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Prevalence of Multidrug Resistant *Bacillus cereus* in Foods and Human Stool Samples in and Around Pantnagar, Uttrakhand

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Abstract

.A total number of 63 samples out of 505 samples were found to be positive for *Bacillus cereus*. The positive samples comprised of 29 samples of human stool, 10 of milk, 23 of meat and meat products origin. These positive isolates were tested against 13 different commonly used antibiotics by the disc diffusion method. Antibiogram pattern of these 63 isolates indicated multidrug resistance. *B. cereus* isolates showed a high rate of resistance to Carbenicillin, Kanamycin and Ampicillin and intermediate frequency of resistance to Amoxicillin and Cephalothin. All the isolates irrespective of their source were resistant to Bacitracin and Penicillin G and sensitive to Aminoglycosides (Gentamycin, Neomycin and Streptomycin) and Chloramphenicol. However, variable resistance patterns were also recorded in *B. cereus* isolates obtained from different origin, which is suggestive of source-associated resistance. It can be inferred from the above study that presence of multidrug resistant *B. cereus* in animal-origin food and environment in such high proportion is of public health significance.

Keywords: Bacillus cereus; meat; stool: antibiotics; resistance; sensitive

Introduction

Food-borne diseases, the major cause of morbidity and mortality are reported to be a serious threat to public health all over the world. Among the organisms responsible for causing foodborne diseases, *Bacillus cereus* has emerged as major foodborne pathogen during the last few decades and is often present in a variety of foods, such as starchy foods (rice), animal origin foods (meat, milk and dairy products) and others viz; vegetables, spices, cake desserts etc (Jay, 2005). In India, occurrence of *B. cereus* has been reported from foods like milk (Garg *et al*. 1977; Chopra *et al*. 1980), meat (Bacchil and Negi, 1984; Bacchil and Jaiswal, 1988) and various other foods (Kamat *et al*. 1989; Meena *et al*. 2000).

Now-a-days, antimicrobial-resistant organisms are a major public health challenge requiring the participation of the entire medical community and public health agencies. *B. cereus* produces a broad-spectrum β-lactamase and it is one of the potential virulence factors that makes the strains resistant to Penicillin, Ampicillin, and even to the 3rd generation of Cephalosporins (Cormican *et al.* 1998). Increasing occurrence of *B. cereus* isolates with multiple drug resistance may pose a significant public health hazard. Reports of resistance in *B. cereus* to Erythromycin and Tetracyclines from the United States and Europe indicate the development of further resistance. Widespread antibiotic usage exerts a selective pressure that acts as a driving force in the development of antibiotic resistance indicating the need to consider the potential transmission of antibiotic-resistant bacteria to humans from the food chain (Faria-Reyes *et al.* 2001).

Considering aforesaid aspect, the objectives of this study were to determine the prevalence of *B. cereus* in various sources in and around Pantnagar along with their antimicrobial agent susceptibility and resistance pattern.

Materials and methods

A total of 505 (n) samples which comprising of 311 human stool, 100 raw milk and 94 meat and meat products samples were collected from different residences, instructional dairy farm and meat shops, respectively, in and around Pantnagar, Uttarakhand. All the samples were examined for the presence of *B. cereus* (Table 1). Meat samples were processed

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for isolation and identification of *B. cereus* as per the methodology of Rhodehamel and Harmon (1998), where as stool and milk samples were processed as per the methodology of Shinagawa (1990) with slight modifications. Each of the collected stool samples was aseptically transferred into 10 ml of Brain heart Infusion broth (BHI) supplemented with polymyxin B and incubated at 35^0C for 24 h. After incubation, a loop full inoculated BHI broth was streaked over polymyxin pyruvate egg yolk mannitol bromothymol blue agar (PEMBA), while milk and meat samples were grown over Mannitol egg yolk polymyxin agar (MYP) and the plates were incubated at 35^0C for 24 h. The typical crenate to fimbriate peacock blue colored colonies (3 to 5 mm) surrounded by blue zone of egg yolk hydrolysis on PEMBA and typical eosin pink colonies, surrounded by zone of egg yolk hydrolysis on MYP were presumptively identified to be *B. cereus*. The suspected colonies were picked up on nutrient agar and identified as *B. cereus*, following the method of Shinagawa (1990). Drug susceptibility pattern of the isolates were determined by the disc diffusion methodology of the Bauer *et al*. (1966) using various antibiotic discs.

