Available Online at www.ijpscr.info
International Journal of Pharmaceutical Sciences and Clinical Research 2021; 1(1):46-51

RESEARCH ARTICLE

Prevalence of *Staphylococcus aureus* and *Escherichia coli* in Sun-cured Meat (*Jerky, Kilishi*) from Retail Outlets in Sokoto, Nigeria

Tukur Nuhu Ibrahim[1], Faleke Olufemi Oladayo[1], Musa Galadima[2]

[1]Department of Veterinary Public Health, Usmanu Danfodiyo University, Sokoto, Nigeria, [2]Department of Microbiology, Lovely Professional University, Phagwara, Punjab, India

Received: 10-12-2020; Revised: 10-01-2021; Accepted: 29-01-2021

ABSTRACT

Pathogenic bacteria have been implicated in many outbreaks of food-borne diseases all over the world. This study is aimed at detecting the presence of *Staphylococcus aureus* and *Escherichia coli* in sun-cured meat, *kilishi* or *jerky* from retail outlets in Sokoto, Nigeria. A total of 81 samples of *kilishi* from ten areas were examined using bacteria culture and biochemical tests. Sixty-eight samples yielded positive for *S. aureus* with a prevalence of 83.9% while *E. coli* had 0.0% prevalence. The rate of contamination with *S. aureus* was higher in four areas which had 100% prevalence whereas other locations had 25%, 60%, and 80%. Due to the high occurrence of the entero-toxin-producing *S. aureus* isolated from *kilishi* samples collected within Sokoto metropolis, it can be deduced that the meat may serve as a source of staphylococcal infection which may invariably affect the health of consumers as meat contributes a major part of food intake of people in the study area. Standard hygienic practices can therefore not be overemphasized in all stages of food production, marketing and service.

Keywords: Jerky, Kilishi, *Staphylococcus aureus*, *Escherichia coli*, enterotoxin

INTRODUCTION

Globally, it is not uncommon that food safety assurances have been of immense concern to public health agencies due to how food markets are regularly gaining global recognition and how foods consumed away from homes have significantly increased (El-hadedy and El-Nour, 2012). Retail of diversified minimally processed meat has increased rapidly in recent years because of busy lifestyles, their freshness, and convenience (Syne *et al*., 2013).

The processing of diversified ready-to-eat meat such as "kilishi" has grown as an important delicacy especially in Northern Nigeria, involves series of steps that begins right from slaughter to butchers table, cutting, slicing or shredding, addition of groundnut sauce (*kuli-kuli* sauce), onions, seasoning, and other spices as well as the drying of the meat. These processes aggravate potential proliferation of food-borne pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus*.

In recent years, significant upsurge in the patronization of these foods have grown all over the world due to their convenience and nutritive significance (Johler, 2013). And therefore, the consumption of *kilishi* may at any point in time bring about a threat to the health of the public due to the potential availability of food-borne pathogens that are capable of causing intoxications, several ailments and may cause death outbreaks (El-hadedy and El-Nour 2012). *S. aureus* is part of the usual bacteria inhabitants of the skin, present on a large part of the general population without resulting in any clinical manifestation (Smetzer and Benken,

***Corresponding Author:**
Musa Galadima
E-mail: waabenya@gmail.com

2013). However, it may also serve as an etiology to wide range of diseases in humans which ranges from mild skin infections to severe diseases such as food poisoning, septicemia, osteomyelitis, or endocarditis, (Francois, 2017). *S. aureus* has also been reported in many Veterinary clinics across the globe causing significant problems in milk-producing animals. Throughout the world, *S. aureus* is a serious etiologic agent of food-borne intoxications. These pathogenic bacteria can poison many foods including sun-cured meat and thereby producing various forms of intestinal toxins (Johler, 2013). *E. coli*, as an intestinal pathogen, is frequently becoming imperative to researchers concerned about the health of the public, especially the psychotropic strains of *E. coli* 0157:H7 that may exponentially proliferate on the prepared meat and vegetables at lower temperatures resulting in gastrointestinal pathologies in humans such as hemorrhagic colitis.[1-5]

Routine microbial detection of food-borne pathogenic organisms such as *S. aureus* and *E. coli* in *killishi* is usually carried out by techniques that are conventional using bacterial culture media, subsequently by detection of bacteria colonies by morphology and biochemical reactions. This technique is tedious and slows (Jayne *et al*., 2001). Besides, they bring about ambiguous outcomes due to a considerate amount of fields of isolates in some fast isolates. Speedy and delicate techniques of detection of food-borne bacteria are imperative for food safety (Mcvey and Chengappa, 2013). However, a significant amount of fine methods of detection have been developed using molecular approach. This confers sensitivity and rapidity; but the use of such techniques in developing countries has been arguably of immense challenge (Adzitey and Ali, 2013).[6-10]

