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Isolation and Identification of Bacteria Associated with Suya Sold in Makurdi Metropolis

RESEARCH ARTICLE

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ABSTRACT

Isolation and identification of bacteria associated with suya sold in Makurdi metropolis were investigated. A total of 10 samples were collected from different selling points in Makurdi and cultured using Nutrient, Mac-Conkey and Salmonella Shigella culture medium. In this study a total number of nine (9) bacteria isolates comprising of four (4) genera of gram positive bacteria; *Bacillus species*, *Staphylococcus aureus*, *Micrococcus* and *Streptococcus species* and five (5) genera of gram negative bacteria; *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella species*, *Proteus species* and *Klebsiella pneumonia* were isolated. The findings of this study expressed issue of serious public health concern as most of the isolates are known to cause various health problems. The study recommends adequate preventive measures to avert the contamination of suya-meat for public consumption.

Keywords: Suya meat, Bacteria contamination.

INTRODUCTION

Bacteria are the oldest, structurally simple, and the most abundant forms of life on earth (Muhammad, 2012). They are also the only organisms with prokaryotic cellular organization. Bacteria are ubiquitous on Earth, and live everywhere eukaryotes do. Many of the different extreme environments in which bacteria are found would be lethal to any other form of life. Bacteria live in hot springs that would cook other organisms, hyper-saline environments that would dehydrate other cells, and in atmospheres rich in toxic gases like methane or hydrogen sulfide that would kill most other organisms.

Bacteria can be characterized properly only when they are grown on a defined medium because the characteristics of these organisms often change, depending on their growth conditions (Muhammad, 2012).

Bacteria cause many diseases in humans, including cholera, leprosy, tetanus, bacterial pneumonia, whooping cough, diphtheria and lyme disease. Members of the genus Streptococcus are associated with scarlet fever, rheumatic fever, pneumonia, and other infections. Tuberculosis

(TB), another bacterial disease, is still a leading cause of death in humans (CDC, 2010). Some of these bacterial diseases are dispersed in food or water, including typhoid fever, paratyphoid fever, and bacillary dysentery (CDC, 2010).

Suya is a popular, traditionally processed, ready to eat Nigerian meat product, which may be served or sold along streets, in club houses, at picnics, parties, restaurants and within institutions (Igene and Mohammed, 1983). It is a mass consumer fast food. Its preparation and sales along streets are usually not done under strict hygienic condition because, they are still done locally (Uzeh *et al*., 2006).

There exist different types of meat products ranging from the industrially processed corned beef, ham, bacon sausage as well as the indigenous Nigerian traditionally processed ready-to-eat meat product such as "balangu" (roasted meat), "kilishi, dan-bu-nama, tsire, jirga, ndako, banda, suya and many more" (Yunusa, 2000).

Suya is a roasted boneless meat of either mutton, beef, or goat that is cooked around a glowing fire in which the meat pieces are stacked on wooden sticks and spiced with peanut cake, spices, vegetable oil, salt or other flavourings. The prepared suya when being sold are usually packaged in newspapers and sometimes in cellophane or nylon bags. According to Uzeh *et al*., 2006, most of the stages of suya preparation, materials used in its preparation and packaging, the handlers and the surrounding environment can serve as source of contaminants to the meat product.
Micro-organisms that occur in suya-meat and other meat products most times are responsible for food borne illnesses. These micro-organisms are *Bacillus species, Clostridium species, Escherichia coli, Salmonella species, Shigella species, Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas, Lactobacillus species, Micrococcus, Mycobacterium species,*etc. Salmonella may be transferred from raw meat to cooked meat by hands, surfaces or utensils (Moshood *et al*., 2012; Uzeh *et al*., 2006).

Suya-meat is vastly sold and consumed within the Makurdi area in the evening and at night and this meat product is likely to be contaminated and contain various pathogenic bacteria. It is for this reason, this research work seeks out to evaluate, isolate and identify the type of bacteria associated with suya-meat sold in Makurdi.

MATERIALS AND METHODS

Study Area

This study was carried out in Makurdi; the capital of Benue State.The city is located in central Nigeria and lies on the south bank of the Benue River and holds the base for the Nigerian Air Force's MiG 21 and SEPECAT Jaguar aircraft squadrons. As of 2007, Makurdi had an estimated population of 500,797.Makurdi is situated at 7.74° North latitude, 8.51° East longitude and 104 meters elevationabove the sea level.
The town is a local trade center for the yams, sorghum, millet, rice, cassava, shea nuts, palm oil, peanuts (groundnuts) and soybeans raised by the people of the surrounding area.
The time zone in Makurdi is Africa/Lagos, morning Sunrise at 06:18 and Evening Sunset at 18:37.

