Prevalence of *Bacillus cereus* in Rabbit Meat Consumed in Burdur-Turkey, Its Enterotoxin Producing Ability and Antibiotic Susceptibility [1][2]

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**INTRODUCTION**

*Bacillus cereus* causes two food-borne syndromes 1. The first syndrome resembles staphylococcal intoxication and is characterised with vomiting, 1-5 h incubation time, and is due to an “emetic” exotoxin of unknown nature. Outbreaks depending on the consumption of rice and other starchy foods are almost exclusively of the emetic type. Food

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**Summary**

This study was undertaken to determine the existence, enterotoxin producing ability and antibiotic susceptibility of *Bacillus cereus* at rabbit meat. *B. cereus* was enumerated by the surface plating method with mannitol egg yolk polymyxin agar. The BCET-RPLA test was used for detecting of diarheal type-enterotoxin. The disk diffusion method was used for antimicrobial sensitivity test. *B. cereus* was found in 18 (36%) of 50 samples of rabbit meat, with the mean contamination level of 2.89x10³ cfu/g in positive samples. The 8 (44.4%) of the total isolates of *B. cereus* was able to produce enterotoxin. While antibiotic resistance of *B. cereus* isolates was found to be 100% penicillin, 94.4% ampicillin, 27.7% streptomycin, 22.2% gentamicin and erythromycin, no resistance was detected to chloramphenicol and vancomycin.

**Keywords:** Rabbit meat, Bacillus cereus, Enterotoxin, Antibiotic resistance

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**Burdur’dada Tüketime Sunulan Tavşan Etlerinde Bacillus cereus Varlığı, Enterotoksin Üreteceği ve Antibiyotik Duyarlılığı**

**Özet**

Bu çalışma, tavşan etlerinde *Bacillus cereus*’un varlığı, enterotoksin üretme özelliği ve antibiyotik duyarlılığının belirlenmesi amacıyla yapıldı. *B. cereus*, egg yolk polymyxin agarda yüzeye ekim yöntemiyle sayıldı. Diarheal tip enterotoksinin tespitinde BCET-RPLA testi kullanıldı. Antimikrobial duyarlılık testi için disk difüzyon metodu kullanıldı. *B. cereus* 50 tavşan eti örneğinin 18’inde (%36), ortalamda 2,89x10³ kób/g düzeyinde bulunuldu. *B. cereus* izolatlarının 8 tanesi (%44.4) enterotoksin üretme özelliğinde bulundu. *B. cereus* izolatlarının %100’unun penisilin, %94.4’nün ampişilin, %27.7’sinin streptomisin, %22.2’sinin gentamisin ve eritromisin dirençli olduğu belirlenmesine karşın, kloramfenikol ve vankomisin direnç sahnetenmıştır.

**Anahtar sözcükler:** Tavşan eti, Bacillus cereus, Enterotoksin, Antibiyotik dirençliliği
intoxications in Japan caused by B. cereus during 1982-2001 were especially the emetic types and the amount of emetic toxin in food poisoning cases due to B. cereus, received of emetic toxin in 1974-1999 in Japan ranged from 0.01 to 1.28 μg/g. The second syndrome, resembling Clostridium perfringens food poisoning, is characterised with diarrhoea, 8-16 h incubation time, and is due to a heat-labile toxin. Nearly all reported meat-borne outbreaks have been of this type. Currently four enterotoxins, able to cause the diarrheal syndrome, have been described: hemolysin BL, nonhemolysin enterotoxin and two enterotoxic proteins; enterotoxin T(bc-D-ENT) and cytotoxin K.

Serious bacterial food poisonings are usually ill-fated combinations of improper food handling and accidents. It is estimated that in the USA food poisoning cases annually cost 2-3 billion dollars and that every year there are 27,000 cases in which B. cereus is involved. Its ability to survive makes B. cereus problematic to essentially all sectors of the food industry. B. cereus has been found in about 25% of the food products sampled, including cream, pudding, meat, spices, dry potatoes, dry milk, spaghetti sauces and rice. As many as 5% of foodborne outbreaks have been connected with B. cereus in the Netherlands, England, France, and the USA. 110 outbreaks were reported in UK during the period 1971-1979. It was reported from some of the other outbreaks that B. cereus posed the greater percentage risk among the pathogens present in street foods consumed in Sao Paulo, Brazil and that B. cereus caused 104 documented food poisoning outbreaks in Taiwan and 50% of the ready-to-eat food items analysed contained B. cereus.

