

Bacteriological Quality Assessment of Meat Pie Sold in Yenagoa Metropolis, Nigeria

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Abstract

This study assessed the bacteria quality of meat pie sold in Yenagoa metropolis, Nigeria. Triplicate samples were collected from seven locations. Analysis was carried out following standard microbiological procedures. Results showed that total heterotrophic bacteria counts, total coliform and total *Staphylococci* counts ranged from 2.52 – 10.73 x 10³ cfu/g, 1.57 – 6.93 x10² cfu/g and 3.767 – 7.600 x10¹ cfu/g. Analysis of variance showed that there were no significance differences (P>0.05) among the various locations for each of the bacteria parameters except for total heterotrophic bacteria count. The total heterotrophic bacteria counts and bacteria of the *Enterobacteriaceae* were within satisfactory level of 10³ and 10² respectively for ready to eat food as specified by Center for Food safety. Bacteria tentatively identified include *Staphylococcus aureus*, *Escherichia coli*, *Micrococcus*, *Bacillus*, *Proteus*, *Pseudomonas*, *Cornebacteria* species. Of all the bacteria isolates, *Staphylococcus aureus* and *Cornebacteria* species had the highest and least occurrence frequency of 48.8% and 5.2% respectively. This suggests that meat pie sold in Yenagoa is mostly contaminated due to handling processes. The quality with regard to bacteria isolates could be improved following quality control protocol in food.

Keywords: Food; Meat Pie; Ready-To-Eat Food; Snacks

Introduction

Food are substances needed by the body for effective functioning of the various system and development [1-4]. Foods are classified according to its readiness to consumption. One of the most notable food classes is ready to eat food including fruits, snacks, cooked foods [1]. Among them snacks are widely consumed in many places in Nigeria including rural and urban settings. According to Hassan., *et al.* [5], Snack is a “high-energy” food and they include doughnut, egg roll, sausage, chin chin, meat pie, puff puff, fried meat or fried and African dish like mio-moi and bean cake etc. Oranusi., *et al.* [6] also listed sandwiches, kebabs, hotdogs, meat pie, salad, doughnuts, takeaway foods and bakery products as ready to eat foods. Like other food sources, snacks have carbohydrate, fat and oil, protein and water as constituent [5].

In the recent times ready-to-eat foods are regarded as potentially hazardous, because such foods can support the growth of pathogens [6]. This is because these foods are stored or preserved at ambient temperature that could encourage the growth of psychrophilic microbes. Also the handling processes are typically carried out in non-aseptic manner especially in the rural area in developing countries like Nigeria. According to Oranusi., *et al.* [6], due to extensive handling involved, they are usually prone to contamination/cross contamination from soil, water, air, storage/distribution facilities, environment and human activities (food handlers and vendors).

Food borne infections and illness is a major international health problem with consequent economic reduction [7]. Food is an essential instrument for health promotion and disease prevention [8]. Food contamination is a major problem associated with food and snacks hawking in our society today [9]. In recent times. Food borne illness is becoming an alarming concern involving a broad range of diseases,

caused by many bacterial, viral, parasitic and chemical contaminants [10]. For instance, diseases such as brucellosis, typhoid fever, diphtheria and tuberculosis have from time to time, been associated with poor pasteurization of milk and meat [11]. Several others have been linked to poor hygienic condition and water used for drinking and other domestic purposes.

The problems of food safety in industrialized world differ considerably from those faced by developing countries [12]. Meat or fish pie is a savory pie that contains filled minced meat or fish and other savory ingredients [13]. Meat pie is one of the commonly consumed snacks by students and other citizens in some part of Nigeria. The fish or meat used for their preparation has the tendency to spoil easily due to the activities of spoilage microbes found in it. These microbes are typically introduced into the meat pie through materials used in their preparation including water and utensils.