Preparation of Culture Media

Nutrient Agar

Nutrient agar is a basic culture medium used as a slant medium to culture pathogens isolated on carbohydrate containing source. To prepare nutrient agar, 7g of the powder was dissolved in 250ml of distilled water in a flat-bottomed flask. The solution was autoclaved at 121˚C for 15 minutes. It was then poured aseptically into petri dishes and allowed to cool and solidify.

Mac-Conkey Agar

Mac-Conkey agar is a differential and low selectivity medium used to distinguish lactose from non-lactose fermenting bacteria. To prepare Mac-Conkey agar, 13g of the powder was dissolvedin 250ml of distilled water in a flat-bottomed flask and then autoclaved at 121˚C for 15 minutes. It was then poured aseptically into petri dishes and allowed to cool and solidify.

Blood Agar

Blood agar is an enriched media from nutrient agar where whole blood is used. To prepare blood agar, 7g of the powdered nutrient agar was dissolved in 250ml of distilled water in a flat-bottomed flask. The solution was autoclaved at 121˚C for 15 minutes. It was allowed to cool to the temperature of 40˚C - 45˚C. Whole blood was then added in the proportion of 2ml:18ml of blood and nutrient agar respectively. It was then poured aseptically into petri dishes and allowed to solidify.

Salmonella Shigella Agar (SSA)

Salmonella shigella agar (SSA) is a selective and differential medium used to isolate salmonella and shigella species. The medium was prepared by dissolving 15.75g of the commercially made powder in 250ml of distilled water in a flat-bottomed flask. It was not autoclaved but heated with frequent agitation to simmer (just below the boiling point). This was poured aseptically into petri dishes and allowed to solidify.

Sample Collection

The samples were collected from 10 randomly selected selling points within Makurdi metropolis; Gyado Villa, Steam Fast Restaurant Villa Suites, 18 plus bar, Jackies Lounge, North Bank SRS Junction, Ostrich Bakery High Level, Balcony Bar, Quararafa Quarters, Wadata Market and NYSC Secretariat. This gave a total of 10 samples to work with in the laboratory for this project work.

The samples were collected in aluminum foil and transported to a place where it was refrigerated at 4˚C till the next day, since the suya-meat is usually sold late in the evening. By day time, the samples were then unopened and safely transported to the laboratory for analysis within 24 hours from the time of collection of the sample.

Sample Preparation

The collected samples were allowed to come to room temperature and then sliced into thin smaller pieces using a sterile blade. 1gram from the blended sample was then placed in 10ml of distilled water and mixed thoroughly to give a good homogenate. Several dilutions were achieved for each prepared samples using 1ml from stock homogenate and 9ml of distilled water for the serial dilution experiment up to three times. This was carried out in order to obtain less bacterial load. The final homogenate for each sample was then cultured on prepared Nutrient, Mac-Conkey and Salmonella Shigella culture medium. The culture was done in the petri dishes by the spread plate method using 0.1ml of the sample from the final dilutions and afterward, the inoculated plates were allowed to set firmly for five (5) minutes and then incubated at 37˚C for 24 hours. The inoculated plates were then removed, the colonies were counted using the colony counter and the growth pattern on the dishes was observed and recorded.

Isolation of Colonies

Distinct colonies were then sub-cultured on freshly prepared Nutrient Agar in order to obtain discrete colonies for isolation and it was incubated at 37˚C for 24 hours.

Identification of Isolates

This was done using the physically and observable characteristics or the morphology of the bacterial growth such as type of growth, colonial appearance, elevation, form, edge and colour of growth as described by Christopher and Bruno (2003) and that of Bergey and John (1993). The isolates from the subculture were then used for Gram staining, Catalase test and hemolysis test for further identification.

Gram Stain Procedure

A drop of distilled water was placed on a clean glass slide. Using a sterile wire loop, a loop of the growth was picked and smeared on the glass slide in the distilled water and left to air dry. Using the Bunsen burner, the glass slide was heat-fixed by gently passing it over the flame for about four (4) times. The glass slide was then placed on the staining bridge and covered with crystal violet for 30 seconds. It was then rinsed with distilled water and Lugols iodine was added to the slide and allowed to stand for 30 seconds after which it was rinsed with distilled water. The slide was then held at an angle of 45˚ to the horizontal and washed with acetone alcohol until no further colour was seen dripping from the slide. The slide was rinsed with distilled water and then counter-stained with neutral red for 1 minute and after which the slide was rinsed with distilled water and allowed to air dry. It was then viewed under the microscope using the oil immersion objective. The bacteria were identified as gram positive or gram negative based on the microscopic view as appearing blue for gram positive or red/pink for gram negative.

