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Surveillance of Multi Drug Resistant *Bacillus cereus* in Retail Meat .

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Abstract

Bacillus cereus **can be considered as one of the most important food borne pathogens that causes 2 distinct poisoning syndromes diarrhea and emesis and responsible for some non – gastrointestinal infection. It also has gained public health concern as it can cause food infection and intoxication in humans. The objectives of the present study were to isolate and identify multidrug resistance "MDR" *B.cereus*. out of 255 retail meat samples .There were 27 (10.6%) positive samples for *B. cereus* isolation. The high prevalence of isolation of *B. cereus* was found in raw beef meat 16.2 %. While rate in raw chicken were 7% . Antibiogram for recovered *B.cereus* showed that all isolates were 100% resistance to Penicillin G, Oxacillin , Sulfamethazole /trimethoprim and Cephalothin as well as multi drug resistance index (MDRI) from beef meat and chicken meat isolates have also same high ranged from 40% to 60 % .**

Conclusion: The antimicrobial resistance *B .cereus* strain has become remarkably become wide spreads in raw meat indicate the poor sanitary condition during processing which may create a health risk for consumers. Also there is need to legislate and enforce lows to limit the prescription and dispensation of antibiotic to only qualified professionals to control the misuse in human and veterinary medicine.

Key words :, *B.cereus* , Retail meat , MDR , MDRI , Antibiogram .
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Introduction

Bacillus is Gram-positive rods able to produce endospores resistant to unfavorable external conditions, **(Nemeckova Irena *et al.*, 2011)** . *B.cereus* is widely distributed in nature and a common contaminant of foods such as rice, spices, meat, cereal, various vegetables, egg and dairy product **(Dikbas ., 2010).**

B.cereus causes two distinct poisoning syndromes diarrhea and emesis and responsible for some non – gastrointestinal infection 2012 **(Khudor *et al.*,2012)**.The diarrheal type of food poisoning is caused by enterotoxins such as hemolysin BL (HBL), non hemolytic enterotoxin (NHE) cytotoxin K (CytK) and enterotoxin FM during produced vegetative growth in the small intestine and emetic type of food poisoning is heat stable and known as cereulide **(Beom Kim *et al.*,2011).**

The occurrence of *B. cereus* as a meat contaminated was reported by many investigators not only in row meat **(Nel *et al.*,2004)** but also in meat products **(Nortje *et al* .,1999)** and meat product additives**(Shinagawa *et al.*,1988).** Poultry is likely contaminated with *B. cereus* during industrial breeding, from dusty housing conditions, from contaminated chicks, or from feed. Feed products are likely sources of *B. cereus,* because some common ingredients such as wheat and wheat products and both meat and vegetable proteins can be positive for *B. cereus,*

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Spores survive feed manufacture and readily colonize the gut of the chicken (**Jadamus *et al.*,2001**).

The use of antimicrobials in both animals and humans can be selected for resistant bacteria populations. In food animals, antimicrobials are used for the control and treatment of bacterial associated infectious diseases as well as for growth promotion purposes , An undesired consequence of antimicrobial use in animals is the potential development of antimicrobial-resistant zoonotic food borne bacterial pathogens and subsequent transmission to human as food contaminants (EFSA) **(Özcelik Berrin and Çitak 2009).**

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 make the strains resistant to Penicillin, Ampicillin, and even to the 3rd generation of Cephalosporins. Increasing occurrence of *B. cereus* isolates with multiple drug resistance may pose a significant public health hazard. 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 **(Tewari Anita *et al.*, 2012).** The goal of this study were to determine the prevalence of *B. cereus* in retail beef and chicken meat with and focus on antimicrobial agent resistance and susceptibility pattern.

Material and methods

Sample:

A Total of 255 retail samples including raw chicken (146) samples and raw beef meat (99) samples were collected from different supermarkets and butchers, all samples collected in serial plastic bags and transport to laboratory in cold chain under aseptic condition.

Isolation and identification of B.cereus:

Immediately after arrived to laboratory 10 g from each sample was aseptically weighted, the sample were homogenized in 90 ml of sterile buffered peptone water BPW (Britan) for 1 min . The sample diluted with BPW and 0.1 ml of dilution was steaks onto surface plating methods with Mannitol Egg Yolk Polymyxin(MYP) agar and plates were incubate at 30 c for 24 h. Typical colony transferred to nutrient agar slant and identification was confirmed by microscopic and biochemical characterization including vogos proskauer , lecithinase production ,nitrate reduction ,motility, lack acid production from mannitol and anaerobic utilization of glucose and hemolysis according to **Guven Kiymet et al .(2005)** .