Rabbit production for meat is a very important livestock activity and rabbit meat is a meat type demanded by people in most mediterranean countries. And also the rabbit meat industry is highly developed in many other countries. Global rabbit meat consumption in 2004 was 1.1 million tonnes. In Turkey, rabbit breeding has been increasing recently and demand for rabbit meat in tourism places is increasing. Although many studies conducted for B. cereus were present on red and white meat, any literature has not been found in Turkey in rabbit meat. It is stated that because of slaughtering the rabbits in nonhygienic and uncontrolled-uncluttered places, the rabbit carcasses are contaminated with pathogen micro-organisms.

By this study, it was aimed to test the rabbit meats for contamination by B. cereus and to determine the diarrheal enterotoxin producing ability and the susceptibility of B. cereus isolates to antimicrobial agents.

**MATERIAL and METHODS**

In this study, 50 samples of New Zealand White Rabbits meat were obtained from different supermarkets, restaurants and butchers in Burdur and Antalya, Turkey. All samples were transported to the laboratory in a cooler.

**Microbiological Analyses**

A 10 g aliquot from each sample was aseptically weighed and diluted in 90 ml of 1/4-strength Ringer solution (Oxoid BR0052, Basingstoke, UK) and homogenised in a Colworth Stomacher Lab-Blender 400 (Seward Medical, London, UK) for at least 2 min. Tenfold dilutions prepared from the initial 1/10 dilution in 1/4-strength Ringer solution were spread plated (two plates per dilution) onto Plate Count Agar (Oxoid CM325) and incubated at 35°C for 48 h to determine the counts of mesophilic aerobic microorganism. The B. cereus was enumerated by the surface plating method with mannitol egg yolk polymyxin (Oxoid CM0929) agar, and the plates were incubated at 30°C for 24 h. Rough and bright pink coloines with zone of egg yolk precipitation were then transferred to nutrient agar. Afterwards the incubation, identification was confirmed by microscopic and biochemical characterization (Gram stain, endospore formation, lecithinase production, catalase reaction and oxidase test, lack of acid production from mannitol and indol, lack of anaerobic utilulization of glucose and ksilose, reduction of nitrate, Voges-Proskauer test, motility, and hemolysis).

**Diarrheal Toxin Production**

Culture filtrates of the isolates were prapered in a brain-heart infusion (BHI) broth (CM 225, Oxoid, Basingstoke, UK). After the incubation at 32°C for 18 h, the culture was centrifuged at 900x g for 10 min at 4°C. The BCET-RPLA test, used for detecting of B. cereus diarrheal type-enterotoxin, was carried out according to recommendations of the manufacturer (Oxoid, TD 950A, Basingstoke, UK).

**Sensitivity Test to Antimicrobial Agents**

The antimicrobial sensitivity test was performed using the disk diffusion method described by the National Committee for Clinical Laboratory Standards. Fresh cultures grown in BHI broth (CM 225, Oxoid, Basingstoke, UK) were used to make bacterial suspensions adjusted to 0.5 McFarland standard. Mueller-Hinton plates (M 105437, Merck, Germany) were seeded using swabs. Antibiotic-impregnated discs of streptomycin (10 μg, BD 231328), tetracycline (30 μg, BD 254728), ampicillin (10 μg, BD 254727), gentamicin (10 μg, BD 254726), chloramphenicol (30 μg, 231274), penicillin G (10 IU, BD 254708), vancomycin (30 μg, BD 254858) and erythromycin (15 μg, BD 254731) were placed on the seeded plates, and following 18 h of growth at 37°C, zones of inhibition were measured. The results were interpreted according to the NCCLS criteria.

**RESULTS**

A total of 50 samples of commercially available rabbit
meat were analysed for the presence of *B. cereus*. Although the incidence of *B. cereus* in different foods has been reported, this study is important that it is the first comprehensive study regarding the existence of *B. cereus* in rabbit meat consumed in Turkey.

*B. cereus* was isolated from 36% of the rabbit meat samples (Table 1) and 44% of the isolates was found to be able to produce diarrhoeal enterotoxin. Mean mesophilic aerobic microorganism and *B. cereus* count was determined as 3.6x10^3 and 2.89x10^3 cfu/g respectively (Table 2). All strains isolated were resistant to penicillin. Ampicillin was the next most common, with seventeen isolates, gentamicin and erythromycin with four isolates and tetracycline with only two isolates. All isolates were sensitive to chloramphenicol and vancomycin (Table 3).