Several studies have been carried out on microbes associated with meat pie and other snacks. Some of these studies include moulds associated with four selected snacks sold in Nnamdi Azikiwe University, Awka and its environs [9], snacks sold in Rivers State University of Science and Technology, Port Harcourt, Rivers state [11], fast food restaurants in Benin City, Edo State [14], puff puff sold on the streets of Lagos state [5], snacks sold in fast food shops in Ota, Ogun State [6], street-vended foods and ready-to-eat vegetables in some Nigerian cities [12], street vended foods in Port Harcourt metropolis, Rivers State [8], snacks sold in abia metropolis, Abia state [13], street foods along Lokoja-Abuja Express Way, Lokoja [15]. Hence this study was designed to assess the bacteriological quality of meat pie sold in Yenagoa metropolis, Nigeria.

Materials and Methods

Field Sampling

The meat pie was bought from vendors in different location in Yenagoa metropolis including Edepie, Igbogene, Akenfa, Agudama-Epie, OpoloTombia, Biogbolo and Amarata in Bayelsa state capital. The sample were obtained in triplicate from each vendors. The meat pie was packaged in sterile Ziploc bag and analysis was carried out approximately three hours after collection.

Sample preparation

About 20 grams of the sample was blended (BLG-450, Binatone, Nigeria) in 180 ml of sterile water. The blender was washed and rinsed sterile and de-ionized water prior to re-use.

Enumeration bacteria counts

The populations of microorganisms in the samples were enumerated using serial dilution pour plate method of Pepper and Gerba [16], Benson [17]. Nutrient, Mannitol Salt Agar, MacConkey agar cooled in water bath at 45°C was poured into the plates. Similarly, prepared Salmonella-Shigella Agar (via boiling) was also cooled in the water bath before pouring into the plates meant for Salmonella-Shigella counts. The plates were stirred and allowed to solidify before incubation at 37°C for 24 - 48 hours. The resultant colonies was counted and expressed as colony forming unit per gram of the meat pie samples.

Tentative identification of the bacteria isolates

Colonies that grew on the different agar media was streaked in Levine's eosin Methylene Blue (EMB) Agar and incubated at 37°C for 24 hours. The presence of small nucleated colonies with greenish metallic sheen indicates *E. coli*, while absence of the sheen with large nucleated colonies indicate *Enterobacter* sp. [16,17]. Growth on the Nutrient Agar was streaked in Mannitol Salt Agar and incubated inverted at 37°C for 24 hours. The presence of yellow pigment indicates *Staphylococcus aureus*. Also, the bacterial pure culture were streaked in Blood Agar, the presence of hemolytic properties indicates *Streptococcus* species. While growth with swarming characteristics suggests *Proteus* species. Biochemical test including gram reaction, citrate, urease, catalase, oxidase, Indole, coagulase, motility, methyl red was carried out using the scheme of Cheesbrough [18] and Benson [17]. The resultant microbial species from biochemical test in this study was compared with those of known taxa using scheme of Cheesbrough [18] and Bergey's Manual of Determinative Bacteriology by Holt, *et al* [19].

Statistical Analysis

Statistical Package for Social Sciences software version 20 was used for the statistical analysis of the various bacteria counts. Descriptive statistics i.e. mean and standard deviation values were expressed. A one-way analysis of variance was carried out at $P = 0.05$ and Tukey HSD Test was used for Post hoc. The percentage occurrence of the bacteria isolates were plotted using Microsoft excel.

Results

The bacteria population in meat pie sold in Yenagoa metropolis, Bayelsa state is presented in Table 1. The total heterotrophic bacteria counts, total coliform and total *Staphylococci* counts ranged from $2.52 - 10.73 \times 10^3$ cfu/g, $1.57 - 6.93 \times 10^2$ cfu/g and $3.767 - 7.600 \times 10^1$ cfu/g. There was no significance difference ($P > 0.05$) among the various locations for each of the bacteria parameters except for total heterotrophic bacteria count. Furthermore, in the total heterotrophic count the sources of the observed difference ($P < 0.05$) were mainly from Location A, E and F. Also, Salmonella-Shigella counts were not detected from any of the meat pie samples.

