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Cultural and Biochemical Characterization of *B. cereus* Isolates and Multidrug Resistant Detection of *B. cereus* Isolates Collected from Various Chicken Shops of Market in and around Anand, Gujarat, India

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A B S T R A C T

A total of 200 chicken meat samples were collected from different chicken shops in and around Anand city. Out of 200 samples 40 were found positive cultural characteristics on selective media mannitol egg yolk polymixin agar (MYPA). All the isolates showed typical pinkish growth surrounded by lacithinase activity on mannitol egg yolk polymixin agar (MYPA). All the isolates were catalase positive and VP test positive. Majority of isolates were nitrate test positive, motile, rhizoid growth producing on nutrient agar and β-haemolytic. Few negative isolates were also detected. Some degree of variability was exhibited in sugar fermentation test. All isolates were tested against different 12 antibiotics. The most effective or sensitive antibiotics were Imipenem, Oxytetracycline, Gentamicin and Vancomycin. All isolates were resistance against Ampicillin and Penicillin-G.

Introduction

Epidemiological studies have indicated food and water main causative agents of food poisoning outbreaks. Food poisoning diseases are continuing problem of high magnitude in all the countries, both, developed and developing. In developing countries, significant proportion of death and illnesses are attributed to gastroenteritis, which are mainly foodborne (Desai and Varadraj, 2010). Foodborne diseases are a worldwide growing health problem involving a wide spectrum of illnesses caused by microbial, viral, parasitic or chemical contamination of food. Food diarrhoeal diseases can lead to serious illnesses and in some cases, leads to death. Some diseases are caused by toxins from the "disease causing" microbe, others by the human body's reactions to the microbe itself. Apart from *Clostridium botulinum, Campylobacter jejuni, Escherichia coli O157:H7, Listeria monocytogenes, Salmonella Spp., Shigella Spp.*, the most frequently isolated bacterial foodborne pathogens are the *Bacillus cereus* group

species (WHO 2007) which include: *B. cereus, B. mycoides, B. pseudomycoides, B. weihenstephanensis, B. thuringiensis, B. anthracis* (Ehling-Schulz *et al.,* 2005; Lindback and Granum, 2006).

Over the past twenty-five years *Bacillus cereus* has been recognized as one of the major food poisoning organisms and reported as the second most dangerous pathogen among *Bacillus* species affecting man and animal. Various types of foods including meat, fish, desserts, sauces, milk and boiled rice have been shown to be contaminated with*B. cereus*. The two types of food borne illnesses, the emitic and diarrhoeal, are caused bytwo distinct enterotoxins (Kamat *et al.,* 1989).The more common manifestation is a diarrhoeal illness with an incubation time of 8-16 h characterized by abdominal pain and diarrhoea. The other is an emetic illness with an incubation time of 1-5 h and characterized by nausea and vomiting. While the emetic type is usually associated with cereal type products such as rice, the diarrhoeal type is more widely associated with many foods (Lattuada and McClain, 1998). Bacillus species are ubiquitary organisms; they can readily be isolated from soil, water, dust and air. At this moment about 50 species have been described. Some Bacillus species have applications in industrial (enzyme-production) or environmental (insecticide) applications and some are pathogenic to man and/or animal (Kramer and Gilbert 1989). The most important pathogenic species belong to the *B. cereus* group which consists of *B. cereus, B. mycoides, B. thuringiensis, B. anthracis* and the recently described *B. weihenstephanensis* (Lechner *et al.,* 1998) and *B. pseudomycoides* (Nakamura 1998).

Materials and Methods

A total 200 samples of chicken meat were aseptically collected from different chicken shop located in and around Anand, Gujarat and transported to the laboratory in an icebox for further study. Each of the aseptically collected chicken meat samples were transferred into 10 ml Brain Heart Infusion broth (BHI), which is supplemented with Polymixin- B. The incubation was carried about 18-24 hours at 35ºC for *B. cereus*.

