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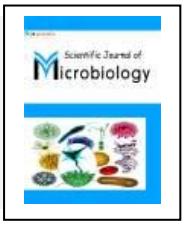

Original article

Characterization and antibiotic susceptibility pattern of *Bacillus cereus* isolates from fried soyabean cake in Zaria, Nigeria

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ABSTRACT

A total of one hundred and fifty fried soyabean cake samples were collected from different vendors in various parts of Zaria, Kaduna state and were assessed for the presence of *Bacillus cereus*. 84 out of the total 150 were positive for B. cereus given a prevalence of 56%. All the samples tested contained more than 10^5 cfu/g of the bacterium with a mean count value of 7.8±7.1 log cfu/g. Isolates were tested for enterotoxin production using the BCET-RPLA kit and 90% of the isolates were found to possess the L_2 fraction of the HBL components. All enterotoxic B. cereus isolates were found to be susceptible to Amikacin (30µg), Ciprofloxacin (5µg), Gentamicin (10µg) and Tetracycline (30µg). 100% resistance was observed against Ampicillin (10µg) and Oxacillin (1µg). The percentage resistance to Bacitracin (10µg), Chloramphenicol (30µg), Erythromycin (15µg) and Vancomycin (30µg) of the enterotoxic isolates are 56%, 33%, 39% and 44% respectively.

1. Introduction

Present economic conditions have resulted in the increasing demand for fast, easy, portable and convenient food supply which has led to the increasing demand of street vended foods. Food vending has become increasingly important in most countries, contributing significant income inflow for households involved in selling these foods

(FAO/WHO, 2005). Snack foods sold on the street are important vehicles for delivery of essential nutrients because of the growing change in eating habits (Henshaw and Agunbiade, 2004), although they are also frequently associated with diarrhoeal diseases which occur due to improper use of additives, the presence of pathogenic bacteria, environmental contaminants and disregard for good manufacturing practices (GMPs) and good hygiene practices (GHPs).

Bacillus cereus is an important cause of foodborne disease worldwide (Clavel et al., 2004; Granum, 2007), although it is probably highly under-reported in official lists of foodborne disease causes. *Bacillus cereus* is a member of the *Bacillus cereus* group which comprises of *B. anthracis, B. thuringiensis, B. mycoides, B. pseudomycoides* and *B. weihenstephanensis*. *Bacillus cereus* is a gram-positive, facultatively aerobic sporeformer whose cells are large rods and whose spores do not swell the sporangium. The ability of this bacterium to produce endospores which can withstand time and harsh environments such as heat, dehydration and other physical stresses accounts for its ubiquitous occurrence in the natural environment as well as for the high frequency of its isolation from various kinds of contaminated raw and processed food products, such as rice, spices, milk and dairy products, vegetables, meat, farinaceous foods, desserts and cakes (Duc et al., 2004).

A number of food poisoning incidents can be attributed to *B. cereus* and this bacterium is known to cause a variety of non-gastrointestinal diseases, as well as two different types of food poisoning which are characterized by either emesis or diarrhoea. The emetic syndrome is an intoxication caused by the *B. cereus* emetic toxin. The emetic toxin is a heat stable small ring forming peptide (Lund et al., 2000) named cereulide, produced in foods before ingestion. The course of the disease is characterised with nausea, vomiting and emesis occurring only a few hours after the meal (Ehling-Schulz et al., 2004). The diarrhoeal syndrome is a toxicoinfection caused by vegetative cells, ingested as viable cells or spores, producing protein enterotoxins in the small intestine (Clavel et al., 2004) and typically characterised with abdominal pain, watery diarrhoea and occasionally nausea. The best investigated enterotoxin is HBL, a three-component haemolysin that consists of two lytic proteins (L_1 and L_2) and one binding component (B) (Beecher and Macmillan, 1991). This toxin, which possesses haemolytic and dermonecrotic activities and increases vascular permeability, is considered the primary virulence factor in diarrhoea for its ability to cause fluid accumulation in rabbit ileal loops (Beecher et al., 1995). As a result of the role of microorganisms in spreading diseases, the need to assess the safety and quality of foods is very important, in order to ensure safety of supply, clean, wholesome and high quality delivery to the public. When the food hygiene system fails, a batch of food is contaminated with high level microbe potential for food borne disease outbreak. The microbiological safety of foods sold in the market is of major concern because of the environment in which they are prepared, often in places that may have poor sanitation, coupled with use of containers which expose the food to numerous potential contaminants such as heavy metals and pesticides.

