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Microbiological Analysis of Ready-To-Eat-Foods Obtained from Bukaterian within the Ekiti State University and Environment, Ado-Ekiti, Nigeria

Peter Oluwayinka Akindele[1*] and Kazeem Adewumi Ibrahim[2]

[1]Department of Microbiology, Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria.
[2]Department of Microbiology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author POA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors POA and KAI managed the analyses of the study. Author POA managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Food-borne diseases are the global public health problem. At random 75 food samples comprising of fifteen each of the five commonly eaten ready-to-eat foods (rice, beans, yam, fufu and meat) were collected from different vendors of the university. Aerobic bacterial count and fungal count were determined by counting the colonies on nutrient agar plates and saboraud dextrose agar plates respectively. The identification of the organisms was determined by their morphology, culture characteristics and biochemical profile. The result obtained revealed that Mean aerobic plate counts ranged from 1.0 x 10^2 cfu/g (rice) to 6.0 x 10^4 cfu/g (meat) and mean fungal count ranged from 1.3 x 10^2 cfu/g (rice) to 5.2 x 10^4 cfu/g (meat). A total of eleven species (spp) of microorganisms including *Escherichia coli, Bacillus cereus, Salmonella* spp*., Clostridium perfringens, Shigella* spp*., Klebsiella* spp*., Proteus* spp*., Staphylococcus aureus, Campylobacter* spp*., Aspergillus* spp. and *Mucor* spp. were isolated from the food samples. *Bacillus cereus* had the highest percentage frequency with (18.12%) while *Campylobacter* spp. had the lowest percentage

**Corresponding author: E-mail: Akindelepeter7@yahoo.com;*

frequency with (1.45%). Fufu had the highest percentage of contamination of 35.51% with lowest in yam and meat which both had 5.8%. Based on the specifications by International Commission for Microbiological Specification for Foods (ICMSF), the level of contaminations was within acceptable microbiological limits except for Meat and Fufu; this could be attributed to inadequate processing, poor handling practices and post-cross contamination which can pose danger to the health of the consumers. It is recommended that regular microbiological quality control programs and good hygiene practices should be encourage.

Keywords: Food vendors; foodborne pathogen; microbiological quality; ready-to-eat foods.

1. INTRODUCTION

Ready-to-eat foods are defined as foods easily purchased from the food vendors such as restaurants or food hunt. These kinds of foods are consumed at the point of sale without any further treatment such as heating. In the south-western part of Nigeria such as Ekiti, Lagos, Ogun, Oyo, Ondo and Osun foods such as rice, beans, fufu, eba, yam flour and plantain are mostly purchased at food vendors. Based on the relatively cheap and easily available of these ready-to-eat foods, its consumption has increased over the few years. Despite the excessive patronage of these food vendors /handlers; it is very essential to ensure the safety level of these foods from contaminants and microorganisms.

Over the years Food borne disease outbreaks linked with Ready-To-Eat foods have been associated with various foodborne pathogens [1, 2]. Foodborne diseases are a major global problem causing considerable morbidity and mortality annually [3]. Bacteria such as *Salmonella species*, *Escherichia coli* and *Staphylococcus aureus* can cause food poisoning and other food-borne diseases such as tuberculosis, typhoid fever and cholera [4]. Some symptoms of food borne illness among others include stomach pain, diarrhea, vomiting, nausea and headache. The global incidence of food borne diseases is difficult to estimate but it has been reported that in 2000 alone, 2.1 million people died from diarrheal diseases. A great proportion of these cases can be attributed to contamination of food and drinking water [5]. In United States, it has been estimated that seven pathogens found in animal product such as *Escherichia coli* 0157:H7, *Listeria monocytogenes*, *Campylobacteria jejuni*, *Clostridium perfringens*, *Salmonella spp*., *Toxoplasma gondii* and S*taphylococcus aureus* account for approximately 3.3-12.3 million cases of food borne illnesses and a record of 3900 deaths each year [6]. Scharff [7] estimated that the total cost of food borne illness in USA is almost $152 billion per annum.

