INCIDENCE OF *BACILLUS CEREUS* STRAINS IN FOOD CONSUMED BY SCHOOL CHILDREN IN ILORIN METROPOLIS

[1]Ayilara, Taibat Tayo, [1]Awe, Sunday and [1]Adedayo, Majekodunmi Racheal
[1]Department of Microbiology, Faculty of Pure and Applied Sciences, Kwara State University, Malete, Nigeria
Correspondence:majekodunmi.adedayo@kwasu.edu.ng; (+234) 8133325627

Abstract:
Foodborne diseases are caused by eating contaminated food. *Bacillus cereus* is a versatile enterotoxigenic agent of foodborne disease. This study investigated the incidence of *Bacillus cereus* in food sold within some school premises within Ilorin metropolis and their hazard analysis critical control point (HACCP). Isolation of *Bacillus cereus* was carried out using food samples collected from some selected school within Ilorin metropolis using Bacara agar. The isolated bacteria were characterized and identified following microbiological techniques. Molecular identification of isolates was done using 16S rRNA sequencing method. Effect of physiochemical parameters (such as incubation period, temperature, pH, UV-light, salt concentration and monosodium glutamate concentration used in cooking) on the growth of the isolates was carried out. Identification of possible points of contamination was assessed from the points of production and sale of foods using well structured questionnaire. Total *Bacillus* count in food samples sold in all the sampled points within the Local Government Areas was significantly different from each other. Cooked Rice and spaghetti were found to have the highest count (4.42 ± 0.72 to 4.75 ± 0.89 and 4.00 ± 0.99 to 4.17 ± 1.03). All the isolates were confirmed to be *Bacillus cereus* with accession number OQ235070, OQ235071, OQ235072, OQ235073, OQ235075 and OQ235076 except one confirmed to be *B. thuringiensis* OQ235074. The isolates were found to have optimum growth between 30 – 35 ^{0}C, pH 3 - 7, incubation period of 12 - 24 hours and hours and maximum growth at 1.0 % monosodium glutamate concentration. Hazard analysis critical control point reveals poor storage system, illiteracy and poor hygienic practices among the handlers. In conclusion, food items sold to school children examined were found to be contaminated with *Bacillus cereus*.

Key words: *Bacillus cereus,* Foodborne diseases, School Children, Ilorin metropolis, Rice and Spaghetti

1. Introduction

Food is very pivotal to the continuous existence of any organism; it includes any substance in liquid or solid form, consumed by an individual. The energy derived from food consumption nourishes and provide basis for cellular metabolism [1].

Food borne disease refers to illnesses contacted through eating food contaminated by pathogenic microbes and or their toxic metabolites during production or processing and distribution lines [2].

Food borne disease (FBD) poses a global challenge to human survival with increase in percentage occurrence annually. Furthermore, is the issue of increase in cost of treatment, reduction in labour efficiency and sometimes death of the consumers. The impact of foodborne disease on consumers have been globally burdensome especially the diarrheal diseases [3].

"Street-vended foods" or "ready to eat foods" refers to food in liquid or solid form prepared and sold in public places and consumed without further processing [4]. This kind of food has become very important due to its immediate availability around the home and workplaces. It is a ready alternative for people during work hours and has become major source of livelihood to low and medium business vendors [5]. Despite the associated benefits of this food, human safety is often at risk [6, 7]. *B. cereus* is among the commonly encountered bacteria that are responsible for instances of reported cases of outbreak of Foodborne diseases [8].

Bacillus cereus is an endospore forming, enterotoxin producing rod commonly associated with food and soil environments [6]. The spores contaminate a variety of food products often during production and distribution, essentially when food is held at ambient condition for a long time before consumption. This bacterium is notorious for producing two forms of toxins which are responsible for the two forms of infections; the diarrheic and vomiting (emetic) type. Both of which are heat labile and heat stable respectively. The hemolysin (BL type) and the non-hemolytic (emetic type) of toxins were thought to be required for full activity of the bacterium. As an endospores former, the endospores produced by the organisms can withstand varying degrees of dehydration, irradiations, temperature and pH relevant to soil and food processing environments [9]. The spores are reported to have affinity for surface adhesion and survival in foods such as grains, nuts, and vegetables, hence, the vegetative cells can be found in both cooked and raw food materials [10]. Ingestion of the organism or the toxins from the

organism can cause severe cases of foodborne disease. Foodborne illness associated with toxin produce by *B. cereus* affect people of all ages but is thought to be more severe in children and aged [10]. Study on the occurrence of this foodborne pathogen in food consumed by pupils in this region is of public health significance; the importance of the knowledge to avoid risk to consumers' health cannot be overemphasized. This work was therefore aimed at investigating the incidence of *B. cereus* strains in food consumed by school children and analyzing the hazard critical control points along processing lines in selected Local Governments Areas within Ilorin metropolis.

2. Materials and Methods

2.1 Sample Collection

Different food samples consumed by school children in twelve selected schools each within Ilorin West, Ilorin East, Ilorin South and Asa all within Ilorin metropolis were randomly collected in sterile coolers under aseptic condition from different food vendors at different schools for a period of three months. The food items collected include cooked rice, spaghetti, bean, puff-puff, meat and fish that are consumed by school children. These food items were transported to the laboratory and microbial analysis were carried out on the food items within 6 hours of collection [11].

2.1.1 Isolation of Bacteria from Food Samples

Precisely 10 g of each food sample was homogenized for a minute with 90 ml of buffered peptone. Serial dilution was prepared and 0.1 ml of each dilute sample was placed in Petri-dishes and pour plate method was done using Nutrient Agar, which was completely mixed by revolving the plates and then left to solidify. After 48 hours of incubation at 37 °C, the plates were checked for the appearance of single colonies growing throughout the medium. The plate count was recorded [12].

2.1.2 Isolation of *B. Cereus* usingBacara Agar Plates

The medium *Bacillus cereus* rapid agar (Bacara) from Oxoid Ltd (Basingstoke, UK) was prepared following manufacturer's instruction, dispensed into Petri-dishes and inoculated with 0.1 ml of the serially diluted samples using spread plate method. Plates were incubated for 24 hours at 30 ^{0}C. Distinct colonies were subcultured repeatedly and pure colonies maintained at 4^{0}C for characterization [13].

2.2 Identification of Isolates

2.2.1Characterization and Confirmation of *Bacillus cereus*

Characterization using morphological appearance of cell, shape and pigmentation were done. The following biochemical tests such as (catalase, heamolysis, citrate utilization, urease production, Voge-Proskauer, lecithinase production, hydrolysis of starch) and cultural characteristic such as Gram's reaction, spore location and motility were determined [14; 15; 16; 17; 18; 19;20;21;22].

