The prevalence of and resistance to antimicrobial agents of *Bacillus cereus* isolates from foodstuffs

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ABSTRACT: The study was aimed at the assessment whether foodstuffs contaminated with *Bacillus cereus* (*B. cereus*) may concurrently be vectors of spreading resistance. The contamination of foodstuffs with *B. cereus* strains was found in 31% of dairy and in 28% of meat products tested. Only one product from skimmed milk was contaminated. High-fat milk products that were heat-treated during the technological process (87 samples), as well as heat-treated meat products (65 samples), were contaminated significantly frequently (63% and 48% of the samples respectively) ($P < 0.01$). Almost all *B. cereus* isolates displayed low susceptibility to ampicillin, cephalothin, and to oxacillin. Except for streptomycin (STR) resistance, resistance to other 8 antimicrobial agents occurred sporadically. The STR resistant isolates came particularly from spreading butter (8 samples) ($P < 0.05$). It was established that the same samples were contaminated with two subpopulations of *B. cereus* with different STR resistances. The frequent occurrence of *B. cereus* in foodstuffs with either fat content and/or subject to heat treatment in processing makes these products risky, however, our study did not confirm that foodstuffs contaminated with *B. cereus* are concurrently vectors of transmissible resistance genes.

Keywords: pathogenic microorganisms; food safety; acquired resistance

Bacteria resistant to antimicrobial drugs which penetrate into the human population with foods of animal origin and rank with direct causative agents of foodborne diseases, represent a possible source of drug resistance for human pathogenic agents (Shryock, 1999; Teuber *et al.*, 1999).

Bacillus cereus (*B. cereus)* causes problems to the foodstuff industry both by deteriorating the products (TeGiffel *et al.*, 1996; Pirttijarvi *et al.*, 2000; Eneroth *et al.*, 2001), and by endangering people's health upon consuming contaminated foods (Granum *et al.*, 1993; Ghelardi *et al.*, 2002). Under certain conditions, strains of this species produce haemolysins, phospholipases C, and also emetic toxins and enterotoxins that cause food poisoning (Rusul and Yaacob, 1995; Andersen-Borge *et al.*, 2001; Agata *et al.*, 2002).

The danger posed by some strains of this microorganism is increased by its ability to adapt to chemical (Willinghan *et al.*, 1996; Ultee *et al.*,

2000), heat (Browne and Dowds, 2001) and cold environment (TeGiffel *et al.*, 1997), as well as by toxin production (Andersen-Borge *et al.*, 2001). The presence of spores in raw milk, their high resistance to the pasteurisation temperature (Lin *et al.*, 1998; Janstova and Lukasova, 2001), and also the adherence of spores and vegetative cells to metal surfaces (Peng *et al.*, 2001; Faille *et al.*, 2001) make *B. cereus* an unwelcome, but very frequent contaminant of dairy products (Grieger *et al.*, 1990; Huang *et al.*, 1999 etc.).

Although formerly considered an apathogenic species or a facultative pathogen, *B. cereus* has been ever more often isolated as an etiological agent in serious brain infections (Chu *et al.*, 2001) and in infections of patients with neutropenia and with carcinoma (Zinner, 1999). Also, it has been found in post-operation and post-traumatic infections of wounds with strains producing the necrotising HBL toxin (Kemmerly and Pankey, 1993) as well as in

other infections. The production of β-lactamases (Kotiranta *et al.*, 2000) is one of potential virulence factors that make the producing strains resistant even to the 3rd generation of cephalosporins (Cormican and Jones, 1995). The latter drugs are effective as sporulation inhibitors in sensitive strains of *B. cereus* (Miyamoto *et al.*, 1997).

Even though Ombui *et al.* (1996) did not prove the link between the presence of plasmids and the antimicrobial drug resistance of *B. cereus* strains isolated from milk, a tetracycline resistance gene on a potential mobile element, plasmid pBC16 (Palva *et al.*, 1990), was demonstrated and sequenced some time ago. It was also demonstrated that resistance to antimicrobial substances methicillin, gentamicin, kanamycin and tetracycline, can be acquired by the transfer of the relevant plasmid between Gram-positive microorganisms, among which *B. cereus* falls (ECDC, 2002). Intraspecies as well as interspecies transfer of resistance was established in the named organisms (Muhammad *et al.*, 1993; Licht *et al.*, 1999 etc.). For example, from *Listeria monocytogenes* to *Staphylococcus aureus* (Lemaitre *et al.*, 1998), from *Enterococcus faecalis* and *Enterococcus faecium* to *Staphylococcus aureus*, *S. hyicus* and *S. intermedius* (Noble *et al.*, 1996).

