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Cross-Sectional Study of Different Strains of *Bacillus cereus* among Pap Sold in Major Towns in Ihiala LGA, Anambra State

Iheukwumere, I.H.[1], Iheukwumere, C.M.[2], Obianom, A.O.[2], Nnadozie, C.H.[1], Okereke, F.O.[3], Onwuasoanya, U.F.[4], Udeagbara, O.E.[5], Unaeze, B.C.[6], Obiefuna, O.H.[1], Ike, V.E.[7], Onyemekara, N.N.[7] and Ihenatuoha, U.A.[8]

[1]Department of Microbiology, Faculty of Natural Sciences, Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria.
[2]Department of Applied Microbiology & Brewing, Faculty of Biosciences, Nnamdi Azikiwe University Awka, Nigeria.
[3]Department of Biological Sciences (Microbiology), Spiritan University, Nneochi, Abia State.
[4]Department of Medical Microbiology and Public Health, Faculty of Medical Laboratory Science, NAU.
[5]Department of Science Laboratory Technology, Federal Polytechnic, Oko Anambra State.
[6]Department of Medical Laboratory, Faculty of Health Sciences, UNIZIK.
[7]Department of Biology, University of Agriculture and Environmental Sciences Umuagwo, Imo State.
[8]Department of Environmental Health, Technology, Federal College of Animal Health and Production Technology, NVRI, VOM, Plateau State.

*Corresponding author email: ik.iheukwumere@coou.edu.ng /ikpower2007@yahoo.com

Abstract

Species of *Bacillus* have long been troublesome to food producers on account of their resistant endospores. *Bacillus cereus* is well known as a cause of food poisoning, and much more is known about the toxin produced by various strains of this species, so that it significance in such episodes is clearer. However, it is still unclear why such cases especially among locally produced foods like pap are so rarely reported. This study was undertaken to evaluate the cross-sectional study of different strains of *Bacillus* species in pap sold at different towns in Ihiala Local Government Area, Anambra State. A total of 700 samples, one hundred samples each from different major towns (A, H, I, O, S, U, Z) were randomly collected from different vendors and shops at different locations and markets in Ihiala L.G.A. and screened for the presence of *Bacillus cereus* using standard microbiological techniques. The prevalence of the different strains encountered in the samples was also determined. The study revealed that 30.43% of the studied samples were positive to *Bacillus cereus, and the occur*rences of the organism were significantly (P<0.05) seen most in town O, and least in town U. *Bacillus cereus* strain FORC60 (BCF60), *Bacillus cereus* strain DQ01 (BCD1), *Bacillus cereus* strain M72-4 (BCM72) and *Bacillus cereus* strain MB1 (BCB1) were encountered in the studied samples, and there occurrences were 22.07 %, 38.03 %, 29.11 % and 10.79 % respectively. The study also revealed that BCD1 was most significantly (P<0.05) distributed among the studied towns in Ihiala L.G.A. From the above study, different strains of *Bacillus cereus* were isolated from pap sold at different towns in Ihiala L.G.A., of which isolate BCD1 was most predominant and distributed strain in the study samples.

Keywords: *Bacillus cereus, Food contamination, Pap (fermented maize gruel), Endospores, Strain prevalence, Ihiala Local Government Area*

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1. Introduction

Bacillus is a Gram-positive, facultative anaerobic and spore-forming rod. *Bacillus* genus includes both food borne pathogens and food spoilage-associated bacteria, such as *B. cereus, B. subtilis, B. licheniformis, B. pumilus, B. weihenstephanensis and B. sporothermodurans*(Gopal et al.

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2015). *B. cereus* is most commonly detected food pathogen from this genus (Logan 2011; Tewari and Abdullah 2015; Gopal et al. 2015). Among other members, *B. licheniformis* can cause an enteric disease and food poisoning in humans. Similarly, strains of *B. subtilis* may occasionally cause food poisoning outbreaks involving foods such as milk powder (Fernández-No et al. 2011; Gopal et al. 2015). *Bacillus* spp. are widely distributed in the environment with soil as the natural habitat (Tewari and Abdullah 2015).

