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Bacteriological Assessment of Ready-To-Eat Foods Sold in Baze University Student Cafeteria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Although foods can be easily contaminated by naturally occurring microbes or pathogens by any means possible, safe eating is considered as a basic human right. In this study, the bacteriological quality of ready-to-eat (RTE) foods sold within the Baze University campus were assessed. Ready-to-eat food samples including spaghetti, fried rice, jollof rice, yam and moi-moi were bought from different major food vending sites to the student and staff community within the university. The Bacterial species were isolated from food samples and the isolates were identified using standard microbiological procedures. The ability of the isolates to produce hemolysins and lyse blood cells was investigated with hemolysis test and the antibiotic susceptibility pattern of the isolates was determined with the following antibiotics: Amoxicillin, Ampicillin, Tetracycline, Ciprofloxacin, Erythromycin and Gentamicin. The sensitivity and resistance of the isolates to these antibiotics

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were observed and recorded. A total of six (6) different bacterial species including *Corynebacterium* spp. (31.6%), *Staphylococcus* spp. (26.3%), *Streptococcus* (10.5%), *Micrococcus* spp. (10.5%), *Lactobacillus* spp. (15.8) and *Micrococcus varians* (5.3%) were isolated and identified. The isolates also showed varying reactions to hemolysis and some were highly resistant to common antibiotics, signaling their tendency to initiate foodborne illness. The study concluded that while these sites are necessary in meeting the needs of the university community, it is important that adequate safety measures be enforced to reduce the spread of pathogenic bacterial contaminants in RTE foods and enhance the wellbeing of consumers.

Keywords: Foods; ready-to-eat; pathogens; Baze university; quality.

1. INTRODUCTION

"Food is considered as a substance in its raw or processed form, consumed by living organisms for nutritional support. Despite the fact that many foods are often contaminated with naturally occurring pathogenic microbes, safe eating is a basic human right. Ready-to-eat (RTE) foods are foods intended by the producer or the manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to an acceptable level, any micro-organisms of concern" [1]. "Foods in this category usually contain raw materials of animal origin, such as eggs, fish, meat and poultry, cooked to allow the lowest internal temperature to reach a minimum temperature, at a minimum required holding time, to destroy microorganisms of public health concern. In an industrial setting, the cooking step is achieved by thermal processing using steam, hot water, microwave, or infrared. Due to improper handling, serving mechanisms or poor packaging, pathogenic microorganisms can find their way into ready to eat foods, capable of causing illness of varying severity" [2].

The rapid rise in population density has increased the production of RTE foods and based on its affordability and accessibility, catering firms or related establishments provide RTE meals at different private and public foundations such as medical facilities, nursing homes, educational institutions, military bases as well as strategic points in public places [3]. "There are many reasons people eat away from home. These include absence from home while travelling, studying, while at work or need for a change both in terms of food type and the location. As a result, many people purchase food from the streets. In Baze University, Abuja, Nigeria for instance, students on campus are not allowed to prepare food themselves within the University, but rather purchase food from cafeterias and canteens making these outlets the only source of RTE foods. Foodborne bacteria such as *Aeromonas* spp., *Bacillus cereus*, *Enterococcus* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella* spp., and *Shigella* spp. have been reported to contaminate RTE foods, using them as vehicles of entry into the human body" [4]. "Despite the available empirical data on foodborne bacterial contamination of RTE foods globally, there is heavy dependence on poorly processed and packaged RTE foods in countries categorized as low and middle income such as Nigeria" [1]. Furthermore, according to a World Bank study, the effects of contaminated food cost low-income and middle-income nations approximately US$ 110 billion annually in lost productivity and medical costs. Such data are necessary to elucidate the broad picture of food safety contaminants in these highly sourced foods. Moreover, the World Health Organization reports a foodborne disease burden of 45% among population who are major consumers of the RTE foods. This study was aimed to ascertain the bacteriological quality of RTE foods vended in Baze University, Abuja, Nigeria, with a view to promoting consumer's health and awareness for best hygienic practices amongst food vendors.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fifty (50) freshly cooked RTE food samples were collected randomly from major food vending locations in Baze University Abuja. Foods purchased include 10 samples each of fried rice, jollof rice, yam, spaghetti and *moi-moi*. Samples were collected between 9am and 5pm which are the most active hours within the campus. The samples were immediately transferred in ice packs to the microbiology lab, Department of Microbiology, Baze University, Abuja for bacteriological analysis.