Many studies have shown that the most common bacteria associated with RTE food are *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni*, *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium perfringens*. A number of observational studies have shown that these foods are sometimes held at improper temperatures, excessively handled by food vendors and sold at very dirty surroundings [8, 9]. In addition, the vendors practice poor personal hygiene and reports of food vendors being carriers and therefore could serve as a potential source of transmission of enteric fevers are many [10].

Most of the food handlers have no proper formal education in food handling which can also promote transmission of food borne illness. The majority of students on Campus do not prepare food themselves or take it along with them to the University. This demand for food gives opportunity to the Cafeterias and canteens to serve as the major vending sites where students purchase food daily. This objective of this study aimed to analyse the bacteriological profile of RTE food produced by various bukaterian in the University premises and to determine whether these foods meets the acceptable microbiological standards and specification for foods.

2. MATERIALS AND METHODS

2.1 Study Area

The study took place at the major food vending zones (north, south, east and west) of Ekiti State University (EKSU), Ado-Ekiti, Ekiti State, Nigeria; where students buy foods within the school hours. EKSU is situated at 7.7105° N and 5.2444° E. The vending sites were selected due to the level of students patronage.

2.2 Sample Size

At random 75 food samples comprising of fifteen each of the five commonly eaten ready-to-eat foods (rice, beans, yam, fufu and meat) were collected from different vendors of the university. The food samples were purchased when freshly prepared and collected into a sterile specimen container between a period of September and October, 2011. The freshly collected samples were immediately transferred in ice packs, under aseptic condition to the Department of Microbiology Laboratory, Ekiti State University, for microbiological analysis within one hour of collection.

2.3 Sample Analysis

Ten (10) grams of each food sample was homogenized with 90 ml sterile normal saline. Further five-fold serial dilutions of the resultant homogenates were made to obtain 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} respectively. From the appropriate dilutions, 0.1 ml was plated in replicate onto different media using pour plate technique. Nutrient agar, Eosin Methylene Blue and Mannitol salt agar (all of Oxoid grade) were inoculated for aerobic plate count. Sabouraud - Dextrose agar (Fluka) was used for isolation of fungi while Salmonella Shigella agar (Fluka) was inoculated after 24 hrs pre-enrichment of sample homogenates in selenite-F broth, for isolation of *Salmonella*. All inoculated plates were incubated at 37°C for 24-48 hrs except, however, Sabouraud-Dextrose agar plates that were incubated at 28°C for 72 hrs. At the end of the incubation periods, colonies were counted using illuminated colony counter (Gallenkamp, Englang). The counts for each plate were expressed as colony forming unit per gram of sample homogenate (cfu/g). Morphological attributes of the colonies on the media were observed; discrete colonies on the different media were isolated and purified by repeated sub-culturing on nutrient agar. Pure cultures were stored on agar slants at 4°C for further characterization.

2.4 Coliform Test

2.4.1 Presumptive test

One (1) ml of each sample homogenate was transferred to sterile test tubes containing Lactose broth and inverted Durham tubes. Incubation was for 24-48 hrs at 37°C before tubes were checked for gas production.

2.4.2 Confirmatory test

A loop full of inoculum from the gas positive tubes was streaked onto Eosin Methylene Blue agar plates. Incubation was at 37°C and 44°C for 24 hrs. After incubation, colonies which showed bluish black colour with green metallic sheen and reddish/brown colonies were noted and isolated on agar slants.

2.4.3 Completed test

Colonies which formed green metallic sheen on Eosin Methylene Blue agar were sub cultured into tubes containing lactose broth and incubated at 37°C for 24 hrs after which the tubes were observed for gas production [11].

2.5 Identification and Characterization of Bacterial Isolates

The isolated bacteria were identified by using their cultural and morphological characteristics on media. This was followed by microscopic examination of the bacterial isolates under the microscope. The cultural features examined included shape elevation, surface edge and consistency. Physiological and biochemical tests were employed to confirm their identification [12].