The subject of this study was the establishing of prevalence of *B. cereus* in isolates obtained from samples of foodstuffs made from cow's milk and from meat, and also their prevalence of resistance to antimicrobial substances. The study was aimed at the assessment whether foodstuffs contaminated with *B. cereus* may concurrently be vectors of spreading resistance.

MATERIAL AND METHODS

Isolation and typing of strains

Samples of dairy foodstuffs (215 samples) and meat foodstuffs (110 samples) were collected under sterile conditions at three dairies and at four meat-processing plants during two years. The samples included dairy and meat products. The dairy group comprised soft fresh cheese (31 samples), soft ripening cheese (10 samples), cheese with low-temperature sub-pasteurised curd (44 samples), cheese with high-temperature sub-pasteurised curd (1 sample), cottage cheese (32 samples), thermised spreading butter (63 samples), thermised cream cheese (24 samples), fresh butter (5 samples) and cream (5 samples). The meat products included heat-treated products (65 samples): soft salamis, frankfurters and meat sausages – and products made without heat treatment (45 samples): fermented dry salamis. Analytical samples of meat and dairy products were taken and prepared for microbiological testing in accordance with standard procedures (ISO 3100-1,2 and ISO 8261). Samples of 0.1 ml each were spread plated immediately, and also, in order to increase the probability of detection, after propagation in meat-peptone broth for 24 and 48 hours at 37°C, inoculated in duplicate onto petri dishes with selective media (Oxoid, Hampshire, U.K.).

From each sample, a maximum of 10 suspect colonies of *B. cereus* were inoculated onto a selective medium containing mannitol, egg yolk, and polymyxin (MYP agar) and from there onto blood agar, and identified using the API 50 CHB system. If several strains of *B. cereus* were isolated from a single sample, resistance was examined in all of them.

The isolated strains were kept in ITEST Cryobanks B (ITEST Hradec Kralove, Czech Republic) at –79°C. Prior to the resistance tests, strains of *B. cereus* were resuscitated on blood agar plates. Then four to five colonies were resuspended in 2 ml of Mueller-Hinton broth (Oxoid, Hampshire, U.K.) and further diluted as recommended by NCCLS (1999) with the same medium under spectrophotometric control to obtain suspensions with a density of approximately 5×10^6 CFU/ml.

Tests of sensitivity to antimicrobial agents

Antimicrobial drugs used for sensitivity tests were selected according to the following criteria. (a) Antimicrobial drugs used for the treatment of bovine mastitis on farms (ampicillin – AMP; ampicillin-sulbactam – AMS; cephalothin – CLT; clindamycin – CLI; neomycin – NEO; oxacillin – OXA; streptomycin – STR; tetracycline – TET). (b) Drugs under regulations limiting the use of selected antibiotics in the treatment of animals (norfloxacin – NOR). (c) Drugs important in the treatment of human infectious diseases (erythromycin – ERY; vancomycin – VAN). In addition, gentamicin – GEN was also included into the tests. Serial twofold dilutions of antimicrobial drug standards (Sigma, St. Louis, MO) (Table 1) were prepared as recommended by Hindler *et al.* (1992). The drugs were diluted with Mueller-Hinton broth supplemented with Ca^{2+}

Table 1. Range of dilution series on microtitre plates, MIC_{50}, MIC_{90}, and MIC range of antimicrobial agents used for *B. cereus* resistance testing

Agents	Concentration range on MP	MIC_{50}	MIC_{90}	MIC range
		(µg/ml)		
Ampicillin	0.25–32	≥32	≥32	8–≥32
Ampicillin; sulbactam	0.5; 0.25–64; 32	8; 4	32; 16	4; 2–≥64; ≥32
Clindamycin	0.25–32	1	2	0.25–8
Cephalotin	0.25–32	≥32	≥32	8–≥32
Erythromycin	0.25–32	0.25	0.25	0.25–4
Gentamicin	0.25–32	2	4	0.25–16
Neomycin	0.5–64	2	4	0.5–16
Norfloxacin	0.5–64	0.5	1	0.5–4
Oxacillin	0.25–32	≥32	≥32	8–≥32
Streptomycin	1–128	8	32	1–≥128
Tetracycline	0.25–32	1	4	0.25–≥32
Vancomycin	0.25–32	1	2	0.5–4

and Mg^{2+} (CAMHB). For OXA, the medium was further supplemented with sodium chloride (20 g/l) as recommended by Tamashiro (1992a).