2.2 Bacteriological Analysis of Food Samples

Twenty (20) grams of each food sample was aseptically taken into a sterile mortar, mashed, and homogenized in 200 mL of peptone water (Hi Media, Maharashtra, India). The homogenized samples were serially diluted by tenfold with maximum recovery diluent (MRD), and 1 mL aliquots were spread/pour plated on the general-purpose nutrient agar (Oxoid, Basingstoke, UK). Duplicate plates for each dilution were spread plated per food sample and incubated at 37^{o}C for 24 h. for the enumeration of total bacterial count (TBC). Thereafter, an assessment of the cultural characteristics (colony colour, elevation, and edges) and examination of cell morphology (colour, shapes, and arrangement) after Gram staining were performed for each isolate. Representative isolates based on differences in the cultural, morphological and staining characteristics were selected after the examinations aforementioned were maintained as pure cultures on nutrient agar slants and kept in the refrigerator at 4^{o}C [5].

2.3 Presumptive Identification of Bacterial Isolates

The bacteria isolates were identified based on standard microbiological methods via catalase, oxidase, citrate, coagulase tests and reaction on Triple Sugar Iron (TSI) using the Bergey's manual of determinative bacteriology [6].

2.4 Determination of Haemolysin Production in Foodborne Isolates

The potential of the isolates to induce any form of foodborne illness was determined by assaying for the production of haemolysin with the methods described by Foulquie-Moreno et al. [7]. These tests, though not definitive, are easy to perform in a resource-scarce setting. A haemolysis test was conducted by streaking each isolate on freshly prepared blood agar base (Oxoid CM0331) supplemented with 7% v/v antibiotics-free human blood and incubated at 37^{o}C for 48 h. After incubation, the production of green-hued zones around the colony was recorded as α-hemolysis while non production of any effect on the blood agar plates was recorded as γ-hemolysis. Lyses of blood around the colony of the test organism was classified as hemolytic (β-hemolysis).

2.5 Antibiotic Susceptibility Pattern of Isolates

Antibiotic susceptibility test was used to determine the appropriate antibiotics that will possibly inhibit the bacteria capable of causing foodborne illness. Antibiotics used include; Amoxicillin, Ampicillin, Tetracycline, Ciprofloxacin, Erythromycin and Gentamycin. This was done using the disc diffusion method described by Raut et al., [8]. Standardized suspension of each isolate in normal saline was spread on the surface of Mueller Hinton Agar plates using sterile swab sticks. After thorough spreading, antibiotic discs were aseptically placed on the surface of the agar plates using sterile forceps. The plates were then incubated at 37^{o}C for 24h. The diameter of zone of inhibition was measured after incubation and the values were recorded in millimetre. The values were compared to CLSI standard and the susceptibility pattern was recorded.

2.6 Data Analysis

Bacteria enumeration was performed in triplicates and values were recorded as mean and standard deviation of mean. Data were analysed using SPSS Statistics package version 20.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were applied to elucidate the distribution of bacteria in the RTE food types.

3. RESULTS AND DISCUSSION

3.1 Bacteria Count in RTE Food Samples

The occurrence of bacterial contamination varied across food samples. Yam had the highest TBC of 5.8 x 10^5 with the lowest TBC of 1.4 x 10^3 per gram observed in spaghetti (Table 1). Some studies have also reported bacteria contamination of RTE foods in numbers higher than 10^3 which poses a significant threat to the health of consumers (Al-Nasiry, 2020; Amare et al., 2019). The higher TBC in boiled yam when compared to other food samples could be as a result of poor handling during processing and/or unhygienic practices during post food processing activities. Other sources of bacterial contamination could also be through water used to wash the equipment and utensils as well as the activities of large number of people in or around the serving area. Also, the time between food preparation and consumption with frequent exposure could be a factor encouraging microbial contamination.

