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Study the *in-vitro* Sensitivity of Most Common Biotypes of *B. cereus* towards Commonly used Antibiotics

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The present investigation describes the *in-vitro* sensitivity of most common biotypes of *B. cereus* of ready-to-eat chicken products (chicken patties and chicken rolls) in Srinagar city during two seasons *viz.* autumn and winter. A total of 120 ready-to-eat chicken products comprising of 60 chicken patties and 60 chicken rolls were tested. *Bacillus cereus* strains were isolated from 25 of chicken patties and 22 of the chicken rolls resulting in prevalence of 41.66% and 36.67%, respectively. The field isolates and the standard strains of *Bacillus cereus* had similar cultural, morphological and biochemical characteristics. The strains of *Bacillus cereus* were higly resistant to ampicillin (100.00%), amoxycillin (100.00%) and ceftrioxone (78.7%). Intermediatte resistance was noted against oxytetracycline (34.00%), nalidixic acid (34.00%) and kanamycin (29.8%). The most effective antibiotics against *B. cereus* included gentamicin (100%), enrofloxacin (100%), erythromycin (100%) followed by streptomycin (80.9%).

Key Words: *Bacillus cereus* strains, Chicken patties, Chicken rolls, Antibiogram.

Ready-to-eat (RTE) chicken products are those that are edible without any additional preparation to achieve food safety or may receive additional preparation for palatability, aesthetic, epicurean, gastronomic or culinary purposes. The expanding population of highly susceptible people such as elderly and immunocompromised individuals and the high consumption of RTE foods due to changes in lifestyle and the global trade food distribution could be the reasons for observed increase of high risk on food poisoning (WHO, 2002). RTE foods may be contaminated with different pathogenic micro-organisms like *Bacillus cereus*, *Clostridium perfringens*, *Salmonella* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus* spp., *Streptococcus* spp. etc. (Yeboah *et al.*, 2010). Among various food-borne pathogens, *Bacillus cereus*, a gram positive, spore forming, aerobic, rod shaped bacterium has been regarded as the most prevalent pathogenic species present in the food. As a soil bacterium, *B. cereus* can spread easily to many foods such as vegetables, eggs, meat and dairy products and is known to cause 25% of food-borne intoxications in humans (FDA, 2007; Larsen and Jorgeusen, 1996).

B. cereus causes two distinct types of gastrointestinal disorders in humans viz; an early "emetic syndrome" and a late "diarrheal syndrome" involving two different types of enterotoxins (Kramer and Gilbert, 1989). The emetic syndrome, a food borne intoxication is caused by preformed *B. cereus* emetic enterotoxin (BCEET) in foods. In contrast, the diarrhoeal syndrome is caused due

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to production of *B. cereus* diarrhoeal enterotoxin (BCDET) during the vegetative growth of bacteria in the foods or in the intestines following ingestion. It has a longer incubation period (12-24 hours) and is characterized by symptoms like diarrhoea, abdominal pain and rectal tenesmus, resembling closely *Clostridium perfringens* type A food poisoning (Adams and Moss, 2007).

B. cereus also produces a large array of other potentially toxic substances/metobolites including haemolysins, phospholipase-C, metalloproteases, collagenases and beta-lactamases (Turnbull *et al*., 2004). It is also emerging as a potential pathogen of serious concern in animals owing to the increasing reports of its role in diseases like, osteomyelitis, middle ear infections, abortions and mastitis (Schiefer *et al.*, 1976).

There are several indications of continuous adaptation and development of resistance by foodborne pathogenic microorganisms to antibiotics. Antibiotic resistant strains of *B. cereus* can get access into the human population through foods of animal origin and are incriminated as the possible source of drug resistance for human pathogenic agents (Shyrock, 1999). The organisms show low susceptibility to β-lactam antimicrobial agents like ampicillin, cephalothin and oxacillin (Schlegelova *et al*., 2003). An overall resistance of 82, 56.7, 53.3, 44, 6.7, 3 and 1 percent to penicillin G, cefotaxime, ceftriaxone, ampicillin, tetracycline, nalidixic acid and Gentamicin, respectively, has been reported by Whong and Kwaga (2007).

Keeping in view the importance of *B. cereus* in foodborne infections and intoxications and the increasing demand of RTE poultry products by general public, the present study was undertaken with *in-vitro* sensitivity of most common biotypes of *B. cereus* towards commonly used antibiotics

MATERIAL AND METHODS

Sampling

Bacteriological quality of 120 ready to eat (RTE) chicken products viz. chicken patties (60) and chicken rolls (60) collected from retail shops of five zones of Srinagar city was studied in two subsequent seasons viz. Autumn and Winter (Table-1) . For this purpose, six samples of each product per zone per season were collected in sterile zip lock sachets and brought to Veterinary Public Health Laboratory in ice for processing within 2-3 hours. The predominant biotypes of *B. cereus* recovered from ready to eat chicken products were subjected to *in vitro* antibiotic sensitivity by disc diffusion method (Bauer *et al*., 1966) and the results were interpreted as per the guidelines of National Committee for Clinical Laboratory Standards (2004). The antibiotics and their respective concentration used in the study are presented in (Table 2).