Microdilution technique for the determination of MIC

Microtitre plates (MP) for tests of sensitivity to antimicrobial agents using the standard microdilution technique were prepared as described by Tamashiro (1992a,b). Ten-microlitre volumes of standardised suspensions of the individual strains were dispensed into MP wells, each containing 100 µl of the appropriate antimicrobial drug dilution (Table 1). The MP's were incubated for 18 h at 35°C and turbidity, indicating the growth of bacteria, was read at 630 nm using the SUNRISE apparatus (Tecan, Grödig, Austria). Every strain was tested on two MP's simultaneously. A reference strain of *Staphylococcus aureus* ATCC 29213 was tested concurrently with each batch of MPs and also once a week during the testing period. The MIC (minimum inhibitory concentration) was defined for every strain as the highest dilution of the antimicrobial drug at which the growth was completely inhibited. From the duplicate measurements, the higher MIC value within tolerance ± one dilution step is always reported.

Evaluation

The prevalence of *B. cereus* were assessed. The inter-group differences found were analysed with the χ^2-test (Armitage and Barry, 1987).

The MIC, the MIC_{50} and MIC_{90} characteristics, and the MIC range were evaluated. MIC_{50} and MIC_{90} include 50% and 90% of strains with the given or lower MIC value.

The isolates were classified as "sensitive" or "resistant" using the interpretation criteria for MIC given in Table 2. No unambiguous interpretation criteria in the evaluation of resistance of *B. cereus* strains to the selected anti-microbial agents have been determined (Table 2). The established and presented MIC_{50} and MIC_{90} values and the MIC range found in the study were therefore used for the selection and "determination" of microbiological interpretation criteria of the resistance of *B. cereus* isolates. The interpretation criteria for the determination of resistance to AMP and OXA were not "determined" in this study because of the high MIC_{50} and MIC_{90} values, exceeding the highest value for these antimicrobial agents in the dilution series on the MP's.

Resistance phenotypes and numbers of strains resistant to the individual antimicrobial drugs were determined and the association of resistance in isolates from the same food sample were assessed.

Table 2. Interpretation criteria for resistance assessment in terms of MIC (MIC breakpoints)

Agents	MIC breakpoints (μg/ml)			
	Jensen *et al.* (2001)	NCCLS (1999)	ECDC (2002)	Criteria in this study
Ampicillin	–	–	2[a]	ND
Ampicillin; Sulbactam	–	–	–	≥64
Clindamycin	–	≥4	–	≥8
Cephalotin	–	16[b], ≥32	–	16 [b], ≥32
Erythromycin	≥8	1–4[b], ≥8	4	≥4
Gentamicin	≥16	≥16	8	≥16
Neomycin	≥16	–	64	≥16
Norfloxacin	–	–	–	≥8
Oxacillin	–	–	–	ND
Streptomycin	≥32	–	64	≥32
Tetracycline	≥16	≥16	16	≥16
Vancomycin	≥16	≥32	4	≥16

[a]note from ECDC (2002): Certain species are inherently resistant

[b]MIC breakpoints for intermediate–resistant strains

ND = the breakpoint was not defined

RESULTS AND DISCUSSION

Prevalence *B. cereus* in selected food samples

Contamination with *B. cereus* strains was recorded in 31% of all dairy products (66 samples), and in 28% of samples (31 samples) of meat products (Table 3). The recorded prevalence of strains in the foodstuffs examined is not at variance with characteristic properties of *B. cereus*. Owing to their surface hydrophobicity (Ronner *et al.*, 1990), vegetative cells and spores of *B. cereus* tend to adhere to fat droplets of the milk (Larsen and Jorgensen, 1997). The presented results (Table 3), indirectly corroborate this fact: products made from skimmed milk (curd cheese) were contaminated in one case only (3.2% of products), while products with higher fat content (even if made with different production technologies) in 10–16% of cases.