2.2.2 Molecular Identification of *Bacillus cereus* Isolates

2.2.2.1 DNA Extraction

Single colonies from overnight culture of the isolates were transferred into broth for 48 hours at 28 ºC. The cultures were centrifuged at 4600 rpm for 5 mins. DNA extraction was according to [23]

2.2.2.2 Polymarase Chain Reaction (PCR)

The DNA extracted was used for PCR amplification with the Gene Amp$^{(R)}$ PCR System (Applied Biosystem USA). The procedure described by [24] was followed.

2.2.2.3 Gel Electrophoresis

Gel electrophoresis of the PCR products was done on a 1 % TAE agarose gel that was stained with ethidium bromide at 80 V for 60 minutes. The resulting bands were viewed under ultraviolet (UV) trans-illumination. Digital images of the Gel were obtained using Alpha imager 2000, Alpha Innotech, San Leandro, CA [25]

2.2.2.4 Purification of PCR Amplified 16S rRNA Gene

The PCR products obtained were purified using ethanol to get rid of residual PCR reagents. The purity of the products were confirmed and quantified according to the method described by [26].

2.3 Effect of Physicochemical Parameters on the Growth of Isolates

2.3.1 Effect of Temperature on Growth of *Bacillus cereus*

Sterile nutrient broth in test tubes (5 ml) was inoculated with 2 ml of isolates in 3 replicates and incubated at 20, 25, 30, 35, 40, 45, and 50 °C for 24 hours. Bacterial growth was measured using turbidity method at 600 nm [9]. All experiments were carried out in triplicates.

2.3.2 Effect of Incubation Periods on the Growth of *B. cereus*

Two (2) ml of test organism was transferred into nutrient broth and was incubated at the temperature of 37 ^{0}C for duration of 24 hours at pH 7 and the differences in the growth pattern were taken [27]. All experiments were carried out in triplicates.

2.3.3 Effect of pH on Growth of *B. cereus*

Nutrient broth containing 2 ml of cultures were prepared and buffered at different pH concentrations of 3, 4, 5, 7, 9, and 12 in conical flasks. They were incubated at 32 ^{0}C on an orbital shaker at 80 rpm for 24 hours; bacterial growth was measured using turbidity method at 600 nm. All experiments were carried out in triplicates [9].

2.3.4Effect of Monosodium Glutamate Concentration on Growth of *B. cereus*

Two millimeter (2 ml) of *B. cereus* culture with OD 600 nm=1.0 which correspond to 10 CFU/ml was added to different concentrations of 2.5, 5.0, 7.5 and 10 % of monosodium glutamate prepared using nutrient broth as diluents. All flasks were incubated at 37 °C with continuous shaking at 150 rpm for 24 hours. Bacterial growth was measured using turbidity method at 600 nm. All experiments were carried out in triplicates [28].

2.3.5 Effect of Ultraviolet Light on the Growth of *B. cereus*

Five millimeter (5 ml) of sterile nutrient broth was inoculated with 2 ml of overnight broth culture of the test organisms. The broth cultures were placed in sterile Petri-dishes and directly exposed to the UV-C light source for 0, 30, 60, 90, 120 and 150 seconds in 3 replicates. The distance between the center of the light and the top of Petri-dish was approximately 15cm. Bacterial growth was measured using turbidity method at 600 nm. All experiments were carried out in triplicates [29].

2.3.6 Effect of NaCl Concentration on the Growth of *B. cereus*

Two (2) ml each of inoculums from overnight broth culture test organism was transferred aseptically to sterile tubes containing different concentration of NaCl (2.5, 5.0 , 7.5 and 10 %), and incubated at 37 ^{0}C for 24 hours, bacterial growth was measured using turbidity method at 600 nm. All experiments were carried out in triplicates [30].

2.4 Identification of Hazard Analysis Critical Control Points

Identification of hazard was carried out using the method of [30]. Collection of information through visitation to both the selling and preparation points was carried out. Several parameters such as sources of water for preparation, rearing of domestic animals, distance from toilet, presences of washing basin, level of personal hygiene, level of environmental sanitation, training attended by vendors, packaging and storage of food product (if applicable) were assessed. All these were done to identify the possible sources of contamination in the production and selling points of the foods. Questionnaires were used to collect information from the vendors.

2.5 Statistical Analysis

Data obtained were statistically analysed using SPSS. ANOVA test under Completely Randomized Design (CRD) and Duncan Multiple Range Test were used to determine Mean and Standard Error of mean and to show significant differences in means. Means were considered significantly different at ($p \leq 0.5$) and denoted by alphabets in superscripts.

3. Results

Bacterial Loads in Food Samples

The result of total bacterialcounts (x 10^3cfu/g) and total *Bacillus* counts (x 10^3cfu/g) of six (6) foods samples(rice, spaghetti, bean, puff-puff, meat and fish) from different school within Ilorin metropolis are as showed in Tables 1 and 2 respectively.

3.1 *Bacillus cereus* Counts in Food Sold in the Local Government Areas

In Ilorin West L.G.A, the isolates from the food sampled differed significantly ($p \leq 0.5$) in total *Bacillus* counts (x 10^3cfu/g). There was no significant difference in *Bacillus* count from Rice and spaghetti (superscript a) but there was in puff puff and other food samples (superscript b), *Bacillus* count in fried fish and beans were not significantly different from each other (superscript c).

In Ilorin South, the food samples differ significantly ($p \leq 0.05$) in total *Bacillus* counts (x 10^3cfu/g). Rice, meat and spaghetti are significantly not different from each other (superscript a) but puff puff (superscript ab) is significantly different from other food samples.

In Ilorin East L.G.A, the food sampled differed significantly ($p \leq 0.05$) in total *Bacillus* count (x 10^3cfu/g). the count in cooked rice is significantly different from other samples (superscript a). While puffpuff and spaghetti were not significantly different from each other (superscript ab) but beans and fried fish are not significant different from each other (superscript c) (Table 2).

In Asa LGA, the food samples differed significantly ($p \leq 0.05$) in total *Bacillus* count (x 10^3cfu/g). Rice and meat were significantly not different from each other (superscript a) but while spaghetti is significant different from other food samples (superscript ab) likewise puffpuff is significantly different from other food samples.(superscript b) but also fried fish and beans are significantly different from each other (superscript c).The total *Bacillus* counts (x 10^3cfu/g) in food samples sold in all local government areas are

significantly different from each other. Methods of preparation and hygienic practices of each of the vendors may influence the total *Bacillus* count (x 10^3cfu/g) in food samples. Furthermore, rice and spaghetti were not significantly different from each other across the L.G.A and beans, fish, meat and puff puff also have similar trend (Table 2.)

