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# Characteristic and Antimicrobial Resistance of *Bacillus cereus* Group Isolated from Food in Poland

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**Key words:** pastry, food safety, toxin, retail food, food control

*Bacillus cereus* is a foodborne pathogen causing food safety issues due to the formation of difficult to eliminate spores and biofilms. The objective of this study was to investigate the occurrence of *B. cereus* (conducted as part of monitoring in 2017–2018) and the presence of a toxin gene in strains isolated from retail products (pastries/cakes; vegetables, spices, delicatessen products) in Poland, and to determine the susceptibility of these microorganisms to different antimicrobial agents. A total of 267 *B. cereus* isolates from food products were examined, of which 95.51% were found positive for the presence of at least one toxin gene, with the highest frequency of the *nhe* gene (91.39%). The *hbl* and *cytK* genes were detected in 53.56% and 44.19% of *B. cereus* strains, respectively. The lowest frequency was found for the *ces* gene (2.62%). The susceptibility of *B. cereus* isolates to 16 antimicrobials was investigated. Ampicillin and penicillin resistance was the most common resistance phenotype and was identified in 100% of the *B. cereus* isolates. In addition, the tested isolates exhibited resistance to: amoxicillin-clavulanic acid (96.25%), cephalothin (67.79%), ceftriaxone (64.42%), rifampicin (46.82%), trimethoprim-sulfamethoxazole (5.62%), quinupristin/dalfopristin (4.87%), chloramphenicol (3.75%), clindamycin (2.62%), teicoplanin (1.87%), erythromycin (1.87%), ciprofloxacin (0.75%), imipenem (0.75%), tetracycline (0.37%), and gentamicin (0.37%). The study results contribute to characterizing the diversity of *B. cereus* isolated from various food products in Poland and their impact on food safety and public health. This study delivers practical information on antibiotic resistance and the frequency of toxin genes among strains isolated from food.

## INTRODUCTION

Bacteria from the *Bacillus cereus* group (or *Bacillus cereus sensu lato*) are aerobic or facultative anaerobic rod-shaped Gram-positive, endospore forming bacteria widespread in the environment [Drobniewski, 1993; Messelhäußer & Ehling-Schulz, 2018]. The group includes at least nine species: *B. cereus sensu stricto, B. thuringiensis*, *B. weihenstephanensis*, *B. mycoides*, *B. pseudomycoides*, *B. anthracis*, *B. toyonensis*, *B. widmanni*, and *B. cytotoxicus* [Ehling-Schulz *et al.*, 2019]. The *Bacillus* species share similar rDNA, high degrees of DNA-DNA relatedness and 16S-23S rDNA intergenic spacers [Hansen *et al.,* 2001; Priest *et al.*, 1994]. The high prevalence of the *B. cereus* group in soil, air, and water, as well as spore production pose problems in the food processing industry [Faille *et al.*, 2007; Rossi *et al.*, 2018]. The primary source of contamination is the soil which contains from $10^2$ to $10^7$ colony-forming units (CFU) of vegetative cells and spores of *B. cereus* in 1 g [Raymond *et al.*, 2010; Brillard *et al.*, 2015].

The *B. cereus* group poses the risk of two types of gastrointestinal diseases – the diarrhoeal and the emetic syndrome [Carlin *et al.*, 2006; Gdoura-Ben *et al.,* 2018; Rodrigo *et al.*, 2021]. The diarrhoeal syndrome is caused by enterotoxin haemolysin BL (Hbl), non-hemolytic (Nhl) and protein cytotoxin K (CytK) produced after ingestion of viable cells or spores. The emetic syndrome is caused by cereulide produced in food before ingestion. In the case of the diarrheal syndrome, *B. cereus* produces toxins in the gastrointestinal tract, while in the case of the emetic toxin, the induction of disease symptoms is related to the presence of a toxin produced in food, and the presence of *B. cereus* cells is not required.

