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Research Article

Incidence and antibiotic susceptibility pattern of *Escherichia coli* and *Staphylococcus aureus* isolated from meat pie sold in a Nigerian North Central town

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ABSTRACT

Background and Objectives: Meat pie is a popular ready-to-eat food sold in Nigeria and is consumed by people of all classes and category. The study aimed to determine the incidence and susceptibility of *Escherichia coli* and *Staphylococcus aureus* isolated from meat pie to antibiotics commonly administered in Makurdi.

Material and Methods: A total of 180 samples were collected and evaluated for bacterial contamination and presence of antibiotic-resistant *Escherichia coli* and *Staphylococcus aureus*. Contaminants were isolated and identified using biochemical test. Antimicrobial susceptibility of isolates was determined using the Kirby-Bauer disc diffusion method.

Results: Eleven bacterial genera was identified. *Bacillus* spp (85%) occurred most frequently, followed by *Staphylococcus aureus* (38.9%), while *Edwardsiella* spp (2.8%) occured the least. *Staphylococcus aureus* was highly resistant to Cloxacillin (87.1%) but highly susceptible to Ofloxacin (88.6%). *Escherichia coli* was resistant to Amoxycillin, Tetracycline, Cloxacillin and Augmentin but susceptible to Gentamicin and Ofloxacin.

Conclusion: Meat pie sold in Makurdi habours *Staphylococcus aureus* and *Escherichia coli* with multiple antibiotic resistance. Regulation of the production and retail process of meat pie is advocated as a possible means of reducing contamination and the risk of transferring antibiotic resistant bacteria to consumers.

Key words: Antibiotic, *Escherichia coli*, meat pie, *Staphylococcus aureus*, susceptibility.

INTRODUCTION

A pie refers to any food or dish that consists of a crust with a filling. Examples include fruit pies, cream pies, custard pies and meat pies [1]. Meat pies and other pies are popular the world over, because they are convenient to stock, sell and consume [2, 3]. They belong to the class of ready-to-eat foods (RTEs) known as pastries. A number of contaminants, including pathogenic bacteria and fungi have been isolated from meat pie by different researchers in different parts of the world [4, 5]. In Nigeria also, studies in Lagos State [6] and Benin City [7] have also reported high bacteria counts as well as the presence of pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus* in meat pie. They are both agents of food borne diseases and their presence in food is considered a risk to the health of consumers [7, 8, 9].

Antibiotic resistance has been reported severally in literature with clinical isolates from human beings. Recent evidences however, suggest that antibiotic resistance traits have entered the microflora of farm animals and the food produced from them [10]. Thus, the food microflora is not separated from its human counterpart in cases of antibiotic resistance. Resistance to antibiotics by *E. coli* and *S. aureus* of clinical and food origins have also been reported in literature [11, 12, 13, 14]. According to [10], the detection, invention and global uses of antibiotics and antimicrobial agents in human and veterinary medicine, agriculture and aquaculture have initiated a 'Darwinian' experiment that has resulted in the survival of resistant microorganisms and the elimination of susceptible ones in the antibiotic environment. Widespread use of antibiotics in food production systems have resulted in the emergence of antibiotic resistant zoonotic bacteria that can be transmitted to humans through the food chain [15].

According to the report of [16], there were 35 food-borne outbreaks in the US between 1973 and 2009, in which the identified bacteria were resistant to at least one antibiotic. Of these outbreaks, 14 (40%) occurred between 2000 and 2009, out of which 26% was from ground beef and 34% from dairy products [16]. A total of 19, 897 people fell sick, 3,061 were hospitalized and 26 deaths resulted from the outbreak for which species of *Salmonella, E. coli, Campylobacter jejuni* and *S. aureus* were responsible [16]. The authors also reported that the responsible bacteria for 31 of the outbreaks displayed resistance to 14 different antibiotics and at least one sulfonamide. Seven of those antibiotics are classified by the WHO as "critically important" to human medicine, while eight of them are among the group of antibiotics classified by the WHO as "highly important" to human medicine. They also reported that fifteen of those outbreaks occurred between 1990 and 2009, in which 19 of the associated bacteria were resistant to at least five antibiotics [16].