3. RESULTS

The result obtained revealed that Mean aerobic plate counts ranged from 1.0×10^2 cfu/g (rice) to 6.0×10^4 cfu/g (meat) as shown in Table 1.

Mean fungal count which ranged from 1.3×10^2 cfu/g (rice) to 5.2×10^4 cfu/g (meat) as shown in Table 2.

Species of Microorganisms detected in different food samples namely: *Escherichia coli, Bacillus cereus, Salmonella* spp*., Clostridium perfringens, Shigella* spp*., Klebsiella* spp*., Proteus* spp*., Staphylococcus aureus, Campylobacter* spp*., Aspergillus* spp. and *Mucor* spp as shown in Table 3.

The percentage frequency of the microorganisms isolated is shown on Fig. 1 where *Bacillus cereus* had the highest frequency of occurrence in all food type with (18.12%) while *Campylobacter* spp. had the lowest frequency of occurrence with (1.45%). *Bacillus cereus* had the highest frequency of occurrence in rice with (14.01%) recorded in rice as shown in Fig. 2. Fig. 3 revealed percentage frequency of contaminated

to uncontaminated food type. The highest percentage frequency of containment food type is recorded in fufu with 35.51% while the lowest percentage frequency of contaminated food type is recorded in yam and meat with 5.8 and 5.8% respectively.

Table 1. Mean aerobic plate counts (cfu/g) of various food sample

Food samples	ZONE A	ZONE B	ZONE C	ZONE D
	ABC	ABC	ABC	ABC
Rice	$4.4 \times 10^3 \pm 0.25$	$5.1 \times 10^3 \pm 0.15$	$2.5 \times 10^2 \pm 0.43$	$1.0 \times 10^2 \pm 0.11$
Beans	$5.6 \times 10^3 \pm 0.19$	$6.0 \times 10^3 \pm 0.43$	$3.3 \times 10^2 \pm 0.15$	$2.4 \times 10^2 \pm 0.24$
Yam	$3.4 \times 10^3 \pm 0.42$	$3.6 \times 10^3 \pm 0.20$	$2.1 \times 10^2 \pm 0.20$	$1.3 \times 10^2 \pm 0.60$
Fufu	$5.2 \times 10^3 \pm 0.22$	$5.5 \times 10^4 \pm 0.19$	$3.8 \times 10^2 \pm 0.72$	$3.1 \times 10^3 \pm 0.12$
Meat	$5.4 \times 10^3 \pm 0.27$	$6.0 \times 10^4 \pm 0.42$	$4.5 \times 10^2 \pm 0.29$	NG

Legend: ABC- Aerobic bacterial count, ZONE A - Campus joint, ZONE B - Satellite, ZONE C - School gate, ZONE D - Faculty of Science

Table 2. Mean fungal counts (cfu/g) of various food sample

Food samples	ZONE A	ZONE B	ZONE C	ZONE D
	FC	FC	FC	FC
Rice	$3.1 \times 10^2 \pm 0.34$	$4.2 \times 10^2 \pm 0.17$	$2.8 \times 10^2 \pm 0.33$	$1.3 \times 10^2 \pm 0.18$
Beans	$4.4 \times 10^2 \pm 0.16$	$5.0 \times 10^2 \pm 0.52$	$2.9 \times 10^2 \pm 0.42$	$2.2 \times 10^2 \pm 0.61$
Yam	$3.5 \times 10^2 \pm 0.44$	$3.5 \times 10^2 \pm 0.20$	$2.2 \times 10^2 \pm 0.71$	$1.5 \times 10^2 \pm 0.90$
Fufu	$3.9 \times 10^2 \pm 0.23$	$4.5 \times 10^2 \pm 0.55$	$3.3 \times 10^2 \pm 0.60$	$2.8 \times 10^2 \pm 0.55$
Meat	$4.4 \times 10^2 \pm 0.32$	$5.2 \times 10^2 \pm 0.42$	$3.4 \times 10^2 \pm 0.15$	$3.6 \times 10^2 \pm 0.52$