Antibiogram for B.cereus isolates:

The antimicrobial sensitivity test was performed using the disk diffusion method on Mueller hinton agar (Oxoid) and the isolate strain were tested for sensitivity to ten antimicrobial erythromycin (E15), vancomycin (VA30) , penicillin G (P10),nalidixic acid (NA30) , oxacillin (OX1) , kanamycin (K30), chloramphenicol (C30) , tetracycline (TE10) , cephalothin (KF30) and sulphamethoxazole / trimethoprom (SXT 25) . The interpretation of these antimicrobial was resistance, intermediate or sensitive based on the size for inhibition zone for individual antimicrobial according to **Bauer et al. (1966).**

Determination of multidrug resistance index (MDRI) for B.cereus isolates:

Resistance to more than four antibiotic was taken an multi drug resistance (MDR) . MDR index (MDRI) of individual isolates was calculated by individual the number of antibiotics to which the isolate was resistance by the total

number of antibiotics to which the isolates was exposed **(Chandran Abhirosh et al., 2008)**, isolates with MDRA values of more than 0.2 or 20% were considered highly resistance. The MDRI was calculated using the following equation .

$$\text{MDR index} = \frac{\text{Number of antibiotics resistance X 100}}{\text{Total number of antibiotics used}}$$

Results

Prevalence of *B. cereus* from different examined samples showed in table (1), in total 27 (10.6%) samples from 255 examined samples were positive for *B. cereus* isolation. The high prevalence of isolation of *B. cereus* was found in raw beef meat 16.2 % and isolation rate in raw chicken samples 7%. On recording the antimicrobial resistance in table (3) of chicken meat samples it was found that all isolates show 100% resistance to Penicillin G, Oxacillin , Sulfamethazole /trimethoprim and Cephalothin; while other antimicrobial resistance Nalidixic acid was 73 % , both erythromycin and tetracycline were 9% . On the other hand recording the antimicrobial resistance of beef meat show 100% resistance to Penicillin G, Oxacillin and Cephalothin while other antimicrobial resistance in beef meat 87.5 % for Sulfamethazole /trimethoprim 62.5% for Nalidixic acid ,37.6% for tetracycline, 18.8% for erythromycin, and 6.3 % for Chloramphenicol. Table (2) showed the antimicrobial resistance and multi drug resistance index (MDRI) of isolated *B. cereus* strain from different meat samples. That MDRI from beef meat and chicken meat isolates have the same high ranged from 40% to 60 %.

Table (1): Prevalence of isolation of B. cereus from different meat samples.

Type of sample	No of examined samples	*B. cereus* +ve samples	
		No	%
Beef meat	99	16	16.2
Chicken	156	11	7
Total	255	27	10.6

Table (2) : Antimicrobial resistance and multi drug resistance index (MDRI) of isolated *B. cereus* strains.

Origin	Isolated *B. cereus* strain	Resistance pattern	Multi drug resistance index (MDRI)
Beef meat samples	1	P,NA,OX,SXT,KF	50 %
	2	P,NA,OX,TE,SXT,KF	60 %
	3	P,NA,OX,SXT,KF	50 %
	4	P,NA,OX,SXT,KF	50%
	5	P,NA,OX,SXT,KF	50 %
	6	E,P,NA,OX,KF	50 %
	7	E,P,NA,OX,SXT,KF	60 %
	8	P,NA,OX,C,SXT,KF	60 %
	9	P,OX,TE,SXT,KF	50 %
	10	P,NA,OX,TE,SXT,KF	60 %
	11	P,OX,TE,KF	40 %
	12	E,P,OX,TE,SXT,KF	60 %
	13	P,OX,TE,SXT,KF	50 %
	14	P,OX,SXT,KF	40 %
	15	P,OX,SXT,KF	40 %
	16	P,NA,OX,SXT,KF	50 %
Chicken samples	1	P,NA,OX,SXT,KF	50 %
	2	P,NA,OX,SXT,KF	50 %
	3	P,NA,OX,SXT,KF	50 %
	4	P,OX,SXT,KF	40 %
	5	P,NA,OX,SXT,KF	50 %
	6	P,OX,TE, SXT,KF	50 %
	7	E,P,NA,OX,SXT,KF	60 %
	8	P,OX,SXT,KF	40 %
	9	P,NA,OX,SXT,KF	50 %
	10	P,NA,OX,SXT,KF	50 %
	11	P,NA,OX,SXT,KF	50 %

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Table (3) antibiotic sensitivity / resistance pattern of *B.cereus* isolates .