This study was carried out to evaluate the presence of *Bacillus cereus* in fried soyabean snack purchased from various outlets and points in Zaria, Kaduna State and characterize the organism isolated in terms of biochemical reaction and antibiotic susceptibility.

2. Materials and methods

2.1. Sampling site and collection

A total of one hundred and fifty fried soyabean cake samples being sold in various sales points in Samaru, Sabon Gari and Zaria city areas of Zaria were enumerated for the viable counts of *Bacillus cereus*. The food samples were collected from the food vendors in sterile disposable polyethylene bags and brought to laboratory for microbiological analysis. Analyses of samples were performed within 60mins of collection.

2.2. Enumeration of bacillus cereus

A homogeneous sample was prepared with 10g of food sample, aseptically transferred into 90mL of sterile 0.1% buffered peptone water. Decimal dilutions up to 10^{-9} were prepared. Aliquots of 0.1mL from 10^{-5} dilutions were surface plated on sterile dried Mannitol Egg-Yolk Polymyxin Agar (MYP) (Oxoid, Hampshire, England) media and incubated at 37°C for 24 h. Media used in our investigation were prepared according to manufacturer's instructions.

2.3. Identification of Bacillus cereus isolates

Suspected pink colonies with zones of egg-yolk precipitate on the medium were picked, purified and identified using the following characteristics: gram stain, cellular and spore morphology, motility, catalase test, utilization of carbohydrates, Voges-Proskauer test, nitrate reduction, urea hydrolysis, indole test, casein degradation; utilization of citrate and growth at 7% NaCl. The presumptive *B. cereus* was confirmed using the Bacillus Microgen identification kit (Microgen Ltd, UK).

2.4. Assay for *B. Cereus* toxin

Production of the L_2 component of HBL by *B. cereus* was tested in culture filtrates using the *B. cereus* Enterotoxin-Reversed Passive Latex Agglutination (BCET-RPLA, Oxoid) kit which was used according to manufacturer's instructions.

2.5. Antibiotic susceptibility

This test was performed by the disc agar diffusion method (CLSI, 2006). Single colony of 24 h old culture was transferred to 5 ml of Nutrient Broth media (Oxoid) and incubated at 37°C for 6 to 8 h. A sterile cotton swab dipped into the nutrient broth growth was applied onto pre-dried Muller-Hinton agar (Oxoid) plate. After drying, the antibiotic discs were placed aseptically and plates were incubated at 37°C for 24 h. The diameter of inhibition zones were measured and recorded according to the standard methods (CLSI, 2006). The organisms were investigated using 10 different antibiotic discs selected from seven (7) classes of antibiotics as follows, Aminoglycoside (Amikacin (30µg), Gentamycin (30µg)); β-lactam (Ampicillin (10µg), Bacitracin (10µg), Oxacilin (1µg)); Chloramphenicol (Chloramphenicol (30µg)); Fluoroquinolones (Ciprofloxacin (10µg)); Glycosides (Vancomycin (30µg)); Macrolides (Erythromycin (15µg)) and Tetracycline (Tetracycline (30µg)). The antibiotic discs used in this study were bought from Oxoid Company.

3. Results

A total of One hundred and fifty fried soyabean cake samples were collected from three different locations (Samaru, Zaria city and Sabon Gari) in Zaria, Nigeria and were analysed. Contamination with *B. cereus* strains was recorded in 56% of all the samples (Table 1). The recorded prevalence from each location was also examined. From each location, a total of fifty samples were collected and subjected to analysis. The incidence of *B. cereus* was highest in Samaru samples (64%) which were closely followed by Sabon Gari samples (58%) and the Zaria city samples was the least with 46%. The viable populations of *B. cereus* enumerated from the food sample from the different locations are summarized in Table 2. The mean viable counts range from a minimum of 7.4 $\log_{10}$ cfu/g from Zaria City samples to a maximum of 7.9 $\log_{10}$ cfu/g from Samaru samples of the fried soyabean cakes.

Table 1
Distribution of Bacillus cereus in "Awara" samples at different selling outlets.