Catalase Test

This was done using Hydrogen peroxide. A loop full of the isolated colonies was picked and suspended in the hydrogen peroxide. Formation of gas bubbles indicated a positive result. This test indicates the production of the enzyme catalase by the bacteria which breaks down hydrogen peroxide to produce water and oxygen gas.

Hemolysis Test

This was done using Blood agar. The bacteria isolates were sub-cultured on freshly prepared blood agar plates. The ability of the bacteria isolates to lyse red blood cells was observed and recorded as Alpha, Beta or Gamma.

RESULTS AND DISCUSSION

Table 1: Morphological, gram stain and biochemical properties of bacteria isolated from suya-meat obtained at Gyado Villa selling point

S/N	Colonial morphology			Gram Reaction	Microscopic Examination	Biochemical Tests		Probable Organism
	Elevation	Colour	Shape			Catalase	Hemolysis	
1	Raised	White	Circular	+	Long rods	+	Beta	*Bacillus species*
2	Raised	White	Irregular	-	Rods	+	Beta	*Pseudomonas aeruginosa*
3	Flat	Golden	Circular	+	Cocci	+	Beta	*Staphylococcus aureus*

Table 2: Morphological, gram stain and biochemical properties of bacteria isolated from suya-meat obtained at Villa Suite selling point

S/N	Colonial morphology			Gram Reaction	Microscopic Examination	Biochemical Tests		Probable Organism
	Elevation	Colour	Shape			Catalase	Hemolysis	
1	Convex	Pink on MacConkey agar	Circular	-	Short rods	+	Alpha	*Escherichia coli*
2	Convex	Creamy pink	Circular	-	Short rods	+	Beta	*Salmonella species*

Table 3: Morphological, gram stain and biochemical properties of bacteria isolated from suya-meat obtained at 18 Plus Bar selling point

S/N	Colonial morphology			Gram Reaction	Microscopic Examination	Biochemical Tests		Probable Organism
	Elevation	Colour	Shape			Catalase	Hemolysis	
1	Convex	Creamy pink	Circular	-	Short rods	+	Beta	*Salmonella species*
2	Raised	White	Circular	+	Long rods	+	Beta	*Bacillusspecies*
3	Raised	Yellow	Irregular	+	Paired cocci	+	Gamma	*Micrococcus*
4	Flat	Golden	Circular	+	Cocci	+	Beta	*Staphylococcus aureus*

Table 4: Morphological, gram stain and biochemical properties of bacteria isolated from suya-meat obtained at Jackies Lounge selling point

S/N	Colonial morphology			Gram Reaction	Microscopic Examination	Biochemical Tests		Probable Organism
	Elevation	Colour	Shape			Catalase	Hemolysis	
1	Raised	White	Irregular	-	Rods	+	Beta	*Pseudomonas aeruginosa*
2	Convex	Pink on MacCon key agar	Circular	-	Short rods	+	Alpha	*Escherichia coli*
3	Convex	Creamy pink	Circular	-	Short rods	+	Beta	*Salmonella species*
4	Raised	White	Circular	+	Long rods	+	Beta	*Bacillus species*
5	Flat	Gray	Irregular	-	Rods	+	Alpha	*Proteus species*
6	Convex	Creamy	Circular	-	Rods	+	Alpha	*Klebsiella pneumonia*

Table 5: Morphological, gram stain and biochemical properties of bacteria isolated from suya-meat obtained at North Bank, SRS Junction selling point

S/N	Colonial morphology			Gram Reaction	Microscopic Examination	Biochemical Tests		Probable Organism
	Elevation	Colour	Shape			Catalase	Hemolysis	
1	Convex	Pink on MacCon key agar	Circular	-	Short rods	+	Alpha	*Escherichia coli*

Table 6: Morphological, gram stain and biochemical properties of bacteria isolated from suya-meat obtained at Ostrich Bakery, High Level selling point

S/N	Colonial morphology			Gram Reaction	Microscopic Examination	Biochemical Tests		Probable Organism
	Elevation	Colour	Shape			Catalase	Hemolysis	
1	Convex	Pink on MacConkey agar	Circular	-	Short rods	+	Alpha	*Escherichia coli*