Antimicrobial Agent	Disk Concentration	Efficacy of antibiotics	*B. cereus* isolates originates from Chicken samples		beef meat samples	
			N	%	N	%
Vancomycin (VA)	30 µg	R	0	0	0	0
		I	0	0	0	0
		S	11	100	16	100
Erythromycin (E)	15 µg	R	1	9	3	18.8
		I	7	64	6	37.5
		S	3	27	7	43.7
Penicillin G (P)	10 IU	R	11	100	16	100
		I	0	0	0	0
		S	0	0	0	0
Nalidixic acid (NA)	30 µg	R	8	73	10	62.5
		I	1	9	2	12.5
		S	2	18	4	25
Oxacillin (OX)	1µg	R	11	100	16	100
		I	0	0	0	0
		S	0	0	0	0
Kanamycin (K)	30 µg	R	0	0	0	0
		I	5	45	2	12.5
		S	6	55	14	87.5
Chloramphenicol (	30 µg	R	0	0	1	6.3
		I	0	0	2	12.5
		S	11	100	13	81.2
Tetracycline (TE)	10 µg	R	1	9	6	37.6
		I	7	64	5	31.2
		S	3	27	5	31.2
Sulfamethazole/ Trimethoprim (SXT)	25 µg	R	11	100	14	87.5
		I	0	0	0	0
		S	0	0	2	12.5
Cephalothin (KF)	30 µg	R	11	100	16	100
		I	0	0	0	0
		S	0	0	0	0

Discussion

On qualitative analysis of 255 retail beef meat and chicken samples, 27 samples (10.6 %) were confirmed to be positive for B. cereus. The prevalence of B.cereus isolation from beef meat was 16 samples (16.2%), while prevalence of B.cereus from chicken meat 11samples (7%). The prevalence of isolation of B.cereus in beef meat nearly similar to study conducted by **Wijnands et al. (2006)** *which B.cereus was (20.8%) in Netherlands also This contamination rate in meat is lower than that observed in by* **Tewari Anita et al.(2012)** *which was 30.85 % but higher than that found by* **Dikbas Neslihan (2010)** *which record 4 samples (5.2%).*

It was expected that it would be present on raw meat. During the slaughtering process, the meat is exposed to various sources of *B. cereus* contamination which include soil, hides, equipment and personnel **Nel et al. (2004).**

On the other hand the isolation of *B.cereus* from chicken meat was detected in 18out of 80 samples tested but the highest incidence of contamination was found in the processed poultry products (12 of 30 samples) while raw meat chicken samples were 5 out 50 (10 %) . This was previously conducted by **Floristean et al. (2007)** and **Dikbas Neslihan (2010)** which is nearly to this study which give prevalence of 7% , but the study observed by **Tahmasebi Hanna et al. (2014)** found that the overall examined chicken meat samples showed 15.6 % which is higher than this study.

The presence of bacterium in raw poultry may be principally due to spores originated in breeding farms, contamination during abattoir processing and post processing handling. 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. The drug resistance problem in human medicine will not be solved if there is a constant influx of resistance genes into the human micro flora via the food chain (**Kursun Ozen et al., 2011**)So far as the sensitivity of the *B. cereus* isolates against various antibiotics is concerned, variations in sensitivity attributes against 10 tested antimicrobial agents were observed in this study. In the present study *B.cereus* isolates showed a high rate of resistance to Penicillin G, Oxacillin which are antibiotic of penicillin group reach (100%) in (beef meat and chicken isolates) which is also reported in previous papers conducted by **Dikbas Neslihan (2010)** which may be the first report demonstrating resistance of *B. cereus* strains isolated from milk, meat, cereal and chicken to penicillin and Oxacillin in Eastern Anatolia Region of Turkey as a consequence of β lactamases action enzymes secreted by *B.cereus*.

