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Bacteriological quality and isolation of *E coli* and antibiotic susceptibility from meat pies sold at University of Abuja, FCT, Abuja, Nigeria

E Godwin*, A M Babayemi
Department of Veterinary Public Health and Preventive Medicine, University of Abuja, Abuja, F.C.T, Nigeria

Abstract
Ready-to-eat foods such as meat pies are susceptible to microbial contamination and street vended foods have become a global health problem. This study investigated the bacteriological quality and as well isolated *E.coli* from meat pies sold at the University of Abuja permanent site and mini campuses, Federal Capital Territory, Abuja. A total of 50 samples of meat pies were purchased from vendors within the campuses. Isolation and enumeration of bacterial colonies was carried out on Nutrient agar and MacConkey agar following standard methods. *E. coli* was isolated and identified using biochemical test. The antibiotic sensitivity of the *E. coli* isolates was determined using the Kirby Bauer disc diffusion method. Total aerobic plate counts ranged from 2 ± 0.33 to 1.84 ± 0.09 log10cfu/g for mini campus and permanent site respectively, while total coliform counts ranged from 1.92 ± 0.09 to 1.6 ± 70.55 log10cfu/g for mini campus and permanent site respectively. Statistical analysis of the mean microbial load showed no significant difference (P>0.05). Findings revealed the overall percentage as 17(34%). Presence of *Escherichia coli* in meat pies sold at permanent site and mini campus (43.3%, 20%) respectively. The antibiotic sensitivity test suggested that the *E.coli* isolates were 17(100%) resistant to Sparfloxacin, 16 (94.22%) to ciprofloxacin, amoxicillin and augmentin but 15 (88.23%) susceptible to gentamycin and 8 (47.05%) to streptomycin. Ready to eat meat pies should be kept and packed aseptically into sanitized containers before retailing. Antibiotic surveillance on ready-to-eat foods such as meat pies should be instituted.

Keywords: *E coli*, Meat pies, antibiotics

Introduction

Ready to eat foods can be described as the status of foods being ready for immediate consumption at the point of sale. Ready to eat foods could be raw or cooked, hot or chilled and can be consumed without further heat treatment (Tsang, 2002) [21]. Different terms have been used to describe such ready to eat foods. These include convenient, ready, instant and fast foods. Examples of such ready to eat foods include pastries, meat pie, sausage, rolls, burger, moin-moin, salad or coleslaw, fried meat, fried chicken, milk and milk products (Caserani and Kinston, 1974) [6]. A general observation of our society shows asocial pattern characterized by increased mobility, large numbers of itinerary workers and less family or home centered activities. This situation however has resulted in more ready to eat foods taken outside home. Thus food vendor services become on the increase and responsibility for good manufacturing practices of food such as good sanitary measures and proper food handling have been transferred from individuals/families' to the food vendors who rarely enforce such practices (Musa and Akande, 2002).

Food-borne illnesses are becoming a global public health concern. These microorganisms are responsible for an estimated 48 million illnesses and 3000 fatalities in the United States each year (Rahman *et al*., 2017) [19]. In contrast, ready-to-eat foods do not require any additional preparation, with the exception of warming, and these ready-to-eat foods are often eaten raw or cold without any additional heat treatment (Bagumire *et al*., 2017; Oje *et al*., 2018) [4, 17]. Because of rapid population expansion and the modern lifestyle, longer working hours, increased women's participation in the labor market, and changes in cooking and eating habits, ready-to-eat foods consumption has surged in recent years. For busy city inhabitants, ready-to-eat foods are convenient. Food-borne outbreaks are known to occur as a result of handling, preparing, and marketing these items (Oje *et al*., 2018) [17]. Ready-to-eat foods meat products are in high demand due to their biological value, reasonable price, agreeable taste, and easy preparation. Meat products are considered excellent sources of high-quality protein, minerals, and vitamins (World Health Organization, 1984) [23]. Moreover, these foods are shelf-stable, flavorful, affordable, and immediately accessible to customers because they do not require a lengthy pre-treatment process (Jaroni *et al*., 2001; Spence *et al*., 2005). The possibility of *Escherichia coli* to induce health risks occurs mainly during the preparation and storage of contaminated ready-to-eat foods meat (Mokhtar *et al*., 2021) [4]. *E. coli* is a widespread species found in the intestines of farm animals, poultry, and humans. The majority of *E. coli* strains are non-pathogenic, but a few are very pathogenic, causing watery and bloody diarrhea; *E. coli* 0157:H7 has been linked to life-threatening diseases such as hemorrhagic colitis (HC), hemolytic uremic syndrome and thrombotic thrombocytopenic purpura (Rahman *et al*., 2017) [19]. *E. coli* have been found to contaminate ready-to-eat foods (Abebe *et al*., 2020) [1]. This species can survive on hands and other surfaces and is readily transferred to foods (Ayamah *et al*., 2021) [3]. Meat pies are susceptible to contamination along the value chain. The presence of bacteria especially enteric organisms is an indication of unhygienic or poor sanitary practices during processing, packaging, storage and marketing of the endpoint product. The meat/minced meat used on meat pie preparation can be contaminated on slaughter process as cattle regarded as the main reservoir of the E.coli in the gastrointestinal tracts, the carcass becomes contaminated