Isolation and identification

A loop full of broth culture was streaked on to mannitol egg yolk polymixin agar (MYPA) and incubated at 35-37 ºC for 24 hr. the typical eosin pink coloured colonies (3-5 mm size) on MYPA surrounded by same colored zone of egg yolk hydrolysis were presumptively identified to be *B. cereus*. All the presumptive colonies of *B. cereus* were further subjected various biochemical tests for identification of *B. cereus* species.

Biochemical characterization of *B. cereus* isolates

All the isolates of *B. cereus* were subjected for various biochemical tests like catalase test, nitrate reduction test, motility test, lecithinase activity, modified Voges-Proskauer (VP) test, haemolysis on 5% sheep blood agar and various sugar (Inositol, dulcitol, fructose, dextrose, sucrose, mannitol and salicin) fermentation test were conducted.

The presumptive *Bacillus cereus* isolates were confirmed and identified by various biochemical tests proposed by Harmon (1979), Schiemann (1978), Tewari and Singh (2015) and Guven *et al.,* (2006).

Antimicrobial resistance of *B. cereus* for various antibiotics

The antibiotic susceptibility tests were performed as per method described by Bauer *et al.,* (1966) to find out the antibiotic

resistance pattern of all *Bacillus cereus* isolates. *In vitro* antibiotic sensitivity test of the isolates was conducted by paper disc diffusion method using the discs supplied by HiMedia Laboratories Pvt. Ltd., Mumbai (India). Isolates were subjected to antimicrobial sensitivity tests against 12 antibiotics.

Antibiotic discs used to test Antimicrobial resistance of *B. cereus* were Amikacin 10 µg, Ampicillin 10 µg, Chloramphenicol 10 µg, Ciprofloxacin 5 µg, Gentamicin 10 µg, Imipenem 10 µg, Methicillin 30 µg, Streptomycin 10 µg, Oxytetracycline 30 µg, Vancomycin 30µg, Penicillin- G 10µg and Polymixin- B 50 µg.

Bacillus cereus isolates were grown in Tryptone soya broth (TSB) (HiMedia) for 12-18 hours. The grown cultures were swabbed on Muller-Hinton agar plates (HiMedia Pvt. Ltd.) with sterile cotton swabs and left for 30 minutes for prediffusion time. Then using an ethanol dipped and flamed forceps different antibiotic discs were placed on the agar surface at about two cm apart. The discs were slightly pressed with the forceps to make complete contact with the medium. The plates were incubated at 37ºC for 18-24 hours. After the incubation period, the diameter of inhibition zones was measured and compared with interpretative chart provided by the manufacturer and zones were graded as sensitive, intermediate and resistant.

Results and Discussion

Isolation on selective media

Out of 200 samples processed 40 samples were found positive cultural characteristics on selective media mannitol egg yolk polymixin agar (MYPA). All the isolates showed typical pinkish growth surrounded by lacithinase activity on selective media MYPA.

In this study, 20 per cent isolates revealed characteristic features of *B. cereus* from poultry meat which is in agreement with the study of Floristean *et al.*,(2008) reported that 22.5% of isolated were positive and 22.4% prevalence recorded by Guven *et al.,* (2006) which is nearly similar to present study. According to Tewari and Singh, (2015) 27.3% prevalence found and 30.9% high prevalence recorded by Tewari *et al.,* (2015) from meat samples.

Biochemical characterization and antimicrobial resistance pattern of isolates

The *Bacillus cereus* isolates were gram positive, large sporulated rod with unswollen sporangium and spores were centrally located.

