Antibiotic Resistant *Bacillus cereus* in Some Selected Foods in Osun State, Nigeria.

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Abstract: Microbiological analyses were carried out on Four hundred samples of five different foods i.e. boiled maize, cooked rice, garden egg, carrot and roasted beef from four towns Osogbo, Ilesha, Ife and Ikirun in Osun State, Nigeria. Food samples were collected from different vendors and examined for *B. cereus*, using *B. cereus* selective Agar Base, egg yolk emulsion and Bacillus selective supplement. *B. cereus* was detected in 202 (50.50%) of 400 food samples. *B. cereus* occurred in 71% of boiled maize, 63.75% cooked rice, 51.25% roasted beef (suya), 40% carrot and 23.75% garden egg. The range of population of *B. cereus* in these foods was 1.3 x 10^2 to 7.5 x 10^5 cfu/g. Boiled maize and cooked rice had highest mean counts of 10^5 cells/g. The level of *B. cereus* contamination of foods examined was higher in Ikirun compare to other towns in the state. From the results of antibiotic susceptibility test, almost all the *Bacillus cereus* strains were susceptible to gentamicin (98.24%) chloramphenicol (96.70%), and sulphamethoxazole (92.92%). The highest level of antibiotic resistance was obtained for ampicilin (70.80%), and amoxicillin (42.23%). The incidence of *B. cereus* foodborne intoxication could be reduced by maintaining high level of hygiene in our environment and good production practices.

[Olawale, Adetunji, Kolawole and Akintobi, Akinsoji, Olubiyi. Antibiotic Resistant *Bacillus cereus* in Some Selected Foods in Osun State, Nigeria. Report and Opinion 2011;3(1):21-25]. (ISSN: 1553-9873). http://www.sciencepub.net.

Keywords: Bacillus cereus, Antibiotic resistant, Food intoxication, Hygiene, Food contamination.

Introduction

Bacillus cereus is a gram positive, spore forming motile aerobic rod bacteria but, grows well anaerobically. It is widespread in nature, being frequently isolated from soil and growing plants {Kramer and Gilbert, 1989} from this natural environment it is easily spread to foods especially those of plants origin. Through cross-contamination, it may then be spread to other foods such as meat products {Kramer and Gilbert, 1989}. The problems in milk and milk products are caused by *B. cereus* which is spread from soil and grass to udders of cows and into the raw milk. Through sporulation *B. cereus* spores survive pasteurization, and after germination the cells are free from competition from other vegetative cells (Anderson *et al*., 1995).

B. cereus causes two different types of food poisoning, the diarrheal type, first recognized after a hospital outbreak (caused by contaminated vanilla sauce) in Oslo, Norway in 1948 (Granum, 1994) and the emetic type described about 20 years later after several outbreaks (rice) in London (Mortimer and McCann, 1994). The diarrheal type of food poisoning is caused by an enterotoxin (s) produced during vegetative growth of *B. cereus* in the small intestine (Granum *et al*., 2003) while emetic toxin is produced by cells growing in the food (Anderson *et al*., 1995). *B. cereus* food borne illness is underreported, as both types of illness are relatively mild and usually last less than 24 hours (Kramer and Gilbert, 1989). However a more severe form of diarrheal type of *B. cereus* foodborne illness that lasts longer has occasionally been reported (Mortimer and McCann, 1994).The dominant type of illness caused by *B. cereus* differs from country to country. In Japan, the emetic type is reported about 10times more frequently than the diarrheal type, whereas in Europe and North America the diarrheal type is reported more frequently (Gramum, 2001). Eating habit probably account for this difference. In Nigeria the picture is quite different in that there is paucity of information concerning the involvement of *B. cereus* in overall picture of foodborne intoxication. However, the organism has been isolated from different dried condiments (Obuekwe and Ogbimi 1989). Nigeria flour-based foods (Yusuf *et al.*, 1992); fermented milk "nono" (Ado and Whong, 1995) cooked and uncooked rice, roasted beef (suya) and spices (i.e garlic and pepper) (Whong *et al*., 2006).