Justification of the study

Despite the fact the Nigerian food and drug agencies have been keeping an eye on the standard of drugs and food vended in within the country; there are, however, no efficient systems to examine food-borne diseases in the country. Millions of cases of food-related poisonings and thousands of deaths (including children) from food-borneintoxications have been reported annually (WHO, 2017). The pathogenic bacteria (*S. aureus*, *E. coli*, and *Salmonella* species) were highly deemed to be behind those deaths. Deleterious microbes or chemicals have also been associated to more than 200 ailments, ranging from diarrhea to tumors (Adeleke, 2009). Akanji, (2017) on threats to safety of foods, discovered that a high majority of workers in Nigeria consume meals served away from their homes and as a result are susceptible to intoxications if these meat products are not handled safely. In the year 2015, there was an epidemic of poisoning from food in Ibadan, Nigeria, associated to *Salmonella typhimurium* in a spread of sandwich that took about 20 lives (Pepple, 2017). The report further noted that the sandwich was kept at room temperature until it was consumed the next day. A different case of food poisoning was reported among some families in Kano, Nigeria, after ingestion of yam flour. And further findings depicted the usage of certain preservatives that inflicted noxious consequence on the people who consumed it (Adeleke, 2009). There is also another record of sixty cases and three deaths from food-borne intoxication with notable gastrointestinal anomalies among people who consumed a certain food during a funeral service (Fatiregun *et al*., 2008). These deaths were associated to contamination of the food during serving, preservation, and processing.

In Nigeria, there are no organized systems for monitoring outbreaks of food-borne intoxication in humans, which explains the inability to ascertain the nature of the outbreaks of death after consumption of certain types of food in many parts of the country. and because of the predicament in depicting an unerring picture of the burden of food-borne disease, there is however some proof that these diseases add-up to general ill-health and death in the Nation, and there are evidences and confirmations of unhygienic food practices by food handlers and caterers in several situations.

Aim of the study

Isolation and identification of *E. coli* and *S. aureus* from sun-cured meat obtained from retail outlets within Sokoto Metropolis.

Objective of the study

The objectives of the study are as follows:

- To isolate and identify *S. aureus* and *E. coli* in sun-cured meat *kilishi* or *jerky* using the conventional qualitative technique.
- To determine the prevalence of *S. aureus* and *E. coli* in sun-cured meat *kilishi.*

MATERIALS AND METHODS

Study area

Sokoto State lies to the uttermost part of northwestern Nigeria, toward the conflux of the Sokoto river and the Rima river at latitude 13°05'N and longitude 05°15'E (Anon*.,* 2018a). It borders Kebbi state through the west and south, Zamfara lies acrossit's south and eastern borders and Niger republic to the north. Sokoto city is the modern day capital of Sokoto State and also the largest city of Sokoto State (Anon., 2018).

Materials

Nutrient agar, Peptone water, eosin methylene blue (EMB) agar, Microscope, Slide and cover slip, Grams reagents, Pipette, Autoclave, Weighing balance, Aluminum foil, Conical flask, Distilled water, Petri dishes, Wire loop, Hot plate, Bunsen burner, Slant bottle, Mannitol salt agar, Simmons citrate agar, Urease agar, Human plasma, 3% hydrogen peroxide, Incubator, and Sample bottle.

Sample collection

A total of 81 representative samples of sun-dried ready to eat meat *kilishi* were obtained from ten different locations within Sokoto metropolis. The areas include:

SAMPLE A: Sultan Abubakar road, SAMPLE B: Aliyu Magatakarda Fly-over, SAMPLE C: Tamaje Area, SAMPLE D: Gawun Nama, SAMPLE E: kwannawa Area, SAMPLE F: Arkilla Area, SAMPLE G: Rungin Sambo area, SAMPLE H: New Market, SAMPLE I: Mabera Area, SAMPLE J: Old Market.

Each representative sample collected from the vendors was wrapped exactly as sold to consumers, placed in another nylon bag and brought to the Veterinary Public Health Laboratory of the Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Nigeria, for bacteriological isolation and identification. Each sample was properly identified alphabetically with the names of the area in which the sample was collected.