**DISCUSSION**

The hygienic status of animals prior, during and after slaughter can be critical to the finished product quality. Meat can be contaminated during processing through contact with the skin of animals; feet and intestinal contents of the animal; floor, equipment and bleeding of the animal and subsequently be distributed via cut or raw meat intended for further processing. Mean mesophilic aerobic microorganism count determined in the present study is lower than finding of Rodriguez-Calleja et al. They found the APC of the rabbit meat ca. 5 log cfu/g at first day of storage and ca. 8 log cfu/g at seventh day of storage and reported that the average shelf life of rabbit carcasses was estimated to be 6.8 days when mean APC, psychrotrophic and pseudomonas numbers were ca. 8 log cfu/g. Discrepancy between the results may be attributed to differences in the slaughtering and storage conditions.

The presence of *B. cereus* in high counts suggests a potential risk to consumer, because of the subsequent production of toxins associated with food poisoning. Besides causing foodborne illness, *B. cereus* is also responsible for the spoilage of a variety of food products. Borge et al. stated that psychrotolerant microorganisms, like *B. cereus*, continue to be spoilage and safety problem in refrigerated foods. The present result that *B. cereus* was isolated from 36% of the rabbit meat samples is in agreement with that of Schlegelova et al. who reported that 28% of the meat products tested was contaminated with *B. cereus*. Guven et al. determined that 22.4% of the meat and meat products contained *B. cereus*. However, Abostate et al. found that the incidence of *B. cereus* in meat luncheon from Cairo was 60%. And they reported that the incidence of *B. cereus* is higher in cooked and meat products than in raw meat samples. Mean viable count of *B. cereus* in our study was determined as 2.89x10^3 cfu/g. Similar result was reported by Guven et al. from beef as 8.0x10^3. But it is conflicted with Agata et al. who found that mean viable count of *B. cereus* in meat and meat products is 2.8x10^4. Hanashiro et al. reported that presence of *B. cereus* in 12.5% of street food samples in counts above 3 log cfu/g indicates a potential risk to the consumer.
B. cereus have the capacity to grow and generate toxin at storage temperatures above 6°C \(^1\) and the numbers of enterotoxigenic B. cereus required to cause food poisoning are ≥10\(^5\)cfu/g \(^9\). The numbers of enterotoxigenic B. cereus found in the present study were lower than last reported numbers. In the present study, 44% of the isolates was found to be able to produce diarrhoeal enterotoxin. This result is similar with those reported by others. Rusul and Yaacob \(^{25}\) stated that 91.8% and 84.5% of the isolates from some selected foods was positive for enterotoxin production both using TECRA and RPLA kits. Guven et al.\(^{20}\) reported that most of the isolates (86.6%) from meat and meat products were able to produce the toxin in culture. Reyes et al.\(^{36}\) found that 29.8% of the isolates from dried milk products were able to produce the diarrhoeal enterotoxin. From all the above mentioned conclusions, it is important to take all precautions for preventing contamination of rabbit meat with B. cereus and its toxin \(^{31}\).

The use of antimicrobial agents for food animals may cause problems in the therapy of infections in animals though the selection for resistance among bacteria pathogenic for animals \(^{32}\). The resistance problem in human medicine will not be solved if there is a constant influx of resistance genes into the human microflora via the food chain \(^{33,34}\). In this study, the antimicrobial susceptibility of the 18 isolates of B. cereus examined by the Standard disk diffusion method are shown in Table 3. The behavior of the isolated strains from rabbit meat to the action of antibiotics showed that all the isolated strains were resistant to penicillin. Ampicillin was the next most common, with tetracycline (98.7%) from meat and meat products were able to produce the diarrhoeal enterotoxin. From all the above mentioned conclusions, it is important to take all precautions for preventing contamination of rabbit meat with B. cereus and its toxin \(^{31}\).

It was concluded that isolation of B. cereus from 18 (36%) of 50 rabbit meat samples and 44% of the isolates found to be able to produce diarrheal enterotoxin are results which should be paid attention. And also, antimicrobial sensitivity test results were alarming because 100% and 94.4% of the isolates were resistant to penicillin and ampicillin. To improve rabbit meat safety and prevent harms to public health, the control of contamination routes at production stage of rabbit meat is an important measure. For this purpose food safety programmes focusing on a farm-to-table approach should be put in to practice in rabbit meat production.

REFERENCES