3.2 Characteristics of Presumptive *Bacillus cereus* Isolates

There were ten isolates of which seven were found to be positive to starch hydrolysis, hemolysis and lecithinase and are therefore considered to be tentatively enterotoxigenic *Bacillus cereus* while other that failed the three physiological characteristic were considered to be other species of *Bacillus* and were removed from further studies (Table 3).

3.3 Molecular Identification of Bacterial Isolates and Phylogenetic Tree of Relationship

Agarose gel electrophoresis showed the positive amplification of 16S rRNA partial gene amplified from the selected bacteria isolate which align with marker primer confirms the isolates to be *Bacillus* species. The presence of approximately 1500bp amplicon size indicates a positive amplification as shown in Figures 1 and 2.The result of molecular characterization of isolated *Bacillus* spp presented in Table 4 shows that isolated *Bacillus cereus* 1,2,3, 4, 6 and 7 align with strain of *Bacillus cereus* with above 97 % identity (Figure 1). However, isolate 5 align and had higher % similarity with *Bacillus thuringiensis* strain (Figure 1).

3.4 Effect of Physiochemical Parameters on the Growth of Isolates

3.4.1Effect of Temperature on the Growth of *Bacillus cereus*

The growth of *Bacillus cereus* at different temperature is presented in Table 5. The mean value of their growth ranges between 1.157 ± 0.005 to 1.036 ± 0.092 at temperature of 25 and 35 ^{0}C. There was no significant difference (P < 0.05) in the growth of all the strains. At 40 ^{0}C, the growth tends to decrease progressively up to 50^{0}C. Lowest mean value of all the growth was observed at 50 ^{o}C having thermal effect on the cell macromolecules leading to cell death.

3.4.2 Effect of pH Change on Growth of *B. cereus*

The growth of the bacteria increased significantly (P < 0.05) as the pH increased from 3 – 12. The optimum growth with OD 660 values of (0.611 ± 0.004) (0.765 ± 0.005) (0.732 ± 0.000) were obtained at pH 3, 5, and 7 respectively. It was observed that pH above 7 tend to inhibit the growth of *B. cereus* strains. Duncan Multiple Range Test (DMRT) comparison at (P < 0.05) showed that the mean values of the growth were significantly different. This is presented in Table 6.

3.4.3 Effect of Incubation Period on *Bacillus cereus*

The growth of the *B. cereus* under different incubation period was presented in Table 6. It ranged between (1.180 ± 0.007) to (0.007 ± 0.003). There were significant differences in growth in all the strains relative to incubation period (P < 0.005). Result is presented in Table 7

3.4.4 Effect of Monosodium Glutamate Concentration on the Growth of *B. cereus*

The effect of monosodium glutamate concentration on the growth of *Bacillus cereus* strains is presented in the Table 8. The growth of bacteria increased significantly (P < 0.05) as the concentration of monosodium glutamate increased. The maximum cell growth with OD 660 value of 1.136 ± 0.004) (0.817 ± 0.007) at 0.5 and 1.0% respectively were achieved. The mean growth values were statistically significant.

3.4. 5 Effect of Duration of Exposure to UV Light on the Growth of *Bacillus cereus*

The lethal effect of UV light exposure on growth at specific period of time is presented in Table 9. The mean values of growth ranged between (1.609 ± 0.07) to (0.010 ± 0.05). The growth rate patterns of all the strains were similar. There was no significant different at (P < 0.05) in all the strain at specific exposure duration.

3.4.6 Effect of Sodium Chlorides Concentration on Growth of *B. cereus*

Growth pattern of *B. cereus* strain in different concentrations of NaCl is presented in Table 10. The growth of the strains increased significantly (P < 0.05) at 2.5%, however, growth decreased progressively up to 10.0%. None of the strain was able to survive 10 % of sodium chloride.

3.5 Identification of Hazard

The results presented in Table 11 shows that out of 48 schools from all the local government, 17(33.3 %) vendors used well water for production of their food while 20(41.7 %) and 12(25.0 %) used tap water and borehole water respectively. Majority 44(91.7 %) and 47(97.9 %) of the vendor do not rear domestic animals and do not have toilet close to selling point and preparatory point.

Similarly, 42(87.5 %) of schools have washing hand basin and 44(91.7 %) have no refrigerator. The result further showed that 30(62.5 %) of the people involved in preparation of food had hygienic training attended. The average level of personal hygiene and environmental sanitation of the people involved in preparation of food were 64.67(±1.83), 57.5(±2.14) and 3(±0.25) respectively.

Table 10 shows cross tabulation of source of water for food production and local government areas. About 50 % of the vendors in Ilorin West use tap water while 20 % of vendors use well water and borehole water. Similarly in Ilorin East LGA, we have the same proportion as in Ilorin West. 41.7 % of vendors in Ilorin South local government use well water and tap water while 6.6 % of schools use borehole water while in Asa LGA 41.7, 25 and 33.3 % of vendors use well water, tap water and borehole water respectively.

Majority is 91.7, 83.3, 100 and 91.7 % of vendors in Ilorin West, South, East and Asa do not rear domestic animals. All the vendors (100 %) do not have toilet close to their selling points except Ilorin East that is 91.7 %. The level of personal hygiene, which is 83.3, 91.7 and 83.3 % in Ilorin West, South and East respectively were above average while 58.3 % of vendors in Asa were about average.

The level of environmental sanitation of 66.7, 25 .0, 41.7 and 58.3 % school in Ilorin West, South, East and Asa LGA respectively are about average. Three quarter (75.0 %) of school vendors in Ilorin South and Ilorin East LGA have ever attended hygiene training while only half (50 %) of vendors in Ilorin West and Asa LGA have never attended any food hygiene training.

Table 1: Total Bacterial Counts (x 10^3 cfu/g) of Food Samples

Food samples	Local Government Areas			
	Ilorin West	Ilorin East	Ilorin South	Asa
	Total Bacterial Count (x 10^3 cfu/g)			
Rice	8.83 ± 1.53^{ab}	6.83 ± 1.03^{b}	7.00 ± 1.04^{ab}	7.00 ± 1.04^{ab}
Fried Fish	7.17 ± 1.47^{c}	8.25 ± 1.49^{ab}	8.00 ± 1.60^{abc}	8.00 ± 1.60^{ab}
Meat	9.25 ± 0.87^{a}	9.42 ± 0.79^{a}	9.25 ± 0.87^{a}	9.17 ± 0.84^{b}
Beans	7.17 ± 1.80^{c}	8.25 ± 2.18^{ab}	8.75 ± 2.05^{ab}	8.42 ± 2.23^{ab}
Puff puff	7.50 ± 1.31^{bc}	7.33 ± 1.44^{b}	7.50 ± 1.31^{bc}	8.42 ± 3.29^{ab}
Spaghetti	7.92 ± 2.61^{abc}	7.83 ± 2.48^{b}	7.58 ± 2.43^{bc}	7.75 ± 2.38^{ab}