The bacterium is also known to cause several non-gastrointestinal diseases which include endocarditis bacteremia and septicemia, respiratory infection, pneumonia, meningitis, wound and ocular infection (Johnson, 1984; Drobniewski, 1993).

The absence of documented cases of *B. cereus* outbreak in Nigeria may be due to lack of awareness of the problem, failure to use appropriate isolation and identification techniques and lack of information on the basic properties of the organism. In the South Western part of Nigeria, common foods of the populace include corn, rice, roasted beef, (suya), carrot and Garden egg. These foods are liable to contamination by *B. cereus* from the soil. It is therefore necessary to investigate the distribution of B. cereus among those foods.

This paper reported the occurrence of B. cereus in various foods in Osun State, Nigeria and the resistivity of the organism to some antibiotics.

Materials and Methods

Twenty (20) samples of each of the following foods; boiled maize, cooked rice, garden egg, carrot and roasted beef (suya) were purchased from retailed outlets in Osogbo, Ilesha, Ife and Ikirun all in Osun State of Nigeria. They were collected into clean, dry high-density polythene bags. The samples were taken to the laboratory within 1hrs of collection and were held refrigerated at 5% until examined.

Enumeration of Bacillus

The method of Whong *et al.*, (2006) was used. 0.1ml aliquots of 10^{-1}, 10^{-2} and 10^{-3} dilutions of each sample were surface plated in duplicates on Bacillus selective Agar (Oxoid). All plates were inverted and incubated at 30^{o}c for 24hrs. Following incubation, the plates were left at room temperature for another day and re-examined. Presumptive *B. cereus* colonies, which were crenate with egg yolk precipitate, were counted. Typical *B. cereus* colonies with lecithinase activity were gram- stained and the gram- positive rods purified on nutrient agar plates were stored on nutrient agar slant until they were required for biochemical tests.

Characterization of *B. Cereus*

The Bacterial isolates were tested for different biochemical characteristics by the method described by Cowan and Steel (1974). Tests done included motility, catalase, methylred reaction, Voges-proskaer reaction, gelation hydrolysis, starch hydrolysis, citrate utilization, beta-hemolysis, hemolysin, acid production from: manitol, arabinose glucose, mannose, xylose and salicin.

Antibiotic Susceptibility Test

Bacillus cereus isolates were tested for susceptibility to 5 antimicrobial agents. The following antibiotics (final concentration) were used; chloramphenicol (cm; 30mg/ml) gentamicin (gm; 10mg/ml), suphamethoxazole (smx; 100mg/ml), amphicillin (Amp; 20mg/ml), amoxillin (amx; 20mg/ml). The plates were prepared by adding the drug to molten Muller-Hinton agar, previously cooled to 45^{o}c. Each plate was inoculated with several isolates using the multipoint inoculation method (Hassani *et al*., 1992) and incubated at 37^{o}c for 24 hrs. A control plate without antibiotic was prepared and similarly inoculated. An isolate was considered to be resistant to an antimicrobial agent if its growth in the medium containing the agent was similar to that on the control plate.

Results

The distribution and densities of *Bacillus cereus* in food samples purchased in four major towns in Osun State was presented in tables 1 and 2. Out of the 400 food samples assayed, 202 (50.50%) were contaminated by *B. cereus* distributed per location as follows; Osogbo (44%), Ilesha (48%), Ife (49%) and Ikirun (61%). The levels of contamination by *B. cereus* varied in the positive samples for the four locations, ranging from 1.3 x10^{2} to 7.5 x 10^{5} cells/g. High levels of contamination by *B. cereus* were obtained in boiled maize, 7.5 10^{5}/g; cooked rice, 4.4 x 10^{5}/; roasted beef (suya), 2.4.x 10^{5}.

The mean counts of *B. cereus* for four locations for carrots were a bit moderate, while those for garden egg were very low.

