) samples done April-August, 2015

Analytes Number of colonies isolated (cfu/g) in the samples			
Location 1/*Suya* samples	**A**	**B**	**C**
Beef	5.6 x 10^2	4.9 x 10^3	3.8 x 10^4
Liver (cow)	6.0 x 10^2	5.2 x 10^3	4.0 x 10^4
Intestine (cow)	8.2 x 10^2	7.4 x 10^3	6.0 x 10^4
Chicken	SM/CONT	SM/CONT	SM/CONT
Location 2/ *Suya* samples	**A**	**B**	**C**
Beef	6.0 x 10^2	5.4 x 10^3	4.4 x 10^4
Liver (cow)	7.2 x 10^2	6.4 x 10^3	5.0 x 10^4
Intestine (cow)	1.0 x 10^3	8.5 x 10^3	7.0 x 10^4
Chicken	SM/CONT	SM/CONT	SM/CONT

KEYS: Location 1= Ogbete Main Market; Location 2 = Oye Emene Market; SM/CONT = Swarmed/Contaminated.

Table 1 showed the result of the total colony count of the isolated *E coli* in the various *suya* samples in the April-August 2015 analysis. *E. coli* were detected in all the samples, except the chicken samples that swarmed with contaminants, so produced no determined result. All the samples showed very high inoculums that were greater than the known infective dose (10 organisms) for the Enteropathogenic strain of *E coli;* (the least total colony count in the April-August 2015 test samples from location 1 was 5.6 x 10.^{2}

Table 2. Average number of E *coli* colonies isolated in each serial dilution of 3 roasted meats (*suya*) samples done September-November 2016

Analytes Number of colonies isolated (cfu/g) in the samples			
Location 1/ *Suya* samples	**A**	**B**	**C**
Beef	6.6 x 10^2	4.8 x 10^3	5.8 x 10^4
Liver (cow)	6.0 x 10^2	5.2 x 10^3	4.6 x 10^4
Intestine (cow)	8.2 x 10^2	7.4 x 10^3	6.0 x 10^4
Chicken	4.4 x 10^2	5. 2 x 10^2	4.6 x 10^2
Location 2/ Suya samples	**A**	**B**	**C**
Beef	7.0 x 10^2	5.4 x 10^3	4.4 x 10^4
Liver (cow)	6.2 x 10^2	6.4 x 10^3	5.0 x 10^4
Intestine (cow)	7.0 x 10^3	8.5 x 10^3	7.3 x 10^4
Chicken	1.0 x 10^3	2.1 x 10^2	3.2 x 10^2

Keys: Location 1= Ogbete Main Market; Location 2 = Oye Emene Market.

Table 2 showed the result of the total colony count of the isolated *E coli* in the various *suya* samples in the September-November 2016 analysis. All the samples showed very high inoculums that is greater than the known infective dose (10 organisms) for the Enteropathogenic strain of *E coli;* (the least total colony count in the April-August 2015 test samples from location 2, and it was 3.2 x 10.^{2}

Table 3. Distributions of the types of suya and their least/highest grades of inoculums/total colony counts in the two locations and test periods

April-August test/Locations	Inoculums' grade	Total colony count	Type of *suya*
I	Least	5.6×10^2	Beef
	Highest	6.0×10^4	Intestine (cow)
2	Least	6.0×10^2	Beef
	Highest	7.0×10^4	Intestine (cow)
September-November test/Locations			
1	Least	4.4×10^2	Chicken
	Highest	6.0×10^4	Intestine (cow)
2	Least	3.2×10^2	Chicken
	Highest	7.3×10^4	Intestine (cow)

Keys: 1 = Location 1 (Ogbete Main Market); 2 = Location 2 (Oye Emene Market).

Table 3 is the summary of the distributions of the various types of *suya* and their determined least/highest grades of inoculums and total colony counts in the test samples from the two locations and test periods

Further, Table 3 showed that all the E.*coli* isolates were at inoculums much greater than the known infective dose (10 organisms) for the Enteropathogenic strains; the least total colony counts found in all the analysis was from chicken *suya* in the September-November 2016 test sample atlocation 2: 3.2×10^2.

The table also showed that two of the four least total colony counts were from the chicken suya, while the other two were from the beef suya.

Also, it showed that all the four highest total colony count in the analysis in the two periods were from the cow intestine suya.

Lastly, it as well indicated that the least average number of E *coli* isolated per gram of meat sample was 3.2×10^2cfu, and it was from the chicken sample, and the highest was 7.3×10^4 and it was from the intestine sample at Location 2, both in the September-November 2016 analysis.

Genomic sequence result of molecular test for *Escherichia coli indicate* 99% identical to *Escherichia coli* strain JJ1897 complete genome

4. Discussions

The results obtained from this study indicated that the *suya* samples were not wholesome in spite of their spicy, delicious and aesthetic look, since all the 48 samples were found contaminated with E *coli* that were probably from biotic origin when viewed from the sources of production, modes of handling and septic forms of vending,.

There is no doubt that this contamination may have been introduced at the point of processing and distribution because observation showed that the animals were usually slaughtered without proper washing, carcass spread on ill-prepared surfaces, cutting of the meat done without proper microbiological aseptic protocols, spicing and roasting were as well done in unhygienic procedures, likewise are the so spicy delicious suya eventually vended uncovered from dusts and flies. Like in this work, poor microbial quality of fruits in their raw state, contaminated water or inadequate hand-washing by fruit vendors and the absence of individual sanitary practices were similarly reported too old to be cited here i in which there was *P. aeruginosa* infection in a hospital via vegetables.