- Values are means ± standard deviation.
- means with different superscript within the same column are significantly different at ($p \leq 0.05$)

Table 2: Total *Bacillus* Counts (x 10^3 cfu/g) of Food Samples

Food Samples	Local Government Areas			
	Ilorin West	Ilorin East	Ilorin South	Asa
	Total Bacterial Count (x 10^3 cfu/g)			
Rice	4.42 ± 0.72^{a}	4.75 ± 0.89^{a}	4.42 ± 0.67^{a}	4.75 ± 0.87^{a}
Fried Fish	2.08 ± 0.99^{c}	3.33 ± 1.03^{c}	2.97 ± 0.84^{b}	3.00 ± 0.85^{c}
Meat	3.42 ± 1.56^{bc}	3.73 ± 0.62^{b}	3.92 ± 0.78^{a}	4.50 ± 0.52^{a}
Beans	1.67 ± 0.49^{c}	3.42 ± 0.94^{c}	2.67 ± 1.13^{b}	2.92 ± 0.79^{c}
Puff Puff	3.83 ± 0.79^{ab}	4.17 ± 0.79^{ab}	3.67 ± 0.65^{ab}	3.75 ± 0.75^{b}
Spaghetti	4.02 ± 1.22^{a}	4.17 ± 1.19^{ab}	4.00 ± 0.99^{a}	4.17 ± 1.03^{ab}

- value and mean ± standard deviation.
- means with different superscript within the same column are significantly different at ($p \leq 0.05$)

Table 3: Morphological and Biochemical Characteristics of Isolated Bacteria

	Morphology Characteristics				Microscopic Characteristics		Biochemical Characteristics					Sugar Fermentation				
Isolates	**Shape**	**Colour**	**Elevation**	**Texture**	**Gram Staining**	**Motility**	**Catalase**	**Citrate**	**Heamolysis**	**Nitrate Test**	**Spore**	**Glucose**	**Lactose**	**Maltose**	**Sucrose**	**Propable Organism**
AA1	Rods	Light pink	Convex	Smooth	+	+	+	+	+	+	+	+	-	+	+	*B. cereus I*
AA2	Rods	Light pink	Convex	Smooth	+	+	+	+	+	+	+	+	+	-	+	*B. cereus II*
AA3	Rods	Light pink	Convex	Smooth	+	+	+	+	+	+	+	-	-	+	+	*B. cereus III*
AA4	Rods	Light pink	Convex	Smooth	+	+	+	+	+	+	+	-	-	+	+	*B. cereus IV*
AA5	Rods	Light pink	Convex	Smooth	+	+	+	+	+	+	+	-	-	+	+	*B. cereus V*
AA6	Rods	Light pink	Convex	Smooth	+	+	+	+	+	+	+	+	+	-	+	*B. cereus VI*
AA7	Rods	Light pink	Convex	Smooth	+	+	+	+	+	+	+	+	+	+	+	*B. cereus VII*
BB1	Cocci	Cream white	Raise	Smooth	+	+	+	+	+	+	+	-	-	-	+	*S. cereus*
BB2	Rods	Light yellow	Convex	Irregular	+	+	+	+	+	+	+	+	+	+	+	*Clostridium Sp.*
BB3	Cocci	Cream white	Raise	Smooth	+	+	+	+	+	+	+	-	+	-	+	*S. cereus*
BB4	Rods	Cream white	Convex	Irregular	+	+	+	+	+	+	+	-	+	+	+	*Listeria Sp.*

KEY: + = POSITIVE

- = NEGATIVE

Table 4: NCBI Blast Showing the Identity of the Sequenced Bacteria Isolates

Isolates	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
AyBio-1	*Bacillus cereus*	2529	35380	99%	0	99.43%	OQ235070
AyBio-2	*Bacillus cereus*	2418	2418	100%	0	98.00%	OQ235071
AyBio-3	*Bacillus cereus*	2507	2507	100%	0	99.14%	OQ235072
AyBio-4	*Bacillus cereus*	2507	2507	100%	0	99.14%	OQ235073
AyBio-5	*Bacillus thuringiensis*	2514	2514	99%	0	99.42%	OQ235074
AyBio-6	*Bacillus cereus*	2549	2549	100%	0	99.71%	OQ235075
AyBio-7	*Bacillus cereus*	2547	2547	100%	0	99.71%	OQ235076

Note: Numbers 1to 7 represents the Isolated *Bacillus* species

Table 5: Effect of Temperature on the Growth of *Bacillus cereus*

Temperature (^{0}C)	Isolates						
	***B. cereus* 1**	***B. cereus* 2**	***B. cereus* 3**	***B. cereus* 4**	***B. cereus* 5**	***B. cereus* 6**	***B. cereus* 7**
20	0.679 ± 0.005^{d}	0.945 ± 0.026^{a}	0.833 ± 0.004^{b}	0.752 ± 0.119^{bcd}	0.734 ± 0.038^{c}	0.733 ± 0.056^{cd}	0.824 ± 0.111^{bc}
25	0.787 ± 0.005^{c}	1.177 ± 0.004^{a}	1.047 ± 0.005^{d}	1.026 ± 0.006^{b}	0.936 ± 0.006^{d}	1.059 ± 0.006^{c}	0.934 ± 0.008^{b}
30	0.927 ± 0.004^{b}	1.157 ± 0.005^{a}	1.033 ± 0.055^{d}	0.991 ± 0.005^{c}	0.959 ± 0.005^{e}	1.027 ± 0.035^{c}	0.981 ± 0.005^{c}
35	0.825 ± 0.022^{e}	0.966 ± 0.033^{ab}	0.893 ± 0.025^{cd}	0.894 ± 0.021^{cd}	0.797 ± 0.021^{f}	1.051 ± 0.092^{a}	0.868 ± 0.006^{cd}
40	0.623 ± 0.085^{abc}	0.683 ± 0.015^{bc}	0.587 ± 0.015^{bc}	0.564 ± 0.015^{cd}	0.623 ± 0.007^{abc}	0.674 ± 0.007^{b}	0.510 ± 0.009^{d}
45	0.471 ± 0.036^{ab}	0.503 ± 0.007^{a}	0.518 ± 0.47^{a}	0.493 ± 0.047^{a}	0.497 ± 0.016^{a}	0.397 ± 0.017^{d}	0.485 ± 0.010^{a}
50	0.225 ± 0.007^{a}	0.188 ± 0.023^{c}	0.197 ± 0.021	0.169 ± 0.016^{b}	0.176 ± 0.018^{b}	0.094 ± 0.012^{de}	0.094 ± 0.012^{d}