RESULTS

Antibiogram of *Bacillus cereus* isolates against some commonly used antibiotics

Sensitivity of all the *B. cereus* isolates against a panel of ten commonly used antibiotics was carried out by disc diffusion method (Bauer *et al*., 1966). Based on National Committee for Clinical Laboratory Standards (NCCLS, 2004) interpretive standards for gram positive and/or aerobic bacteria,

Table 1. Scheme of sample collection

Sample type	Season	East zone	West zone	North zone	South zone	Central zone	Total
		Nishat/ Shalimar	Qamarwari/ Bemina	Hazratbal/ Zakura	Rambagh/ Jawaharnagar	Khayam/ Fateh Kadal	
Chicken Patties	Autumn	6	6	6	6	6	30
	Winter	6	6	6	6	6	30
Chicken Rolls	Autumn	6	6	6	6	6	30
	Winter	6	6	6	6	6	30

Table 2. Antibiotics and their respective concentration used for antibiotic sensitivity testing of *B. cereus* isolates

S. No.	Antibiotic	Concentration (mcg)
1	Ampicillin	10
2	Amoxycillin	30
3	Ceftrioxone	30
4	Erythromycin	15
5	Enrofloxacin	10
6	Gentamycin	10
7	Kanamycin	30
8	Oxytetracycline	30
9	Nalidixic acid	15
10	Streptomycin	10

the antibiotics to which most of isolates were susceptible included gentamicin, enrofloxacin, erythromycin and streptomycin in order. On the contrary most of isolates were resistant against ampicillin, amoxycillin and ceftrioxone. Less than 50 per cent resistance was recorded for oxytetracycline (34%), nalidixic acid (34%) and kanamycin (29.8%).The results are depicted in Table 3.

DISCUSSION

The discovery of antibiotics in the 20th century marked a watershed in the treatment of infections. The ability to treat the infections in the pre-antibiotic era stimulated advances in medical

Table 3. Antibiogram of *Bacillus cereus* isolates against some commonly used antibiotics

Antibiotic	No.of isolates susceptible/ No. tested	No. of isolates with intermediate resistance/ No. tested	No.of isolates resistant/ No. tested
Gentamicin	47/47 (100)	-	-
Enrofloxacin	47/47 (100)	-	-
Erythromycin	47/47 (100)	-	-
Streptomycin	38/47 (80.9)	4/47 (8.5)	5/47 (10.6)
Oxytetracycline	28/47(59.6)	3/47 (6.4)	16/47(34)
Nalidixic acid	25/47 (53.2)	6/47(12.8)	16/47 (34)
Kanamycin	26/47 (55.3)	7/47 (14.9)	14/47 (29.8)
Ceftrioxone	4/47(8.5)	6/47(12.8)	37/47(78.7)
Ampicillin	-	-	47/47 (100)
Amoxycillin	-	-	47/47 (100)

(Fig in parenthesis indicate the percentage)

fields and enlarged the scope of medical care. However, the rapid emergence of resistance to antibiotics amongst pathogens is threatening the present and future medical advances. The infectious diarrheal diseases are responsible for considerable morbidity and mortality, with enteropathogens having developed a high level of resistance to antibiotics especially in developing countries. Therefore, it is important to carry out the regular surveillance programmes against the antibiotic resistance amongst the isolates. *B. cereus* isolates from various foods, and clinical and environmental sources, have shown high degree of susceptibility to gentamicin, ciprofloxacin, erythromycin, streptomycin, dehydro-streptomycin and enrofloxacin (Johnson, 1984; Weber *et al*., 1988 and Turnbull *et al*., 2004). In agreement to the reports of other workers, the *B. cereus* isolates from ready-to-eat chicken products, in the present investigation, were highly susceptible to gentamicin, erythromycin, enrofloxacin and streptomycin in order. Most of the isolates were, however, resistant to ampicillin (100%), amoxycillin (100%) and ceftrioxone (78.7%) which is similar to the findings of Whong and Kwaga (2007) and Luna *et al*. (2007). The isolates

were intermediately resistant to oxytetracycline (34%), kanamycin (29.8%) and nalidixic acid (34%), which is supported by the findings of Kolawole and Akinsnji (2011). However, Weber *et al*. (1988) and Wong *et al*. (1988) reported a higher susceptibility against, erythromycin and tetracycline compared to the findings of the present study. The variability in the resistance pattern of the isolates could be due to difference in the use of different antibiotics in given geographical locations. The antibiotic resistance/susceptibility pattern was almost similar among the isolates from different sources.

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