



## Microbiological Assessment of Ready-to-Eat Foods (RTEs) for the Presence *Bacillus* species

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### Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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### ABSTRACT

This study aimed to microbiologically assess some ready-to-eat (RTE) foods for presence of *Bacillus* species. Hygienic conditions of the food vending sites were also considered. Samples were purchased from nine sale points. Sixty ready-to-eat (RTE) food samples (Buns, Meat pie, Egg roll, White rice, Jollof rice and Fried rice) were randomly purchased from cafeteria, canteens and various food vending sites within and around the institution. Immediate microbiological analysis was carried out to assess the microbiological quality of the foods using general purpose (Nutrient agar) for total viable counts, and selective/differential isolation media (HiCrome *Bacillus* agar - HiMedia) for assessment of *Bacillus* species. Bacteria isolated with percentage occurrence include *Bacillus cereus* (11.26%), *Bacillus subtilis* (21.85%), *B. megaterium* (24.59%) and *B. thuringiensis* (25.83%). Other bacilli such as *Bacillus stearothermophilus*, *B. licheniformis*, *B. amyloliquefasciens*, *B. mycoides*, *B. brevis*, *B. polymyxa* and *B. laterosporus* constituted 16.56%. Total viable count (TVC) varied significantly among samples, especially with the buns ( $134.00 \pm 0.10$ ) samples in week six. Similar observation was made for differential bacilli count at week 6 ( $62.00 \pm 0.04$ ). TVCs for samples were observed to be within the satisfactory and marginal range for standard plate count (SPC); while differential *Bacillus* counts were observed within the marginal to unsatisfactory range. No matter how seemingly clean or unkempt the preparatory environment for these foods, *Bacillus* species were still present at all sample sites. The research reiterated that ready-to-eat foods (RTEs) rarely meet microbiological standards for quality.

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## 1. INTRODUCTION

Based on the report of European Food Safety Agency [1], 1–33% of food-borne poisonings are caused by *B. cereus*. Food poisoning is caused with presence of bacteria in food due to improper food preparation or cooking process and exposure of food to temperatures of 30°C. Common food poisonings are usually mild, but deaths due to food poisoning are also reported. Food poisoning occurs within 48 hours after consumption of contaminated food or drink. The symptoms include nausea, vomiting, diarrhoea and abdominal pain. Most cases of food poisoning are caused by bacteria, viruses or toxins and chemicals [2].

Dairy products, fatty foods, bread, cakes and pastries, seafood can easily be contaminated with *Bacillus* spp. *B. cereus* can cause food poisoning resulting in gastroenteritis. *Bacillus* species are ubiquitous in nature and can be found in soil or in a variety of dried foods such as grains, legumes, starches and spices as vegetative cells and endospores [3]. According to Food and Drug Administration of the United States, food poisonings due to *B. cereus* group have two different clinical syndromes, diarrheal and emetic (vomiting) syndrome. The emetic type causes vomiting after 0.5–6 h of ingestion [4] and diarrhoeal type causes abdominal pain and diarrhoea after 8 to 16 h of consumption. The diarrheal syndrome has been associated with a wide variety of food including meats, milk, vegetables and fish. The emetic syndrome has been generally associated with rice products, starchy foods such as potato, pasta, noodles, spaghetti, pastry and cheese products [5,6].

Some of *B. cereus* outbreaks are under reported as the illness associated with these bacteria limit itself and does not become severe. A recent survey on culture practices for outbreaks of apparent food borne illness showed that 20% of state public health laboratories do not make *B. cereus* testing routinely available. The survey also found that most of food handlers (in food stalls and restaurants) were unaware that cooked rice was a potentially hazardous food [7].

There has been very little study on the occurrence of *B. cereus* and *Bacillus* species in rice products and pastries in Nigeria. The aim of this study was to isolate *B. cereus* and other *Bacillus* species from some ready-to-eat foods, determine microbial load in these foods as a

pointer to their microbiological quality at point of sale, and also their prevalence. Hygienic conditions at sampling points would also be highlighted.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection and Analysis

Sample collection and analysis was carried out according to the methods of Cheesbrough [8] and Fawole and Oso [9]. A total of sixty RTE food samples (10 samples each of 3 different types of pastry products – Egg roll, Meat pie, and Buns; 10 each of different rice products – White rice, Jollof rice and Fried rice) were purchased from different food vending sites and cafeterias in and around the Federal University of Technology, Akure (FUTA), within a period of ten weeks. Food samples purchased were appropriately labelled and transferred to the laboratory for immediate analysis.