Results

Analysis of all 505 samples of human stool, raw milk and meat and meat products origin revealed the presence of *B. cereus* in 63 samples (12.47%). Twenty three isolates were recovered from 311 human stool samples (7.4%), which showed typical crenate to fimbriate peacock blue colored colonies surrounded by blue zone of egg yolk hydrolysis on PEMBA. Eleven out of 100 raw milk (11%) and 29 of 94 meat origin samples (30.85%) produced typical pink colonies on MYP (Table 1).

All the 63 isolates were tested against thirteen different commonly used antibiotics and the result of sensitivity patterns was determined on the basis of zone of inhibition (as per the interpretative chart) as summarized in Table 2. All the isolates were resistant to Bacitracin and Penicillin G, but susceptible to Streptomycin except 3 isolates of meat origin that were found resistant to Streptomycin. Out of 63 isolates of *B. cereus* 41, 52 and 41 isolates displayed resistance against Amoxicillin, Ampicillin, and Cephalothin, respectively. Only 5 of the meat and 1 of the milk origin isolates were sensitive to Carbenicillin, whereas the rest of the isolates were resistant to this antibiotic. Chloramphenicol displayed susceptibility for all isolates except 3 of meat origin. All isolates except 7 of meat and 1 of milk origin showed intermediate sensitivity pattern to Kanamycin. *B. cereus* isolates showed variable degree of susceptibility to Gentamycin (20, 17 and 7 from human stool, meat and milk, respectively), Neomycin (18, 22 and 7 from human stool, meat and milk, respectively) and Nalidixic acid (12, 12 and 8 from human stool, meat and milk, respectively). For Erythromycin, 3 of human stool sample isolates were susceptible and 14 and 6 isolates from meat and milk were resistant to this antibiotic.

Table1. Prevalence of *B. cereus* in the samples collected from various sources

Samples	No. of samples examined	Samples positive for *B. cereus*	
		No.	Percent
Human stool	311	23	7.4
Meat and meat products	94	29	30.85
Raw milk	100	11	11
Total	505	63	12.45

Discussion

On qualitative analysis of 311 stool samples of apparently healthy individuals, 23 samples (7.4%) were confirmed for presence of *B. cereus*. Result of this study is in agreement with Hassan and Nabbut (1995), who also reported 7% *B. cereus* in normal stool samples while, Ghosh (1978) found 14% prevalence of *B. cereus* in stool sample in two studies conducted on British population. Turnball and Kramer (1985), however, reported a higher prevalence of 25 to 40% in normal stool specimen harboring *B. cereus*, whereas, Volkava (1971) reported relatively very less prevalence (0.35%) in stool samples. This variation might be because of type of diet taken by individuals. The higher isolation rate might be expected in that group whose diet is mainly rice and other cereals (Kramer and Gilbert, 1989). Another factor for the variation might be the stage of the stool sample collection after taking B. cereus laden diet, as according to the Ghosh, (1978) excretion of *B. cereus* probably lasted <2 weeks in healthy individuals.

Out of 94 meat and meat product samples analyzed, 29 (30.85%) were found to contain for B. cereus. This finding is in agreement with Schlegelova *et al*. (2003) who reported 28% of

meat samples contaminated with *B. cereus*. While, Kamat *et al.* (1989) however, found 80% of chicken and meat products contaminated with *B. cereus*. While, Kamat *et al*. (1989) however, found 80% of chicken and meat products contaminated with *B. cereus*. The results of analysis revealed that only 11% of milk samples (n=100) were positive for *B. cereus*, which indicate the hygiene level of the animal and dairy farm from where samples were collected. Similar findings were also reported by previous workers (Ahmad *et al.,* 1983; Ziemann and Schutz, 1992 and Odumerer *et al*., 1997) who reported 7 to 10% of incidence in raw milk, respectively. A higher incidence of 37% of *B. cereus* in raw milk was also reported by Martin *et al.* (1962). So far as the sensitivity of the *B. cereus* isolates against various antibiotics is concerned, variations in sensitivity attributes against 13 tested antimicrobial agents were observed in this study. *B. cereus* showed a high rate of resistance to Carbenicillin, Kanamycin and Ampicillin; and intermediate frequency of resistance to Amoxicillin and Cephalothin but all of them were uniformly resistant to Bacitracin and Polymyxin G. These findings were broadly in agreement with the work of Meena *et al.* 2000; Schlegelova *et al*. 2003 and Anamika *et al*. 2004.

