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Characterization and Antibiotic Susceptibility Patterns of Members of Enterobacteriaceae Isolated from Ready-to-eat Fufu Sold in Calabar, Southern Nigeria.Ntui Okam Njok^{1*}, Maurice Mbah¹, Mathias Okang Ajaba^{1,2}, Emmanuel Effiong Bassey¹Department of Medical Parasitology and Entomology, Faculty of Medical Laboratory Science, University of Calabar, Calabar, Nigeria¹, Department of Medical Bacteriology, Mycology and Virology, Faculty of Medical Laboratory Science, University of Calabar, Calabar, Nigeria²Author for correspondence*: njokntui20@gmail.com/+234-813-437-5344/ ORCID ID: <https://orcid.org/0000-0003-2029-5611>. <https://dx.doi.org/10.4314/sokjmls.v8i4.8>**Abstract**

Microbial contamination of vended foods is of public health importance due to the potential of becoming a reservoir of foodborne pathogens and resistant strains of bacteria. This study was conducted to investigate the occurrence of Enterobacteriaceae in Ready-To-Eat (RTE) Fufu, and their antibiotic susceptibility profile. Two hundred (200) Fufu samples were purchased randomly from different locations in Calabar metropolis. Samples were analyzed following standard bacteriological protocols. Bacterial counts were enumerated using the Center of Food Safety guidelines for ready-to-eat foods. Susceptibility pattern of the isolated Enterobacteriaceae were performed using the Kirby-Bauer disk diffusion method in line with the Clinical Laboratory Standard Institute (CLSI) guidelines. Out of 200 samples analyzed, 29.5% yielded count $<10^2$ Cfug (satisfactory), 49.0% yielded count $10^2 - 10^4$ Cfug (borderline) and 21.5% yielded count $>10^4$ Cfug (unsatisfactory). The most prevalent isolate was *Klebsiella pneumoniae* (25.9%), followed by *Citrobacter freundii* (22.2%), *Escherichia coli* (14.8%), *Enterobacter cloacae* (14.8%), *Burkholderia cepacia* (14.8%) while the least isolated Enterobacteriaceae was *Salmonella* sp (7.4%). All members of Enterobacteriaceae isolated were 100% resistant to augmentin and amoxicillin. The most effective drugs against the isolates were gentamicin, streptomycin and sparfloxacin. Multidrug resistance was detected among 81.6% of the isolates. This study showed that Fufu sold in Calabar metropolis were unsatisfactory in terms of microbial

contamination. Hence, surveillance studies of resistant pathogens in foods, increased education and training of food vendors on sanitation, food handling and safety practices in the region is recommended.

Keywords: Enterobacteriaceae, Ready-to-eat fufu, Antibiotic pattern, Calabar.

Introduction

Fufu is a popular cassava food mostly consumed in Africa sub-regions. The production of fufu by most local producers follow no microbiological standard. Hence, the presence of pathogenic microorganisms in fufu is a threat to public health (Inetianbor *et al.*, 2017; Tarh *et al.*, 2023). Fufu can serve as a reservoir of foodborne pathogens and resistant strains of bacteria (Adejunj *et al.*, 2017). Fufu is traditionally processed on a household scale and has not been industrialized (Igbinosa *et al.*, 2020). A lot of problems and constrains are associated with the current processing methods which include; delay in processing fresh roots, the odorous nature, its smelling nature and time taken for processing valuable quality of the end product and poor shelf life (FAO, 2010).

The quality of fufu vary from one location to another and maybe attributed the processing methods (Cardo *et al.*, 2015). Fufu is one of the Street vended ready - to - eat food (Mbah *et al.*, 2012). Ready-to-eat foods receive high patronage from general public due to its affordability, nutritional value and availability (Inetianbor *et al.*, 2017). Due to the conditions in

which these food vendors operate, it's possible that some ready-to-eat items are contaminated with microbial pathogens.

Enterobacteriaceae are a large family of Gram-negative bacteria which is made up of diverse members (Rehab *et al.*, 2023). Some members of *Enterobacteriaceae* such as *Salmonella*, *Klebsiella*, *Shigella*, *Escherichia coli* etc. are opportunistic organisms implicated in many disease conditions (Rehab *et al.*, 2023). According to the U.S. National Healthcare Safety Network, 30% of hospital-acquired infections were caused by Gram-negative bacteria, and 70% were the reason behind infections in intensive care units (Peleg and Hoper, 2010). Healthcare community Researchers are facing crisis globally with the emergence and rise of drug resistance conferred by bacteria (Aslam *et al.*, 2021). According to the global antimicrobial resistance and use surveillance system (GLASS) report published by the World Health Organization [WHO] in 2021, antimicrobial resistance is one of the top ten global public health concerns that threaten humans (WHO, 2021).