#### 2.1.1 Total viable counts (TVC)

Total Viable Counts (TVC) - Microbiological analysis was carried out using the serial dilution technique with pour plating onto Nutrient agar (NA).

#### 2.1.2 Microbiological analysis

Microbiological analysis for *Bacillus* species was done using serial dilution technique with spread plating onto HiCrome *Bacillus* Agar (HiMedia M1651-500G, India), a selective / differential isolation media, for assessment of *Bacillus* species. The fourth dilution was used for plating onto these media. Culture media were prepared according to manufacturer's specification and sterilization of materials was done in an autoclave at 121°C for 15 minutes.

### 2.2 Preservation of Bacteria Isolates

Discrete colonies isolated and purified by repeated sub-culturing were preserved according to Olutiola et al. [10], on slants at 4°C for further characterization.

### 2.3 Classical Identification of Bacterial Isolates

Identification of isolates was performed based on their morphological, and biochemical

characteristics, as described in Bergey's Manual of Systematic Bacteriology [11].

### 3. RESULTS

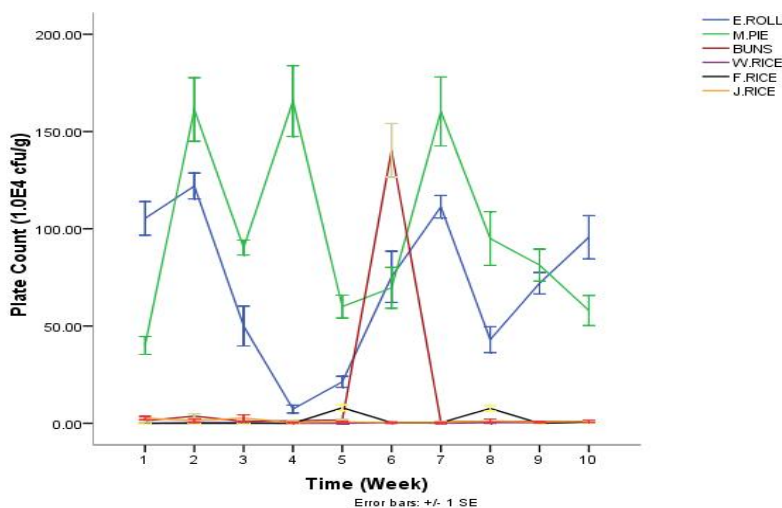
Table 1 showed the count range from TVC and differential *Bacillus* count. Meat pie and fried rice samples as having the highest number of samples contaminated by *B. cereus*, as shown in Table 1; followed by Egg roll, Buns, Jollof rice; and least, White rice. TVC for all samples ranged from 100 – 197 x 10<sup>4</sup> cfu/gram of food samples tested. TVC for egg roll, meat pie and buns snacks fluctuated significantly, compared to viable counts of samples from white rice, fried rice and Jollof rice which were mostly within the range of 0 – 11 x 10<sup>4</sup> cfu/g. TVCs for samples were observed to be within the satisfactory and marginal range as depicted in Fig. 1. Bacilli counts were observed within the marginal to unsatisfactory range as shown in Fig. 2. Differential bacilli count ranged from 0 – 6 x 10<sup>5</sup>

cfu/g of food samples tested. RTE food samples in the sixth week of sampling showed an unusually high bacilli count (6 x 10<sup>4</sup> cfu/g). Table 2 depict the percentage occurrence of various tests bacilli isolated in this study from ready-to-eat (RTE) foods such as Buns, Meat pie, Egg roll, White rice, Jollof rice and Fried rice. *B. thuringiensis* showed the highest occurrence of 25.83%, followed by *B. megaterium* (24.59%), *B. subtilis* (21.85%); other bacilli (16.56%) e.g. *B. stearothermophilus*, *B. mycoides*, *B. amyloliquefaciens* etc. and *B. cereus* (11.26%). Table 3 showed organisms isolated and foods sampled per site. This table also made an attempt to describe the hygienic conditions of the kitchen and personal involved in the production and sale of various RTE foods. Observed conditions were not satisfactory. *Bacillus* species were isolated from all sites. Hygienic conditions observed in sample sites B, D and I were particularly notable.