It is thus clear from the foregoing, that antibiotic resistance in food-borne pathogens is becoming a silently emerging problem and should start receiving attention. Hence, this work was undertaken to determine the incidence and susceptibility of *E. coli* and *S. aureus* isolated from meat pie to antibiotics commonly administered in Makurdi, Benue State, Nigeria.

MATERIAL AND METHODS

Sample collection

The research was a cross-sectional study carried out between March and July 2013. Meat pie samples were obtained from the three main sources of meat pie sold in Makurdi identified as eateries, supermarkets and street hawkers spread across the five major areas of the town namely Ankpa ward, Wadata, High Level, Wurukum and NorthBank. Thirty (30) samples were obtained from each source, making a total of ninety (90) meat pie samples. The filling and crust of each sample were analyzed separately owing to the difference in their composition, bringing the overall sample size for analysis to one hundred and eighty (180). Samples were transferred to the laboratory in icepack container and analyzed within three (3) hours for the presence of bacteria.

Bacteriological analysis

Ten (10) grams of the sample (filling or crust) were weighed into 90 ml of sterile normal saline and homogenized in a sterilized electric blender. A loopful of the stock preparation was streaked on the surface of Nutrient agar (Titan Biotech Ltd.), Mannitol salt agar (Oxoid), Eosin methylene blue agar (Titan Biotech Ltd.) and MacConkey agar (Titan Biotech Ltd.) and incubated at 37^{o}C for at least 18 hours. Colonies with bright yellow appearance on Mannitol salt agar were presumptively identified as *Staphylococcus aureus* and were selected for further biochemical identification, while colonies with greenish metallic sheen on EMBA and lactose fermenters on MacConkey agar were suspected to be *Escherichia coli* and were selected for further identification. Salmonella-Shigella agar was used for the isolation of *Salmonella* spp and *Shigella* spp, with pre-enrichment in Rappaport-Vassiliadis (RV) Enrichment Broth (Oxoid). Other bacterial contaminants were also isolated and identified.

Identification of isolates

The morphological and cultural characteristics of colonies on the various media used were recorded. Colonies with similar morphological characteristics where selected, sub-cultured on both nutrient agar and the other selective media, and discrete colonies obtained were used for identification tests. The Gram reaction of each isolate was determined. Catalase, coagulase, indole (Oxoid, Indole Kovac's reagent), citrate utilization (Oxoid, Simmon's citrate agar), oxidase (Oxoid, Microbact Oxidase strips), lysine decarboxylase (Oxoid, Taylor modification), sugar fermentation (Oxoid, TSI), slide motility and hydrogen sulfide production (Oxoid, TSI) tests were performed on the various isolates [7, 17].

Antibiotic susceptibility

Antimicrobial susceptibility was determined using the Kirby-Bauer disc diffusion method. Gram-positive and Gram-negative discs supplied by Abtek Biologicals London, were used. The Gram-positive disc contained streptomycin (10µg), gentamicin (10µg), tetracycline (10µg), chloramphenicol (10µg), erythromycin (5µg), cotrimoxazole (25µg), cloxacillin (5µg) and augmentin (30µg), while the Gram-negative disc had amoxicillin (25µg), gentamicin (10µg), tetracycline (25µg), cotrimoxazole (25µg), nalidixic acid (30µg), nitrofurantoin (200µg), ofloxacin (5µg) and augmentin (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 diameter of inhibition was read and interpreted in reference to the Clinical Laboratory Standards Institute Performance Standards for Antimicrobial Disk Susceptibility Tests [18, 19]. *E. coli* ATCC 25922 *and Staphylococcus aureus* ATCC 25923 were used as reference control strains.

