Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 14, No. (1) : 2012 : 13-18

INCIDENCE AND ANTIBIOTIC SENSITIVITY OF *BACILLUS CEREUS* ISOLATED FROM READY TO EAT FOODS SOLD IN SOME MARKETS IN PORTHARCOURT, RIVERS STATE, NIGERIA

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(Received 20 August 2011; Accepted 30 September 2011)

Key words : *Antibiotic sensitivity, Bacillus cereus isolates, Microbiological standards, Ready-to-eat indigenous*

Abstract - Sixty-four food samples of "cooked rice", "masa", "agidi" and "epiti" purchased from local markets in Obio-Akpor Local Government Area of Port Harcourt, Nigeria were examined microbiologically. Plate count analysis on mannitol egg-yolk bromothymol blue polymyxin B (MYP) agar revealed that "cooked rice" had the highest frequency of occurrence of *Bacillus cereus* isolates (29.51%), "masa" (26.23%), "agidi" (22.95%) and "epiti" had the least frequency of 21.31%. All *Bacillus cereus* isolates tested were found to be susceptible to rifampin (30µg), chloramphenicol (20µg), erythromycin (30µg), ciprofloxacin (10µg), streptomycin (30µg), gentamycin (10µg) and lincocin (30µg) and 100% resistance against norfloxapin (30ug), floxapen (30µg) and ampiclox (30µg). The study unveils the presence of *Bacillus cereus* in food samples sold in Port Harcourt especially in 'cooked rice" and "masa" which may pose serious threat to the health of consumers and should not be ignored.

INTRODUCTION

Present economic conditions have resulted in situations, where food vending has become increasingly important in most countries; contributing significant income inflow for households involved in selling these foods (Mosupye and vonholy, 1999; FAO/WHO, 2003; FAO/WHO, 2005). Hygiene and sanitation practices obtained during preparation and marketing of these foods provide ample opportunities for the proliferation of these food with food-borne pathogens (Desai and Varadaraj, 2009). Among the food- borne pathogens, strains of *Bacillus cereus* are of significance because of its ubiquitous nature and ability to occur in a wide range of foods (Ehling-schulz, *et al*., 2004a; Oguntoyinbo and Oni, 2004; Reyes *et al*., 2007).

The unique properties of *Bacillus cereus* includes heat resistant endospore forming ability, toxin production in varieties of foods and the psychro-trophic nature makes the organism a prime cause of public health hazard (Griffith and Schraft, 2002; Dierick *et al*., 2005). *Bacilli cause* symptoms leading to illness, relatively mild lasting up to 24h, but could be severe leading to hospitalisation and in some cases death. The organism is found in a wide range of habitat including air, water, dust and soil, from where spores are introduced into cereal crops, vegetables, animal hair, fresh water and sediments. Raw plant foods especially rice, potatoes, beans, peas, and spices are the most common sources of *Bacillus cereus* (Eglezos *et al*., 2010). The occurrence of the organism within the environment enables it to enter the food chain through raw materials and is a major problem in convenience foods and mass catering (Beathe and William, 2000; Jensen *et al*., 2005; Guinebretiere *et al*., 2006). Because 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 microbes potential for food borne disease outbreak, its distribution cannot be controlled, changes in

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public health and deterioration and increases susceptibility rate (Sahota *et al*., 2008).

In several studies that have been conducted on the incidence of food-borne pathogens, emphasis had been on *Bacillus cereus* (Yusuf *et al.,* 1992; Umoh and Odoba, 1999; Umar *et al*., 2006; Desai and Varadaraj, 2009). 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. Some of the food samples have been tested for various microorga-nisms of public health concern and *Bacillus cereus* was among them (Tomlins *et al.*, 2004).

This study was carried out to evaluate the presence of *Bacillus cereus* in ready- to -foods purchased from markets in Port Harcourt and characterize the organism isolated in terms of biochemical reaction and antibiotic susceptibility.

