Short Communication

Antibiograms of *Bacillus cereus* isolates from some Nigerian Foods

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ABSTRACT

A total of 300 isolates of *B cereus* from nine of some Nigeria food were tested for their sensitivity to ten commonly used antibiotics. An average of 25 isolates were obtained per food sample. All *B. cereus* isolates tested were found to be susceptible to ciprofloxacin (5 mg), chloramphenicol (30 mg), ofloxacin (10 mg) and less than 10% were resistance to gentamicin (10 mg) and nalidixic acid (10 mg). Overall resistance to penicillin G (82%), cefotaxime (56.7%), ceftriaxone (53.3%) and ampicillin (44%) were most frequent, whereas isolates were least resistance to tetracycline (6.7%) nalidixic acid (3%) and gentamicin (1%). The predominant antimicrobial resistance patterns irrespective of food source were cefotaxime, penicillin, ampicillin and ceftriaxone (23%), penicillin (16.3) and cefotaxime, penicillin and ceftriaxone (10.3%).

Keywords: *Bacillus cereus* isolates, antimicrobial agents, Nigerian foods.

INTRODUCTION

Bacillus cereus is a gram-positive, spore-forming, facultatively anaerobic bacterium. Differentiation of *B. cereus* from its closely related organisms depends upon the absence of toxin crystal (from *B. thuringiensis*), hemolysis activity (from *B. anthracis*), and nonrhizoid growth and motility from *B. mycoides* (Yamada *et al*., 1991). *B.cereus* causes two principal types of food poisoning, emetic and diarrhoeal syndromes. The emetic type is effected by a small cyclic heat-stable peptide, which cause vomiting a few hours after ingestion and is most frequently associated with farinaceous foods such as fried and boiled rice dishes, macaroni and pasta based foods (Lund, 1990; Drobniewski, 1993).

Diarrhoeal types are attributed to enterotoxins, a group of heat-labile proteins causing abdominal pain and diarrhoea after incubation for 8 to 16 hours and vegetative growth of the bacteria in the intestine (Granum, 2001). Diarrhoeal syndrome is most commonly associated with proteinaceous foods such as meat-based dishes, vegetables and salad (Lund, 1990; Brooks *et al*; 1995). B. *cereus* is a soil bacterium and has been isolated from cooked and uncooked rice, meat, vegetables, spices "kunun zaki", milk and dairy products and infant formulae (Kim *et* al., 2000; Whong *et al*., 2006). This paper reports the antibiotic resistance patterns of *B. cereus* isolated from various Nigerian foods to ten antimicrobial agents.

MATERIALS AND METHODS

Sources of Bacillus Isolates

A total of three hundred (300) previously identified *B. cereus* isolates from fermented milk (Nono), sweetened cereal beverage (kunun zaki), cooked and uncooked rice, roasted beef (suya), spinach (*Amaranthus hybridus*), ginger (*Zingiber officinale*), garlic (*Allium sativum*), and pepper (*Capsicum annuum*) were purchased from retail outlet in Shika, Samaru and Sabon gari markets, Zaria (Whong *et al*., 2006).

Isolation and Enumeration of *Bacillus cereus*

A 0.1ml aliquots of dilution 1:10; 1:100 and 1:1000 were surface plated in duplicates on *Bacillus cereus* selective Agar.(oxoid, cm617) The plates were incubated at 30^{0}C for 24 h. Following incubation, the plates were examined, left at room temperature for another day and re-examined. Presumptive *Bacillus cereus* colonies were crenated, about 5 mm in diameter and had a distinctive rough turquoise to peacock blue colour surrounded by egg yolk precipitate of the same colour were counted. The total number of colony forming unit (cfu) per ml or g of specimen of presumptive *B. cereus* was determined. Representative colonies of *B. cereus* with lecithinase activity were gram-stained. The catalase and gram-positive rods purified on nutrient agar plates were stored on nutrient agar slants until they were required for biochemical tests.