Table 1. Total bacterial count (TBC) of RTE foods sold in different cafeteria of Baze University campus, Abuja, Nigeria

RTE Food type	Mean TBC (CFU/g)	SD (CFU/g)
Jollof rice	1.9×10^4	1.3
Fried rice	1.5×10^4	1.8
Yam	5.8×10^5	2.7
Spaghetti	1.4×10^3	1.3
Moi moi	2.0×10^4	1.1

SD =Standard Deviation

3.2 Occurrence of Bacteria Isolates in RTE Foods

A total of 35 different bacteria isolates were recovered from the RTE food samples sold in different cafeteria in Baze University based on their cultural and morphological characteristics. The distribution had jollof rice and fried rice recording 5 and 7 different bacteria species respectively. The highest number of bacteria contaminants was observed in Yam with 10 different bacteria isolates while 8 and 5 isolates were recovered from spaghetti and moi moi respectively.

3.3 Presumptive Identification of Bacterial Isolates

Based on the presumptive identification of the isolates, the bacteria are members of five (5) different species including *Corynebacterium* (28.5%), *Staphylococcus* (25.7%), *Streptococcus* (20.0%), *Micrococcus* (14.4%) and *Lactobacillus* (11.4%). The percentage occurrence is presented in Fig. 1. The occurrence of some of the specie has been reported in some studies based on microbial evaluation of RTE [9,10]. Specie of *Staphylococcus*, *especially S. aureus* is one of the major bacterial agents causing foodborne disease in humans worldwide. The lethal enterotoxin produced by *S. aureus* are common causes of food poisoning. *S. aureus* produces staphylococcal enterotoxins (SEs) in contaminated food, and ingestion of SEs containing food can induce severe symptoms, including vomiting and high fever with/without nausea and diarrhea, with rapid onset in typically less than 8 hours (usually between 3 and 4 hours). Enumeration of *S. aureus* in food matrices is a more sensitive measure for food hygiene practices rather than testing solely for the presence of enterotoxins which could give a false sense of security when the toxins are not yet produced. Food handlers are the most carriers and sources of *S. aureus* especially at nasal areas and thus serving as vehicle of enterotoxigenic *S. aureus* in restaurants and food outlets [11]. “The presence of *S. aureus* or its enterotoxins in processed food is generally an indication of poor sanitation. Studies have described different incidents of infections, where species of *Corynebacterium* are isolated as the etiological factor” [12-14]. Some strains of this species are reported to produce a strong exotoxin. An example of this is *Corynebacterium diptheriae* and are responsible for causing diphtheria [15]. “Furthermore, *Streptococci* are known to colonize the mucosal surfaces of mouth, intestinal tract, nasal passages and pharynx of human and other animals. The presence of *Streptococci* in drinking water or food matrices indicates faecal contamination. In most Streptococcal food poisoning cases, the food was allowed to stand at room temperature for several hours between preparation and consumption. The contamination of the food is most often the result of poor hygiene, handling of the food by infected people” [16]. Although *Micrococcus* is not considered to be pathogenic, some strains can cause various types of infections as opportunistic pathogens. They are normal flora of the skin of warm- blooded animals including humans and frequently contaminate foods of animal origin [17]. Similarly, the pathogenicity of *Lactobacillus* is rare. They are involved in the production of various food products through fermentation but their occurrence in RTE foods in this study could be through post processing activities.