Products with high fat content and heat-treated products (thermised cream cheese, spreading butter) were 54 and 65% contaminated with *B. cereus* strains. Also, heat treated meat products were significantly more contaminated with *B. cereus* strains than unheated fermented dry salami (*P* < 0.01). At the temperatures of 73°C, used for mild milk pas-

teurisation, 75°C, used during the homogenisation of spreading butter, and 65–70°C, used in the production of thermised cream cheeses (Grieger *et al.*, 1990), as well as during the heat treatment of some meat products (70°C for 10 minutes) (Steinhauser, 1995), most of the contaminating microflora is eliminated. However, spores of *B. cereus* are activated at these temperatures. The findings of spores in milk, but also in the animal environment are not unusual (Larsen and Jorgensen, 1997; Jensen *et al.*, 2001 etc.). Genetic typing of isolated strains of *B. cereus* showed that the spore content of milk was strongly associated with the degree of contamination of the teats with soil (Christiansson *et al.*, 1999). It was also established that spores of *B. cereus* in raw milk were the main source of milk contamination found after pasteurisation (Lin *et al.*, 1998), even if additional contamination may occur during the processing in dairy industry (TeGiffel and Beumer, 1998).

Prevalence of resistance of *B. cereus* to antimicrobial agents

Almost all isolates of *B. cereus* collected from both commodities displayed low susceptibility to β-lactam antimicrobial agents. MIC_{50} and MIC_{90} at-

Table 3. Food samples contaminated with *B. cereus*

Foodstuff	Number of samples examined	Number of contaminated samples *B. cereus*
Dairy products		
Soft fresh cheese[NT]	31	5
Soft ripening cheese[NT]	10	1
Cheese with low-temperature sub-pasteurised curd[NT]	44	5
Cheese with high-temperature sub-pasteurised curd[NT]	1	–
Cottage cheese[NT]	32	1
Fresh butter[NT]	5	–
Cream[NT]	5	–
Cream cheese[T]	24	13
Spreading butter[T]	63	41
Total	215	66
Meat products		
Heat treated	65	31
Unheated fermented dry salami	45	–
Total	110	31

[NT]non–thermised dairy products

[T]thermised dairy products

tained values of ≥32 and ≥32 μg/ml for AMP, ≥32 and ≥32 μg/ml for CLT, and ≥32 and ≥32 μg/ml for OXA (Table 1). These values or this resistance phenotype of *B. cereus* strains are also documented in studies by other authors (Stec, 1990; Ombui *et al.*, 1996; Jensen *et al.*, 2001). The resistance may have been caused by enzymatic modification of agents; this mechanisms, however, does not explain the resistance to OXA. Three forms of β-lactamases were demonstrated in *B. cereus* strains, while strains isolated from clinical cases were usually resistant even up to the third generation of cephalosporins (Kotiranta *et al.*, 2000). In this study, five isolates of *B. cereus* did not manifestly react to the presence of the β-lactamase inhibitor in the combination product Ampicillin; Sulbactam, and the respective MIC was of ≥64; 32 μg/ml in these isolates. We can thus assume that enzymes with different substrate specificity, their overproduction and/or other mechanisms of resistance were involved.

Except for the STR resistance, resistance to other antimicrobial agents occurred only sporadically (Table 4), as registered in the MIC range for individual substances (Table 1). One isolate from spreading butter was polyresistant, namely in to STR and ERY (BC 966) in addition to β-lactam antimicrobials. Isolates resistant to STR (18 isolates) originated from dairy products (13 samples), particularly from samples of spreading butter (8 samples) (*P* < 0.05). Streptomycin is a constituent of a number antimicrobial preparations used for the treatment of mastitis in cows (Schlegelova *et al.*, 2002), but also for the protection of plants (Sundin *et al.*, 1995). One can thus speculate whether the strains of *B. cereus* isolated from dairy products originated from milk or were only carriers of genes of resistance to antimicrobial agents used in the therapy of animals. Repeated isolations of *B. cereus* from the same sample and the testing of isolates for resistance have established that almost all samples were contaminated with two subpopulations of *B. cereus* differing in their resistances to STR (Table 5). This fact shows that more than one colony should be tested for resistance, particularly in samples of mixed origin.