Legend: FC- Fungal count, ZONE A - Campus joint, ZONE B - Satellite, ZONE C - School gate, ZONE D - Faculty of Science

Table 3. Microbial isolates from food samples

Food samples	Microorganisms isolated
Rice	*Bacillus cereus, Salmonella* spp., *Escherichia coli, Staphylococcus aureus, aureus, Klebsiella* spp., *Proteus* spp., *Aspergillus* spp. and *Mucor* spp
Beans	*Escherichia coli, Bacillus cereus, Salmonella* spp., *Shigella* spp., *Klebsiella* spp., *Proteus* spp., *Staphylococcus aureus*, *Aspergillus* spp. and *Mucor* spp.
Yam	*Escherichia coli*, *Staphylococcus aureus, Klebsiella* spp., *Clostridum perfringens* and *Aspergillus* spp.
Fufu	*Escherichia coli, Bacillus cereus, Salmonella* spp., *Clostridium perfringens, Shigella* spp., *Klebsiella* spp., *Proteus* spp., *Staphylococcus aureus, Aspergillus* spp. *and Mucor* spp.
Meat	*Campylobacter* spp., *Clostridium perfringens* and *Bacillus cereus*

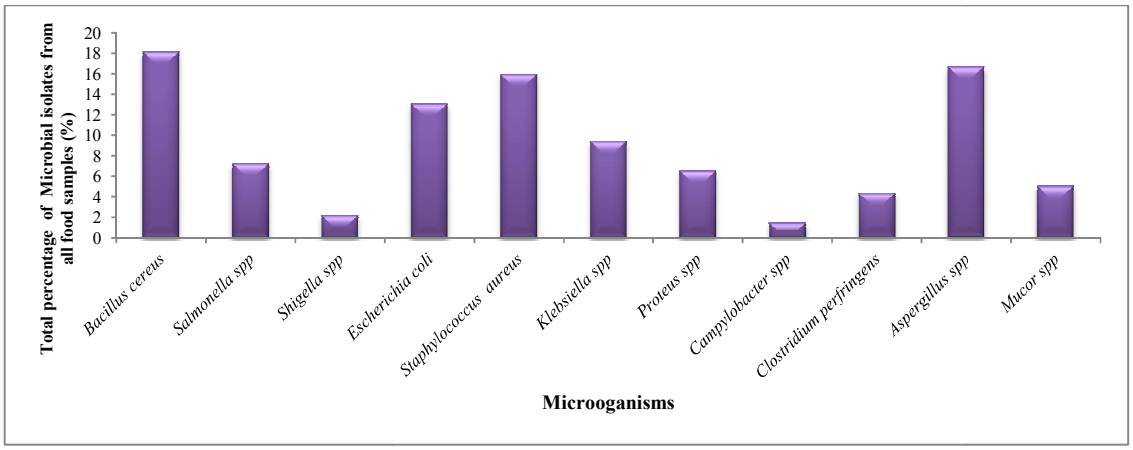

Fig. 1. Total percentage microbial isolates from all food samples

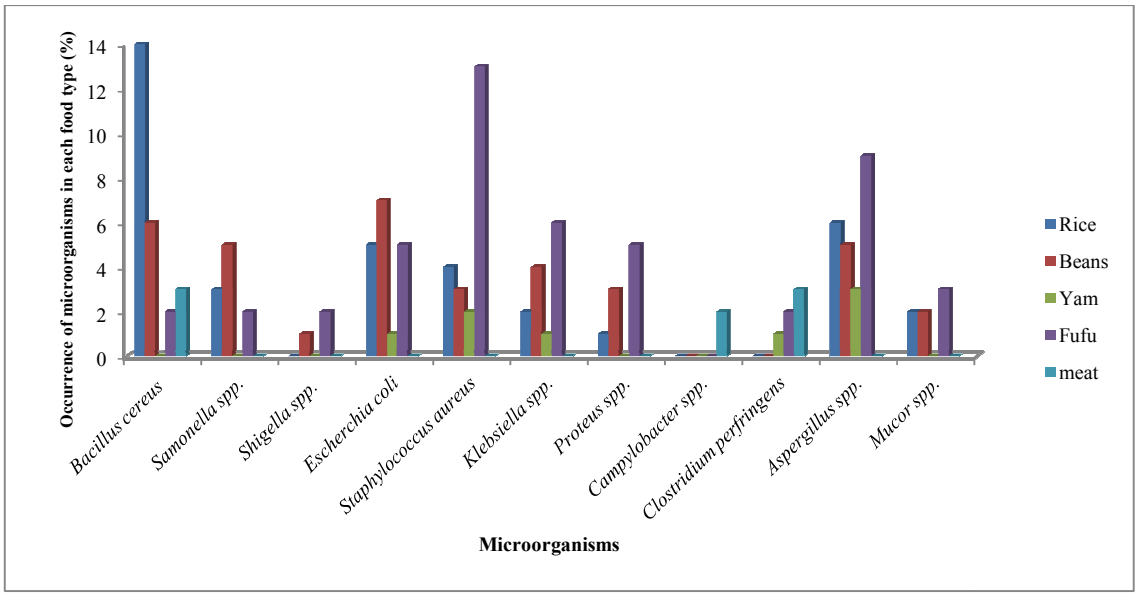

Fig. 2. Frequency of occurrence of microorganism in each food type

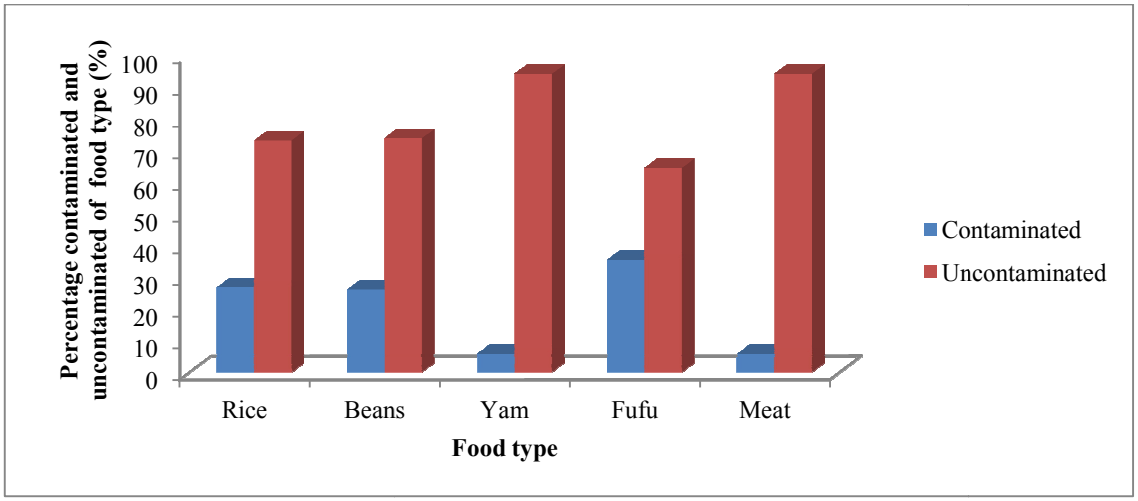

Fig. 3. Percentage frequency of contaminated to uncontaminated food type

4. DISCUSSION

Foodborne diseases are a major global problem causing considerable morbidity and mortality annually [3]. The most common known causes of foodborne diseases are pathogenic bacteria. In this study, the aim was to analyse the microbiological profile of RTE food produced by various bukaterian within Ekiti State University, Ado-Ekiti.