Resistance of cephalothin (one of antibiotics from first generation of Cephalosporins) in this study reach 100% from beef meat isolates but it disagree with results of **Tewari Anita et al. (2012)** which give intermediate frequency of resistance in meat isolates (65.5 %) .These strains pose a significant health risk for consumer, transmission of antibiotic resistance to other microorganisms in the gastrointestinal being possible (**Floristean et al., 2007**). In this study *B.cereus* isolates which originates from beef meat showed susceptible to Kanamycin and Vancomycin (antibiotics from aminoglycoside and glycopeptides group) with (100 %) this result was similarity to those of **Luna et al. (2007)** who observed that *B.cereus* was susceptible to Aminoglycosides, Chloramphenicol, Clindamycin, Erythromycin, Tetracycline and Vancomycin . but in this study sensitivity of Chloramphenicol and Erythromycin and tetracycline originated from Chloramphenicol, macrolide tetracycline group beef meat is lower than the previous papers 81.2 , 43.7 and 31.2 % ; respectively .Variable resistance pattern of Nalidixic acid is one of quinolone group was recorded in *B. cereus* isolates obtained from beef meat in this study were 62.5 % and 73% from chicken samples which is higher than that recorded by **Tewari Anita et al. (2012)** which the recorded resistance was 48.3% from meat. The complete resistance to Sulfamethazole/ Trimethoprim in all *B.cereus* in this study for chicken meat isolates was 100 % it was similar to that reported by **Dikbas Neslihan (2010)** but meat examined in this paper reach to 100% is higher than that observed in is study which is resistance of beef meat was 87.5 %.

Cephalothin (1st generation of Cephalosporins) resistance in chicken samples was 100% which is similar to the study conduct by **Floristean et al. (2007)** .Our result revealed that sensitivity to one drug of aminoglycoside, macrolide and tetracycline group (kanamycin ,Erythromycin & tetracycline respectively) in chicken samples were 55,27 and 27 % respectively while in study that detected by **Floristean et al. (2007)** showing effective against all isolates (Erytromycin, Tylosin, Cotrimoxazole, Flumequin, Kanamycin, Gentamicin, Enrofloxacin, Oxolinic acid, Apramycin, tetracycline, Doxacilin) , also sensitive to drug from chloramphinicol group (Chloramphinicol) which is similar to this which showing 100% sensitivity to Chloramphinicol from chicken samples .

B.cereus sensitivity on drug of glycopeptides group (vancomycin) recorded in this study was (100%) in chicken samples which is similar to conducted by **Kursun Ozen et al. (2011)** who reported that all isolates were sensitive to this antibiotics . Bacteria resistant to antimicrobial drugs, which spread into the human population from foods of animal origin, rank as the direct causative agents of food borne diseases and represent a possible source of drug resistance of human pathogenic agents **(Schlegelova et al., 2003).**

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الملخص العربى

تمت هذه الدارسة لتحديد درجة تواجد ميكروب الباسيلس سيريس ومقاومته للمضادات الحيوية في لحوم الدجاج والابقار المجمعة من المحلات الكبري حيث يعتبر ميكروب الباسيلس سريس واحد من اهم الميكروبات التي تنتقل عن طريق الطعام حيث يسبب نوعين من متلازمة التسمم الغذائي (الاسهال – القئ) بالاضافة الي انه مسئول ايضا عن بعض العدوي الخاصة بغير عدوي الجهاز الهضمي . حيث انه استحوذ علي اهتمام الصحة العامة بسبب تلوث الطعام والتسمم الغذائي في الانسان

وتقوم هذه الدارسة علي عزل ميكروب الباسيلس سريس و تحديد المضادات الحيوية المقاومة للباسيلس سريس حيث تم عزل 27 عترة من 255 عينه من اللحوم بنسبه 10.6 % وقد وصل اعلي معدل لعزل الميكروب في لحوم الابقار النية الي 16.2 % بينما كان معدل العزل في لحوم الدجاج النية 7 % .

باجراء اختبارات الحساسية لعترات الباسلس سريس وجد ان جميع المعزولات مقاومة بنسبة 100 % للبنسيلين – الاوكسيسيللن – السلفاميثازول / ترايميثوبريم – سيفالوسين كما ان معامل المقاومة للمضادات الحيوية للعترات المعزولة من لحوم الابقار والدواجن لها نفس النسبة العالية و تتراوح ما بين 40 % - 60 % .