* Significant difference exists at ($p \leq 0.5$)
*Mean value with different superscript in the same column are significantly different

Table 6: Effect of pH on Growth of *Bacillus cereus*

pH	Isolates						
	***B. cereus* 1**	***B. cereus* 2**	***B. cereus* 3**	***B. cereus* 4**	***B. cereus* 5**	***B. cereus* 6**	***B. cereus* 7**
3	0.611 ± 0.004[b]	0.448 ± 0.004[a]	0.743 ± 0.005[c]	0.839 ± 0.006[c]	0.611 ± 0.005[a]	0.663 ± 0.005[c]	0.565 ± 0.006[c]
5	0.666 ± 0.005[ab]	0.510 ± 0.003[b]	0.765 ± 0.005[a]	0.746 ± 0.004[a]	0.668 ± 0.004[b]	0.617 ± 0.005[b]	0.634 ± 0.004[b]
7	0.732 ± 0.004[a]	0.676 ± 0.004[ab]	0.551 ± 0.007[b]	0.532 ± 0.004[b]	0.442 ± 0.005[c]	0.558 ± 0.004[a]	0.515 ± 0.006[a]
9	0.049 ± 0.004[d]	0.092 ± 0.003[c]	0.028 ± 0.004[d]	0.028 ± 0.006[d]	0.025 ± 9,994[d]	0.031 ± 0.004[d]	0.052 ± 0.005[d]
10	0.027 ± 0.005[C]	0.028 ± 0.004[d]	0.024 ± 0.004[d]	0.037 ± 0.005[d]	0.050 ± 0.004[d]	0.037 ± 0.006d	0.030 ± 0.005[d]

***** Significant difference **exists** at $P < 0.05$.
Mean value with different superscripts in the same column are significantly different.

Table 7: Effect of Incubation Period on the Growth of *B. cereus* at pH of 7.0

Incubation Period (h)	Isolates						
	***B. cereus* 1**	***B. cereus* 2**	***B. cereus* 3**	***B. cereus* 4**	***B. cereus* 5**	***B. cereus* 6**	***B. cereus* 7**
0	0.007 ± 0.003[a]	0.405 ± 0.012[c]	0.162 ± 0.009[c]	0.532 ± 0.005[c]	0.191 ± 0.009[c]	0.370 ± 0.067[c]	0.237 ± 0.004[a]
4	0.532 ± 0.059[c]	1.060 ± 0.009[b]	0.944 ± 0.008[ab]	0.890 ± 0.006[b]	0.798 ± 0.007[a]	0.790 ± 0.004[b]	0.750 ± 0.007[b]
8	0.594 ± 0.014[c]	1.065 ± 0.008[b]	0.925 ± 0.014[ab]	0.989 ± 0.007[ab]	0.798 ± 0.003[b]	0.805 ± 0.007[ab]	0.758 ± 0.007[b]
12	0.765 ± 0.021[b]	1.169 ± 0.007[a]	1.654 ± 0.009[a]	1.007 ± 0.003[a]	0.914 ± 0.008[ab]	0.988 ± 0.004[ab]	0.943 ± 0.005[ab]
16	0.787 ± 0.021[b]	1.176 ± 0.007[a]	1.059 ± 0.006[a]	1.009 ± 0.004[a]	0.922 ± 0.006[ab]	0.995 ± 0.003[ab]	0.949 ± 0.005[ab]
20	0.800 ± 0.020[ab]	1.180 ± 0.007[a]	1.067 ± 0.006[a]	1.030 ± 0.006[a]	0.934 ± 0.006[ab]	1.005 ± 0.006[a]	0.954 ± 0.006[ab]
24	0.784 ± 0.010[b]	1.184 ± 0.006[a]	1.050 ± 0.005[a]	1.607 ± 0.003[a]	0.945 ± 0.009[ab]	1.058 ± 0.007[a]	0.943 ± 0.006[ab]

***** = Significant difference exists at $P < 0.05$
Mean value with different superscript are significantly different.

Table 8: Effect of Monosodium Glutamate Concentration on the Growth of *B. cereus*

Concentration %	Isolates						
	***B. cereus* 1**	***B. cereus* 2**	***B. cereus* 3**	***B. cereus* 4**	***B. cereus* 5**	***B. cereus* 6**	***B. cereus* 7**
0.5	1.017 ± 0.004^{ab}	0.880 ± 0.006^{b}	1.014 ± 0.004^{b}	1.059 ± 0.003^{b}	1.059 ± 0.006^{ab}	1.018 ± 0.011^{b}	1.029 ± 0.004^{b}
1.0	2.727 ± 0.005^{a}	2.615 ± 0.003^{a}	2.692 ± 0.006^{a}	2.658 ± 0.005^{a}	2.747 ± 0.005^{a}	2.709 ± 0.008^{a}	2.742 ± 0.004^{a}
1.5	0.797 ± 0.215^{bc}	0.441 ± 0.115^{c}	0.572 ± 0.005^{c}	0.387 ± 0.006^{b}	0.602 ± 0.212^{b}	0.462 ± 0.012^{c}	0.441 ± 0.002^{c}
2.0	0.370 ± 0.034^{c}	0.366 ± 0.006^{d}	0.357 ± 0.003^{d}	0.382 ± 0.006^{b}	0.389 ± 0.003^{c}	0.353 ± 0.035^{d}	0.328 ± 0.005^{d}

* = Significant difference exists at P <0.05
Mean values with different superscripts in the same column are significantly different.

Table 9: Effect of Duration of Exposure to UV Light on the Growth of *B. cereus*

Duration Sec.	Isolates						
	***B. cereus* 1**	***B. cereus* 2**	***B. cereus* 3**	***B. cereus* 4**	***B. cereus* 5**	***B. cereus* 6**	***B. cereus* 7**
0	0.609 ± 0.07^{c}	1.314 ± 0.006^{a}	0.897 ± 0.006^{b}	1.208 ± 0.005^{bc}	1.202 ± 0.005^{bc}	1.151 ± 0.006^{a}	1.307 ± 0.007^{b}
30	1.214 ± 0.008^{ab}	0.829 ± 0009^{b}	0.750 ± 0.056^{a}	0.784 ± 0.014^{a}	0.817 ± 0.006^{a}	0.927 ± 0.031^{b}	0.944 ± 0.005^{a}
60	1.061 ± 0.008^{b}	0.648 ± 0.047^{c}	0.659 ± 0.003^{ab}	0.748 ± 0.014^{b}	0.718 ± 0.008^{b}	0.768 ± 0.045^{dc}	0.897 ± 0.004^{b}
90	0.859 ± 0.131^{a}	0.521 ± 0.105^{d}	0.478 ± 0.057^{c}	0.665 ± 0.010^{c}	0.546 ± 0.008^{c}	0.546 ± 0.009^{c}	0.650 ± 0.043^{c}
120	0.492 ± 0.068^{d}	0.239 ± 0.080^{e}	0.320 ± 0.035^{d}	0.447 ± 0.012^{d}	0.327 ± 0.033^{d}	0.322 ± 0.033^{d}	0.469 ± 0.042^{d}
150	0.010 ± 0.005^{e}	0.050 ± 0.030^{f}	0.162 ± 0.006^{e}	0.173 ± 0.058^{e}	0.193 ± 0.041^{e}	0.193 ± 0.041^{e}	0.231 ± 0.031^{e}

* = Significant difference exists at P < 0.05
Mean value with different superscripts in the same column are significant different.