We can state that "sensitive" strains of *B. cereus* (but resistant to β-lactam antimicrobials), and also polyresistant strains, predominantly contaminated

Table 4. Number of *B. cereus* isolates from selected foodstuff samples resistant to individual antimicrobial agents

Agents	Number of isolates resistant to antimicrobial agents	
	number of isolates from meat products	number of isolates from dairy products
	n = 33	*n* = 96
Ampicillin	–	–
Ampicillin; Sulbactam	–	5
Clindamycin	2	1
Cephalotin	33	96
Erythromycin	1	4
Gentamicin	1	–
Neomycin	–	2
Norfloxacin	–	1
Oxacillin	–	–
Streptomycin	3	18
Tetracycline	1	1
Vancomycin	2	1

Table 5. Resistance (R) and sensitivity (S) of individual isolates of *B. cereus* from repeated isolations from the same foodstuff sample

Sample number	Sensitivity and/or resistance of *B. cereus* isolates	Food sample
1 417	R-(STR. NEO); S	spreading butter
1 419	S; S; S	thermised cream cheese
1 444	S; R-STR; S; S	soft fresh cheese
1 447	S; S	soft fresh cheese
1 510	R-STR; S; S; S; S; R-STR; R-STR; S; S	spreading butter
1 512	S; R-STR; R-STR; S; R-STR	spreading butter
1 513	S; R-STR; R-STR	spreading butter
1 515	S; S; S; S; S; S; S	thermised cream cheese
1 566	R-STR; S	soft fresh cheese

Abbreviations:
NEO = neomycin; STR = streptomycin; S = the isolates sensitive to all the microbial agents used in this study with exception of β-lactam antimicrobial agents

foodstuffs with high fat content and also foodstuffs heat-treated in the technological process. The presence of *B. cereus* strains resistant to β-lactam antimicrobial agents (AMP, AMS, CLT and OXA), to STR, and sporadically to other substances (CLI, ERY, NEO and TET), suggests that meat or dairy products may be, under certain conditions, vectors of resistance to antimicrobial agents via this pathogenic microorganism. However, our study did not confirm that foodstuffs contaminated with *B. cereus* are important vectors of transmissible resistance genes.

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REFERENCES

Agata N., Ohta M., Yokoyama K. (2002): Production of *Bacillus cereus* emetic toxin (cereulide) in various foods. Int. J. Food Microbiol., *73*, 23–27.

Andersen-Borge G.I., Skeie M., Sorhaug T., Langsrud T., Granum P.E. (2001): Growth and toxin profiles of *Bacillus cereus* isolated from different food sources. Int. J. Food Microbiol., *69*, 237–246.

Armitage P., Barry G. (1987): Statistical methods in medical research. 2th ed. Blackwell Scientific Publications, Oxford. 559 pp.

Browne N., Dowds B.C.A. (2001): Heat and salt stress in the food pathogen *Bacillus cereus*. J. Appl. Microbiol., *91*, 1085–1094.

Christiansson A., Bartilsson J., Svensson B. (1999): *Bacillus cereus* spores in raw milk: Factors affecting the contamination of milk during the grazing period. J. Dairy Sci., *82*, 305–314.

Chu W.P., Que T.L., Lee W.K., Wong S.N. (2001): Meningoencephalitis caused by *Bacillus cereus* in a neonate. Hong Kong Med. J., *7*, 89–92.

Cormican M.G., Jones R.N. (1995): Antimicrobial activity of cefotaxime tested against infrequently isolated pathogenic species (unusual pathogens). Diagn. Microbiol. Infect. Dis., *22*, 43–48.

ECDC (2002): Opinion of the Scientific Committee on Animal Nutrition on the criteria for assessing the safety of micro-organisms resistant to antibiotics of human clinical and veterinary importance. European Commission Directorate C – Scientific Opinions.

Eneroth A., Svensson B., Molin G., Christiansson A. (2001): Contamination of pasteurized milk by *Bacillus cereus* in the filling machine. J. Dairy Res., *68*, 189–196.

Faille C., Fontaine F., Benezech T. (2001): Potential occurrence of adhering living *Bacillus* spores in milk product processing lines. J. Appl. Microbiol., *90*, 892–900.