Bacillus spores are devoid of metabolic activity and are refractory to extreme environmental conditions such as heat, freezing, drying and radiation. These spores can be transmitted through processed, pasteurized and heat-treated food products. Most strains of bacillus are mesophilic, having an optimal temperature between 25 ℃ and 37 ℃, and neutralophilic, preferring neutral pH, but some have been found to grow in environments with much more extreme conditions. *B. cereus* has been isolated from a variety of foods, particularly Ready–to–eat foods such as cooked rice and mixed salad. *B. cereus* can cause food poisoning even at very low doses, with more than 10^3*B. cereus* g^{-1} is considered unsafe for consumption. Two types of illness have been attributed to the consumption of foods contaminated with *B. cereus*. The first and better known is characterized by abdominal pain and non-bloody diarrhoea; it has an incubation period of 4-16 hours following ingestion with symptoms that last for 12- 24 hours. The second, which is characterized by an acute attack of nausea and vomiting, occurs within 1-5 hours after consumption of contaminated food; diarrhoea is not a common feature in this type of illness (Sandra et al, 2020).

While *B. cereus* vegetative cells are killed during normal cooking, spores are more resistant. Viable spores in food can become vegetative cells in the intestines and produce a range of diarrheal enterotoxins, so the elimination of spores is desirable. In wet heat (poaching, simmering, boiling, braising, stewing, pot roasting, steaming), spores require more than 5 minutes at 121 ℃ (250 ℉) at the coldest spot to be destroyed. In dry heat (grilling, broiling, baking, roasting, searing, sautéing), 120 ℃ (248 ℉) for 1 hour kills all spores on the exposed surface. This process of eliminating spores is very important, as spores of *B. cereus* are particularly resistant, even after pasteurization or exposure to gamma rays. *B. cereus* and other members of *Bacillus* are not easily killed by alcohol; they have been known to colonize distilled liquors and alcohol-soaked swabs in numbers sufficient to cause infection.

In Nigeria, the majority of outbreaks of foodborne diseases go unreported, unrecognized or un-investigated and may only be noticed after major health or economic damage. In such conditions, detecting the causative agents of such outbreaks creates a vital pathway for controlling the disease. Studies have shown that one–third of total pediatric admissions in hospitals are due to diarrheal diseases and 17% of all deaths in indoor pediatric patients are diarrhoea-related (Park, 2011). In such a scenario the sure shot identification of the causative agent becomes very difficult, and few reports pointed to *Bacillus cereus*.

Bacillus cereus has reported from a wide variety of foods other than meat and milk, such as desert mixes, infant foods, seafood, coca/chocolates, pulses cereals, vegetables and rice (Choo *et al*., 2007; Rahmati and Labbe, 2008; Desai and Varadaraj, 2009; Kim *et al*., 2013; Tewan and Abdullah, 2015) but there was paucity or no documented information on the occurrences of *Bacillus cereus* in pap, which is a common infant food sold at Ihiala L.G.A and its environs.

2. Methodology

Sample Collection and Handling

A total of 700 samples, one hundred samples each from different major towns (A, H, I, O, S, U, Z) were randomly collected from different vendors and shops at different locations and markets in Ihiala L.G.A., each sample in different container. The pap samples were collected with sterile containers. The containers were thoroughly washed with detergent, rinsed with water, and then rinsed with 70% ethanol and finally rinsed three times with distilled water. The samples were carefully labelled and then kept in a disinfected cooler to maintain the temperature and stability of the number of isolates. The samples were transported to the laboratory for immediate analysis.

Isolation of Organisms: Five grams (5.0g) of the pap sample was weighed using an electronic weighing balance (DC-300), and the sample was aseptically dissolved in 15.0 ml of sterile normal saline in a 50 ml beaker (Pyrex), then up the volume to 50.0 ml before serial dilution. One milliliter aliquot was aseptically transferred into a sterile test tube (Pyrex) containing 9.0 ml of the diluent (sterile normal saline). This was carefully boiled at 100℃, and allowed to cool at room temperature. A one-tenth millilitre of the prepared sample was plated on Petri dishes (60 mm OD × 55 mm ID × 13mm high) containing Nutrient Agar medium (ReadyMED). All the plates in triplicates were incubated at an inverted position at 37±2˚C for 24-48 h.

Characterization and identification of the isolates: The isolates were sub-cultured on nutrient agar (Biotech), and incubated in an inverted position at 37±2˚C for 24 h. The isolates were characterized and identified using their colonial and morphological descriptions as described in the study published by Iheukwumere *et al*. (2018), biochemical reactions as described in the study published by Iheukwumere *et al.* (2020) and molecular characterization as described in the study published by Gabriela *et al.* (2014). The colonial description was carried out to determine the colours of the isolates on agar media plates, their sizes, edges, consistencies and optical properties of the isolates.