Hbl is a thermostable enterotoxin. Haemolysin consists of three components: binding protein B (37 kDa) encoded by the *hblA*, and lytic components: L1 (38 kDa) encoded by *hblC* and L2 (46 kDa) encoded by *hblD* [Ehling-Schulz *et al.*, 2006]. The non-haemolytic enterotoxin Nhe toxin comprises three components: NheA – 41 kDa, NheB – 39 kDa, NheC – 105 kDa coded, respectively, by the *nheA*, *nheB*, and *nheC* genes [Dietrich *et al.,* 2021; Lindbäck *et al.*, 2004]. Poisonings caused by *B. cereus* strains capable of producing Nhe and Hbl toxins are manifested by watery diarrhoea and abdominal pain, which appear from 8 to 16 h after ingestion of contaminated food [Ehling-Schulz *et al.*, 2019; Schoeni & Wong, 2005]. Protein CytK (34 kDa) has the capability to form pores in lipid bilayers. Moreover, it has been shown that CytK is highly toxic towards human intestinal

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epithelial cells and can be responsible for severe food poisoning and also for necrotic enteritis [Hardy *et al.*, 2001]. The diarrhoea syndrome is frequently associated with the consumption of contaminated foods such as dairy products (*e.g.* puddings, raw and pasteurized milk) [EFSA, 2016; Gdoura-Ben *et al.*, 2018; Zhao *et al.*, 2020], soups, meat products [EFSA, 2016; Gdoura-Ben *et al.*, 2018], stews [Borge *et al.*, 2001; Shah *et al.,* 2019], sauces and vegetables [EFSA, 2016; Gdoura-Ben *et al.*, 2018]. The other sources of these bacteria include flour [EFSA, 2016; N'guessan *et al.*, 2019], pasta [EFSA, 2016; Gdoura-Ben *et al.*, 2018; Juneja *et al.*, 2019], confectionery (cakes) [EFSA, 2016], seafood [EFSA, 2016; Gdoura-Ben *et al.*, 2018], herbs and spices [EFSA, 2016; Gdoura-Ben *et al.*, 2018].

The emetic form of food poisoning involving *B. cereus* is the result of intoxication with emetic toxin (cereulide) [Marxen *et al.*, 2015]. Cereulide is one of the most resistant (heat-stable and acid-resistant) enterotoxins that stays active after being subjected to the temperature of 121°C for 90 min and at pH 2–11 for 2 h [Rajkovic *et al.*, 2008; Rouzeau-Szynalski *et al.,* 2020]. The amount of produced cereulide is related to the conditions of microbial growth, pH, temperature, and oxygen availability [Rouzeau-Szynalski *et al.,* 2020]. The optimal temperature for toxin formation is 21°C [Berthold & Doroszkiewicz, 2009]. However, significantly reduced cereulide production is observed at 8–10°C and above 35°C. The presence of oxygen in the environment significantly increases the amount of toxin formed. Characteristic symptoms of intoxication, *i.e.* nausea and vomiting, are observed 30 min to 6 h after ingestion of toxin-contaminated foodstuffs [Ehling-Schulz *et al.*, 2005; Glasset *et al.*, 2016]. Cereulide is found in food products, including pasta, rice, milk, and dairy products [Rouzeau-Szynalski *et al.,* 2020]. Symptoms may persist for about 24 h [Li *et al.,* 2021]. They often resemble poisoning with staphylococcal enterotoxin. Poisoning can lead to acute liver failure, haemolytic uremic syndrome, cerebral oedema or even death. The vomiting dose is 0.02–1.28 $\mu$g cereulide. The toxic dose for an adult human is 400–500 $\mu$g cereulide [Berthold & Doroszkiewicz, 2009]. Symptoms persist for 6 to 24 h after food intake, reminiscent of staphylococcal enterotoxin poisoning.

Most cases of foodborne outbreaks caused by the *B. cereus* group have been associated with concentrations exceeding $10^5$ CFU/g. However, there have been cases of emetic and diarrhoeal illness reporting levels of *B. cereus* ($10^3$ and $10^5$ CFU/g) [EFSA, 2016].

The objective of this study was to investigate the occurrence of *B. cereus* (conducted as part of monitoring in 2017–2018) and the presence of toxin genes in strains isolated from retail products (pastries/cakes; vegetables, spices, delicatessen products), and to determine the susceptibility of these microorganisms to different antimicrobial agents.