through faeces (Karch *et al*., 2005) [13]. Ground or tenderized meats are riskers than intact cuts of meat because bacteria can be mixed throughout the meat in the grinding process or during tenderization (Gansheroff *et al*., 2000) [10]. New bacteria can be introduced to the meat or food from the environment, through handling and processing (Clevence *et al*., 2009). Bacteria will spread into the centre of the food, where they will be less easily destroyed on cooking (Clevence *et al*., 2009). The pathogen reduction should be available during the production process such as heating/cooking (Karch *et al*., 2005) [13].

Point of sale practices by vendors has also been reported as a major source of food contamination. It is required that food safe for human consumption should be displayed in a clean environment and in containers that are insect and dust proof. Serving of food at retail points should also be done with sanitized utensils (WHO, 2002). Unfortunately, vendors of meat pie often use bare hands to serve, wear no aprons and leave their hair uncovered. As a result, the same hands used for collecting money are used to package the food for buyers (Falola *et al*., 2011; Yuksek *et al*., 2009) [8, 25]. Bacteria are also introduced into food through aerosols released during vendor–client communication at the point of sale (Annan-Prah *et al*., 2011) [2]. Bacteria in the released aerosol from saliva settle on the food and are carried away by the buyer unknowingly. Sometimes, flies and insects are not totally prevented from making contact with the food. This could lead to cross contamination (Tambekar *et al*., 2008) [22]. The most frequent known causes of foodborne diseases are pathogenic bacteria. In this study, the goals aimed at analyzing the microbiological quality as well as isolating *E.coli* from meat pies produced by various vendor within University of Abuja campuses.

Methodology

Study area

The study was conducted in University permanent site and mini Campuses Gwagwalada Federal Capital Territory. The Federal Capital Territory (FCT), which was formed in 1976. Abuja has an estimated human population of 1,405,201 according to 2006 census (NPC, 2006). It lies between latitude 8.25 and 9.20 North of the equator, and longitude 6.45 and 7.39 East of Greenwich -Meridian. The Federal Capital Territory has a land mass of approximately 7,315km^2 of which the actual city occupies 275.3km^2. Gwagawalada is located in the southern part of the Federal Capital Territory and it serves as one of the area councils within Abuja. It houses significant educational institutions, including the University of Abuja, and is known for its agricultural activities, such as farming and livestock rearing (Federal Capital Territory Administration, 2018). Gwagwalada area council has and estimated human population of 157,770 according to the 2006 census (National Population Commission, 2006).

Sample collection

Fifty meat pie samples were collected from University of Abuja permanent site and Mini campuses using a convenience sampling method. All samples were collected aseptically in labeled sterile sample bags (polythene bags) and transported to the Department of Veterinary Public Health and Preventive Medicine Laboratory, University of Abuja for analyses.

Sample Analyses

Ten (10) grams each of meat pie sample was weighed using electronic weighing balance and placed in a sterile sample bag and a total of 90ml of peptone water was added to the sample and homogenized in a stomacher for 2 minutes. Tenfold Serial dilution up to 10^7 were used for both total aerobic plate and coliform counts. A volume of 0.1ml from the final dilution was spread on nutrient agar for total aerobic counts and on MacConkey agar for coliform counts and Eosin Methylene Blue and incubated at 37^0C for 24hr. The number of colonies that grew on the incubated plates were counted using a colony counter and the results expressed as Colony Forming Units per milliliter (CFU/g) (ISO, 2013; A.P.H.A, 2015), while metallic green sheen colonies on Eosin Methylene Blue were Gram's stained according to the method described by (Cheesbrough, 2010) [5] and subsequently stored at 4^0C in the refrigerator for further identification by standard methods.