Sample processing

One gram of *killishi* from each sample was measured and placed with 10 ml of prepared peptone water and homogenized using a stomacher. Serial dilution was conducted using distilled water to make a four-fold serial dilution. Ten test tubes containing 9 ml of distilled water were used, 1 ml of the prepared stock was placed in the first test tube and subsequently taken again and added to the next test tube until it gets to the last test tube, achieving maximum dilution required. The four-fold diluted sample was used to inoculate the culture media (Cheesbrough, 2006).[11,12,14-16]

Media preparation

EMB agar (37 g/l)

3.7 g of E.M.B agar was measured using a weighing balance and poured into a clean conical flask. 100 ml of distilled water was added to the agar, the brim of the conical flask was sealed tight with cotton wool, aluminum foil, and masking tape.[13] The conical flask was placed on a hot plate; the mixture was homogenized by gentle heating and swaying. The mixture was sterilized in an autoclave for 15 min sat 121°C. The media were then allowed to cool and poured gently into the Petri dishes (Cheesbrough, 2006).

Nutrient agar (28 g/l)

2.8 g of nutrient agar was taken and poured into a clean conical flask, 100 ml of distilled water was

poured to the flask, and the brim was sealed tight with cotton wool, aluminum foil and tape. The mixture was homogenized on a hot plate by gentle heating and swaying. The media were autoclaved for 15 min at a temperature of about 121°C. After the media have cooled, it was poured into the respective Petri dishes and slant bottles (Cheesbrough, 2006).

Peptone water (28 g/l)

2.8 g of peptone broth powder was measured using a weighing balance and poured into a clean conical flask; 100 ml of distilled water was added. The flask's brim was sealed tight with cotton wool, aluminum foil, and masking tape. The flask was placed on a hot plate and homogenized by gentle heating and swaying. The media were sterilized at 121°C for 15 min (Cheesbrough, 2006).

Mannitol salt agar (11.1 g/l)

11.1 g of Mannitol salt agar was poured into a conical flask and 100 ml of distilled water was added. The mixture was stirred gently on a hot plate until a homogeneous mixture was obtained. The mixture was autoclaved at 121°C for 15 min. The media was left to cool and poured into the Petri dishes. The media were allowed to solidify (Cheesbrough, 2006).

Isolation and identification

A sterile swab stick was placed in the fourth-fold dilution solution, the Petri dish was opened gently by tilting the cover a little bit, and the sticks were used to inoculate by forming streaks on the surface of the solidified media. The Petri dishes were placed in the incubator which was set at 37°C for 24 h. The morphological characteristics and presence or absence of growth on the media were recorded and the colony growths were transferred into prepared slant bottles containing nutrient agar and stored in the refrigerator. Colonies were taken for further subculture in mannitol salt agar and also for biochemical tests (coagulase and catalase tests), (Ochei & Kolhaktar, 2008).

Gram staining

A wire loop was used to prepare a smear on a clean glass slide and a flame was used to heat-fix the smear, crystal violet was poured and rinsed with water after 1 min. This was followed by the addition of Gram's iodine and also rinsed with water after 1 min. About 95% alcohol was used to decolorize the smear for about 5 min and immediately rinsed with distilled water. Safranin was finally added to counter stain for about 45 s, followed by rinsing with distilled water. The smear was allowed to dry and mounted on the microscope by adding oil immersion for viewing (Ochei and Kolhaktar, 2008).

Biochemical tests

Coagulase test

With the aid of a wire-loop, an emulsification of a suspected colony was carried out on a clean and grease-free slide with a drop of water. The suspensions were made on separate glass slides to serve as positive and negative control. A drop of human plasma was added on the positive control slide. Clumping of the colonies visible within 10 s depicts a positive coagulase reaction (Ochei and Kolhaktar, 2008).

Catalase test

A small amount of colony growth was transferred on a clean glass slide containing a drop of water with the aid of a wire-loop (properly flamed), it was emulsified to form a suspension.[13] One drop of 3% hydrogen peroxide was gently added on the suspension. Immediate evolution of oxygen bubbles was observed for a positive test while absence of these bubbles reflected a negative test (Ochei and Kolhaktar, 2008).

RESULTS

Table 1. Portrays 100% prevalence in samples collected from three locations J, K and F while location A presented a lower prevalence of 25%. The colonial morphology of each positive sample as shown in Figure 1; depicts a change in color of the media from a pink to a golden yellowish color. Figure 2; reflects a trend in the level of *Staphylococcus aureus* contamination in cultured samples from different locations in sokoto metropolis.