Table 7: Morphological, gram stain and biochemical properties of bacteria isolated from suya-meat obtained at Balcony selling point

S/N	Colonial morphology			Gram Reaction	Microscopic Examination	Biochemical Tests		Probable Organism
	Elevation	Colour	Shape			Catalase	Hemolysis	
1	Convex	Creamy pink	Circular	-	Short rods	+	Beta	*Salmonella species*
2	Flat	Golden	Circular	+	Cocci	+	Beta	*Staphylococcus aureus*
3	Convex	Pink	Circular	-	Short rods	+	Alpha	*Escherichia coli*

Table 8: Morphological, gram stain and biochemical properties of bacteria isolated from suya-meat obtained at Quararafa Quarters selling point

S/N	Colonial morphology			Gram Reaction	Microscopic Examination	Biochemical Tests		Probable Organism
	Elevation	Colour	Shape			Catalase	Hemolysis	
1	Flat	Gray	Irregular	-	Rods	+	Alpha	*Proteus species*
2	Raised	White	Circular	+	Long rods	+	Beta	*Bacillus species*

Table 9: Morphological, gram stain and biochemical properties of bacteria isolated from suya-meat obtained at Wadata Market selling point

S/N	Colonial morphology			Gram Reaction	Microscopic Examination	Biochemical Tests		Probable Organism
	Elevation	Colour	Shape			Catalase	Hemolysis	
1	Raised	White	Circular	+	Long rods	+	Beta	*Bacillus species*
2	Flat	Milky	Circular	+	Cocci in short chains	-	Alpha	*Streptococcus species*
3	Convex	Creamy	Circular	-	Rods	+	Alpha	*Klebsiella pneumonia*
4	Flat	Gray	Irregular	-	Rods	+	Alpha	*Proteus species*
5	Convex	Creamy pink	Circular	-	Short rods	+	Beta	*Salmonella species*

Table 10: Morphological, gram stain and biochemical properties of bacteria isolated from suya-meat obtained at NYSC Secretariat selling point

S/N	Colonial morphology			Gram Reaction	Microscopic Examination	Biochemical Tests		Probable Organism
	Elevation	Colour	Shape			Catalase	Hemolysis	
1	Flat	Golden	Circular	+	Cocci	+	Beta	*Staphylococcus aureus*
2	Convex	Pink	Circular	-	Short rods	+	Alpha	*Escherichia coli*
3	Raised	Yellow	Irregular	+	Paired cocci	+	Gamma	*Micrococcus*

Table 11: Frequency of occurrence of the bacteria isolated from suya-meat at 10 different selling locations in Makurdi metropolis

S/N	Bacteria Isolates	Frequency	Percentage (%)
1	*Bacillus species*	5	16.67
2	*Pseudomonas aeruginosa*	2	6.67
3	*Staphylococcus aureus*	4	13.33
4	*Escherichia coli*	6	20.00
5	*Salmonella species*	5	16.67
6	*Micrococcus*	2	6.67
7	*Proteus species*	3	10.00
8	*Klebsiella pneumonia*	2	6.67
9	*Streptococcus species*	1	3.33
Total		30	100

The results show that Jackies Lounge selling point had the highest number of isolates with six (6) different genera of bacteria (Table 4). Five (5) genera of bacteria were isolated from Wadata Market selling point (Table 9), while four (4) different genera of bacteria were isolated from 18 Plus Bar selling point (Table 3). Three (3) genera of bacteria were isolated from Gyado villa, Balcony and NYSC Secretariat selling points (Tables 1, 7 and 10 respectively). Two (2) genera of bacteria were also isolated from Steam Fast and Quararafa Quarters selling points (Tables 2 and 8 respectively). One (1) genus of bacteria was isolated from both North Bank and Ostrich Bakery selling points (Tables 5 and 6 respectively).

From Table 11, *Escherichia coli* had the highest frequency of 6 (20.00%). Secondly, *Bacillus species* and *Salmonella species* had the frequency of 5 (16.67%). *Staphylococcus aureus* is third with the frequency of 4 (13.33%). *Proteus species* had a frequency of 3 (10.00%). *Pseudomonas aeruginosa*, *Micrococcus* and *Klebsiella pneumoniae* all had a frequency of 2 (6.67%), while *Streptococcus species* is least with a frequency of 1 (3.33%).