The contamination level was significantly higher for food samples from Ikirun as compared to other three towns, while lower contamination was recorded from Osogbo (Table 1).

Antibiotic Susceptibility of the *Bacillus cereus* Isolates.

The incidence of antibiotic resistance among *B. cereus* strains is presented in Table 3. The mean proportion of *Bacillus cereus* isolates resistant to at least one antibiotic was 25.03%. A very high percentage was susceptible to gentanicin (98.24%). The percentage of chloraniphenicol resistant, sulphamethoxazole- resistant and Amoxicillin resistant isolates were; 3.30%, 8.20% and 42.23% respectively. More than 70% of the *Bacillus cereus* strains examined exhibited resistance to Ampicillin. Significant differences in the susceptibility of antibiotics depending on the source of isolate were recorded in (Table 3). Strains isolated from Ikirun exhibited higher resistance 21.71% than those isolated from other towns. The percentage of *B. cereus* exhibited resistance to antibiotic in Ilesha, Osogbo and Ife were 14.61%, 14.9% and 19.00% respectively.

Table 1: Distribution of *Bacillus cereus* in different foods in Osun State.

Food samples (Number)	Number and Percent of samples containing *B. cereus* in different locations				
	Osogbo *B. cereus* n (%)	Ilesha *B. cereus* n (%)	Ife *B. cereus* n (%)	Ikirun *B. cereus* n (%)	Total Location *B. cereus* n (%)
A. Boiled Maize (20)	12 (60)	13 (65)	16 (80)	17 (90)	59 (71.25)
B. Cooked Rice (20)	9 (45)	10 (50)	14 (70)	18 (90)	51 (63.75)
C. Garden egg (20)	5 (25)	7 (35)	3 (15)	4 (20)	19 (23.75)
D. Carrot (20)	8 (40)	10 (50)	6 (30)	8 (40)	32 (40.0)
E. Roasted beef (20)	10(50)	8 (40)	10 (50)	13 (65)	41 (51.25)
Total 100	44 (44)	48 (48)	49 (49)	61 (61)	202 (50.50)

Table 2: Mean count of *Bacillus cereus* for five (5) foods from different locations

Food Samples	**Locations** Osogbo	Ilesha	Ife	Ikirun
Boiled Maize	4.5×10^2	1.2×10^4	7.5×10^5	3.1×10^5
Cooked rice	7.5×10^3	4.4×10^5	1.6×10^4	2.2×10^5
Garden Egg	1.8×10^2	5.2×10^3	3.0×10^2	1.2×10^3
Carrot	1.3×10^2	6.4×10^2	2.1×10^4	2.4×10^3
Roasted beef	2.1×10^3	2.4×10^5	4.7×10^3	2.4×10^4

Table 3: Percentage of antimicrobial resistance (R) and antimicrobial susceptibility (S) of *Bacillus cereus* isolates from 4 different Towns.

Antibiotic	**B. cereus from Osogbo N = 44**		**B.cereus from Ilesha N = 48**		**B. cereus from Ife N = 49**		**B. cereus from Ikirun N = 61**		**B. cereus from all sources N = 202**	
	(R%)	(S%)	(R%)	(S%)	(R%)	(S %)	(R%)	(S %)	(R%)	(S%)
CM	2.51	97.49	3.56	96.44	2.15	97.85	4.60	95.40	3.30	96.70
GM	1.50	98.50	1.80	98.20	1.64	98.36	2.01	97.99	1.76	98.24
SMX	6.55	93.45	6.05	93.95	7.15	92.85	8.20	91.80	7.08	92.92
AMP	30.34	69.66	33.10	66.90	35.50	64.5	39.61	60.39	70.80	29.20
AMX	33.60	66.40	28.55	71.45	48.54	51.46	54.15	45.85	42.23	57.77
TOTAL	**14.90**	**85.10**	**14.61**	**85.34**	**19.00**	**81.00**	**21.71**	**78.29**	**25.03**	**74.97**