3.4 Determination of Haemolysin Production in Foodborne Isolates

The ability of the isolates to produce haemolysin and lyse blood cells were analysed. Isolates showed varying haemolytic reactions with 37% of the isolates showing Alpha (α) haemolysis while 32% showed Beta (β) hemolysis and 31% were observed to have no hemolytic reaction which is Gamma (Y) hemolysis (Fig. 2). Gram-positive bacteria are known to exhibit toxigenic properties with their ability to break down red blood cells [18]. Although molecular confirmation of

haemolytic properties of bacteria is necessary for assertions, the phenotypic presentation of haemolysin production is a presumptive test for toxicity. "This makes the presence of these organisms in RTE foods sold in campus a thing of concern for the safety and wellbeing of consumers. More Epidemiological risk-based studies of RTE foods are necessary to ensure the safety of RTE food consumers, especially in immunocompromised individuals or people with weaker immune system such as children and infants. The underestimated health risks, loss of RTE food quality, and economic losses associated with the presence of haemolytic organisms could result in direct consequences on consumers and vendors of RTE foods, such as severe gastroenteritis and loss of livelihood, respectively" [1].

3.5 Antibiotic Susceptibility Pattern of Isolates

The isolates showed varying reactions to the different antibiotics used in the study. All the isolates were resistant to Amoxicillin while 47%, 16% and 11% were sensitive to Gentamicin, Ampicillin and Tetracycline respectively. Also, some isolates fell within the intermediate range for Erythromycin and Tetracycline. Surprisingly, the percentage sensitivity was lower than that of resistance as observed for all isolates in the current study (Fig. 3).