Biochemical identification of *E coli*

Purified colonies were subjected to motility, indole test, methyl-Red test, voges Proskauer test, simmon citrate test and Sugar fermentation tests fructose, sucrose, glucose, maltose, raffinose, arabinose mannose, mannitol, sorbitol, galactose, inulin and lactose (Cheesbrough, 2010; Purkayastha *et al*., 2010) [5, 18].

Antimicrobial susceptibility

Antimicrobial susceptibility was determined using the Kirby-Bauer disc diffusion method (1966). The antibiotics tested includes amoxicillin (30µg), gentamicin (30µg), septrin (30µg), sparfloxacin (10µg), ciprofloxacin (30µg), chloramphenicol (30µg), pefloxacin (30µg), augmentin (10µg), tarivid (10µg) and streptomycin (30µg). An equivalent of conventionally standardized pure culture of test isolate was evenly streaked on agar surface, after which antibiotic impregnated discs were placed on the agar surface using sterile forceps. The plates were then incubated at 37^{o}C for at least 18 hours. The zone of inhibition diameter of each antibiotic was measured and interpreted as resistant, intermediate and susceptible in reference to the Clinical Laboratory Standards Institute (CLSI, 2020).

Results

Table 4.1 shows the log10 TAPC of the two locations with a range of 2 ± 0.33 (cfu/g) and 1.84 ± 0.09 (cfu/g). Meat pie samples from mini campus had the highest log10 TAPC of 2 ± 0.33 (cfu/g), while meat pie samples from permanent site had the least TAPC of 1.84 ± 0.09 (cfu/g). The mean log10 TCC of the two locations is on the range of 1.92 ± 0.09 and 1.6 ± 70.55 (cfu/g). Meat pie samples from mini campus had the highest TCC of 1.92 ± 0.09 (cfu/g) while permanent site had the least TCC of 1.6 ± 70.55 (cfu/g). There was no significant difference at P≤0.05.

Table 1: The mean Total Aerobic Plate and Total Coliform Counts from University of Abuja Mini Campus and Permanent site Gwagwalada Federal Capital Territory, Abuja

Location	APC	TCC
MC	2 ± 0.33	1.92 ± 0.09
PS	1.84 ± 0.09	1.6 ± 70.55

TCC P value = 0.1470, APC P value = 0.2728. No significant difference

Key: PS (Permanent site), MC (Mini campus)

Detection of *E. coli* from Meat Pies

Out of the fifty meat pies samples from mini campus and permanent site 17 isolates were positive for *E. coli*.

Table 2: Frequency of isolation of *E.coli* from Mini Campus and Permanent site University Abuja, Federal Capital Territory

Location	Total sample collected	Frequency of occurrence	Percentage
PS	30	13	43.3%
MC	20	4	20%
Total	50	34	

Key: PS (Permanent site), MC (Mini campus)