Ghelardi E., Celandroni F., Salvetti S., Barsotti C., Baggiani A., Senesi S. (2002): Identification and characterization of toxigenic *Bacillus cereus* isolates responsible for two food-poisoning outbreaks. Fems Microbiol. Lett., *208*, 129–134.

Granum P.E., Brynestad S., Kramer J.M. (1993): Analysis of enterotoxin production by *Bacillus cereus* from dairy products, food poisoning incidents and non-gastrointestinal infections. Int. J. Food Microbiol., *17*, 269–279.

Grieger C., Holec J., Burdova O., Krcal Z., Lukasova J., Matyas Z., Pleva J. (1990): Hygiena mlieka a mliečnych výrobkov. Príroda, Bratislava and SZN Praha. 397 pp.

Hindler J., Hochstein L., Howell A. (1992): Preparation of routine media and reagents used in antimicrobial sensitivity testing, sec. 5.19, p. 1–30. In: Isenberg H.D. (ed.): Clinical Microbiology Procedures Handbook. Am. Soc. Microbiol., Washington. 183 pp.

Huang C.C., Lai S.H., Shin S.L., Liau S.M., Peng J.S. (1999): A study on the spoilage of aseptic packaged long life milk caused by *Bacillus cereus*. J. Chin. Soc. Anim. Sci., *28*, 249–260.

ISO 3100-1 (1991): Meat and meat products – Sampling and preparation of test samples. Part 1: Sampling.

ISO 3100-2 (1988): Meat and meat products – Sampling and preparation of test samples. Part 2: The preparation of test samples for microbiological examination.

ISO 8261 (2001): Milk and milk products – General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination.

Janstova B., Lukasova J. (2001): Heat resistance of *Bacillus* spp. spores isolated from cow's milk and farm environment. Acta Vet. Brno, *70*, 179–184.

Jensen L.B., Baloda S., Boye M., Aarestrup F.M. (2001): Antimicrobial resistance among *Pseudomonas* spp. and the *Bacillus cereus* group isolated from Danish agricultural soil. Environ. Int., *26*, 581–587.

Kemmerly S.A., Pankey G.A. (1993): Oral ciprofloxacin therapy for *Bacillus cereus* wound infection and bacteremia. Clin. Infect. Dis., *16*, 189.

Kotiranta A., Lounatmaa K., Haapasalo M. (2000): Epidemiology and pathogenesis of *Bacillus cereus* infections. Microb. Infect., *2*, 189–198.

Larsen H.D., Jorgensen K. (1997): The occurrence of *Bacillus cereus* in Danish pasteurized milk. Int. J. Food Microbiol., *34*, 179–186.

Lemaitre J.P., Echchannaoui H., Michaut G., Divies C., Rousset A. (1998): Plasmid-mediated resistance to antimicrobial agents among *Listeriae*. J. Food Prot., *61*, 1459–1464.

Licht T.R., Christensen B.B., Krogfelt K.A., Molin S. (1999): Plasmid transfer in the animal intestine and other dynamic bacterial populations: the role community structure and environment. Microbiology, *145*, 2615–2622.

Lin S., Schraft H., Odumeru J.A., Griffiths M.W. (1998): Identification of contamination sources of *Bacillus cereus* in pasteurized milk. Int. J. Food Microbiol., *43*, 159–171.

Miyamoto T., Yamaguchi K., Abu Sayed M., Sasahara R., Honjoh K., Hatano S. (1997): Penicillin-binding protein

sensitive to cephalexin in sporulation of *Bacillus cereus*. Microbiol. Res., *152*, 227–232.

Muhammad G., Hoblet K.H., Jackwood D.J., Bech-Nielsen S., Smith K.L. (1993): Interspecific conjugal transfer of antibiotic resistance among *Staphylococci* isolated from the bovine mammary gland. Am. J. Vet. Res., *54*, 1432–1440.

NCCLS (1999): National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved Standard M31-A. NCCLS, Wayne, USA.

Noble W.C., Rahman M., Karadec T., Schwarz S. (1996): Gentamicin resistance gene transfer from *Enterococcus faecalis* and *E. faecium* to *Staphylococcus aureus*, *S. intermedius* and *S. hyicus*. Vet. Microbiol., *52*, 143–152.