MATERIALS AND METHODS

Sampling site and collection

A total of sixty four food samples being sold in various sales points in urban markets at Mile I, Mile III, Oil mill, and Rumuokoro areas of Port Harcourt were enumerated for the viable counts of *Bacillus cereus*. The food samples comprised of Sixteen samples each of "cooked rice", "masa", "agidi" and "epiti"(cereal preparation) were collected from the food vendors in sterile disposable polyethylene bags brought to laboratory for microbiological analysis. Analysis of samples were performed within 60mins of collection

Table 1. Ingredients of the food samples analysed

Samples	Ingredients
Cooked rice	Rice, meat, groundnut oil, Seasonings(salt, onions, pepper, tomato paste)
Masa	Rice and raw milk
Agidi	Powdered corn grain & seasonings
Epiti	Powdered corn grain, plantain flour, palmoil, seasonings.

Enumeration of *Bacillus cereus*

A homogeneous sample was prepared with 10g of food sample, aseptically transferred into 90mL of tryptone broth to give an initial 1:10 dilution. Aliquots of 0.1mL from 10-5dilutions were surface plated on sterile dried Mannitol egg-yolk bromothymol blue polymyxin B (MYP) agar plates. The plates were incubated at 37C for 24h examined, then left for another 24h at room temperature and re-examined. Sixteen presumptive colonies of *Bacillus cereus* were randomly selected and counted based on characteristic colony feature. The colonies were purified on freshly prepared MYP agar plates. After incubation, typical colonies of bluish green - blue colonies with zones of egg yolk precipitate on the medium were picked, streaked out to obtain pure cultures and finally maintained on nutrient agar slopes in the refrigerator (4C). The presumptive isolates were identified by morphological characteristics. The morphological test includes appearance of cell, shape and pigmentation. Further identification of the organisms using biochemical characteristics are Gram's reaction, position of spores, motility, citrate utilisation, oxidase, indoleproduction, urease, voges-proskauer reaction, hydrolysis of starch, nitrate reduction, gelatine hydrolysis, production of gasandacid from glucose, sucrose, mannitol and lactose (Cappuccino and Sherman, 2004).

Antibiotic sensitivity test

All *Bacillus cereus* isolates were tested for their sensitivity to antibiotics by means of a disc diffusion method (Bauer *et al.,* 1966). The organisms were investigated using antibiotics disc containing rifampin (10μg), chloramphenicol (20μg), erythromycin (30μg), ciprofloxacin (10μg), streptomycin (30μg), gentamycin (10μg)), floxapen (30μg), ampiclox (30μg), norfloxapin (30ug),and lincocin (30μg). The antibiotics disc were spaciously placed on Mueller - Hinton agar plates previously seeded with 6h-broth cultures of Bacillus cereus isolates. The plates were incubated at 37^{o}C for18-24h. The different zones of inhibition were measured to the nearest millimetre and interpreted as sensitive, moderate sensitive and resistant based on the interpretation table recommended by the disc manufacturer (Oxoid, 1998).

RESULTS

The results of *Bacillus cereus* in some cereal based ready to eat foods sold in Port Harcourt is depicted in Fig. 1 and Table 2. Among the various markets sampled, Oil mill market had the highest microbial load of the organism, followed by rumuokoro, mile 3 and finally mile 1 market. A total of sixty-four food samples "cooked rice", "masa", "agidi" and "epiti" were assessed. The average count from the food samples showed that about 7.2 x 105cfu/mL were obtained from cooked rice, 6.4 x105cfu/mL from masa, 5.6 x 105cfu/mL from agidi and 5.3 x 105cfu/mL from epiti. Sixteen presumptive colonies of *Bacillus cereus* isolated revealed an irregular edge, large, flat, rough bluish green to blue colonies with zones of egg yolk precipitate. Antibiotic sensitivity test of the isolates determined by the disc diffusion method in accordance with the instructions of the antibiotics disc manufacturer are shown in Table 3. The isolates were highly resistant to ampiclox, norfloxacin & floxapen, sensitive to erythromycin, chloramphenicol, streptomycin rifampin and ciprofloxacin and less sensitive to lincocin and gentamycin.