The presumptive *Bacillus cereus* isolates were confirmed and identified by various biochemical tests viz., catalase test, Haemolysis on blood agar, Voges-Proskauers test, nitrate reduction test and sugar fermentation test. The presumptive isolates showing positive catalase test, Voges-Proskauer test, positive nitrate reduction test, positive for anaerobic utilization of sugars by *Bacillus cereus*. According to present study, all the isolates were gram's positive bacilli, catalase positive, all isolates had egg yolk reaction and VP test positive. Total 85% isolates were motility test positive, 92.50% isolates were nitrate test positive, 95% isolates were β- haemolytic. According to the work of Tewari and Singh (2015), all the isolates showed typical growth on mannitol egg yolk polymyxin agar and found positive for motility but some degree of variability was exhibited in nitrate, haemolysis and Voges-Proskauer test. In present study some degree of variability also exhibited in nitrate reduction test and motility test.

All the 40 isolates were further tested for various sugar fermentation test. Out of 40

isolates 28 (70%) isolates fermented sugar Salicin and produced acid. Fructose were utilized by 36 isolates (90%). 22 (55%) fermented dextrose and produced acid. None of isolates were utilized mannitol and dulcitol. Eighteen isolates (45%) utilized sucrose and produced acid. Only two isolates (5%) produced acid from inositol utilization. According to the study of Guven *et al.*, (2006), Floristean *et al.*, (2008) and Tewari and Singh (2015) none isolates produced acid from mannitol fermentation which is correlated with present study that zero percent isolates utilized mannitol.

Schiemann (1978) reported that 46% isolates utilized sucrose which is in agreement with the finding of present study where 45% isolates were acid producer from sugar and it contrast with 90.1% with the study of Shinagawa (1990). But 30% salicin fermented by isolates which was contrast with present study and utilized by 70% isolates. Hafeez *et al.*, (2012) also indicate the higher amount of *Bacillus cereus* isolates utilized salicin.

All 40 isolates were tested for *in- vitro* antimicrobial resistance pattern against 12 commonly used antimicrobial drugs. The resistant pattern of *B. cereus* to the various antibiotics encountered was highest towards Penicillin- G (100%) and Ampicillin (100%) followed by methicillin (55.00%). In present study sensitivity was highest for Imipenem (100%), Oxytetracycline (100%), Vancomycin (100%), Gentamicin (100%), followed by Amikacin (80.00%), Streptomycin (75.00%), Chloramphenicol (75.00%), Polymixin- B (72.50%), Ciprofloxacin (67.50%), Methicillin (42.50%).In the study of Aklilu *et al.*, (2016), Floristean *et al.*, (2008) *B. cereus* isolates showed 100% resistance towards beta lactam antibiotics (Penicillin G, Ampicillin) which was relevant in present study all isolates were resistant to ampicillin and penicillin- G. According to Tewari *et al.*, (2012) showing 82.8% and Rather *et al.*, (2012) showing 91.75% of resistance toward ampicillin. Low resistant 33.33% according to Tahmasebi *et al.*, (2014) was contrast to this present study. Floristean *et al.*, (2008) and Schiemann (1978) recorded that Oxytetracycline was 100% sensitive for *B. cereus* isolates which was similar to present study showing 100% sensitivity.

In conclusion, total 200 poultry meat samples were processed. Out of 200, 40 (20%) samples were found to be Positive for *B. cereus*, which need strict monitoring and surveillance for effective measures for hygiene and sanitary practice. All the isolates found gram positive, catalase positive, produced egg yolk reaction and positive for VP test. 95% of isolates were beta-haemolytic on 5% sheep blood agar. 92.50% isolates were nitrate test positive and 85% of isolates were motile on motility media. Maximum (90%) of isolates were utilized fructose. Salicin, Dextrose, Sucrose and Inositol were also utilized by *B. cereus* at 70%, 55%, 45% and 5% respectively. None of the isolates were utilized Mannitol and Dulcitol. The high percentage resistance (100%) of bacterial isolates to ampicillin and Penicillin- G while high sensitive (100%) towards Gentamicin, Imipenem, Vancomycin and Oxytetracyclin. It will be helpful as guidelines for clinical approach in forms of antibiotic therapy.

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