Prevalence and Distribution of the Isolates in the Stream Samples

The number of each bacterial isolate in each sampling area was enumerated, and these were calculated in percentage of the occurrences. The bacteria that appeared in each sample location were detected and recorded as described in the study published by Iheukwumere *et al* (2020).

Statistical Analysis

The results of the data generated were expressed as mean, percentage and Table; Data were analyzed by two-way Analysis of Variance (ANOVA) to determine the significance of the main effects and interactions at a 95 % confidence level. Pair wise comparison of the mean was done by the Student "t" test as described in the study published by Iheukwumere*et al* (2018).

3. Results

The present study revealed that 3.43% of the total pap samples showed the presence of *Bacillus cereus* as shown in Table 1. The study also revealed that town O recorded the highest occurrences of *Bacillus cereus* among the total pap samples collected from the seven towns in Ihiala L.G.A.

Town U recorded the lowest occurrences of the test isolate as seen in table 1. The occurrences of *Bacillus cereus* in the pap samples collected from town O was significantly (P<0.05) most when compared to towns A, S, U and Z.

The prevalence of different strains of *Bacillus cereus* in the studied pap samples Collected from seven different towns in Ihiala L.G.A revealed that BCD1 significantly (P<0.05) recorded the highest occurrences among the detected strains (Table 1). This was followed by BCM72, BCF60, and BCB1 was the least shown in table 6. It was also observed that BCD1 was significantly (P<0.05) distributed most in towns H, I an Z and non-significantly (P>0.05) distributed most in towns A, S and U as shown in table 7. BCM72 was distributed most in town O. The cultural and morphological characteristics, the biochemical and nucleic acid verification were presented in Tables 2-5).

Table 1: Prevalence of *Bacillus cereus* in the studied samples across the studied towns.

Town	Number	P(%)	N(%)
A	100	22(22.00)	78(78.00)
H	100	41(41.00)	59(59.00)
I	100	37(37.00)	63(63.00)
O	100	47(47.00)	53(63.00)
S	100	21(21.00)	79(79.00)
U	100	14(14.00)	86(86.00)
Z	100	31(31.00)	69(69.00)
Total	700	213(30.43)	487(69.57)

Table 2: Cultural and morphological characteristics of the isolates

Parameter	G	M	P	R
Appearance on Nutrient Agar	Cream white	White white	Gray white	Cream white
Elevation	Flat	Flat	Flat	Flat
Edge	Rhizoids	irregular	irregular	irregular
Surface	Rough	Rough	Rough	Rough
Size	Medium	Medium	Medium	Large
Capsule	-	-	-	-
Gram Reaction	+	+	+	+
Shape	Rods	Rods	Rods	Rods
Length	Shorts	Shorts	Short	Shorts
Endospore	+	+	+	+
Position of spore	Central	Central	Central	Central
Bulging	-	-	-	-
Motility	+	+	+	+

Table 3: Biochemical characteristics of the isolates

Parameter	G	M	P	R
Catalase	+	+	+	+
Citrate	+	+	+	+
Gelation	+	+	+	+
Methyl red	-	-	-	-
Voges Proskauer	-	-	-	-
Indole	-	-	-	-
Oxidase	+	+	+	+
Urease	-	-	-	-
Hydrogen sulphide	+	+	+	+
Glucose	+	+	+	+
D-mannitol	-	-	-	-
Lactose	+/-	-	+/-	-
Sucrose	+	+	+	+/-
Maltose	+/-	+	+/-	+
Sorbitol	+/-	+	+/-	+/-
Inositol	+	+	+/-	+/-
Xylitol	+/-	+	+/-	+
Dulcitol	+/-	+	+	+/-

Table 4: Nucleic acid verification using Nanodrop

Sample ID	Conc (mg/ml)	260nm	280nm		260/280
G	166.40	3.5146	1.9312	1.82	
M	172.20	3.6142	1.9750	1.83	
P	164.30	3.4762	1.9100	1.82	
R	178.10	3.6561	1.9870	1.84	

Table 5: Molecular characteristics of the isolates

Parameter	G	M	P	R
Max score	7498	115001	11158	15784
Total score	7498	11501	11158	15784
Query cover (%)	100	100	100	100
E-value	0.0	0.0	0.0	0.0
Identity (%)	100	100	100	100
Accession number	CP020383	CP097351	CP119300	CP09171
Description	*Bacillus cereus* Strain FORC60 (BCF60)	*Bacillus cereus* Strain DQ01 (BCD1)	*Bacillus cereus* Strain M72-4 (BCM72)	*Bacillus cereus* Strain MB1 (BCB1)