Location	Total Heterotrophic Bacteria, $\times 10^3$ cfu/g	Total coliform, $\times 10^2$ cfu/g	Total Staphylococci counts, cfu/g	Samonella-Shigella, Log cfu/g
A	$2.52 \pm 1.27a$	$3.94 \pm 3.03a$	$60.00 \pm 37.80a$	ND
B	$5.67 \pm 3.55ab$	$6.93 \pm 1.80a$	$62.67 \pm 26.76a$	ND
C	$5.60 \pm 1.70ab$	$2.57 \pm 1.07a$	$64.67 \pm 24.79a$	ND
D	$6.24 \pm 3.28ab$	$2.86 \pm 1.87a$	$52.33 \pm 30.02a$	ND
E	$3.25 \pm 1.98a$	$3.06 \pm 1.66a$	$37.67 \pm 37.50a$	ND
F	$10.73 \pm 1.26b$	$6.73 \pm 1.76a$	$76.00 \pm 21.07a$	ND
G	$5.87 \pm 3.26ab$	$2.68 \pm 1.79a$	$51.00 \pm 22.61a$	ND

Table 1: Bacteria population in meat pie sold in Yenagoa metropolis, Bayelsa state, Nigeria.

Each value is expressed as mean \pm standard error ($n = 3$); The same letters along the column is not significantly different at $P > 0.05$ according to the Tukey HSD Statistics.

Figure 1 presents the bacteria isolated from the meat pie samples vended in some locations in Yenagoa metropolis. The bacteria isolates are *Staphylococcus aureus*, *Escherichia coli*, *Micrococcus*, *Bacillus*, *Proteus*, *Pseudomonas*, *Corneycacteria* species. Of all the bacteria isolates, *Staphylococcus aureus* and *Corneycacteria* species had the highest and least occurrence frequency of 48.8% and 5.2% respectively.

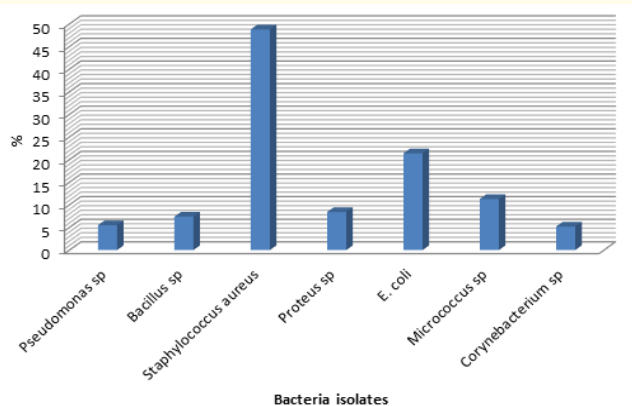


Figure 1: Occurrence frequency of bacteria found in meat pie vended in Yenagoa metropolis, Bayelsa state.

Discussion

In the total heterotrophic bacterial counts, total coliform and total *Staphylococci* counts, there was no significance difference ($P > 0.05$) in most of the parameters. Variation could be due to similarity in handling, personal hygienic level of processors and vendors. The trend in this study have been variously reported in ready to eat food sold in Yenagoa metropolis including gari [20], Suya [21], groundnut [22], sliced fruits viz: water melon, paw-paw and pineapple [4], apple and cucumber [2], kunu drink [23]. This trend in ready to eat food could be due to life style of processors.