The bacteria isolates were identified by their colonial morphology, gram reaction, microscopic view and biochemical tests using the criteria given by Christopher and Bruno (2003) and that of Bergey and John (1993) as *Bacillus species*, *Pseudomonas species*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella species*, Micrococcus, *Proteus species*, *Klebsiella pneumoniae* and *Streptococcus species*.

A total number of nine (9) bacteria isolates comprising of four (4) genera of gram positive bacteria; *Bacillus species*, *Staphylococcus aureus*, *Micrococcus* and *Streptococcus species* and five (5) genera of gram negative bacteria; *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella species*, *Proteus species* and *Klebsiella pneumonia* were isolated.

The observed results from the Suya samples analyzed, were in line with reports by Moshood *et al*., (2012) and that of Egbebi and Seidu (2011) who reported similar genera of bacteria isolated from Balangu (roasted meat product) sold in Bauchi and suya sold in Ado/Akure respectively.

Similarly, findings in this project are in correspondence with the findings of Eke *et al*., (2013); the incidence of *Staphylococcus aureus*, *Escherichia coli* and *Bacillus species* as the incidence of *Staphylococcus aureus* observed in this study agrees with the assumption that it is commonly found on hands, skin and clothing of suya sellers.

The number of bacteria genera isolated from the samples could be attributed to the fact that suya-meat contains an abundance of nutrients required for growth of bacteria in adequate quantity making the product (suya-meat) a house for the pathogens. The significant rise in bacteria load could be as a result of additional contamination arising from the spices used resulting from poor handling of spices when being prepared. Contaminations could also have arisen from slaughter procedures from the slaughter house/abattoir, and from hands and clothing of the sellers.

CONCLUSION

The findings from this research work are of serious public health concern as most of the isolates are known to cause various health problems. *Escherichia coli* which is a normal flora of the human intestinal tract can be pathogenic and can be an important cause of enteric illness when dislodged from its normal habitat. *Staphylococcus aureus* and *Bacillus species* among others have been known to be implicated in food borne illnesses. The presence of *Salmonella species* is of great importance because it is known as the pathogen that causes typhoid fever.

The possible source of contamination of the suya-meat should be taken into consideration as most of these organisms such as *Escherichia coli*, *Salmonella species* and *Streptococcus species* are known to be transmitted via faecal-oral route. This then suggest contact of the suya-meat with faeces by means of hands, the water used in washing the meat, from the cloth of the handler and/orcontaminated materials. *Pseudomonas aeruginosa* is widely spread in soil and water, it may have been introduced in the spices used. *Staphylococcus aureus* can be from the nose of the handlers.*Bacillus species* are known to be spore formers (particularly *Bacillus cereus*) with heat resistant spore and can be found in the air and the spices used. Flies can also cause contamination by continuous contact with the meat product.

REFERENCES

[1]. Bergey, D. and John, G. H. (1993). Bergey's Manual of Determinative Microbiology, 9th edition. Published by Lippinkott, Williams, and Wilkins. Pg1 – 9.

[2]. Center for Disease Control (CDC) (2010). Guide book on antimicrobial chart.

[3]. Christopher, K. and Bruno, E. (2003). Identification of bacterial species. Pages 103-130.

[4]. Egbebi, A. O. and Seidu, K. T. (2011). Microbiological evaluation of Suya (dried smoked meat) sold in Ado and Akure, South West Nigeria. *European Journal of Experimental Biology*. 1 (4): 1 – 5.

[5]. Eke, S. O., Irabor, J. I., Okoye, M., Aitufe, O. F. and Ekoh, S. N. (2013). The microbial status of commercial 'suya' meat products in Ekpoma, Edo, Nigeria. *International Journal of Community Research*. 2(1): 18-21.

[6]. Igene, J. O. and Mohammed I. D. (1983). Consumer's attitude towards 'suya meat' product. Ann.Borno. Pg1.

[7]. Moshood, A. Y., Tengku, H. A. and Ibrahim, H. (2012). Isolation and identification of bacteria associated with balangu (roasted meat product) sold in Bauchi, Nigeria. *Journal of Pharmacy*. 2 (6). 38 – 48.
[8]. Muhammad, I. (2012). Bacterial cell structure. KMU Publishers. Pg3 – 22.
[9]. Uzeh, R. E., Ohenhen R. E. and Adeniji O. O. (2006). Bacterial Contamination of Tsire-Suya, a Nigerian Meat Product. *Pakistan Journal of Nutrition*. 5 (5): 458-460.
[10]. Yunusa, A. I. (2000). Curing and smoking meat for home food preservation. Meat science press. Pages 175 – 175.