Table 10: Effect of Sodium Chlorides Concentration on Growth of *B. cereus*

Concentration %	Isolates						
	***B. cereus* 1**	***B. cereus* 2**	***B. cereus* 3**	***B. cereus* 4**	***B. cereus* 5**	***B. cereus* 6**	***B. cereus* 7**
2.5	0.246 ± 0.008[a]	0.026 ± 0.007[a]	0.196 ± 0.005	0.216 ± 0.005[a]	0.188 v 0.004[c]	0.228 ± 0.15[a]	0.252 ± 0.014[a]
5.0	0.054 ± 0.012[d]	0.041 ± 0.006[a]	0.063 ± 0.005[d]	0.056 ± 0.011[d]	0.065 ± 0.007-d	0.073 ± 0.012[d]	0.032 ± 0.014[d]
7.5	0.004 ± 0.014[e]	0.005 ± 0.011[d]	0.016 ± 0.007[e]	– 0.009	– 0.007	– 0.004	– 0.008
10.0	– 0.007	– 0.007	– 0.008	– 0.008	– 0.007	– 0.009	– 0.006

* = Significant exist at $P < 0.05$
Mean values with different superscripts in the same column are significant different.

Table 11: Frequency and Percentage of Characteristics of Variable of Selling Points and Preparation Points in the LGAs Selected.

Variables	Frequency/Percentage (%)	Total (%)
Sources of Water		
Well	16 (33.3)	
Tap	20 (41.7)	48 (100)
Borehole	12 (25.0)	
Domestic Animal Reared		
No	44 (91.7)	48 (100)
Yes	4 (8.3)	
Proximity to Toilet		
No	47 (97.9)	48 (100)
Yes	1 (2.1)	
Use of Washing Basin		
No	6 (12.5)	48 (100)
Yes	42 (87.5)	
Training Attended		
No	18 (37.5)	48 (100)
Yes	30 (62.5)	
Use of Refrigerator		
No	44 (91.7)	48 (100)
Yes	4 (8.3)	

Table 12: Hygiene Parameters Based on School and Local Government Area

Parameters of school/Type		Local Government Area			
		Ilorin West	**Ilorin South**	**Ilorin East**	**Asa**
		Count (%)	***Count (%)***	***Count (%)***	***Count (%)***
Water Sources	Well Water	3 (25.0)	5 (41.7)	3 (25.0)	5 (41.7)
	Tap Water	6 (50.0)	5 (41.7)	6 (50.0)	3 (25.0)
	Borehole Water	3 (25.0)	2 (16.6)	3 (25.0)	4 (33.3)
Domestic Animal Reared	No	11 (91.7)	10 (83.3)	12 (100.0)	11 (91.7)
	Yes	1 (8.3)	2 (16.7)	0 (0.0)	1 (8.3)
Proximity to Toilet	No	12 (100.0)	12 (100.0)	11 (91.7)	12 (100.0)
	Yes	0 (0.0)	0 (0.0)	1 (8.3)	0 (0.0)
Use of Washing Basin	No	2 (16.7)	1 (8.3)	2 (16.7)	1 (8.3)
	Yes	10 (83.3)	11 (91.7)	10 (83.3)	11 (91.7)
Level of personal Hygiene	Below Average	1 (8.3)	0 (0.0)	1 (8.3)	1 (8.3)
	Average	1 (8.3)	1 (8.3)	1 (8.3)	4 (33.3)
	Above Average	10 (83.3)	11 (91.7)	10 (83.3)	7 (58.3)
Level of Environmental Sanitation	Below Average	2 (16.7)	4 (33.3)	2 (16.7)	2 (16.7)
	Average	2 (16.7)	5 (41.7)	5 (41.7)	3 (25.0)
	Above Average	8 (66.7)	3 (25.0)	5 (41.7)	7 (58.3)
Training Attended	No	6 (50.0)	3 (25.0)	3 (25.0)	6 (50.0)
	Yes	6 (50.0)	9(75.0)	9 (75.0)	6 (50.0)
Availability of refrigerator	No	11 (91.7)	11 (91.7)	10 (83.3)	12 (100.0)
	Yes	1 (8.3)	1 (8.3)	2 (16.7)	0 (0.0)
Total		12 (100.0)	12 (100.0)	12 (100.0)	12 (100.0)

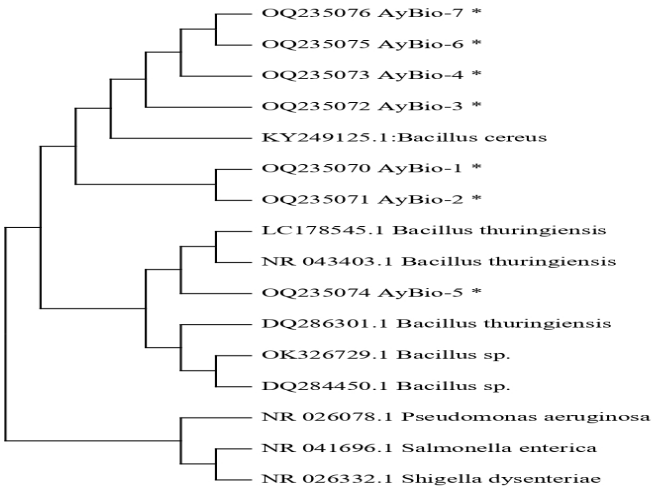

Figure 1: An Unrooted Phylogenic Tree Showing the Relationship of *Bacillus cereus* Strains