Based on quality, the bacteria density in the meat pie is within the acceptable limit ($\leq 10^3$) recommended for ready to eat food as specified by International commission of Microbiological Specification in Food [21,22,24,25]. Similarly, the aerobic colony count is within satisfactory level of 10^4 for cooked foods chilled but with minimum handling prior to sale or consumption; canned pasteurised foods requiring refrigeration (such as whole pies, sausage rolls, samosas, flans, quiches, chicken portions; canned ham requiring refrigeration, pasteurised foods including fruit juice and soups; desserts) and bakery and confectionery products without dairy cream, powdered foods (such as cakes without dairy cream, soup powders, milk powder, powdered dairy products, other reconstituted powdered foods ready to eat after reconstitution or warming) as reported by Center for Food Safety [26] and Gibert., *et al.* [27]. Typically total heterotrophic bacteria count sometimes known as total viable counts or total colony count is used to assess quality in food.

However, the bacteria that belong to the family of *Enterobacteriaceae* is used to assess hygienic level of the products including handling [8]. Furthermore, the coliform counts observed in this study is within the maximum level $<10^2$ for heat treated food, fishes, and cheeses ready to eat food [26].

Among the coliforms, fecal coliform which is basically assessed using *E. coli* is frequently studied because its presence suggest poor personal hygiene, handling and preservation/storage techniques [26]. The dominance of the *Micrococcaceae* group of bacteria i.e *S. aureus* and other bacteria such as *Bacillus* and *Pseudomonas*, *Proteus* species in the meat pie could be due to their ability to thrive under harsh environmental conditions. *S. aureus* had the highest occurrence. Some of these bacteria isolates are environmental contaminants and opportunistic pathogens and have been implicated to cause food borne diseases [5]. Also, *S. aureus* being a normal body microorganism could have also been introduced into the samples through unclean hands and mouths of the vendors where they attempt to open the packaging material by blowing air into it in order to open it [8].

Like *S. aureus*, *Enterobacteriaceae* bacteria in the meat pie could be due to poor handling and partial cooking of the snacks (especially on the hurriedly fried buns which brown-crust exterior could be misinterpreted to imply complete cooking) and or use of water of low quality [11]. Some of these organisms might be picked up during grinding, mixing, kneading and molding [5]. Specifically, the occurrence of *Staphylococcus aureus* could be due to cross contamination of using unhygienic utensils and environment and/ or contamination from skin and mixture during production processes [5].

Based on the bacteria isolates, the trend in this study has some similarity with previous studies on some snacks. Kigigha and Uge [11] reported bacteria occurrence in pastry snacks (Moin-moin and Buns) hawked in the Rivers State University of Science and Technology, Port Harcourt (around Student Residential area, Faculty of Science, Faculty of Engineering and the Old Administrative area) as *Staphylococcus* sp. (50.0%); *Bacillus* and other Gram positive rods (23.3%); *Escherichia coli* (6.7%); *Proteus* species (10.0%); *Enterococcus* species (3.3%); *Flavobacterium/ Xanthomonas* species (3.3%) and *Pseudomonas* species (3.3%), (exterior), and *Staphylococcus* species (63.3%); *Bacillus* and other Gram positive rods (6.3%); *E. coli* (10.0%); *Proteus* species (6.7%); *Enterococcus* species (3.3%); *Flavobacterium/ Xanthomonas* species (3.3%) and *Pseudomonas* species (6.7%) (interior).

Bukar., *et al.* [28] reported percentage occurrence of bacteria in snacks vended in Maiduguri as *Bacillus subtilis* (21.43%), *Staphylococcus aureus* (50%), *Staphylococcus epidermidis* (7.14%) and *Corynebacteria* species (21.43%). Hassan., *et al.* [5] reported *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella bongori*, *Klebsiella pneumonia* and many *Bacillus* species as bacteria found in puff puff (ready to eat snacks) vended in ten selected local governments (Ikorodu, Island, Ikeja, Mushin, Oshodi, Ebute Meta, Shomolu, Ketu, Ojota, Surulere) in Lagos State.