Key:
(R%) = Percentage resistance
(S%) = Percentage Susceptible
CM = Chloramphenicol
GM = Gentamicin
SMX = Sulphamethoxazole

AMP	=	Ampicillin
AMX	=	Amoxicillin

Discussion

The *B. cereus* population greater than 10^5 cell/g was observed in boiled maize, cooked rice and roasted beef. This result agreed with Obukwe and Ogbimi (1989) and Yusuf *et al.*, (1992)'s report with high load of *B. cereus* (> 10^5/ml or g) in Nigeria foods and food condiments. The high level of contamination by *B. cereus* may be attributed to the ubiquitous distribution of this organism in the environment (Kramer *et al.*, (1982). The possible sources of contamination could be from the soil during harvesting the atmosphere which is known to harbour the spores of the organism in larger numbers, improper handling of both the raw materials and finished products, the water used in processing and the manner the finished products were displayed in the market for sale (Bryan, 1992)

Hazardous levels or infective doses of 10^7, 10^5 and 10^3cfu/ml or g are required to cause foodborne illness for adults, children and infants respectively. The mean counts of *Bacillus cereus* found in boiled maize, cooked rice and "suya" were lower than the hazardous level or infective dose for adults (Gilbert and Kramer, 1986). However, the mean counts were higher than the hazardous levels for children and for infants (Becker *et al.*, 1994).

Low mean counts of *B. cereus* were obtained from garden egg purchased at Osogbo and Ife; and carrot from Osogbo and Ilesha. The low mean counts that were found may be attributed to proper cleaning with tap water and lack of heating processes.

There were significant variations in mean counts of *B. cereus* from the food samples purchased at the four locations. The level of contamination by *Bacillus cereus* from boiled maize, cooked rice, and roasted beef (suya) purchased at Ikirun, Ilesha and Ife may be considered safe for consumption by adults but not for infants and children. However all the food purchased from Osogbo may be considered safe for consumption by all. The contamination of roasted beef may be through contaminated spices and seasoning which are normally added to it (Lund, 1990). Other sources of contamination of "Suya", boiled maize and rice may included environment (dust), handing by vendors, flies, trays and other containers. The heat treatment given to these food items may be insufficient to kill spores and improper holding after roasting or boiling allows the spores to germinate resulting in vegetative cells which multiply (Goepfert *et al.*, 1972).

Antibiotic resistant

More than 25% of the *B. cereus* strain exhibited resistance to at least one antibiotic. This was in line with the report of Milch, (1985). Resistance to antibiotics is not surprising considering how frequently these drugs have been used in treating the disease caused by pathogenic microorganisms. It is well known that misuse of antibiotic will promote an increase of the proportion of resistant bacteria (Morinigo *et al.*, 1990). The misuse and abuse of antibiotics for treatment of infection caused by gram-positive and gram – negative microorganisms may explain the spread of resistance among clinical strains (Milch *et al.*, 1985).

Conclusion

Bacillus cereus may be present in most foods at low level without being hazardous. However problems develop when cooked foods are stored at room temperature or if the temperature is abused for a longtime. Under these conditions, the organism grows to high numbers, releasing toxin during growth in the food and in the intestinal tract (Doyle, 1988). From the observation made in tables 1 and 2 three of foods purchased at Ilesha, Ife and Ikirun contained counts of *B. cereus* at contamination levels that may be of health hazard to infants and children but not for adults. It is apparent from the data in Table 3 that about 25% of the *B. cereus* strains exhibited resistance to at least one antibiotic. Almost all the isolates were susceptible to gentamicin (98.24%), chloramphenicol (96.70%), and sulphametoxazole (92.92%). The highest levels of antibiotic resistance were obtained for ampicillin (70.8%) and amocixillin (42.23%).

In view of the fact that the emergence of strains resistant to antimicrobial agents are brought about by their contact with such agent during treatment, it is therefore, important that people should avoid utilization of antibiotics where avoidable, to prevent the increase of antibiotic resistance level.

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Date of submission: 5th October, 2010