**Table 1. Range for total viable and differential bacilli counts**

Food sample	Number of samples	Contaminated by <i>B. cereus</i>	Range for microbial count (cfu/g)	Range for bacilli count (cfu/g)
Buns	10	2	100 – 134 x 10 <sup>4</sup>	100 – 6 x 10 <sup>5</sup>
Egg roll	10	3	4900 - 10 <sup>4</sup>	5 – 2 x 10 <sup>4</sup>
Meat pie	10	4	15600 – 197 x 10 <sup>4</sup>	≤ 2 x 10 <sup>4</sup>
White rice	10	1	≤ 10 <sup>4</sup>	200 - 10 <sup>4</sup>
Fried rice	10	4	100 – 11 x 10 <sup>4</sup>	100 – 2 x 10 <sup>4</sup>
Jollof rice	10	2	100 – 6 x 10 <sup>4</sup>	≤3 x 10 <sup>4</sup>
Total	60	16		

Key: *B. cereus* = *Bacillus cereus*, cfu/g = colony-forming unit per gram



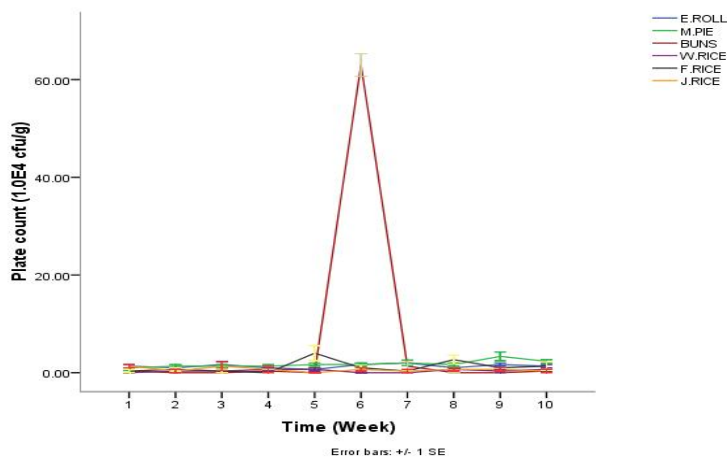
**Fig. 1. Total viable count (TVC) for ready-to-eat food samples**

Key: E.roll= Egg roll, M.pie = Meat pie, W.rice = White rice, F.rice= Fried rice, J.rice = Jollof rice; cfu/g= colony-forming unit per gram

**Table 2. Percentage distribution and occurrence of bacilli**

Organism(s)	Description on HiCrome media	Count (% distribution)	Site(s) detected
<i>Bacillus cereus</i>	Deep blue coloured centre surrounded by pinkish hue	17 (11.26)	SOS (A), SAAT (B), Cakes and Pastries (C), H, G and Tayo's Kitchen (I)
<i>Bacillus thuringiensis</i>	Light blue centre spread, with pinkish hue around	39 (25.83)	AROMA restaurant (D), WOLEPH (E), F and I
<i>Bacillus subtilis</i>	Green, little raised and entire edge	33 (21.85)	Stateline junction (F), Chicken republic (G), WOLEPH (E), Tayo's Kitchen (I)
<i>Bacillus megaterium</i>	Yellow, with entire edge	37 (24.59)	SOS (A), SAAT (B), Cakes and Pastries (C), F and H
Other bacilli	Pink, cottony white spread etc.	25 (16.56)	ATOLAGBE (H), Chicken Republic (G), AROMA restaurant (D), Cakes and Pastries (C), Tayo's Kitchen (I)

Key: A = School of Sciences (SOS), B = School of Agriculture and Agricultural Technology (SAAT), C = Cakes and pastries restaurant, D = Aroma restaurant, E = Woleph restaurant, F = Stateline junction food vending site, G = Chicken republic restaurant, H = Atolagbe complex food vending site, and I = Tayo's kitchen and pastries



**Fig. 2. Differential bacilli count for ready-to-eat food samples**

Key: E.roll= Egg roll, M.pie= Meat pie, W.rice= White rice, F.rice= Fried rice, J.rice = Jollof rice; cfu/g= colony-forming unit per gram