Oluwafemi, *et al.* [12] reported the occurrence of *Micrococcus*, *Pseudomonas*, *Bacillus* species, *Staphylococcus aureus*, *Escherichia coli* in street foods and ready-to-eat vegetables (including cooked rice, boiled and roasted maize, plantain chips, puff-puff, lettuce, cabbage, spring onions, cucumber, carrot and tomatoes) in some Nigeria cities. Akusu, *et al.* [8] reported *Bacillus*, *Proteus* species, *Staphylococcus aureus*, *Pseudomonas* species, *Shigella* species, *Enterobacter* species and *Streptococcus* species from some street vended ready to eat food including egg burger, packaged fried rice, meat pie, vegetable salad in Port Harcourt metropolis, Rivers state. Ike, *et al.* [13] reported *Staphylococcus aureus*, *Escherichia coli*, *Bacillus* species, *Pseudomonas* species, *Clostridium* species, *Enterococcus* species, *Klebsiella* species in some locally vended snacks (meat pies, fish pies, plantain chips and potato chips) sold in some location in Aba metropolis, Abia state. Madueke, *et al.* [15] reported *Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus* sp., *Enterobacter* sp, *Escherichia coli*, *Shigella dysenteriae*, *Klebsiella*, *Pseudomonas*, *Micrococcus*, *Flavobacterium* species in some street foods (including fried yam, fried potato, fried plantain, akara, fish and suya) vended along Lokoja-Abuja express way, Lokoja, Kogi state. Oluwafemi and Simisaye (2006) reported *E. coli*, *Streptococcus*, *Clostridium*, *Klebsiella*, *Shigella*, *Pseudomonas*, *Lactobacillus* species and *S. aureus* in sausages sold in Abeokuta and *Salmonella*, *Proteus*, *Shigella*, *S. aureus*, *Klebsiella* and *Lactobacillus* species in sausages sold in Benin-City.

Adebeshin [29] reported *Staphylococcus aureus*, *Staphylococcus epidermidis* sp, *Clostridium perfringens*, *Klebsiella pneumoniae*, *Streptococcus* sp, *Bacillus* sp, *Proteus* sp, *Escherichia coli*, *Shigella*, *Pseudomonas* species in sausage and meat pie vended in Abeokuta, Nigeria. Fowoyo and Baba-Ali [30] reported *Streptococcus*, *Bacillus*, *Staphylococcus*, *Flavobacterium*, *Proteus*, *Escherichia coli*, *Klebsiella* sp., *Salmonella* sp. and *Pseudomonas aeruginosa* in some ready to eat food (namely fried chicken, fried rice, meat pie and cake) from four different fast food restaurants in Lokoja, Kogi state. Oranusi, *et al.* [6] reported *E. coli*, *S. aureus*, *Bacillus cereus*, *Enterococcus*, *Klebsiella* species, *Pseudomonas* species in some ready to eat foods sold in Ota, Ogun State. Monday, *et al.* [31] reported *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella*, *Salmonella* species in some ready-to-eat foods (rice and moimoi) sold by vendors in Federal Polytechnic, Bali campus, Taraba State.

Most of the bacterial isolates that occurs in ready to eat food consumed in Nigeria are microbes of medical importance. This isolates usually arises from contamination from the environment and poor hygienic status of the handlers. As such the need to practice improves hygiene in food.

Conclusion

Snacks are consumed by people irrespective socioeconomic status, sex, age etc. Snacks are sold in different location including street, public place including schools, hospitals, motor park etc. The type of snacks depends on individuals. One of the commonly consumed snacks is meat pie. This study assessed the microbiological quality of meat pie vended in some locations in Yenagoa metropolis, Bayelsa state, Nigeria. The study found that the total heterotrophic bacteria counts and *Enterobacteriaceae* counts are within satisfactory level in ready to eat meat pie as recommended by Centre of Food safety. Furthermore, high occurrence of *Staphylococcus aureus* suggests poor handling. Some of the isolates found in the meat pie are bacteria of medical importance. Based on the findings of this study, we suggest improved personal hygiene and handling processes by vendors of meat sold in Yenagoa metropolis.

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