Data analysis

Data analysis was performed using Statistical Package for Social Sciences (SPSS) version 20. Descriptive statistics were computed using Cross tabulation to obtain percentages.

RESULTS

Table 1 shows a list of bacteria isolated. Of the samples examined, *Bacillus* spp. was the most isolated (85%, n=153), followed by *Staphylococcus aureus* (38.9%, n=70). *Escherichia coli* were found as contaminant in 10% (n=18) of the samples while *Edwardsiella* spp was found in only 2.8% (n=5) of the samples. No salmonellae were isolated.

The susceptibility of *Staphylococcus aureus* isolated from meat pie obtained from hawkers, supermarkets and eateries in Makurdi was tested with 12 different antibiotics (Table 2). The result reveals that 88.6% (n=62) of the *Staphylococcus aureus* isolates tested were susceptible to Ofloxacin while 87.1% (n=61) were resistant to Cloxacillin. Intermediate results of 17.1% (n=12) to Streptomycin, 2.9% (n=2) to Nalidixic acid and 8.6% (n=6) to Nitrofurantoin were observed.

Five out of the eighteen *Escherichia coli* isolates were subjected to antibiotic susceptibility test. As presented in Table 2, 80% (n=4) of the isolates were found to be most susceptible to Gentamicin and Ofloxacin. All the isolates were resistant to Amoxycillin (100%, n=5), Tetracycline (100%, n=5), Cloxacillin (100%, n=5) and Augmentin (100%, n=5). Intermidiate result was observed with Nalidixic acid (20%, n=1). Resistance to erythromycin and streptomycin was observed in 80% (n=4) and 60% (n=3) of the isolates respectively.

Table 1: Frequency of bacterial isolates from fillings and crusts of meat pie samples (n = 180)

Isolate	Frequency in samples	Percentage (%)
Bacillus Spp	153	85.0
Staphylococcus aureus	70	38.9
Staphylococcus Spp	40	22.2
Klebsiella Spp	34	18.9
Proteus Spp	32	17.8
Enterobacter Spp	23	12.8
Pseudomonas Spp	21	11.7
Escherichia coli	18	10.0
Shigella Spp	9	5.0
Citrobacter Spp	9	5.0
Edwardsiella Spp	5	2.8

DISCUSSION

The results of this study demonstrate that meat pie sold in Makurdi are highly contaminated with different species of bacteria. Similar studies [5, 7, 20, 21, 22, 23] have implicated most of the bacteria isolated in this study, in meat pie and other ready-to-eat foods. The high level of contamination is an indication that adequate attention may not have been paid to hygiene in the production process and in the retailing of the final product. From investigations, addition of the fillings into the pastry before baking, is mostly done manually by meat pie producers and this practice may have contributed to bacterial contamination.

Table 2: Antibiotic susceptibility of *Staphylococcus aureus* isolated from meat pie sold by hawkers, eateries and supermarkets in Makurdi (n = 70)

Antibiotic (µg)	Sensitive (%)	Resistant (%)	Intermidiate (%)
Amoxicillin	15 (21.4)	55 (78.6)	0 (0)
Gentamicin	60 (85.7)	10 (14.3)	0 (0)
Tetracycline	55 (78.6)	15 (21.4)	0 (0)
Chloramphenicol	49 (70.0)	21 (30.0)	0 (0)
Erythromycin	21 (30.0)	49 (70.0)	0 (0)
Cotrimoxazole	43 (61.4)	27 (38.6)	0 (0)
Streptomycin	46 (65.7)	12 (17.1)	12 (17.1)
Cloxacillin	9 (12.9)	61 (87.1)	0 (0)
Nalidixic Acid	11 (15.7)	57 (81.4)	2 (2.9)
Nitrofurantoin	56 (80.0)	8 (11.4)	6 (8.6)
Ofloxacin	62 (88.6)	8 (11.4)	0 (0)
Augmentin	16 (22.9)	54 (77.1)	0 (0)