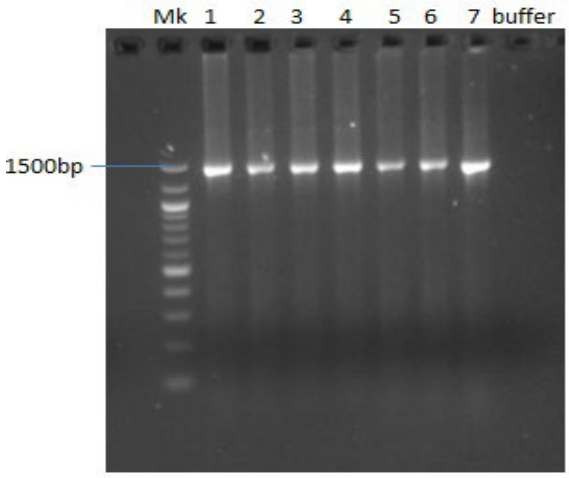

Figure 2: Agarose Gel Electrophoresis
Note: Numbers 1 to 7 represents the Isolated *Bacillus* species

3.6 Discussion of Results

The findings in this study reveal the high incidence of *Bacillus cereus* in some of the food examined. The presence of pathogenic bacteria such as *Bacillus cereus* in food for immediate consumption is a serious menace to the health of the masses both young and old. The ability to produce toxin and the resistance of the endospores to antimicrobial agents enhance pathogenicity in this bacterium. The isolated *Bacillus cereus* strains in this research conform morphologically and by similarities in the sequenced 16S rRNA to referenced *Bacillus cereus.* Previous studies have identified this organism in many ready to eat food [32]. The occurrence and total high count of this bacillus in the food sampled presents a high risk to the health of the intended consumers (Table 2).

This finding agrees with literature on the occurrence of *Bacillus cereus* in many of street vended food sold in public places [11]. Cooked Rice and spaghetti were the two food samples mostly contaminated in this study. These two food items were often the most consumed among food pupils in many primary schools across the country. The food served with beans and fish or meat is very nutritious to the body as it contains balanced nutrients. However, microbiological safety of the food and the health of consumers are often poorly prioritized during processing and distribution.

Sources of contamination to these ready to eat food spanned through occurrence of spores in raw materials from the farm, processing equipment and utensils, hands of food handlers and serving cutleries [33], this findings was corroborated by the report on HACCP studied. The ability of the spores to survive adverse conditions enhances its chance of surviving processing conditions and it invariably get consumed by the masses and thus causes disease [34], occurrence of the spore in bakery raw materials, products and processing lines has been reported [35]. *Bacillus cereus is* very ubiquitous and is found in environments where they could easily become foodborne [32]. The nutrient components in such food serve as the medium for the growth of the bacterium and hence, the vehicle for oral ingestion. Other authors with similar records on incidence of *Bacillus cereus* in food for ready consumption were [32, 34, 36, 37].

Growth of this bacterium in many of the conditions examined such as different temperature, pH, NaCl, UV light and incubation period corroborate the fact that the organism can survive over a wide range of seemingly adverse conditions. Thus, it invariably gets consumed and infection occurs [37] (Tables 5-10).

Poor hygiene and sanitation practices on the part of the vendors expose these ready to eat food to contamination (Tables 11-12). Most of the vendors are illiterate or semi- literate people who have little knowledge of food safety and food associated hazards. The place and process of production, means of distribution and serving the food to the consumers, the packaging materials and availability of potable water were identified as hazard critical points [38] through which *Bacillus cereus* and other pathogens could gain access into food and thrive [39].

Holding temperature, exposure to flies, dust and bioaerosol along the road where these food items are sold and purchased could contribute to the occurrence of *Bacillus cereus* [40]. The high incidence of this food borne pathogen recorded in this research calls for careful selection of raw materials, proper hygiene

during processing, right food storage conditions, and education of food handlers. There is an urgent need to monitor food sold in public places to mitigate epidemics of toxicosis from *Bacillus cereus* among school children (and staff members) in these local government areas.

Conclusion

The findings in this research indicates occurrence of *Bacillus cereus* in some of the food samples examined with highest occurrence in cooked rice and spaghetti. The high incidence of contamination of foods with *Bacillus cereus* highlights public health risks; furthermore, the inability of the strains to grow at a temperature lower than 20 ^{0}C indicates that storage temperature is critical for controlling growth of *Bacillus cereus* in food samples.

Recommendations

It is recommended that; to avoid incidence of *Bacillus cereus* at the stage of consumption, cooked foods should be eaten soon after cooking, or kept above 40 ^{o}C.

REFERENCES

[1] Arfaoui L, Mortada M, Ghandourah H, Alghafari W (2021) Food safety knowledge and self-reported practices among Saudi women. *Current Nutrition and Food Science17*(8), 891-901

[2] Biggel M, Jessberger N, Kovac J, Johler S (2022) Recent paradigm shifts in the perception of the role of *Bacillus thuringiensis* in foodborne disease. *Food microbiology*, 104025

[3] WHO World Health Organization (2016). Foodborne Diseases: A focus for health education, 53rd World Health Assembly Geneva

[4] Trafialek J, Drosinos EH, Laskowski W, Jakubowska-Gawlik K, Tzamalis P, Leksawasdi N, Kolanowski W (2018) Street food vendors' hygienic practices in some Asian and EU countries–A survey .*Food control* 85 212-222

[5] Opara D, Johnny A (2014) The economy and safety of street-vended foods in Uyo, Akwa Ibom State. *World*6(1) 79-86

[6] Tewari A, Abdullah S(2014) *Bacillus cereus* food poisoning: International and Indian perspective. *Journal of Food Science and Technology*522500-2511

[7] Van Cauteren D, Le Strat Y, Sommen C, Bruyand M, Tourdjman M, Da Silva NJ, Desenclos JC (2017) Estimated annual numbers of foodborne pathogen–associated illnesses, hospitalizations, and deaths, France, 2008–2013 *Emerging infectious diseases*23(9) 1486

[8] Liu C, Yu P, Yu S, Wang J, Guo H, Zhang Y, Ding Y (2020) Assessment and molecular characterization of *Bacillus cereus* isolated from edible fungi in China*BMC microbiology20*(1) 1-10

[9] Gauvry E, Mathot AG, Couvert O, Leguérinel I, Coroller L (2021) Effects of temperature, pH and water activity on the growth and the sporulation abilities of *Bacillus subtilis* BSB1. *International Journal of Food Microbiology*337 108915

[10] Jovanovic J, Ornelis VF, Madder A, Rajkovic A. (2021) *Bacillus cereus* food intoxication and toxicoinfection. *Comprehensive Reviews in Food Science and Food Safety*20(4), 3719-3761

[11] Odeleye AOO, Marsh DTJ, Osborne MD, Lye GJ, Micheletti M (2014) On the fluid dynamics of a laboratory scale single-use stirred bioreactor. *Chemical Engineering Science*111299-312