#### 4. DISCUSSION

Microbiological assessment of foods cannot be overemphasized as results provide scientists and researchers information as to the quality and possible risk(s) to consumer health. It is essential to note that in this study, total viable counts (TVCs) for ready-to-eat (RTE) food samples collected over a period of 10 weeks varied significantly week after week. This calls for serious concern, given the fact that the world as we know it seeks out 'fast foods' as alternative to preparation of meals at home. The concept and lifestyle of patronizing eateries or fast food joints is predicted to further rise in the years ahead due to the ever increasing busy schedules. TVCs showed significant variation in microbiological quality of the RTE foods sampled. This may be attributed to inconsistency in practice of good

personal and food preparatory hygiene [12,13]. Hygienic practices at sampling sites varied significantly from week to week, and most sample sites fell below standard. These could be responsible for the wide variety of *Bacillus* species associated with samples / sites. Also, these microorganisms are ubiquitous, and often associated with soil, air, water, and vegetation. Hence, at no point in time can RTEs be regarded as 'safe' for consumption, following this is an attendant risk to the health of consumers [14]. TVCs for samples were observed to be within the satisfactory and marginal range for standard plate count (SPC) as stipulated by ICMSF [15] guidelines for determining microbiological quality of RTE foods. It is however pertinent to mention that TVC derived from food sample analysis is dependent on certain factors [16].

**Table 3. Organisms isolated per site and hygienic conditions observed**

Sample site	Foods sampled	Organisms isolated	Hygienic condition(s) observed
A	Buns, Egg roll, Meat pie	<i>B. cereus</i> , <i>B. megaterium</i> , <i>B. polymyxa</i> , <i>B. licheniformis</i> , <i>B. amyloliquefaciens</i>	Foods stored in not so greasy show glass with a yellow light bulb inside. Show glass frequently opened to sell foods to consumers, and talking usually follows such action.
B	Buns, Egg roll, Meat pie	<i>B. cereus</i> , <i>B. megaterium</i> , <i>B. polymyxa</i> , <i>B. sphaericus</i> , <i>B. stearothermophilus</i> , <i>B. mycoides</i>	Foods stored in not so greasy show glass with a yellow light bulb inside. Show glass frequently opened to sell foods to consumers, and talking usually follows such action. Sometimes sneezing.
C	Fried rice, Jollof rice	<i>B. cereus</i> , <i>B. megaterium</i> , <i>B. licheniformis</i> , <i>B. amyloliquefaciens</i> , <i>Enterobacter dissolvens</i> , <i>Corynebacterium striatum</i>	Open kitchen exposed to dirt, and dust from the roadside. Prepared foods are immediately transferred to and kept in coolers prior to purchase. At point of sale for eat-in or take-away talking ensues. Presence of flies. Well water used
D	White rice	<i>B. thuringiensis</i> , <i>B. brevis</i> , <i>B. licheniformis</i> , <i>B. laterosporus</i> , <i>Enterobacter amnigenus</i>	Open kitchen exposed to dirt, and dust from the roadside. Prepared foods are transferred to and kept in coolers prior to purchase. At point of sale for eat-in or take-away talking ensues. Presence of flies. Pumped well water used.
E	White rice	<i>B. thuringiensis</i> , <i>B. subtilis</i> , <i>B. laterosporus</i> , <i>B. licheniformis</i> , <i>Enterobacter amnigenus</i>	Open kitchen exposed to dirt, and dust from the roadside. Prepared foods are immediately transferred to and kept in coolers prior to purchase. At point of sale for eat-in or take-away talking ensues. Presence of flies. Unclean hands. Well water used
F	Buns, Egg roll	<i>B. thuringiensis</i> , <i>B. subtilis</i> , <i>B. megaterium</i> , <i>B. brevis</i> , <i>B. mycoides</i> , <i>B. sphaericus</i> ,	Poor. RTE Foods openly exposed on trays, and to dust from roadside, prior to purchase, and sometimes for a long period of time. Talking while cooking. Infrequently washed hands. Soot from firewood around.
G	Fried rice, Jollof rice	<i>B. cereus</i> , <i>B. subtilis</i> , <i>B. amyloliquefaciens</i> , <i>Corynebacterium striatum</i>	Standard average kitchen. Sometimes talking while serving. Some RTE foods were stored in refrigerators. Cleaning-in-place (CIP) practiced.
H	Buns, Egg roll, Meat pie	<i>B. cereus</i> , <i>B. megaterium</i> , <i>B. subtilis</i> , <i>B. licheniformis</i> , <i>B. laterosporus</i>	Foods stored in not so greasy show glass. Show glass frequently opened to sell foods to consumers, and talking usually follows such action.
I	Egg roll, Meat pie	<i>B. cereus</i> , <i>B. thuringiensis</i> , <i>B. subtilis</i> , <i>B. brevis</i> , <i>B. sphaericus</i> , <i>B. licheniformis</i> , <i>B. amyloliquefaciens</i> , <i>B. stearothermophilus</i>	Tight kitchen space close to road side and exposed to dirt and dust. Presence of rats. Infrequent hand washing. RTE foods kept in show glass. Show glass frequently opened to sell foods to consumers, and talking usually follows such action.