In this study, the mean aerobic bacterial counts ranged from 1.0 x 10^2 cfu/g (rice) to 6.0 x 10^4 cfu/g (meat) (Table 1) while Table 2 revealed the mean fungal count which ranged from 1.3 x 10^2 cfu/g (rice) to 5.2 x 10^4 cfu/g (meat). Microbial loads of the food samples especially Meat and fufu was higher than stipulated. This may arise due to improper handling of foods and unhygienic measures taken by the food vendors. These microbes may be introduce during chain of its production, processing, and storage as suggested by Oranusi et al. [10] that the high level of contamination of coleslaw could be associated with extensive handling and mixing during processing via food handlers, utensils and from the environment. The isolation of *Escherichia coli, Bacillus cereus, Salmonella*

spp., *Clostridium perfringens, Shigella* spp., *Klebsiella* spp., *Proteus* spp., *Staphylococcus aureus, Campylobacter* spp., *Aspergillus* spp. and *Mucor* spp as contaminate corroborate with the suggestion of Nichols et al. [13], Mensah et al. [14], Idowu [15], Taulo et al. [16], Ajao and Atere [17] and Oranusi and Braide [18] which reported that microorganisms was implicated in ready-to-eat foods. Presence of moulds *Mucor* sp and *Aspergillus* spp. isolated in the food sample can be introduce through dust and soil as they disperse in the form of spores which is abundant in the environment [19]. In this study, the presence of moulds in food samples is of serious health concern as also suggested by Makun et al. [20] which reported that is of serious public health concern as these fungi have all been implicated with the production of mycotoxin. The occurrence of *Bacillus cereus* and *Clostridium perfringens* in this study collaborate as suggested by Rajkowski and Bennett [21] that these bacteria are associated with the production of toxin; diarrheal and emetic in food, which causes food poisoning. These bacteria are found in dust, soil and raw food and can survives normal cooking as a heat resistant spore. These heat-resistant spores may have survived processing while vegetative cells were eliminated.

The presence of *S. aureus* in RTE food is an indication of poor hygiene practices. *S. aureus* in RTE food is associated with cross contamination occurring during processing and storage or through the contamination of raw ingredients.

In this study, the detection of coliform bacteria (*Shigella* spp., *Salmonella* spp*, Proteus* spp. and *Klebsiella* spp.) and *E. coli* shows the possibility of fecal contamination microorganism as also suggested by Adams and Moss [22] but in contrast with Tambekar et al. [23] were *Salmonella* or *Shigella* species were not detected. In the present study, *Bacillus cereus, Salmonella* spp., *Escherichia coli, Staphylococcus aureus, Klebsiella* spp., *Proteus* spp., *Aspergillus* spp. and *Mucor* spp demonstrates a potential health risk as these organisms are pathogenic and have been implicated in food borne diseases. Hence, Wagner [24] and Osamwonyi et al. [25] suggested that it is mandatory that foods must be free from contaminations as much as possible. Based on the specifications by International Commission for Microbiological Specification for Foods (ICMSF) [26], the level of contaminations was within acceptable microbiological limits except for Meat and Fufu; this could be attributed to inadequate processing, poor handling practices and post-cross contamination which can pose danger to the health of the consumers. Foodborne illness can be prevented by good hygiene practices such as the use of Hazard Analysis Critical Control Point (HACCP) application in the chain of food production, processing and storage. Educating the food handlers/ food vendors on food safety practices and strict supervision of ready-to-eat foods sold to students and staff in the University should be properly investigated by the relevant authorities to prevent epidemics of food borne illness within the university and environment.

5. CONCLUSION

Based on the specifications by International Commission for Microbiological Specification for Foods (ICMSF), the level of contaminations was within acceptable microbiological limits except for Meat and Fufu; this could be attributed to inadequate processing, poor handling practices and post-cross contamination which can pose danger to the health of the consumers. Foodborne illness can be prevented by good hygiene practices such as the use of Good Manufacturing Practices (GMP) and Hazard Analysis Critical Control Point (HACCP) application in the chain of food production and processing. It is recommended that regular microbiological quality control programs and education of the food handlers/ food vendors on food safety practices should be encourage. Strict supervision of ready-to-eat foods sold to students and staff in the University should be properly investigated by the relevant authorities to prevent epidermics of food borne illness within the university and environment.

ETHICAL APPROVAL

Relevant University officials gave approval.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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