DISCUSSION

This study shows high occurrence of *S. aureus* with a prevalence of 83.9% whereas that of *E. coli* was 0.0%. This may be associated with the fact that both *E. coli* and *S. aureus are* mesophilic bacteria and may not survive the high temperatures used in the preparation of *kilishi* (Medvedova *et al*., 2012) However, due to the ubiquitous nature of *S. aureus* (Richardson, 2015), it can contaminate the meat through unhygienic practices during meat handling and packaging even after the meat has been grilled. *E. coli* being water coliform would require moisture to grow and therefore may not survive on the sun-dried ready-to-eat meat (Hiramatsu *et al*., 2005).

The prevalence of *S. aureus* (83.9%) obtained from this study is significantly higher than the values obtained by Okwori *et al.*, (2009) from some cities within Northern Nigeria (including Sokoto) with 40% prevalence. Ribah and Manga, (2018), also isolated *S. aureus* from *kilishi* as well as the environmental ubiquity of *S. aureus* from *kilishi* within Kebbi and Sokoto state and obtained a prevalence of 15.51%. The two studies highlighted above both attributed their source of *S. aureus* contamination in *kilishi* to low level of hygiene by handlers of the meat as well as the environmental ubiquity of *S. aureus.* Table 1 shows the prevalence of positive isolates obtained from different locations within Sokoto metropolis reflecting a high occurrence in four areas with 100%, 90%, 87.5%, and 80% prevalence, perhaps due to the unsanitary and unhygienic practices observed during the processing of *kilishi* in these areas by meat handlers and water suppliers. However, other areas had only 25% prevalence perhaps due to the

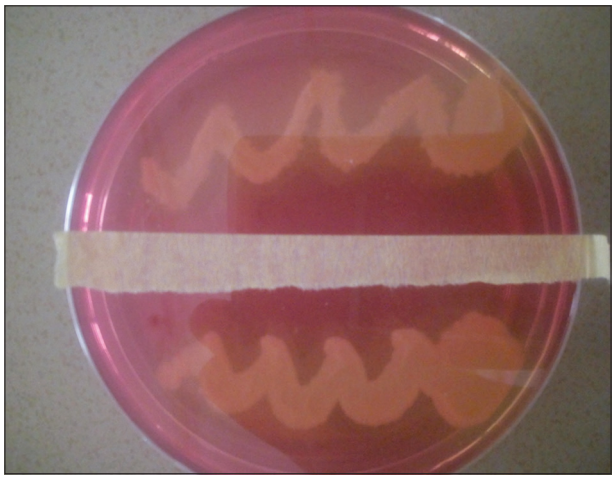

Figure 1: *Staphylococcus aureus* colonies change the color of Mannitol Salt agar from pink to yellowish color.

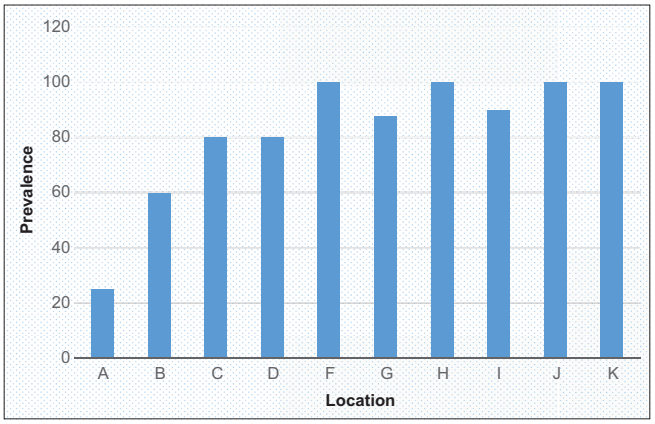

Figure 2: Sokoto Metropolis.

Table 1: The values obtained from isolation and identification of isolates from sun-dried ready to eat meat obtained from retail outlets in Sokoto metropolis

S. No	Location	Number of samples collected	Total culture positive	Culture positive for *Staphylococcus aureus*	Culture positive for *Escherichia coli*	Culture with mixed positive	Prevalence(%)
1.	A	8	2	2	-	-	25
2.	B	5	3	3	-	-	60
3.	C	5	4	4	-	-	80
4.	D	10	8	8	-	-	80
5.	F	8	8	8	-	-	100
6.	G	8	7	7	-	-	87.5
7.	H	10	10	10	-	-	100
8.	I	10	9	9	-	-	90
9.	J	7	7	7	-	-	100
10.	K	10	10	10	-	-	100
TOTAL		81	68	68	-	-	83.9

hygienic measures put in place by the meat handlers during the processing of the meat in these locations. These measures include the use of customized nets that prevents the entrance of flies and other contaminants when the meat is dried, also, the use of nylon gloves during meat packaging was also observed (Raji, 2018).[17-21]

It is recommended that standard hygienic practices should be employed during production and after processing of sun-cured meat *kilishi*. Therefore, public health workers and food regulation agencies should accurately strategize to prevent possible upsurge of food-borne diseases by frequently making sure that safe and hygienic conditions in the production, storage, and handling of *kilishi* are employed.