In the present study, *B. cereus* was found to be susceptible to Aminoglycosides (Gentamycin, Neomycin and Streptomycin) and Chloramphenicol. Similarly, Luna *et al.* (2007) observed that *B. cereus* was susceptible to Aminoglycosides, Chloramphenicol, Clindamycin, Erythromycin, Tetracycline and Vancomycin. Variable resistance pattern was recorded in *B. cereus* isolates obtained from human stool (30.4%) and meat and ready-to-eat meat products (48.3%) samples against Nalidixic acid, whereas, none of the isolates from milk was found resistant to this antibiotic. Similarly, in case of erythromycin, 73.91% isolates from human stool were susceptible, while 48.3 and 54.5% from meat and ready-to-eat meat products samples were resistant to erythromycin. In agree-

Table 2. Antibiotic sensitivity/ resistance pattern of *B. cereus* isolates

Antibiotics	Efficacy of antibiotics	*B. cereus* isolates originated from		
		Human stool (%)	Meat (%)	Milk (%)
Amoxicillin	R*	17 (73.9)	16 (55.2)	8 (72.8)
	I*	1 (4.3)	0 (0)	1 (9.1)
	S*	5 (21.7)	13 (44.8)	2 (18.2)
Ampicillin	R	19 (82.6)	24 (82.8)	9 (81.8)
	I	0 (0)	0 (0)	1 (9.1)
	S	4 (17.4)	5 (17.2)	1 (9.1)
Bacitracin and polymyxin G	R	23 (100)	29 (100)	11 (100)
	I	0 (0)	0 (0)	0 (0)
	S	0 (0)	0 (0)	0 (0)
Carbenicillin	R	23 (100)	22 (75.9)	10 (90.9)
	I	0 (0)	2 (6.9))	0 (0)
	S	0 (0)	5 (17.2)	1 (9.1)
Cephalothin	R	14 (60.9)	19 (65.5)	8 (72.8)
	I	4 (17.4)	4 (13.8)	0 (0)
	S	5 (21.7)	6 (20.7)	3 (27.3)
Chloramphenicol	R	0 (0)	3 (10.3)	0 (0)
	I	4 (17.4)	8 (27.6)	0 (0)
	S	19 (82.6)	18 (62.1)	11 (100)
Erythromycin	R	6 (26.1)	14 (48.3)	6 (54.5)
	I	14 (60.9)	9 (31.0)	2 (18.2)
	S	3 (13.0)	4 (13.8)	3 (27.3)
Gentamycin	R	0 (0)	6 (20.7)	2 (18.2)
	I	3 (13.0)	6 (20.7)	2 (18.2)
	S	20 (86.9)	17 (58.6)	7 (63.7)
Kanamycin	R	0 (0)	0 (0)	0 (0)
	I	0 (0)	7 (24.1)	1 (9.1)
	S	23 (100)	22 (75.9)	10 (90.9)
Nalidixic acid	R	7 (30.4)	14 (48.3)	0 (0)
	I	4 (17.4)	3 (10.3)	3 (27.3)
	S	12 (52.2)	12 (41.4)	8 (72.8)
Neomycin	R	0 (0)	0 (0)	0 (0)
	I	5 (21.7)	7 (24.1)	4 (36.4)
	S	18 (78.3)	22 (75.9)	7 (63.7)
Streptomycin	R	0 (0)	0 (0)	0 (0)
	I	0 (0)	3 (10.3)	0 (0)
	S	23(100)	26 (89.7)	11 (100)

R*= Resistant, I*= intermediate, S*= Sensitive

ment to this observation, Wong *et al*. (1988) also found source-associated resistance pattern, which might have important implication in selection of therapy in cases of outbreaks.

All these findings indicate the need to consider the potential transmission of multidrug resistant bacteria to humans from the food chain, more particularly through contamination of milk, meat and meat products and via faeces to food. It is concluded that prevalence of *B. cereus* was less frequent in the transitory intestinal flora of apparently healthy population of either sex or age in Pantnagar, while moderately higher prevalence rate was observed in meat and raw milk. But the possible presence of multidrug resistant and toxigenic B. cereus in animal-origin food and environment in high proportion is of public health significance.

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