Further, presence of*E. coli* and other coliform bacteria is generally an indication of faecal contamination of the water often used by vendors for washing their utensils and hands before processing [16]. So also had high occurrence of thermo-tolerant coliforms in 25% of samples been found as evidence of this [17]. As in their work [16,17], the visit to the abattoirs at Oye Emene Market and Ogbete Main Market and the data presented here, suggest that suya could become contaminated with food borne bacteria by factors such as processing utensils in unhygienic conditions since meat under procession, and after processing as suya, are left uncovered, trays are left open in unsuitable places for buyers, and irregular hand-washing by the vendors. Besides, suya-meat processors as well as the vendors, used untreated stream water??? to wash their hands and the knives used for cutting the sampled suya. Likewise, like in the work of Little and Mitchell (2004), this study could be associated with the general poor sanitary environmental conditions (visited too) under which the meat were handled [18]. Similarly, Okonkwo *et al* in Nigeria reported high contamination by pathogenic bacteria of sea food processors and the water used [19]. Further, findings from this research is similar to those of many other authors on different food items [20-24]; and Kumar and Ganguli which reported *E. coli* and *Salmonella* species as being responsible for outbreaks involving pre-cut fruits [25]. Furthermore, Wanyenya *et al*. (2004) further emphasized that cross-contamination of food during preparation has been identified as an important factor associated with food-borne illness; we also indicated this earlier [26].

Although lots of *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination [27]. Therefore, the implication of the E *coli* contaminants in this study cannot be overemphasized since the isolates could have been any one of the highly toxigenic, pathogenic or haemolytic strains, though which was not specifically determined simply because of unavailability of the test kits then. Possible serious ailment in humans from such food infections includes various forms of gastroenteritis; and, at times sequelae urinary tract infections,and neonatal meningitis.In rare cases, virulent strains are also responsible for haemolytic-uremic syndrome, peritonitis,

mastitis, septicemia and gram-negative pneumonia [28]. According to Griffin *et al*. (1991), intestinal mucosa-associated E.*coli* is observed in increased number in the inflammatory bowel diseases, Crohn's disease and ulcerative colitis. Invasive strains of E*coli* exist in high numbers in the inflamed tissue, and the number of bacteria in the inflamed regions correlates to the severity of the bowel inflammation.

What is also very worrisome is the ignorance of the food handlers that they are great potential threats to the health of the public; so in a related survey of retail establishment, Allwood *et al.* reported that only 52% of the food handlers knew how to wash their hands [29]. Like us in this study, Chukwu *et al*, found about 100% of pre-cut fruit vendors were unfamiliar with the importance of maintaining personal and utensil hygienic standards [30].

Lastly, but most importantly, another serious factor that was considered in this study was the issue of infective dose of the isolates. Medical Dictionary for the Health Professions and Nurses (2012), defined infective dose (ID) as: that quantity of a pathogen (measured in number of organisms) that is necessary to cause infection in a susceptible host. According to Greiger, *et al* due to the low infective dose of *E coli* 0157:H7, an inoculum of fewer than 10-100 colony forming units (cfu) is enough to cause very serious infection [31]. Most worrisome is that the detected number of inoculums in all the suya samples were much more than the known required infective dosage of a strain of *E. coli*; therefore, all the isolates were potentially potent.. For example, Enteroheamorrhagic strains of *Escherichia coli* require an infective dose of only about ten cells [31]. The lowest quantity of total colony count/inoculum detected in this study was 3.2 x 10^2 (320), which is thirty-two times higher than the required infective dose. The highest total colony count/inoculum was 7.3 x 10^4 (73,000), which is seventy-three thousands times more than required to potentially cause infection to produce any of those diseases or syndromes stated above. In the same line of this research, risk assessment and impact of food borne pathogens on the health of different populations was one of the goals identified in the Presidential (USA) Food Safety Initiative three-year plan [31]. This study intended the same goal. Lastly, Mahendra and Babu indicated that determination of infective dosage entail estimation of dose-response relationship for food borne pathogens to humans, either by feeding studies or from outbreaks; our research work applied inoculum size (as TCC) via Pour-Plate Method in relativity to a known or already determined infective dose and this method is an indirect and more convenient, relative risk assessment that required no ethical or medical clearance

5. Conclusions

In conclusion, results showed that "*suya*" sold to the public in those locations were highly contaminated, probably of faecal or biotic origin, and are therefore not fit for human consumption; the findings thus, demonstrated that the microbiological quality of those suya sampled indicated eminent high risk of food borne illness.

The study also underscores the need for improved surveillance system on "*suya*" products, to enforce good food hygiene practices, health education and enlightenment of retailers (in order to avoid contamination of retailed *suya*, as well as public health education and enlightenment of consumers on good food hygiene practices

6. Recommendations

There is a need to enforce good food hygiene practices to avoid contamination of retailed suya.The Nation's food agencies should look closer into the productions and retails of *suya*. Likewise, because the world is now what may be termed a "global entity or village" due to lots of immigrations, emigrations and easier travels, food administration and control should no longer be left only to nationals, such as National Agency for Food and Drug Administration and Control (NAFDAC) in Nigeria; but international organizations such as World Health Organization and Food and Agricultural Organization (as in FAO/WHO, 2003 charter) should come much more closer to what individual nationals do, in order to avoid repeat of the ongoing "history" and "geography" of COVID-19. Lastly, geographical coordinates should be a paradigm in environmental microbiology (Amadi, *et al*, 2020); it greatly assists follow-up of precise locations, and it is simple, cost-less, and requires no special technical know-how or training.

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