Table 6: Occurrences of different strains of *Bacillus cereus* in the studied pap samples

Isolate	Number	Percentage
BCF60	47	22.07
BCD1	81	38.03
BCM72	62	29.11
BCB1	23	10.79
Total	213	100.00

Table 7: Distribution of different strains of *Bacillus cereus* in the pap samples collected from different towns

Town	BCF60 (%)	BCD1 (%)	BCM72 (%)	BCB1 (%)	Total (%)
A	6 (2.82)	8 (2.76)	5 (2.35)	3 (1.41)	22 (10.33)
H	7 (3.29)	18 (8.45)	13 (6.10)	3 (1.41)	41 (19.25)
I	6 (2.82)	16 (7.51)	14 (6.57)	1 (0.47)	37 (17.37)
O	10 (4.69)	15 (7.04)	18 (8.45)	4 (1.88)	47 (22.07)
S	5 (2.35)	6 (2.82)	5 (2.35)	5 (2.35)	21 (9.86)
U	4 (1.88)	5 (2.35)	4 (1.88)	1 (0.47)	14 (6.57)
Z	9 (4.23)	13 (6.10)	3 (1.41)	6 (2.82)	23 (10.80)
Total	47 (22.07)	81 (38.03)	62 (29.11)	23 (10.80)	213 (100.00)

4. Discussion

The presence of *Bacillus cereus* in the studied pap samples could be traced from the production practices, water used, packaging, wrapping materials, transportation, poor handling and sanitary conditions. Similar findings were reported by many researchers (Immerseel *et al.*, 2002; Jones and Richardson, 2004; Alshawabkeh, 2006; Maciorowski *et al.*, 2007). Researchers have shown that the transportation of equipment can also harbour bacterial pathogens and this contributes to the contamination of pap samples (Primm, 2008). Pap contaminated by spore-forming bacteria pathogenic to humans can contribute to human food-borne illness through the pap-food-human chain. This shows that the production of pap requires microbiological safety regulations to escape microbial contamination of the product. A similar deduction was drawn by different researchers (Davies and Wales, 2010; Chowdhuri *et al.*, 2011; Fredrick and Huda, 2011). The highest counts of *Bacillus cereus* in the pap samples could be attributed to human activities during processing, water sources, transportation and packaging of the pap. Ali *et al.* (2014) stated that the presence of pathogenic strains of bacteria in foods and their by-products is not only a potential threat of cross-contamination but can also lead to an infectious dose for handlers and consumers.

The presence of *Bacillus cereus* strain FORC60 (BCF60), *Bacillus cereus* strain DQ01 (BCD1), *Bacillus cereus* strain M72-4 (BCM72) and *Bacillus cereus* strain MB1 (BCB1) in the studied samples supported the findings of many researchers (Choo *et al.*, 2007; Rahmati and Labbe, 2008; Desai and Varadaraj, 2009; Fagerlund *et al.*, 2010; Park, 2011; Kim *et al.*, 2013; Rahimi *et al.*, 2013; Tewan and Abdullah, 2015). Tewan and Abdullah (2015) reported that *B. cereus* is well-known as the common cause of food poisoning. Several studies have reported that *B. cereus* produces one emetic toxin (ETE) and three different enterotoxins; hemolysin BL (HBL), non-haemolytic enterotoxin (Nhe) and cytotoxin K (CytK) which are responsible for the diarrhoeal type of food poisoning (Fagerlung *et al*., 2010; Tewan and Abdullah, 2015).

5. Conclusion

The study revealed the presence of *Bacillus cereus* in the studied pap samples, *and the occur*rences of the organism were significantly seen most in town O, and least in town U. *Bacillus cereus* strain FORC60 (BCF60), *Bacillus cereus* strain DQ01 (BCD1), *Bacillus cereus* strain M72-4 (BCM72) and *Bacillus cereus* strain MB1 (BCB1) were encountered in the studied samples. The study also revealed that BCD1 was most significantly distributed among the studied towns in Ihiala L.G.A. From the above study, different strains of *Bacillus cereus* were isolated from pap sold at different towns in Ihiala L.G.A., of which isolate BCD1 was the most predominant and distributed strain in the study samples.

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Conflict of interests
The authors declare that they have no conflict of interests.

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Ethical approval
Not applicable

Authors Contributions
All contributed towards the study design, experiment execution, data analysis, and manuscript drafting.

Availability of Data and Materials
All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

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