Table 3: Antibiotic susceptibility of *Escherichia coli* isolated from meat pie sold by hawkers, eateries and supermarkets in Makurdi (n = 5)

Antibiotic	Sensitive (%)	Resistant (%)	Intermediate (%)
Amoxycillin	0 (0)	5 (100)	0 (0)
Gentamicin	4 (80.0)	1 (20.0)	0 (0)
Tetracycline	0 (0)	5 (100)	0 (0)
Chloramphenicol	3 (60.0)	2 (40.0)	0 (0)
Erythromycin	1 (20.0)	4 (80.0)	0 (0)
Cotrimoxazole	3 (60.0)	2 (40.0)	0 (0)
Streptomycin	2 (40.0)	3 (60.0)	0 (0)
Cloxacillin	0 (0)	5 (100)	0 (0)
Nalidixic Acid	3 (60.0)	1 (20.0)	1 (20.0)
Nitrofurantoin	3 (60.0)	2 (40.0)	0 (0)
Ofloxacin (5)	4 (80.0)	1 (20.0)	0 (0)
Augmentin (30)	0 (0)	5 (100)	0 (0)

In this study, *Bacillus* spp was found to be the most frequently isolated contaminant and was present in both the crusts and fillings of samples. The occurrence of *Bacillus* spp was higher in samples obtained from eateries and hawkers. *Bacillus* spp in meat pie raises an issue of concern since some species are known to cause food poisoning by preformed toxins in food or by the production of enterotoxins in the small intestine [24].

Being a spore-former, it could have come from the soil, water or the gut flora of humans since it is ubiquitous and hence, abundant in the environment.

The level of contamination by *Escherichia coli* found in this study is similar to that of [25] who reported *Escherichia coli* contamination in about one-third of 153 ready-to-eat products. *Escherichia coli* has also been associated with traveler's diarrheoa and hemorrhagic colitis. Its presence in food is therefore considered a threat to human health, and an indication of gross contamination [4, 21]. As suggested by [7] and [17] the presence of *Escherichia coli* in meat pie could be due to faecal contamination of the water sources and raw materials used during the production. *Staphylococcus aureus* occurring in meat pie is also worrisome. A staphylococcal toxin dose of less than 1 microgram in contaminated food will produce symptoms of staphylococcal intoxication [26]. The incidence of *Staphylococcus aureus* suggests excessive human handling, since the bacteria occurs as a normal flora of the human and animal skin [7].

The antibiotic susceptibility test for *Staphylococcus aureus* isolates showed high rate of resistance to cloxacillin, amoxycillin, augmentin, nalidixic acid and erythromycin. The bacterium was susceptible to gentamicin, ofloxacin, nitrofurantoin tetracycline, chloramphenicol, streptomycin and cotrimoxazole. These results corroborate reports of resistance to erythromycin and cloxacillin [14, 27] and susceptibility to chloramphenicol, tetracycline and gentamicin [28, 29] by *Staphylococcus aureus* in various ready-to-eat foods. These antibiotics which the organism is susceptible to are likely to be effective in treating staphylococcal food infections within Makurdi.

Resistance of *Escherichia coli* isolates to amoxicillin, tetracycline, augmentin, cloxacillin and erythromycin, and susceptibility to gentamicin, ofloxacin, chloramphenicol, cotrimoxazole, nalidixic acid, streptomycin and nitrofurantoin partly agrees with the report of [13]. The rates of resistance to selected antibiotics and hence, the occurrence of antibiotic resistance in food contaminants observed in this study raises an issue of public health concern. Much more worrisome is the fact that the trend may be on the increase, hence placing the population at risk of resistance to antibiotics by food borne pathogens. Antibiotic resistance observed in these isolates may have come from the microflora of farm animals and the food produced from them. Evidence in literature suggests that resistance to antibiotics by food-borne pathogens can be transferred from one bacterium to another by conjugation [10]. The resulting mutant strains can contaminate raw materials and be ingested through contaminated food [27]. Also, the indiscriminate use of antibiotics by veterinarians on the farm and by humans, have been fingered as possible reasons for antibiotic resistance in food borne pathogens [28].