Furthermore, WHO has declared carbapenem-resistant and third-generation cephalosporin-resistant *Enterobacteriaceae* as a critical priority for research and development (WHO, 2022) Multi-antibiotic resistance in *Enterobacteriaceae* is an increasing problem, with the strains being resistant to most available antibiotics (Partridge, 2015). β -lactams, fluoroquinolones, and aminoglycosides are the three main groups of antibiotics that *Enterobacteriaceae* confer resistance to. In general, bacteria follow more than one mechanism to resist antibiotics, including (1) enzymatic modification of the drug, (2) modification of the structure of the target, (3) efflux pumps, and (4) reduction in the penetration of the drug due to changes in cell wall permeability (Anderson, 2015; Partridge, 2015).

Food infected with antibiotic-resistant pathogenic bacteria poses a serious risk to the general public's health (Nyenje *et al.*, 2012; Basse *et al.*, 2022). In addition to infecting individuals, these illnesses also pose a risk for spreading antimicrobial resistance, and they are

capable of spreading antibiotic-resistant elements to both unrelated and related bacterial species. There is paucity of research establishing the resistance profile of bacteria isolates from ready-to-eat foods. Hence, this study was conducted to investigate the occurrence of *Enterobacteriaceae* in Ready-To-Eat (RTE) Fufu, and their antibiotic susceptibility patterns.

Materials and Methods

Sampling Method

This research was a cross sectional study conducted between August 2022 and January 2023. A total of 200 samples of locally fermented ready-to-eat fufu were purchased from different location within Calabar Metropolis. The fufu were obtained in ten (10) major markets. Samples were collected in sterile bags, kept on ice packs and transported immediately in a container to the laboratory for processing. One gram of each sample was transferred into 10ml of sterile distilled water, and then homogenized appropriately. One milliliter was aseptically transferred into 9 ml of sterile distilled water in a sterile test tube to make a factor of 10^{-1} . This was further diluted up to 10^{-6} dilutions. Spread plate technique was employed for the enumeration of bacteria (Table 1). For the isolation of *Enterobacteriaceae*, distinctive colonies from nutrient agar plates were sub-cultured into MacConkey agar. Characteristics lactose and non-lactose fermenters were purified and stored in nutrient agar slant for further tests. Characterization and identification were carried out using conventional microbiological and Analytical Profile Index (API) 20E identification system.

Antibiotic susceptibility patterns of the isolated *Enterobacteriaceae* were determined by Kirby-Bauer method (Basse *et al.*, 2022) using 10 commercial antibiotics, according to the Clinical Laboratory Standard Institute (CLSI) (2017) Septrin (SXT, 30 μ g), Chloramphenicol (CH, 30 μ g), Sparfloxacin (SP, 10 μ g), Ciprofloxacin (CPX, 30 μ g), Amoxicillin (AM, 30 μ g), Augmentin (AU, 10 μ g), Gentamycin (CN, 30 μ g), Pefloxacin (PEP, 30 μ g), Tarivid (OFX, 10 μ g) and Streptomycin (S, 30 μ g).

The 24 hours' broth culture of each test organism was suspended in saline solution (0.85% NaCl) and adjusted to match a turbidity of 0.5 McFarland Standard. The standardized inoculums were seeded on to the surfaces of already prepared Mueller Hinton agar plates using sterile cotton swabs. The seeded plates were left to stand for about 30 minutes then the antibiotic disks were aseptically placed on the

surfaces of the seeded plates with the aid of sterile forceps. They were then inverted and incubated at 37°C for 24 hours. After incubation, any clear circular zones of growth inhibition around the immediate vicinity of any disk indicated susceptibility to that antibiotic agent. These inhibition zone diameters were measured, and the results interpreted based on the CLSI (2017) recommendation.

Table 1. Microbiological guidelines for indicator organisms in Ready-to-eat food

| Categories | Colony forming unit (cfu)/g | Description |
|------------|-----------------------------|----------------|
| 1 | $< 10^2$ | Satisfactory |
| 2 | $10^2 - < 10^4$ | Borderline |
| 3 | 4 | Unsatisfactory |

Source; Microbiological Guidelines for Food. Ready-to-eat Food in General and Specific Food (2014).