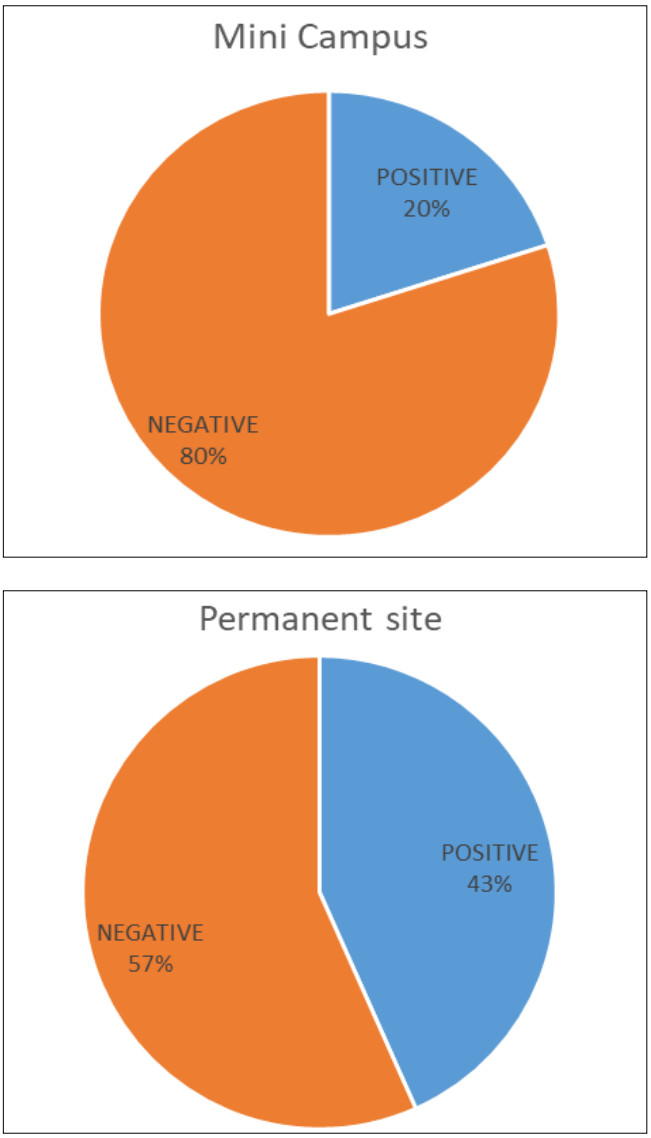

Fig 1: Pie Chart on the Frequency of occurrence of *E. coli* from Meat Pies Sold at Permanent Site and Mini Campus, University of Abuja, Federal Capital Territory ABUJA

A total of 17 isolates were tested against 10 different antibiotics. All the isolates showed (100%) resistance to sparfloxacin, with (94.22%) resistance to ciprofloxacin, amoxicillin and augmentin, (82.35%) to perfloxacin, (82.35%) to tarivid, (76.47%) to septrin, (11.76%) to chloramphenicol, (5.88%) to gentamycin and streptomycin. Susceptibility were observed for gentamycin (88.23%), (47.05%) for streptomycin, (17.64%) for tarivid and chloramphenicol as shown in Table:3

Table 3: Antibiotic Susceptibility of Isolates of *E.coli* from Meat Pies Sold at Mini Campus and Permanent Site University of Abuja

Antibacterial agent	Disk potency (µg)	Susceptible (no%)	Intermediate (no%)	Resistant (no%)
Septrin	30	2 (11.76%)	2 (11.76%)	13 (76.47%)
Chloramphenicol	30	3 (17.64%)	12 (70.59%)	2 (11.76%)
Sparfloxacin	10	0 (0%)	0 (0%)	17 (100%)
Ciprofloxacin	30	1 (5.88%)	0 (0%)	16 (94.22%)
Amoxicillin	30	0 (0%)	1 (5.88%)	16 (94.22%)
Augmentin	10	0 (0%)	1 (5.88%)	16 (94.22%)

Gentamycin	30	15 (88.23%)	1 (5.88%)	1 (5.88%)
Perfloxacin	30	2 (11.76%)	0 (0%)	14 (82.35%)
Tarivid	10	3 (17.64%)	0 (0%)	14 (82.35%)
Streptomycin	30	8 (47.05%)	8 (47.05%)	1 (5.88%)

Key: Septrin (SXT), Chloramphenicol (CH), Sparfloxacin (SP), Ciprofloxacin (CPX), Amoxicillin (AM), Augmentin (AU), Gentamycin (CN), Perfloxacin (PEF), Tarivid (OFX), Streptomycin (S)

Table 4: Antibiotic Resistance Profile

Isolates	Resistance pattern	MARI
PS 30	SXT, CH, SP, CPX, AM, AU, CN, PEF, OFX, S	1.0
PS 6	SXT, CH, SP, CPX, AM, AU, PEF, OFX	0.8
PS 21	SXT, SP, CPX, AM, AU, PEF, OFX	0.7
PS 24	SXT, SP, CPX, AM, AU, PEF, OFX	0.7
PS 25	SXT, SP, CPX, AM, AU, PEF, OFX	0.7
PS 26	SXT, SP, CPX, AM, AU, PEF, OFX	0.7
PS 27	SXT, SP, CPX, AM, AU, PEF, OFX	0.7
PS 28	SXT, SP, CPX, AM, AU, PEF, OFX	0.7
PS 29	SXT, SP, CPX, AM, AU, PEF, OFX	0.7
MC 11	SXT, SP, CPX, AM, AU, PEF, OFX	0.7
MC 12	SXT, SP, CPX, AM, AU, PEF, OFX	0.7
PS 19	SP, CPX, AM, AU, PEF, OFX	0.6
PS 20	SP, CPX, AM, AU, PEF, OFX	0.6
MC 20	SXT, SP, CPX, AM, AU	0.5
PS 17	SXT, SP, CPX, AM, AU	0.5
PS 11	SP, AM, AU,	0.3
MC 13	SP, CPX,	0.2