CONCLUSION

The study reveals that consumption of unhygienically prepared or retailed meat pie could pose a great risk to the health of consumers due to the high incidence of bacterial contamination observed. The high occurrence of *Staphylococcus aureus*, *Escherichia coli* and *Bacillus* spp in meat pie samples is worrisome since some are toxin producers that cause food poisoning. Antibiotic susceptibility test revealed high rates of resistance by *Staphylococcus aureus* and *Escherichia coli* to some selected antibiotics. Antibiotic resistance in food borne pathogens is hence, a seriously emerging issue of importance as resistance to antibiotics may be spreading to food borne pathogens at a higher rate than is generally thought. Continuous monitoring of antibiotic resistance trends in bacteria from food sources, especially ready-to-eat foods should be established as it could help in the formulation of control strategies.

LIMITATIONS

Only meat pie samples randomly obtained from street hawkers, supermarkets and eateries in Makurdi were analyzed in this study. Hence, findings may not hold general applications to other parts of the Benue state or to other forms of ready to eat foods.

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AUTHOR'S CONTRIBUTION

GAO- conceived the study; **GAO, EUU and ETA-** designed the study methodology and performed the research; **GAO, AC and PA-** completed the first and second draft manuscript; **GAO and EUU-** performed data analysis; **GAO-** revised the final manuscript.

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CONFLICT OF INTEREST: None declared.

REFERENCES

1. Kowtaluk H. Food For Today. McGraw-Hill: New York; 2006. 649pp.
2. Sultan WJ. Practical Baking. 5th ed. Van Nostrand Reinhold: New York; 1990. 425pp
3. Bertram P. Fast Food Operations. Barrie and Jenkins: London; 1975. 223pp.
4. Annan-Prah A, Amewowor DHAK, Osei-Kofi J, Amoono SE, Saka SYE, Ndadi HA. Street foods: Handling, hygiene and client expectations in a World Heritage Site Town, Cape Coast, Ghana. Afr J Microbiol Res 2011; 5(13): 1629-1634.
5. Feglo P, Sakyi K. Bacterial contamination of street vending food in Kumasi, Ghana. J Med Biomed Sci 2012; 1(1): 1-8
6. Falola AO, Olatidoye OP, Balogun IO, Opeifa AO. Microbiological quality analysis of meat pies sold by street hawkers: A case study of mainland local government area of Lagos, Nigeria. J Med Appl Biosci 2011; 2: 1-8.
7. Clarence YS, Nwinyi OC, Shalom CN. Assessment of bacteriological quality of ready-to-eat food (Meat pie) in Benin City metropolis, Nigeria. Afr J Microbiol Res 2009; 3(6): 390-395.
8. Cencil GBT, Karma M, Rositto PV, Morgante RA, Cullor JS. Enterotoxin production by *Staphylococcus aureus* isolated from mastitic cows. J. Food Prot 2003; 66(9): 1693-1696.
9. Prescott ML, Harley PJ, Donald AK. Microbiology. 6th ed. Mc Graw Hill: New York; 2005. 992pp.
10. Teuber M. Spread of antibiotic resistance with food borne pathogens. Cell Mol Life Sci 1999; 56: 755-763.
11. Aminu RF, Umeh EU. Antibiotic susceptibility of enteric bacteria isolated from ready-to-eat meat balangu in Kogi State, Nigeria. Afr J Microbiol Res 2014; 8(39): 3536-3539.
12. Aly MEA, Essam TM, Amin MA. Antibiotic resistance profile of *Escherichia coli* strains isolated from clinical specimen and food samples in Egypt. Intl. J. Microbiol. Res., 2012; 3(3): 176-182.
13. Jeyasanta KI, Aiyamperumal V, Patterson J. Prevalence of antibiotic resistant *Escherichia coli* in sea foods at Tuticorin Coast, Southeastern India. Adv Biol Res 2012; 6(2): 70 - 77.
14. Daka D, G/silassie S, Yihdego D. Antibiotic resistant *Staphylococcus aureus* isolated from cow's milk in the Hawassa area, South Ethopia. Ann Clin Microbiol Antimicrob 2012; 11(26): 1-6.
15. Walsh C, Fanning S. Antimicrobial resistance in food-borne pathogens a cause for concern. Curr Drug Targets 2008; 9(9): 808–15.