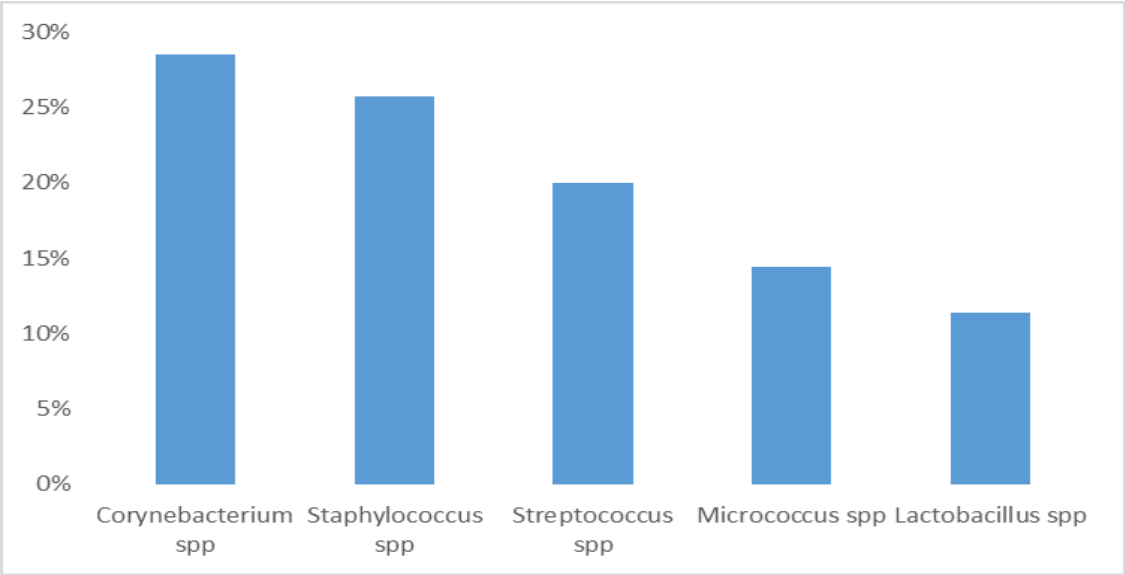

Fig. 1. The percentage occurrence of bacteria in ready-to eat foods

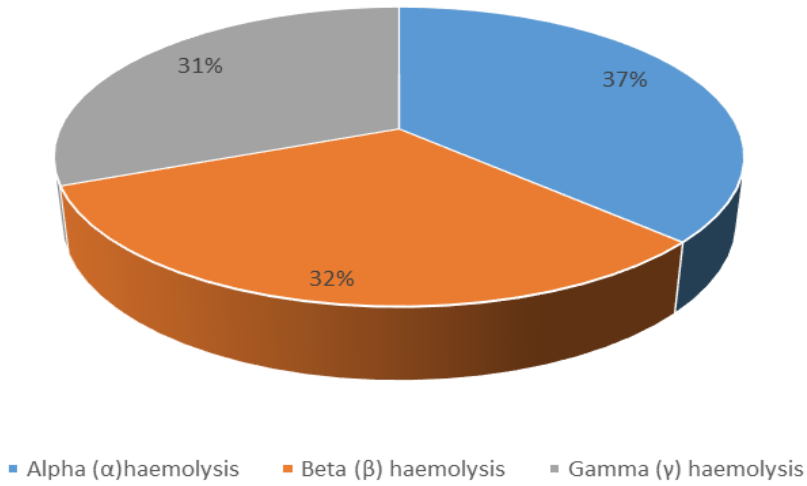

Fig. 2. Haemolytic pattern of bacteria isolates

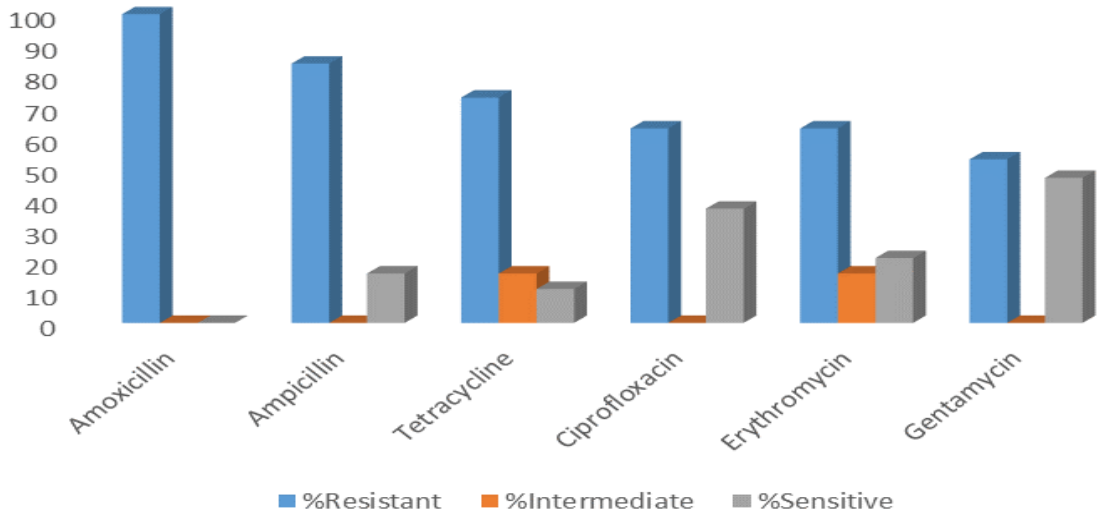

Fig. 3. Antibiotic susceptibility pattern of bacteria isolates

Antibiotics have been used as important treatment of infectious disease. Food is a common vector for delivering antibiotic resistant organisms to human and the occurrence of resistance of isolates to commonly used antibiotics in this study represent a major concern for the wellbeing of consumers. The antibiotic susceptibility pattern observed in this study is similar to the report of Cole and Singh [19,20] on the microbial occurrence and antibiotic resistance of ready-to-go food items [21,22].

4. CONCLUSION

In conclusion, RTE foods in this study presented an unsatisfactory microbial quality as bacterial contaminants were found in all the food types from all the vending sites. Furthermore, the production of hemolysin and resistance ability of these isolates to common antibiotics are major points of concern. Hence, the consumption of these RTE foods could pose risks of foodborne illnesses to consumers, especially students who heavily depend on RTE foods within the campus in numerous times on a daily basis. This suggests that routine surveillance on the cafeteria within the campus should be done regularly as well as education to food processors and handlers to enhance food quality and safeguard consumer health. This will further support the actualization of good health and wellbeing as one of the United Nations sustainable development goals in the country. Further research on the evaluation of all food items sold within the campus would be necessary in establishing the hygienic level of consumed foods within the campus and potential risks on consumers.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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