Key: School of Sciences (SOS), B = School of Agriculture and Agricultural Technology (SAAT), C = Cakes and pastries restaurant, D = Aroma restaurant, E = Woleph restaurant, F = Stateline junction food vending site, G = Chicken republic restaurant, H = Atolagbe complex food vending site, and I = Tayo's kitchen and pastries; B=Bacillus

Differential *Bacillus* counts (Fig. 2) were observed within the marginal to unsatisfactory range according to ICMSF [15]. Differential bacilli counts for *B. cereus* and other pathogenic bacilli were expected to be less than  $10^4$  cfu/g for RTE

foods to be considered satisfactory, and safe for consumption. The presence of *Bacillus* spp. in this study was expected. The isolation of *B. cereus* and other *Bacillus* species corroborates the finding of Mensah et al. [12], Idowu [17] and

Taulo et al. [18] in which these microorganisms were implicated in ready-to-eat foods. Similarly, Lee et al. [19] and Sandra et al. [20] demonstrated the presence of *B. cereus* in rice products. VonHolly and Makhoane [21] also reported on the microbiology of ready-to-eat foods. These microorganisms are known to cause food borne intoxications and infections. It is therefore paramount that their numbers in foods be kept at barest minimum. This could be achieved by good manufacturing practice (GMP), good personal hygiene; and regular health inspection of small, medium and large scale RTE food producers / processors / outfits at local, state and national levels. The examination for the presence of pathogens in ready to eat foods contributes to food safety [22,23].

All cooked rice samples were contaminated with *B. cereus*. Oh and Cox [24] had earlier submitted that in rice-based foods *B. cereus* was the dominant bacterium. In addition, Sandra et al. [20] also detected the presence of *B. thuringiensis* in RTE rice. Due to their ubiquity they have been found in a wide variety of foods. Spores of these microorganisms also have little or no competition with vegetative cells, and would survive heating treatments to which foods are subjected, as well as storage conditions [25]. The level of contamination of cooked rice samples with *B. cereus* in this study was within the unsatisfactory range according to Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods [26].

## 5. CONCLUSION

This study has demonstrated that some of the popular types of ready-to-eat foods that are sold on the streets within and outside the Federal University of Technology, Akure (FUTA) are contaminated, and do not meet the required quality levels. The research has reiterated that ready-to-eat foods (RTEs) rarely meet microbiological standards for quality. The world seeks out fast foods as alternative to preparation of meals at home. The concept and lifestyle of patronizing eateries or fast food joints is predicted to further rise in the years ahead due to the ever increasing busy schedules. This demand for food gives opportunity to the cafeterias, canteens and food vendors to serve as the major vending sites where students and staff purchase food on a daily basis. Hence, it is recommended that a closer and stringent supervision of ready-to-eat foods sold to students in the University should be carried out by

relevant authorities to prevent possible outbreak of food borne illness. Some of *B. cereus* outbreaks are under reported as the illness associated with these bacteria limit itself, and does not become severe.

A recent survey on culture practices for outbreaks of apparent food-borne illness showed that 20% of state public health laboratories do not make *B. cereus* testing routinely available. The survey also found that most of food handlers (in food stalls and restaurants) were unaware that cooked rice was a potentially hazardous food. Cooked rice and other ready to eat food samples used in this study are displayed and served at room temperature that allows the spores of *B. cereus* to grow. Other sources for *Bacillus* contamination are dirty hands or cooking stuffs (e.g. knife), handling foods without gloves and flies / insects contamination. Almost all kind of foods have been implicated in *B. cereus* food borne poisoning. However, a majority of reported outbreaks were linked to the consumption of heat treated foods and frequently occurred in restaurant and catering establishments. Failure in refrigeration was frequently suspected. The major control measures are to control temperature and to establish Hazard analysis and Critical Control Points (HACCP) system. The number of spores in other processed foods must be kept as low as possible by proper cleaning and disinfection of equipments. Rapid cooling is necessary to prevent germination and growth of *B. cereus* spores. Control measures for *B. cereus* would contribute to control other *Bacillus* spp. This work puts forward the need to make test for *Bacillus* species part of normal routine checks and analyses for ready-to-eat foods.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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