Data Analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) software version 20 and results were presented on percentage. Samples contamination rate was interpreted using the Microbiological Guidelines for Food. Ready-to-eat Foods in General and Specific Food items (2014).

Results and Discussion

The mean aerobic bacterial counts (ABC) of ready-to-eat (RTE) fufu collected from different location in Calabar was enumerated following the Center for Food Safety guidelines (Table 1). Of the 200 fufu samples analyzed, 29.5% yielded count $<10^2$ Cfu/g (satisfactory), 49.0% yielded count $10^2 - 10^4$ Cfu/g (borderline) and 21.5% yielded count 10^4 Cfu/g (unsatisfactory) respectively. There were no significant differences in the bacterial counts of samples from different points ($P > 0.05$). Although there is little or no documented outbreak of foodborne diseases in Nigeria, many patients who visited healthcare facilities complained of stomach

upset. The isolation and high rate of unsatisfactory (10^2 Cfu/g) bacterial count recorded in 21.5% (Table 2) of Ready-To-Eat (RTE) fufu sampled in the study area call for concern. This study corroborated previous findings (Mbah *et al.*, 2012). The demand for RTE food is on the increase especially among manual laborers, students and civil servants because of its affordability and easily accessibility. The high rate of contamination recorded in the present study may be attributed to the environment in which the food was processed and the unsanitary or unhygienic status of the vendors. Studies showed that food handlers are the most vehicles for the transfer of pathogenic organisms from their body parts to food (Tarch, *et al.*, 2023). Lack of potable water, long-distance transportation of the product, processing techniques and unhygienic environment could have contributed to this phenomenon (Inetianbor *et al.*, 2017). Similar findings on microbial biodiversity in ready-to-eat fufu had been earlier reported (Inetianbor *et al.*, 2017).

Table 2: Contamination rate of ready-to-eat fufu sold in Calabar

| Location | No of samples | Standard viable bacterial count on ready -to-eat-food | | | No of isolates |
|---------------------|---------------|---|---|--|----------------|
| | | Satisfactory (10^2 Cfu/g) | Borderline (10^2<math><10^4</math>Cfu/g) | Unsatisfactory (>10^4Cfu/g) | |
| Crutech gate market | 20 | 10 | 8 | 2 | 4 |
| Goldie market | 20 | 5 | 11 | 4 | 6 |
| Akim market | | 9 | 6 | 5 | 5 |
| Marian market | 20 | 5 | 11 | 4 | 4 |
| Palm street market | 20 | 4 | 12 | 4 | 4 |
| Watt market | 20 | 4 | 11 | 5 | 8 |
| Timber market | 20 | 3 | 10 | 7 | 7 |
| Beach market | 20 | 7 | 9 | 4 | 5 |
| 8 miles market | 20 | 6 | 9 | 5 | 5 |
| Atakpa market | 20 | 6 | 11 | 3 | 4 |
| Total | 200 | 59 (29.5) | 98 (49.0) | 43 (21.5) | 52 |

The most predominant isolates were *Klebsiella pneumoniae* (25.9%), followed by *Citrobacter freundii* (22.2%), *Escherichia coli*, *Enterobacter cloacae* and *Burkholderia cepacia* with 14.8% isolation rate each while *Salmonella* sp (7.4%) was the least encountered bacteria (Figure 1). This finding is similar to (Ewanfo *et al.*, 2017) who reported the presence of coliform bacteria in commercial ready-to-eat-fufu sold in Benin city, Nigeria. In another study (Igbinsosa *et al.*, 2022). reported the presence of *E. coli*, *S. typhimurium*, *K. pneumoniae*, *Proteus mirabilis*, *Enterobacter* sp in Akoko area of Ondo state, Nigeria, which is in line with the present study (Inetianbor *et al.*, 2017) reported the presence of *E. coli* and *Salmonella* sp in ready-to-eat-fufu sold in Ghana. The diversity of bacteria in the presence study concurred previous report in the study area (Mbah *et al.*, 2012).The isolated bacteria are well-known pathogens linked to a number of disease outcomes, and the fact that they were found in the analyzed Fufu raises the possibility that other intestinal pathogens, including

toxigenic *E. coli* (0157:H7), *Listeria* sp, *Campylobacter* sp, etc. may also be present in the samples (Nyenje *et al.*, 2012). Thus, the presence of these organisms in the Fufu may present a health risk to those with underlying medical disorders, children, and pregnant women. The presence of *E. coli* and *E. cloacae* indicates faecal contamination and are often transferred via faecal-oral-route, through ingestion of contaminated foods or water. These organisms are implicated in high prevalence of gastroenteritis, nausea, fever, urinary tract infection etc. Also, *Salmonella* sp is an enteric pathogen responsible for enteric fever, food poisoning and bacillary dysentery (Brooks *et al.*, 2023). *Burkholderia cepacia* is an environmental isolate which can be transferred to human via contaminated foods or water and is implicated in cystic fibrosis, a severe respiratory infection (Odigie *et al.*, 2018). *K. pneumoniae* isolated from the samples causes pneumonia, bloodstream infection, surgical site infection and meningitis (Odigie *et al.*, 2018).