REFERENCES

1. El-hadedy D, El-Nour SA. Identification of *Staphylococcus aureus* and *Escherichia coli* isolated from Egyptian food by conventional and molecular methods. J Genet Eng Biotechnol 2012;10:129-35.
2. Francois P. Evolution of *Staphylococcus aureus*. Genomic Research Laboratory, Geneva University Hospital; 2017. Open Access Government. Available from: http://www.opengovernmentaccess.org. [Last accessed on 2018 May 24].
3. World Health Organization. Available from: http://www.who.int/news-room/fact-sheets/detail/e-coli. [Last accessed on 2018 Jul 26].
4. Fatiregun AA, Oyabode OA, Oladokun L. Investigation of an outbreak of food poisoning in a resource-limited setting. Trop J Health Sci 2010;17:1177-4153.
5. Smetzer MS, Beenken KE. Veterinary Microbiology Textbook. 2nd ed., Vol. 8. Kayser Publishers; 2013. p. 25-56.
6. Cheesbrough M. Laboratory Practice in Tropical Countries Part II. Cambridge: Cambridge University Press; 2006. p. 71-3.
7. McVey DS, Kennedy M, Chengappa MM. Veterinary Microbiology Textbook. 3rd ed. Hoboken, New Jersey: Wiley-Blackwell; 2013. p. 62-75.
8. Ochie J, Kolhaktar A. Medical Laboratory Sciences, Theory and Practical. 1st ed. New York: Tata McGraw-Hill Education Pvt. Ltd.; 2008. p. 811-32.
9. Okwori AE, Obioha C, Olabode AO, Etukudo NS, Lugos MD, Turay AA. Bacteriology of dried meat kilishi hawked in Some Northern Nigerian citties. Niderian J Biotechnol 2009;20:1-7.
10. Pepple N. Environment and food poisoning: Food safety knowledge and practicen among food vendors in Garki, Abuja, Nigeria. J. Health Educ Res Dev 2018;5:217.
11. Raji AI. Bacteriological quality of dried sliced beef kilishi sold in Illorin Metropolis. J Appl Sci Environ Manag 2006;10:93-6.
12. Ribah MI, Manga SS. Prevalence of *S. aureu*s in some street vended ready-to-eat meat products in Birnin Kebbi metropolis: A Potential food safety threat. J Environ Toxicol Public Health 2018;3:25-9.
13. Olsvik O, Wasteson Y, Lund A, Hornes E. Pathogenic *E. coli* found in food. Int J Food Microbiol 1991;12:103-13.
14. Richardson LA. From friend to foe: Toxicity trade-offs govern *Staphylococcus aureus* infection severity. PLoS Biol 2015;13:E1002230.
15. Jayne DL, Roberson JR, Fluinger F. Use of EMB agar to differentiate *E. coli* other Gram-negative Mastititis Pathogen Ivet Diagn Invest 2001;13:273-5.
16. Syne S, Ramsubhag A, Adesiyun AA. Microbiological Hazard analysis of ready-to-eat meat processed at food plant in Trinidad, West Indies. Infect Ecol Epidemiol 2013;3:20450.
17. Adzitey F, Huda N, Ali GR. *Staphylococcus aureus*. 3 Biotech 2013;3:97-107.
18. Medvedova A, Valik L. *Staphylococcus aureus* Characterization and Quantitative Growth Description in Milk Cheese Production. London: IntechOpen; 2012.
19. Anonymous. 2018a. http://www.sokotoen.wikipedia.org. [Last accessed on 2018 Jul 25].
20. Anonymous. Sokoto City; 2018b. Available from: http://www.britannica.com/place/sokoto-Nigeria. [Last accessed on 2018 Jul 25].
21. Hiramatsu R, Matsumuto M, Sakae K, Miyazaki Y. Ability of Shiga-toxin producing *Escherichia coli* to survive in a desiccation mode system in dry foods. Appl Environ Microbiol 2005;71:6657-63.