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RESEARCH ARTICLE

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

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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 diarrheal 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 $2.89x10^3$ 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*

Burdur'da Tüketime Sunulan Tavşan Etlerinde *Bacillus cereus* Varlığı, Enterotoksin Üretme Yeteneği 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. Diarel tip enterotoksinin tespitinde BCET-RPLA testi kullanıldı. Antimikrobiyel duyarlılık testi için disk difüzyon metodu kullanıldı. *B. cereus* 50 tavşan eti örneğinin 18'inde (%36), ortalama $2.89x10^3$ kob/g düzeyinde belirlendi. Toplam *B. cereus* izolatlarının 8 tanesi (%44.4) enterotoksin üretebilme özelliğinde bulundu. *B. cereus* izolatlarının %100'nün penisilin, %94.4'nün ampisilin, %27.7'sinin streptomisin, %22.2'sinin gentamisin ve eritromisine dirençli olduğu belirlenmesine karşın, kloramfenikol ve vankomisine direnç saptanmamıştır.

Anahtar sözcükler: *Tavşan eti, Bacillus cereus, Enterotoksin, Antibiyotik dirençliliği*

INTRODUCTION

Bacillus cereus causes two food-borne syndromes [1]. The first syndrome resembles staphylococcal intoxication and is characterised with vomiting, 1-5 h incubaton 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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intoxications in Japan caused by *B. cereus* during 1982-2001 were especially the emetic types [2] and the amount of emetic toxin in food poisoning cases due to *B. cereus*, received during 1974-1999 in Japan ranged from 0.01 to 1.28 µg/g [3]. 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 [4,5]. Currently four enterotoxins, able to cause the diarrheal syndrome, have been described: hemolysin BL, nonhemolytic enterotoxin and two enterotoxic proteins; enterotoxin T(bc-D-ENT) [6] and cytotoxin K [7].

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 [8] and that every year there are 27.000 cases in which *B. cereus* is involved [9]. Its ability to survive makes *B. cereus* problematic to essentially all sectors of the food industry [10]. *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 [11]. As many as 5% of foodborne outbreaks have been connected with *B. cereus* in the Netherlands, England, France, and the USA [12]. 110 outbreaks were reported in UK during the period 1971-1979 [4]. 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 [13] 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* [14].

Rabbit production for meat is a very important livestock activity [15] and rabbit meat is a meat type demanded by people in most mediterranean countries [16]. And also the rabbit meat industry is highly developed in many other countries [15]. Global rabbit meat consumption in 2004 was 1.1 million tonnes [17]. In Turkey, rabbit breeding has been increasing recently and demand for rabbit meat in tourism places is increasing [18]. Although many studies conducted for *B. cereus* were present on red and white meat [19,20], 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 [15,21].

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 Zeland 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 l/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 l/4-strength Ringer solution were spread plated (two plates per dilutiuon) onto Plate Count Agar (Oxoid CM325) and incubated at 35°C for 48 h to determine the counts of mesophilic aerobic microorganism [22]. 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 transfered 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 utulization of glucose and ksilose, reduction of nitrate, Voges-Proskauer test, motility, and hemolysis) [23,24].

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) [25].

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 [26]. 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 food 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 erytromycin with four isolates and tetracycline wiht only two isoletes. 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 [27]. 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 [15]. Mean mesophilic aerobic microorganism count determined in the present study is lower than finding of Rodriguez-Calleja *et al.*[28]. 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 [13]. Besides causing foodborne illness, *B. cereus* is also responsible for the spoilage of a variety of food products. Borge *et al.*[24], 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 Schlegevola *et al.*[29] who reported that 28% of the meat products tested was contaminated with *B. cereus*. Guven *et al.*[20] determined that 22.4% of the meat and meat products contained *B. cereus*. However, Abostate *et al.*[27] 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 processed (ground beef) meat 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.*[20] from beef as $8.0x10^3$. But it is conflicted with Agata *et al.*[3] who found that mean viable count of *B. cereus* in meat and meat products is $2.8x10^6$. Hanashiro *et al.*[13] 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.

Table 1. *The distribution of positive samples according to the B. cereus count*

Tablo 1. *Pozitif numunelerin B. cereus sayılarına göre dağılımı*

Rabbit Meat Sample		Positive Sample		Distribution of Positive Samples					
				≤10^2 cfu/g		10^2-10^3 cfu/g		≥10^4 cfu/g	
N	%	N	%	N	%	N	%	n	%
50	100	18	36	12	24	4	8	2	4

Table 2. *Mesophilic aerobic microorganism and B. cereus counts in rabbit meat samples*

Tablo 2. *Tavşan eti örneklerinde mezofilik aerobik mikroorganizma ve B. cereus sayıları*

Microorganism	Range	Cfu/g
Mesophilic Aerobic Microorganism	Mean	$8.0x10^4$
	Min	$3.6x10^3$
	Max	$1.0x10^6$
B. cereus	Mean	$2.89x10^3$
	Min	$2.0x10^2$
	Max	$2.6x10^4$

Table 3. *Antibiotic resistance profile of B. cereus isolated from rabbit meat*

Tablo 3. *Tavşan etlerinden izole edilen B. cereus'ların antibiyotik dirençlilik profili*

Antibiotic	R	I	S
Streptomycin	5 (%27,7)	4 (%22.2)	9 (%50)
Tetracycline	2 (%11.1)	6 (%33.3)	10 (%55.5)
Ampicillin	17 (94.4)	1 (%5.5)	0
Gentamicin	4 (%22.2)	1 (%5.5)	13 (%94,4)
Chloramphenicol	0	1 (%5.5)	17 (94.4)
Penicilin G	18 (%100)	0	0
Vancomycin	0	0	18 (%100)
Erythromycin	4 (%22.2)	2 (%11.1)	12 (%66.6)

R: *Resistant* ***I:*** *Intermediate* ***S:*** *Sensitive (**R:** Dirençli **I:** Orta **S:** Duyarlı)*

B. cereus have the capacity to grow and generate toxin at storage temperatures above 6ºC [1] and the numbers of enterotoxigenic *B. cereus* required to cause food poisoning are ≥10^5cfu/g [25]. 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 were 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*.[30] 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 seventeen isolates, gentamicin and erytromycin with four isolates and tetracycline wiht only two isoletes. All isolates were sensitive to chloramphenicol and vancomycin. Similar results were reported by Rusul and Yaacob [25] who stated that *B. cereus* isolates from some selected foods were resistant to ampicilin (98.8%), cloxallin (100%) and tetracycline (61%) and susceptible to chloramphenicol (87%), erythromycin (77.4%), gentamycin (100%) and streptomycin (98.7). Guven *et al*.[20] stated that the isolates from meat and meat products showed a high resistance to oxacillin and amoxicillin, with all of them being susceptible to vancomycin. Schlegevola *et al*.[29] reported that all *B. cereus* isolates from meat and dairy products displayed resistance to cephalotin. In the illumination of these results, it could be said that *B. cereus* found to be resistant to a variety of antibiotics has become a major public health problem.

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 diarrhoeal enterotoxin are results which should be paid attention. And also, antimicrobial sensitivity test results were alarming because 100% and 94.4% of the isoletes were resistant to peniciline and ampicilin. 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.

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