Locations	No of samples collected	Samples positive for *B. cereus*	
		(n)	(%)
Samaru	50	32	64
Zaria City	50	23	46
Sabon Gari	50	29	58
Total	150	84	56

The 84 isolates characterized as *B. cereus* were tested for diarrhoeal enterotoxin production and 76 strains (90.5%) tested positive to the production of haemolytic fraction L_2 of the HBL genes while only 8 strains (9.5%) were found to be negative using the BCET-RPLA test kit. Table 3 shows the antibiotic sensitivity of the 76 enterotoxigenic strains of *B. cereus* isolated from fried soyabeans cake samples in Zaria. All the isolates were found to be susceptible to amikacin and gentamicin while only five strain each were found resistant to both ciprofloxacin and tetracycline respectively thus making the percentage susceptible isolates to both ciprofloxacin and tetracycline to be 93% (n=71) respectively. In the same respect, all the tested isolates were found to be resistant to both ampicillin and oxacillin (n=76; %=100). Chloramphenicol, erythromycin and vancomycin were also found to be

effective against a reasonable percentage (67, 61 and 57 respectively) of the tested isolates. More than half of the isolates were found resistant to bacitracin.

Table 2

Range and mean of Bacillus cereus counts (log10) in "awara" samples at different selling outlets.

Location	No of samples	Log10 cfu/g	
		Mean±SEM	95% CI
Samaru	50	7.9±7.5	7.3-8.2
Zaria city	50	7.4±6.6	7.2-7.5
Sabon Gari	50	7.8±7.3	7.5-8.0
Total	150	7.8±7.1	7.5-7.9

F=2.092, df= 2, p>0.05; SEM=Standard Error of Mean

Fig. 1 shows the percentage susceptibility of the isolates to the classes of antibiotic used. Aminoglycosides were found to be more effective on all the enterotoxic strains with 100% susceptibity closely followed by tetracycline and fluoroquinolones with both having Percentage susceptibility of 93.4% each. β-lactam antibiotics (43.4%) showed the least effect on the enterotoxigenic strains tested.

Table 3

Antibiotic sensitivity of enterotoxic B. cereus from fried soyabean samples; n=76

Antibiotic used (Class/Structural group)	Disc content (µg)	Number (%) resistant organisms	Number (%) susceptible organisms
Amikacin (Aminoglycosides)	30	0 (0)	76 (100)
Ampicillin (β-lactam penicilin)	10	76 (100)	0 (0)
Bacitracin(β-lactam polypeptides)	10	43 (57)	33 (43)
Chloramphenicol (Chloramphenicol)	30	25 (33)	51 (67)
Ciprofloxacin (Fluoroquinolones)	5	5 (7)	71 (93)
Gentamicin (Aminoglycosides)	10	0 (0)	76 (100)
Erythromycin (Macrolides)	15	30 (39)	46 (61)
Oxacillin (β-lactam penicilin)	1	76 (100)	0 (0)
Tetracycline (Tetracycline)	30	5 (7)	71 (93)
Vancomycin (Glycosides)	30	33 (43)	43 (57)

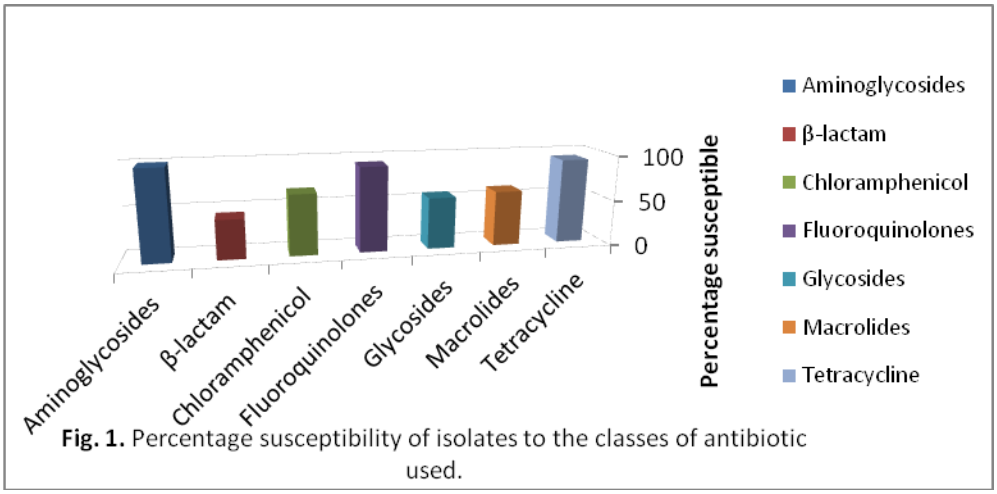

Fig. 1. Percentage susceptibility of isolates to the classes of antibiotic used.