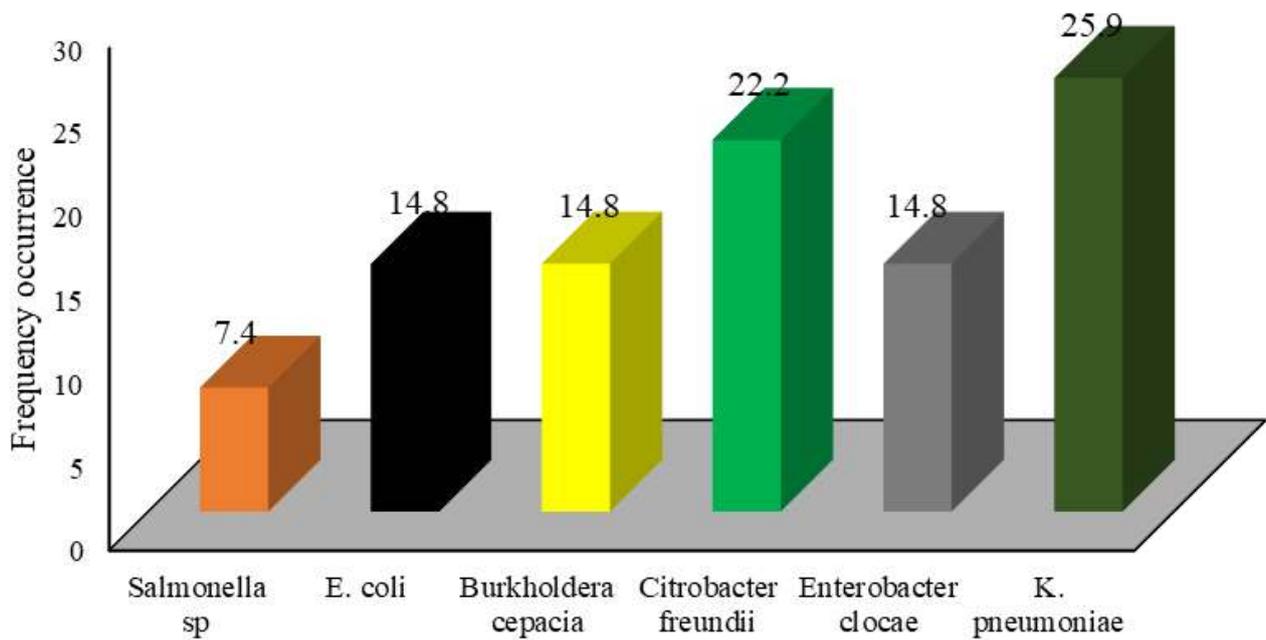


Figure 1: Percentage occurrence of bacterial species

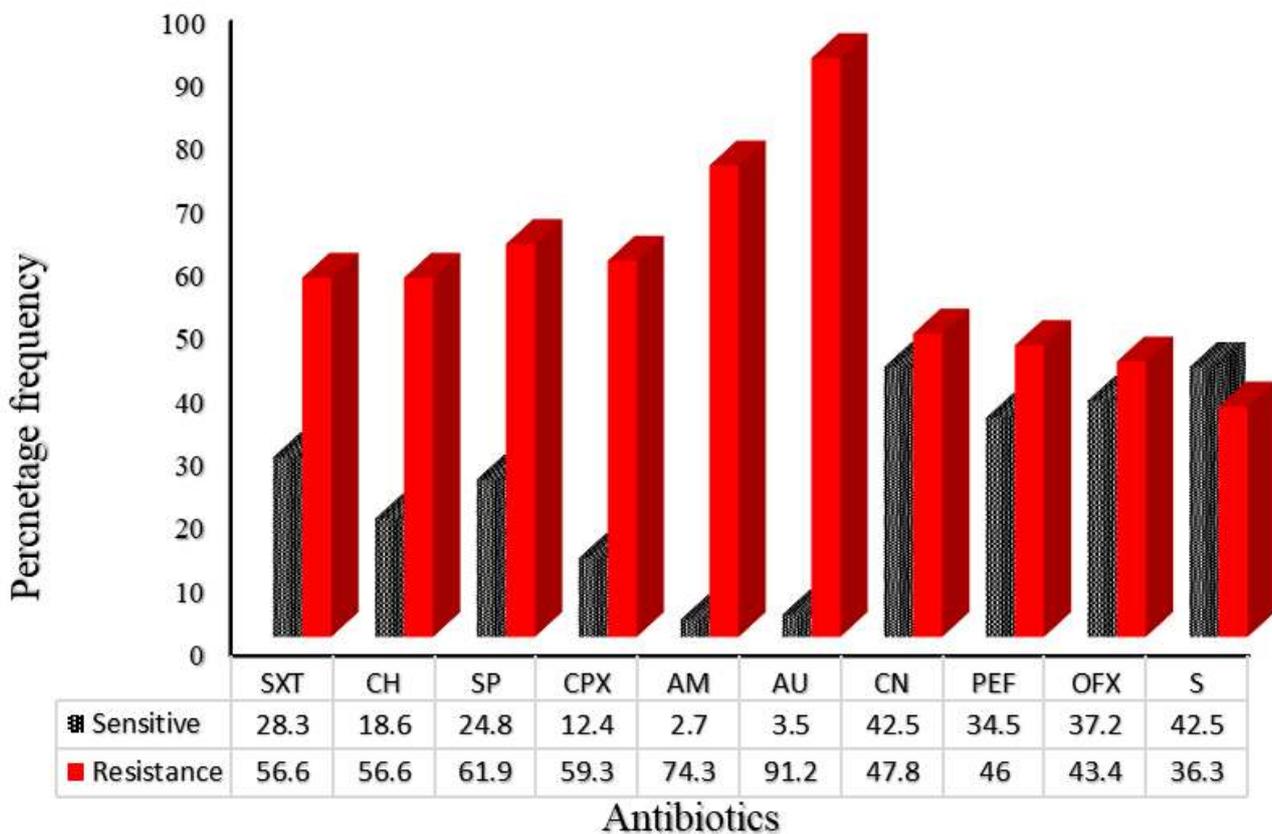


Figure 2: Percentage sensitive and resistance of bacterial isolates

Key: SXT: Septrin; CH: Chloramphenicol; SP: Sparfloxacin; CPX: Ciprofloxacin; AM: Amoxicillin; AU: Augmentin; CN: Gentamycin; PEF: Pefloxacin; OFX: Tarivid; S: Streptomycin.

Multi-drug resistance (MDR) and the production of extended-spectrum β -lactamase (ESBL) among the family Enterobacteriaceae is a great concern (Bassey *et al.*, 2022). Most of the isolated Enterobacteriaceae demonstrated varying degrees of susceptibility and resistance to the used antibiotics (Figure 2). In this study, *E. coli* showed extremely high resistance to Augmentin and Amoxicillin (100%), Chloramphenicol, Sparfloxacin, and Seprin (75%) which may be a sign of ESBLs producing strains. This study corroborated earlier documented reports (Igbiosa *et al.*, 2020). However, Pefloxacin and Streptomycin were the most effective drugs against *E. coli* isolates. Similar study in Tamale, Ghana found Gentamicin and Chloramphenicol as the potent drugs against *E. coli* and *Salmonella* sp (Adetunji *et al.*, 2017). In all, all the test isolates demonstrated a high degree of resistance on β -lactam antibiotics. The high resistance rate observed among members of Enterobacteriaceae support earlier assertion that majority of multidrug resistant isolates in clinical and environmental samples are Gram negative bacteria (Bassey, 2017; Odigie, 2018). Gram negative bacteria possessed outer membrane in addition to cell wall. This membrane prevents many substances from entering into the cell. Multi-drugs resistant strains pose serious health effects with attendant treatment failure, prolong hospital stay and increase cost of treatment (Kapoor, 2017).

Conclusion

This study showed that RTE Fufu sold in Calabar were unsatisfactory in terms of bacterial contamination. This is attributed to improper handling, inappropriate methods of foods storage, and poor water quality used in production. The isolated multi-drug resistant foodborne pathogens may serve as a reservoir for the trade and transmission of resistant strains to consumers as well as a frequent cause of bacterial food poisoning. Hence, surveillance studies of resistant pathogens in foods, increased education and training of food vendors on sanitation, food handling and safety practices in the region is recommended.

Competing Interests

Authors have declared that no competing interests exist

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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