Key: Septrin (SXT), Chloramphenicol (CH), Sparfloxacin (SP), Ciprofloxacin (CPX), Amoxicillin (AM), Augmentin (AU), Gentamycin (CN), Perfloxacin (PEF), Tarivid (OFX), Streptomycin (S), Permanent site (PS), Mini campus (MC)

Discussion

Foodborne diseases are the foremost global problem causing considerable morbidity and mortality each year (Hanson *et al.*, 2012). The bacterial load of the meat pies samples in this study showed values ranging from 2 ± 0.33 to 1.84 ± 0.09 log10cfu/g for total aerobic plate counts and 1.92 ± 0.09 to 1.6 ± 70.55 log10cfu/g for total coliform counts. Bacterial counts from this present study agrees with the reports of Olusola *et al*., (2016) [16] who reported similar reports from street foods sold in Akure Metropolis, Nigeria. The presence of high number of viable bacteria increases the chance of meat spoilage and shortened shelf life. Coliforms are indicator of food and water quality, the presence of *E. coli* is an indication of fecal contamination. *E. coli* are normal flora of the intestines of both humans and animals and have been identified as a leading cause of food borne illness all over the world (Hussein, 2007) [11]. A percentage of 34% of *E.coli* recorded in this study is lower than (Ezeh *et al.*, 2017) who recorded 39% prevalence from meat pie in Anambra State. Similarly, Obande *et al*., (2018) recorded 10% from meat pie sold Nigerian North Central town. The differences could be due to method of isolation, location, environmental hygiene, personal hygiene and method of distribution, package and point of sale. From our investigations, filling of pastry is usually done manually before baking and if necessary hygienic process is not observed, it can serve as source of contamination. Contrarily to popular believe that bacteria can be destroyed during baking process. Dlusskaya *et al.*, (2011) shows that some member of *Enterobacteriaceae* are heat resistance making bacteria viable even after baking. Noteworthy to this study, the presence of *E.coli* in the ready- to- eat food sample analysed portrays a significant public health hazard. The antimicrobial sensitivity test of the 17 isolates revealed 17 (100%) resistance to sparfloxacin, 16 (94.22%) to ciprofloxacin, augmentin and amoxicillin, 14 (82.35%) to perfloxacin and tarivid and 13 (76.47%) to septrin respectively which partly agrees with the reports of (Obande *et al*., 2018: Bako *et al*., 2024). Susceptibility pattern of isolates showed 15 (88.23%) to gentamycin and 8 (47.05%) to streptomycin which agrees with Obande *et al*., (2018) who reported similarly pattern of susceptibility to streptomycin 46 (65.7), gentamycin 60 (85.7). The multiple antibiotic (MAR) index ranged from 0.2 to 0.8 with all the isolates completely resistant to all antibiotics showing a 1.0 MAR index.

Conclusion and recommendation

The research demonstrates that the meat pies sold for human consumption in university of Abuja permanent site and mini campus, based on the specifications by International Commission for Microbiological Specification for Foods (ICMSF, 1996) are grossly contaminated above the acceptable permissible level in ready-to-eat food such as meat pies. Isolates of *E coli* are resistance to multiple antibiotics. This may pose greater risk to the population at large, since some of this meat pies may have been distributed outside the study area. This study thus recommend the need for intensive surveillance of *E.coli* in ready-to-eat foods. Producers and vendors should adhere to hygienic practices during production, packaging, storage, distribution and handling during sells. Improve personnel hygiene, proper storage systems and package during distribution. The results also underscore the necessity to sternly control the usage of antimicrobial therapy in human, animals and agricultural sectors in Nigeria. Regulation of the production and retail process of meat pies should be advocated as a possible means of reducing contamination and the risk of transferring antibiotic resistant bacteria to consumers.

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Conflict of Interest: The authors declare no conflict of interest

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