4. Discussion

The public health significance of *Bacillus cereus* is of high concern, in view of the organism being implicated in a number of food poisoning outbreaks, worldwide. The prevalence of *B. cereus* recorded in this study was more or less similar to those reported by other researchers who had worked with other ready-to-eat food sold on the street (Oguntoyinbo and Oni, 2004; Roy et al., 2007; Das et al., 2009). Fried soyabean cake, provide a source of readily available and nutritious meals for the consumers; however, the safety and microbiological quality of these foods should be the first priority, since they do not receive any further heat treatment before consumption. Despite product profile, which could be unfavourable for survival and multiplication of this organism, the result of this research may be due to the prevailing environmental conditions combined with post processing practices.

The unacceptable and potentially hazardous *B. cereus* count established in over 50% of screened fried soyabean cake samples implies extreme contamination and potential health risk of these street food samples. This findings correlate with earlier studies (Olukoya et al., 1991; Mensah et al., 2002; Yahoah – Manu et al., 2010 and Sandra et al., 2012) and Agwa et al., 2012 also had a similar observation in cooked rice and Masa stating that the organism is a normal flora of rice. The high incidences of *B. cereus* contamination encountered in this study could be explained by the ubiquitous distribution of this organism and its ability to form endospores, a view also suggested by Kotiranta et al., (2000) and McKillip, (2000). Poor storage conditions, unsanitary and largely unhygienic nature of the food preparations and service areas are good indicators of the state of environment in which they are prepared or served do provide suitable conditions for these bacteria to gain access to food and strive. This agrees with other researchers report (Ehirim et al., 2001; Omemu and Aderoju, 2008). In most cases, fried soyabean cake vendors do not have adequate bathing facilities, atimes starts their day without taking a bath, and therefore the snack and its ingredients are exposed to repeated contamination from unwashed hands and materials used for wrapping such as outdated newspapers and re-usable polyethylene bags coupled with the fact that the majority of the production/selling outlets are located beside waste disposal points and dusty roads or streets with human and vehicular traffic which encouraged multiple contaminations due to the deposition of bioaerosol on exposed food products, transfer from dirty hands, utensils and flies (Yassin and Almouqatea, 2010).

The HBL enterotoxin is the primary cause of diarrhoea infection caused by *B. cereus* from contaminated foods and in this present study, over 70% of the *B. cereus* were found to be positive for the proteic sub-unit L_2 (HBLC). This was not unusual, as various studies have reported high percentages of diarrhoeal enterotoxin production among *Bacillus cereus* strains (Pirttijarvi et al., 1996) and this observation is in agreement with other previous works (Granum et al., 1996; Souza and Abrantes, 2009). Expression of BCET was detected in more number of isolates in this study than from other reports (Ombui et al., 1997; Beattie and Williams, 2000; Banerjee et al., 2011).

All the enterotoxic *Bacillus cereus* exhibited high degree of susceptibility to amikacin, gentamycin, ciprofloxacin and tetracycline. The finding of this study corresponds with those obtained by other researchers (Umar et al., 2006; Whong and Kwaga, 2007). Previous works have shown that *Bacillus cereus* were highly susceptible to streptomycin, chloramphenicol, erythromycin, ciprofloxacin, and less susceptible to ampicillin, ampiclox, cotrimazole, and cloxacillin (Umar, et al., 2006; Lunal et al., 2007). The resistance pattern shown by the isolates in this study against β-lactam (ampicillin and oxacillin) antibiotics is not unusual as this may be caused by the production of β-lactamase by *B. cereus* strains (Sabath and Abraham, 1965).

Some of the *Bacillus cereus* in this study demonstrated some degree of resistance to chloramphenicol, erythromycin, tetracycline and vancomycin; contrary to the report of Logan and Tumbull, (2003). These variations in the percentages may be due to the differences in the concentrations of antimicrobial agents used, differences in the source of isolates, drug resistance transfer and the overall wide spread use of the antibiotics in the environment. The development of drug resistance may be due to the use of these drugs in medical and veterinary practice to treat infections and misuse of the drugs in the society, such practices can lead to drug resistance strains.

6. Conclusion

The present study has attempted to establish an alarmingly high prevalence of the potentiality of isolates of *B. cereus* to harbour toxigenic traits capable of causing diarrhoeal coupled with the fact that food samples sold in streets are operating in less than an acceptable and satisfactory environment which calls for concern. The findings

based on the antibiotic susceptibility shows that aminoglycosides (amikacin and gentamycin), cipfloxacin, tetracycline and chloramphenicol are more effective against the *B. cereus* isolates.

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