16. DeWaal CS, Roberts C, Catella C. Antibiotic resistance in food borne pathogens: evidence of the need for a risk management strategy, Center for science in the public interest White Paper 2011, January 25.
17. Edema MO, Osho AT, Diala CI. Evaluation of microbial hazards associated with the processing of Suya (a grilled meat product). Sci Res Essays 2008; 3(12): 621-626.
18. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disc Susceptibility Tests; Approved Standard, 9th ed., M2-A9 2006; 26(1): 1-37.
19. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disc Susceptibility Tests; 22nd informational supplement, M100-S22 2012; 32(3): 1-184.
20. Warapa M, Wipawadee O, Siriporn S, Nitaya P, Phattraphorn C, Tanaporn B. Risk evaluation of popular ready-to-eat food sold in Bangkok. As. J. of Food Ag-Ind 2010; 3(1): 75-81.
21. Tambekar DH, Jaiswal VJ, Dhanorkar DV, Gulhane PB, Dudhane MN. Identification of microbiological hazards and safety of ready-to-eat food vended in streets of Amravati City, India. J App Biosci. 2008; 7: 195–201.
22. Que-King W, Shu-Ling H, Tong RC. Microbiological Quality of Ready-To-Eat Food Products in Southern Taiwan. J Food Drug Analysis 2005; 14(1): 68-73.
23. Obande GA, Azua ET. Extent of microbial contamination of nono, fresh cow milk and yoghurt sold in Makurdi, Benue State, Nigeria. J Microbiol Biotechnol Res 2013; 3(3): 6-14.
24. European Food Safety Authority. Opinion of the Scientific Panel on Biological Hazards on *Bacillus cereus* and other *Bacillus* spp in foodstuffs. EFSA Journal 2005; 175: 1-48.
25. Hosein A, Muñoz K, Sawh K, Adesiyun A. Microbial Load and the Prevalence of *Escherichia coli*, *Salmonella* spp. and *Listeria* spp. in Ready-to-Eat Products in Trinidad. The Open Food Science Journal 2008; 2: 23-28.
26. Food and Drug Administration (2013). (Cited 25 March, 2014) Bad Bug Book: Foodborne Pathogenic Microorganisms and Natural Toxins Handbook, *Staphylococcus aureus*. Available from: http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm070015.htm.
27. Mirzaei H, Farhoudi H, Tavassoli H, Farajli M, Monadi A. Presence and antimicrobial susceptibility of Methicillin-Resistant *Staphylococcus aureus* in raw and pasteurized milk and ice cream in Tabriz by culture and PCR techniques. Afr J Microbiol Res 2012; 6(22): 6224-6229.
28. Sasidharan S, Prema B, Yoga LL. Antimicrobial drug resistance of *Staphylococcus aureus* in dairy products. Asian Pac J Trop Biomed 2011; 130-132.
29. Konuku S, Rajan MM, Muruhan S. Morphological and biochemical characteristics and antibiotic resistance pattern of *Staphylococcus aureus* isolated from grapes. Int J Nutr Pharmacol Neurol Dis 2012; 2(1):70-73.

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