Ombui J.N., Mathenge J.M., Kimotho A.M., Macharia J.K., Nduhiu G. (1996): Frequency of antimicrobial resistance and plasmid profiles of *Bacillus cereus* strains isolated from milk. East. Afr. Med. J., *73*, 380–384.

Palva A., Vigren G., Simonen M., Rintala H., Laamanen P. (1990): Nucleotide sequence of the tetracycline resistance gene of pBC16 from *Bacillus cereus*. Nucl. Acids Res., *18*, 1635.

Peng J.S., Tasi W.C., Chou C.C. (2001): Surface characteristics of *Bacillus cereus* and its adhesion to stainless steel. Int. J. Food Microbiol., *65*, 105–111.

Pirttijarvi T.S., Andersson M.A., Salkinoja-Salonen M.S. (2000): Properties of *Bacillus cereus* and other bacilli contaminating biomaterial-based industrial processes. Int. J. Food Microbiol., *60*, 231–239.

Ronner U., Husmark U., Henriksson A. (1990): Adhesion of bacillus spores in relation to hydrophobicity. J. Appl. Bacteriol., *69*, 550–556.

Rusul G., Yaacob N.H. (1995): Prevalence of *Bacillus cereus* in selected foods and detection of enterotoxin using TECRA-VIA and BCET-RPLA. Int. J. Food Microbiol., *25*, 131–139.

Schlegelova J., Babak V., Klimova E., Lukasova J., Navratilova P., Sustackova A., Sediva I., Rysanek D. (2002): Prevalence of and resistance to anti-microbial drugs in selected microbial species isolated from bulk milk samples. J. Vet. Med. B., *49*, 216–225.

Shryock T.R. (1999): Relationship between usage of antibiotics in food-producing animals and the appearance of antibiotic resistant bacteria. Int. J. Antimicrob. Agents, *12*, 275–278.

Stec E. (1990): Sensitivity to antibiotics and selected compounds of *Bacillus cereus* isolated from food products and cases of food poisoning. Rocz. Panstw. Zakl. Hig., *41*, 269–272.

Steinhauser L. (1995): Hygiena a technologie masa. LAST, Brno, Czech Republic. 643 pp.

Sundin G.W., Monks D.E., Bender C.L. (1995): Distribution of the streptomycin-resistance transposon Tn5393 among phylloplane and soil bacteria from managed agricultural habitats. Can. J. Microbiol., *41*, 792–799.

Tamashiro L. (1992a): Broth microdilution MIC testing, sec. 5.2., p. 1–29. In: Isenberg H.D. (ed.): Clinical Microbiology Procedures Handbook. Am. Soc. Microbiol., Washington. 183 pp.

Tamashiro L. (1992b): Preparation of broth microdilution MIC trays, sec. 5.20., p. 1–20. In: Isenberg H.D. (ed.): Clinical Microbiology Procedures Handbook. Am. Soc. Microbiol., Washington. 183 pp.

TeGiffel M.C., Beumer R.R. (1998): Isolation, identification and characterization of *Bacillus cereus* in the dairy industry. Tijdschr. Diergeneeskd., *123*, 628–632.

TeGiffel M.C., Beumer R.R., Leijendekkers S., Rombouts F.M. (1996): Incidence of *Bacillus cereus* and *Bacillus subtilis* in foods in the Netherlands. Food Microbiol., *13*, 1096–1100.

TeGiffel M.C., Beumer R.R., Granum P.E., Rombouts F.M. (1997): Isolation and characterization of *Bacillus cereus* from pasteurised milk in household refrigerators in the Netherlands. Int. J. Food Microbiol., *34*, 307–318.

Teuber M., Meile L., Schwarz F. (1999): Acquired antibiotic resistance in lactic acid bacteria from food. Antonie van Leuwenhoek, *76*, 115–137.

Ultee A., Kets E.P., Alberda M., Hoekstra F.A., Smid E.J. (2000): Adaptation of the food-borne pathogen *Bacillus cereus* to carvacrol. Arch. Microbiol., *174*, 233–238.

Willinghan E.M., Sander J.E., Thayer S.G., Wilson J.L. (1996): Investigation of bacterial resistance to hatchery disinfectants. Avian Dis., *40*, 510–515.

Zinner S.H. (1999): Changing epidemiology of infections in patients with neutropenia and cancer: emphasis on gram-positive and resistant bacteria. Clin. Infect. Dis., *29*, 490–494.

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