Characterisation of *Bacillus cereus*

Characterisation of *B. cereus* isolates were based on motility test, hemolysis on sheep blood cells, methyl red reaction, Voges-Proskauer reaction, gelatin hydrolysis, starch hydrolysis, citrate utilization, hemolysin *Bl*-production and acid production from mannitol, arabinose, glucose, mannose, xylose and salicin. These tests were carried out as described by Cowan and Steel (1974); Harmon (1978); Beecher and Wong (1994).

Antibiotic susceptibility testing

The antibiotic discs (oxoid) and concentrations tested were as follows: ofloxacin (10 mg), cefotaxime (30 mg), penicillin G (10 units), ampicillin (10 mg), gentamicin (10 mg), tetracycline (30 mg), nalidixic acid (30 mg), ceftriaxone (30 mg), ciprofloxacin (5 mg) and chloramphenicol (30 mg).

Susceptibility testing was conducted by the disc diffusion method of Bauer *et al*. (1966). *Bacillus cereus* ATCC 14579 and *Staphylococcus aureus* ATCC 17079 were provided by Dr. M.M. Lecadet, Louis Pasteur's Institute, France and Professor P.F. Olurinola, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, served as positive and negative controls, respectively, for each test run. The antibiotic disc were spaciously placed on inoculated Mueller-Hinton agar plates. The plates were inverted and incubated aerobically at 37^{0}C for 16 – 18 h. Zones of inhibition were measured to the nearest millimeter and interpreted as sensitive, intermediate and resistant based on the interpretation table recommended by the disc manufacture (oxoid, 1998).

RESULTS AND DISCUSSION

All the *Bacillus cereus* isolates were susceptible to ciprofloxacin, chloramphenicol, ofloxacin and less than 10% were resistant to gentamicin and nalidixic acid. No isolates was simultaneously resistant to all ten antimicrobial agents (Table 1). Majority of *B. cereus* isolates were resistant to penicillin (80.0%), cefotaxime (56.7%), cefriaxone (53.3%) and ampicilin 44.0%. There were twenty-two patterns of resistance (Table 2). Our studies indicate that *B. cereus* isolates

were highly susceptible to of loxacin (100%), ciprofloxacin (100%), chloraphenicol (100%), gentamicin (99.0%), nalidixic acid (97.0%) and tetracycline (93.3%). The *B. cereus* isolates were less sensitive to cefotaxine (56.7%), penicillin G (82.0%) and ceftriaxone (53.3%). The findings of the present study regarding susceptibilities to these agents are similar to those obtained in other countries (Schiemann, 1978; Chung and Sun, 1986; Wong *et al.*, 1988; Drobniewski, 1993). Previous work has shown that the antimicrobial susceptibility of *B. cereus* isolates were highly susceptible to chloramphenicol, erythromycin, streptomycin and tetracycline and less sensitive to co-trimoxazole and ampicilin.(Umoh *et al;* 1995) The variations in the values of percentage susceptibilities in this study may be attributed to the differences in the concentration of antimicrobial agents used, differences in the sources of isolates, drug resistance transfer and the wide spread use of the antibiotics in the environment. The development of drug resistance is multifactorial. The high percentage of isolates resistant to cefotaxime, ceftriaxone, penicillin G and B-lactam antimicrobial agents may be due to the frequent use of these agents in sublethal doses in medical and veterinary practices to prevent or treat infections. This could be a reflection of use and misuse of antibiotics in the society. Such practices could lead to development of drug resistant strains. The antimicrobial resistance patterns of *B, cereus* from foods is useful in epidemiological studies (Kwaga and Adesiyun,1984). There was a higher proportion of single and multiple resistance patterns among the *B. cereus* isolates obtained from foods and spices. The multiple resistance patterns that were observed may provide a picture that these antimicrobial agents are highly abused or used at sublethal dose in this environment and also due to the differences in the sources of the isolates. The effectiveness of any antibiotics decreases in the absence of a rational utilization policy, which unfortunately is virtually non-existent in Nigeria (Bulger and Sherris, 1968).