Table 2. Cumulative plate count and total percentage from various food samples

S/No	Sample tested	No. of samples	Total counts (x106cfu/g)	% total
1	CR	16	72	29.51
2	MS	16	64	26.23
3	AG	16	56	22.95
4	EP	16	52	21.31
	Total	64	244	100

Keys: CR = cooked rice, MS = masa,
AG = agidi; EP = epiti

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. The isolation of Bacillus cereus from all the food samples could be explained by the ubiquitous distribution of this organism and its ability to form endospores (Kotiranta *et al.*, 2000; McKillip, 2000). A total of 64 samples (cooked rice, masa, agidi and epiti) were analysed. From the results obtained in the study, there were high contamination levels of *Bacillus cereus* found in cooked rice (7.2×105cfu/g) this is not surprising because the organism is a normal flora of rice followed by Masa (6.4×105cfu/g), a cereal preparation from rice which harbour the organism naturally with total percentage count of 26.23%. Masa was followed by agidi with 5.6 × 105 cfu/g and a total percentage count of 22.95% and lastly epiti (5.3×105cfu/g) with total percentage count of 21.31%. The variations in the value of bacterial count percentages may be attributed to numerous contaminations potential of the organism, as most of the food samples were prepared in places with poor sanitation practices, poor storage and transport conditions. In most cases, food vendors do not have adequate bathing facilities, atimes starts their day without taking a bath, therefore foods and ingredients are exposed to repeated contamination from unwashed hands and materials used for wrapping such as leaves, newspaper and reusable polyethylene bags. Purchasing ready-to-eat foods from markets exposes one to a considerable risk of public health due to poor hygiene practices (Mosupye and Vonholy, 1999; Ehirim *et al.*, 2001; FAO/WHO, 2003; FAO/WHO, 2005). Agidi and epiti which had the lowest percentage of the isolates could be attributed to the method of processing the corn grain and plantain (drying on moderately clean floor and machine milling) (AOAC, 1995). The limited time & temperature exposure during agidi preparation is equally insufficient to destroy *Bacillus cereus* spores. *Bacillus cereus* food poisoning is principally associated with temperature abuse during the storage of food. Temperature abuse can result in spore germination and multiplication of the vegetative cells leading to hazardous levels of vegetative cells or toxins in the food at the time of consumption (Granum, 2001). The extreme resistance of the spores of this pathogen to heat, desiccation, sanitizers and irradiation contributes to their distribution, survival and persistence within the entire food chain from raw agricultural commodities to finished retailed products (Eglezos *et al.*, 2010)

The results of the antibiotic susceptibility of the isolates are shown in Table 2. The antibiotic susceptibility was determined by the disc agar diffusion method in accordance with the instructions of the antibiotic disc manufacture (Oxoid). All the *Bacillus cereus* isolates were susceptible to Streptomycin (100%) Chloramphenicol (100%), Rifampin (100%), Erythromycin (100%) and Ciprofloxacin (100%) Gentamycin (100%) and less sensitive to Lincocin (82%).

The findings of the present study correspond

Table 3. Antimicrobial sensitivities of *Bacillus cereus* isolates from food sample

Antibiotics (conc/µg)	Food samples			
	Zones of inhibition in diameter mm status			
	CR	AG	EP	MS
Rifampin (10µg)	32(S)	38(S)	32(S)	29(S)
Erythromycin (30µg)	28(S)	23(R)	26(S)	32(S)
Ampiclox (30µg)	-	-	-	-
Chloramphenicol (20µg)	40(S)	31(S)	31(S)	30(S)
Norfloxacin (30µg)	-	-	-	-
Streptomycin (30µg)	34(S)	40(S)	34(S)	26(S)
Gentamycin (10µg)	20(MS)	30(S)	24(S)	28(S)
Lincocin (30µg)	20(MS)	28(S)	30(S)	20(MS)
Floxapen (30µg)	-	-	-	-
Ciprofloxacin (10µg)	27(S)	23(S)	30(S)	27(S)

Bauer *et al.*, (1966)

Keys: CR = Cooked rice ; AG = Agidi ; S = Sensitive ; EP = Epiti ; R = Resistant;
MS = Masa ; MS = Moderately Sensitive