[12] Khaled HA, Ward-Shaher K (2021)Isolation and identification of bacteria of the genus lactobacillus and counting their numbers from the honey bee *Apis mellifera* stomachs. *Plant Archives21*(1) 1746-1750

[13] Karim A, Islam MA, Khalid ZB, Yousuf A, Khan MMR, Faizal CKM (2021) Microbial lipid accumulation through bioremediation of palm oil mill effluent using a yeast-bacteria co-culture. *Renewable Energy*176106-114

[14] James C, Natalie S (2014) *Microbiology.A laboratory manual*. Pearson Education

[15] Reiner K (2010) Catalase test protocol. *American Society for Microbiology*1-6

[16] Lippi G, Blanckaert N, Bonini P, Green S, Kitchen S, Palicka V, Plebani M (2008) Haemolysis: an overview of the leading cause of unsuitable specimens in clinical laboratories. *Clinical Chemistry and Laboratory Medicine46*(6) 764-772

[17] Karki G (2018) Herpes simplex virus (HSV): Structure and genome, mode of transmission, pathogenesis, infection, laboratory diagnosis and treatment

[18] Brink B. Urease test protocol. *American Society for Microbiology*1-7

[19] Leber A (2016) Synergism testing: broth microdilution checkerboard and broth macrodilution methods. *Clinical Microbiology Procedures Handbook* 1 5-16

[20] Dawadi S, Thapa R, Modi B, Bhandari S, Timilsina AP, Yadav RP, Parajuli N (2021) Technological advancements for the detection of antibiotics in food product *Processes9*(9) 1500.

[21] Tille PM, Bailey S (2014) Diagnostic Microbiology. *Misouri: Elsevier*202-927

[22] Shields P, Cathcart L (2011) Motility test medium protocol. *American Society for Microbiology*

[23] Menge JN, Liu YJ, Shen X, Wang J, Xu ZK, Ding Y, Xu ZL (2022) Detection of emetic Bacillus cereus and the emetic toxin cereulide in food matrices: Progress and perspectives *Trends in Food Science and Technology*

[24] Ayanto RT (2021) *Plasmid PCF10-Mediated Enterococcus faecalisHeterogenous Tower-like Biofilm Structures Influence Biological Properties of the Biofilms*, Temple University

[25] Wang Y, Li B, Liu Y, Huang X, Zhang N, Yang Y, Yang Y (2021)Investigation of diverse bacteria encoding histidine decarboxylase gene in Sichuan-style sausages by culture-dependent techniques, polymerase chain reaction–denaturing gradient gel electrophoresis, and high-throughput sequencing. *LWT*139, 110566

[26] Villette R, Autaa G, Hind S, Holm JB, Moreno-Sabater A, Larsen M (2021) Refinement of 16S rRNA gene analysis for low biomass biospecimens. *Scientific Reports*1*1*(1) 1-12

[27] Nayak AK Panda SS, Basu A, Dhal NK (2018) Enhancement of toxic Cr (VI), Fe, and other heavy metals phytoremediation by the synergistic combination of native *Bacillus cereus* strain and *Vetiveriazizanioides L*. *International Journal of Phytoremediation*20(7) 682-691

[28] Diaaeldin IM, El-Shourbagy GA, Hassan HM, Sayed-Ahmed A, Eman T (2022) Microbiological quality of fried chicken breast strips manufactured with monosodium glutamate substitutes. *Zagazig Journal of Agricultural Research49*(3) 351-360

[29] Abraha HB, Choi Y, Hyun W, Lee JW, Kang HS, So MS, Kim KP (2022) Prevalence, diversity and UV-light Inducibility potential of Prophages in Bacillus subtilis and their possible roles in host properties. *Viruses14*(3) 483

[30] Lastochkina O, Pusenkova L, Yuldashev R, Babaev M, Garipova S, Blagova DY, Aliniaeifard S (2017) Effects of *Bacillus subtilis* on some physiological and biochemical parameters of *Triticumaestivum* L.(wheat) under salinity. *Plant Physiology and Biochemistry*121 80-88

[31] Lateef A, Azeez MA, Suaibu OB, Adigun GO (2021) A decade of nanotechnology research in Nigeria (2010–2020): a scientometric analysis. *Journal of Nanoparticle Research*23 1-27

[32] Mwove J, Imathiu S, Orina I, Karanja P (2021)Microbial quality and safety of ready-to-eat street-vended foods sold in selected locations in Kenya. *Journal of Food and Dietetics Research*1(2) 34-40

[33] Saad SA, Hamed NA, Ali AH Mahran SA (2020) Identification of the Gene Responsible for Cereulide Production of *Bacillus Cereus* in Some Meat Products. *Alexandria Journal for Veterinary Sciences66*(1)

[34] Amadi LO, Nwankwo CC (2021) Culture-Dependent Evaluation of Microbial and Proximate Composition of Ready-to-Eat (RTE) African Salad Sold at Nkpolu-Oroworukwo Ultra-Modern Market (Mile 3, Diobu), Port Harcourt, Rivers State, Nigeria. *South Asian Journal of Research in Microbiology* 32-40

[35] Oluwapelumi OB, Oluwasegun SA, Funmilayo AJ, Yomi AR (2020) Evaluation of Bacterial Contamination of Ready-To-Eat Foods Sold in Ado-Ekiti, Ekiti State, Nigeria. *American Journal of Biomedical Sciences12*(3)

[36] Vidic J, Chaix, C, Manzano M, Heyndrickx M (2020) Food sensing: detection of *Bacillus cereus* spores in dairy products. *Biosensors10*(3) 15

[37] Elegbeleye JA, Gervilla R, Roig-Sagues AX, Buys EM (2021) Ultraviolet-C inactivation and hydrophobicity of Bacillus subtilis and Bacillus velezensis spores isolated from extended shelf-life milk. *International Journal of Food Microbiology*349 109231

[38] Cho TJ, Kim SA, Kim HW, Park SM, Rhee MS (2020) Changes in consumers' food purchase and transport behaviors over a decade (2010 to 2019) following health and convenience food trends. *International Journal of Environmental Research and Public Health17*(15) 5448.

[39] Makinde OM., Ayeni KI, Sulyok M, Krska R, Adeleke RA, Ezekiel CN (2020) Microbiological safety of ready-to-eat foods in low-and middle-income countries: A comprehensive 10-year (2009 to 2018) review. *Comprehensive Reviews in Food Science and Food Safety*19(2) 703-732.

[40] Castrica M, Andoni E, Intraina I., Curone G, Copelotti E, Massacci FR, Balzaretti CM (2021) Prevalence of *Listeria monocytogenes* and *Salmonella* spp. in Different Ready to Eat Foods from Large Retailers and Canteens over a 2-Year Period in Northern Italy. *International Journal of Environmental Research and Public Health18*(20)10568