CONCLUSION

Based on the results of the *in vitro* testing, the most effective antimicrobial agents against *B. cereus* isolates were notably ciprofloxacin, ofloxacin (tarivid), chloramphenicol, gentamicin and nalidixic acid.

Table 1: Antimicrobial susceptibility tests of *B. cereus* isolated from different foods and spices.

Antibiotic (potency in ug)	Number (%) of *B, cereus* resistant to selected antimicrobial agents from different foods and spices.									
(%) of	**FM**	**SCB (n* = 43)**	**CR (n = 25)**	**UR (n = 47)**	**RB (n = 45)**	**SN (n = 46)**	**GG (n = 48)**	**GL (n = 14)**	**PP (n = 20)**	**Total no (n = 12) resistant isolates (n=300)**
Ofloxacin (10 ug)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Cefotaxime (30ug	27(62.8)	9(36)	29(61.7)	22(48.9)	34(73.9)	28(58.3)	9(64.3)	8(40.0)	4(33.3)	170(56.7)
)Penicillin G (10 units)	35(81.4)	20(80)	46(97.9)	42(93.3)	34(73.9)	35(72.9)	7(50.0)	17(85)	10(83.3)	24(82.0)
Ampicillin (10ug)	13(30.2)	12(48)	36(76.6)	27(60.0)	12(26.1)	9(18.8)	5(35.7)	11(55)	7(58.3)	132(44.0)
Gentamicin (10ug)	0(0.0)	0(0.0)	3(6.4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(1.0)
Tetracycine (30 ug)	1(2.3	1(8)	3(6.4)	2(4.4)	5(10.9)	3(6.3)	1(7.1)	0(0.0)	3(25.0)	20(6.7)
Nalidixic acid (30ug)	0(0.0)	1(4)	0(0.0)	3(6.7)	0(0.0)	3(6.3)	1(7.1)	0(0.0)	1(8.3)	9(3.0)
Ceftriaxone (30 ug)	23(53.5)	12(48)	33(70.2)	28(62.2)	24(52.2)	18(37.5)	4(28.6)	10(50)	8(66.7)	160(53.3)
Ciprofloxacin (5ug)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Chloramphenicol (30ug)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

**n = Number of isolates tested. FM = Fermented milk, SCB = sweetened cereal beverage, CR = cooked rice, UR = uncooked rice, RB = Roasted beef,*
SN = Spinach, GG = Ginger, GL = Garlic, PP = Pepper.

Table 2: Patterns of antimicrobial resistance of 300 isolates of *B. cereus* from various foods and spices to ten selected antimicrobial agents.

Resistace pattern to antimicrobial agents	Number of isolates (%)
Ctx, P Amp, Cro	69(23.0)
P alone	49(16.3)
Ctx, P, Cro	31(10.3)
P, Amp, Cro	29(9.7)
Ctx, P	23(7.7)
Ctx, Cro	20(6.7)
P, Amp	16(5.3)
Ctx alone	11(3.7)
Ctx, P, Amp	6(2.0)
Ctx, P, Amp Na, Cro	3(1.0)
P, Amp, Te	3(1.0)
P, Amp, G, Te	3(1.0)
P, Cro	3(1.0)
Ctx, P, Amp, Te, Cro	3(1.0)
Ctx, P, Na, Cro	2(0.7)
Ctx, P, Te, Cro	2(0.7)
Ctx, Te, Cro	1(0.3)
P, Amp, Na	1(0.3)
P, Te, Na	1(0.3)
Te alone	1(0.3)
P, Te	1(0.3)
Ctx,	1(0.3)
AmpSensitive to all antimicrobial agents tested	21(7.0)

Ctx = Cefotaxime, P = Penicillin G, Amp = Ampicillin, Cro = Ceftriaxone, Na = Nalidixic acid, Te = Tetracycline, G = Gentamicin

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