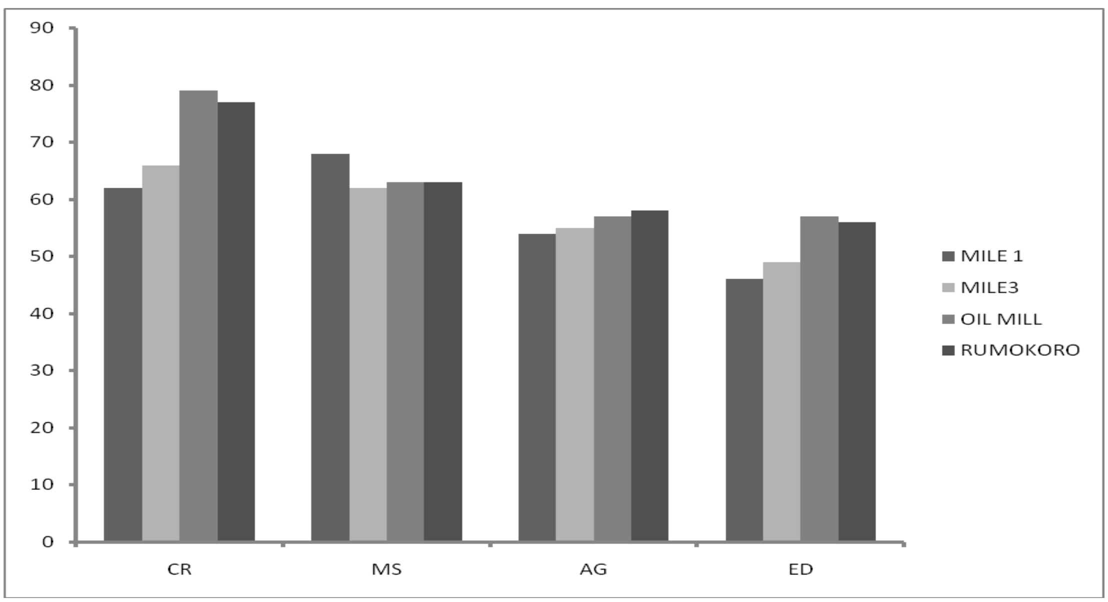

Fig. 1 Distribution of the food samples within the markets analyzed

with those obtained by other researchers (Umar *et al*., 2006; Whong and Kwaga, 2007). Previous works have shown that antimicrobial susceptibility of *Bacillus cereus* were highly susceptible to streptomycin, chloramphenicol, erythromycin, ciprofloxacin, and less susceptible to ampicillin, ampiclox, cotrimazole, cloxacillin (Umar, *et al.*, 2006). 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.The antimicrobial pattern of resistance of Bacillus cereus from foods is useful in epidemiological studies but its effectiveness decreases due to the negligence in utilisation policy

(Whong and Kwaga, 2007).

The following strategies should be undertaken to control this pathogen: ensuring adequate temperature (75°C) is reached during cooking of food, hot foods should be kept hot (maintained at a temperature greater or equal to 63°C) and cold foods should be kept cold (maintained at temperature less than or equal to 5°C) to prevent multiplication and toxin production. Training to help food vendors to comply with regulations and implement safe food handling practice; improve in the equipments used to prepare and serve foods and education campaign to increase consumer's awareness about nutrition and safety of foods.

CONCLUSION

Food samples sold in markets are operating in less than an acceptable and satisfactory environment; efforts should be made to improve the safety of street-vended foods. Illness associated with these food products may be under reported, overlooked and ignored, a few of the affected seek medical attention owing to the mild nature and short duration of symptoms. This must be taken seriously as might lead to the release of toxins into the body causing severe damage to the internal organs and can eventually lead to death. Based on the result, the effective antibiotic against *Bacillus cereus* isolates are streptomycin, chloramphenicol, rifampin, erythromycin, cipfloxacin and gentamycin. Food-borne problems pose a serious threat to human health, the economy of individuals, families and nations. To ensure the safety of food, Hazard Analysis and Critical Control Point (HACCP) must be applied from the primary producer to final consumption and its implementation. Their control requires effort on the part of the Government, food industry (vendors) and consumers to meet the challenges of the future.

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