## MATERIALS AND METHODS

### Collection of samples and selection of isolates

The food samples were collected from 2004 to 2018 as part of the official control and monitoring program in Poland. The samples were examined at the microbiological laboratory of Provincial Sanitary and Epidemiological Stations using the method specified in PN EN ISO 7932:2005, accredited by the Polish Centre of Accreditation. In short, 10 g of each food sample was taken in an aseptic manner and homogenized in 90 mL of buffered peptone water (BPW, Biomaxima, Lublin, Poland). An aliquot of 0.1 mL of the initial suspension and further decimal dilution were transferred to mannitol egg yolk polymyxin agar plates (MYP Agar, Oxoid, Basingstoke, United Kingdom). After incubation for 24 – 48 h at 30°C, typical colonies were counted and then subjected to the haemolysis reaction test. In total, 267 *B. cereus* strains analysed in this study were collected in the years: 2004 – 11 strains; 2005 – 3; 2006 – 15; 2007 – 17; 2008 – 12; 2009 – 7; 2010 – 9; 2011 – 2; 2012 – 15; 2013 – 24; 2014 – 15; 2016 – 3; 2017 – 57; 2018 – 77. Strains isolated from the following food groups: heat-treated pastries, or non-heat-treated cream (240), delicatessen products (24), vegetables (2), and spices (1) (Supplementary Table S1), were sent to our laboratory, *i.e.*, the National Institute of Public Health NIH – National Research Institute, for further studies. Strains were recovered from −80°C brain heart infusion broth (BHI, Oxoid) with 20% glycerol (Merck, Darmstadt, Germany) into plate count agar (PCA, Biomaxima) and stored at 4°C.

### Extraction of DNA from *B. cereus* group strains

The genomic DNA was extracted from *B. cereus* cells using the Chelex-100 resin-based technique (Bio-Rad, Hercules, CA, USA). Single colonies grown on plate count agar (PCA, Bio-Rad) were suspended in 100 $\mu$L of a 5% chelating resin solution. Bacterial cells were suspended with the use of a sterile loop in a Chelex solution, incubated at 99°C for 15 min. Suspensions were cooled on ice for 2 min and centrifuged at 16,162×*g* at room temperature for 2–3 min. The DNA-containing supernatant was used as a template for the molecular analysis.

## PCR AMPLIFICATION OF 16S rDNA

PCR amplification of 16S rDNA for the detection of *B. cereus* strains was performed according to the procedure described by Hansen *et al.* [2001] with modifications. The following oligonucleotides were used: 5'-TCG AAA TTG AAA GGC GGC-3', 5'-GGT GCC AGC TTA TTC AAC-3' (Genomed, Warsaw, Poland). The final 25 $\mu$L of the PCR mixture contained a 2.5 $\mu$L DreamTaq buffer (10× concentrated, Thermo Fisher Scientific, Waltham, MA, USA), 3.75 $\mu$L of a dNTPs mix (2 mM, Thermo Fisher Scientific), 1 $\mu$L of $MgCl_2$ (1.25 mM, Thermo Fisher Scientific), 1 $\mu$L of each primer (10 $\mu$M), 0.25 $\mu$L of DreamTaq polymerase (1U, Thermo Fisher Scientific), 1 $\mu$L of DNA and water for molecular biology tests (Bio-Rad). The PCR was performed under the following conditions: 95°C–10 min, 30× (94°C–15 s, 63°C–45 s, 72°C–2 min), 72°C–2 min. Amplified PCR products were analysed on 1.5% (*w/v*) agarose gel (Prona, Narew, Poland) in a Tris-borate-ethylenediaminetetraacetic acid (TBE) buffer (1×) containing 0.15 $\mu$g/mL of Midori Green Advance DNA Stain (Genetics, Düren, Germany). Gels were run at 120 V for 1 h and photographed with a digital camera.

A GeneRuler™ 100 bp DNA Ladder (Thermo Fisher Scientific) was used as a molecular weight marker. The expected PCR product size was 288 bp. Affiliation to the *B. cereus* group by 16S rDNA amplification reaction was confirmed for all the analysed strains.

### *B. cereus* toxin identification using multiplex PCR

The PCR amplification of *nhe*, *hbl*, *cytK* and *ces* toxin genes was carried out according to the procedure described by Ehling-Schultz *et al.* [2006] with modifications in primers and $MgCl_2$ concentration. Primers synthesized by Genomed (Poland) were used (Table 1). The final 25 $\mu$L reaction mixtures contained 0.6 $\mu$L of a mix of oligonucleotide primers: cesR (100 mM), cesF (100 mM), nheR (150 mM), nheF (150 mM), cytKF (200 mM), cytKR (200 mM), hblF (500 mM), hblR (500 mM); 2.5 $\mu$L of a DreamTaq buffer (10× concentrated, Thermo Fisher Scientific); 2.5 $\mu$L of a dNTP mix (2 mM, Thermo Fisher Scientific); 1 $\mu$L of $MgCl_2$ (1.25 mM, Thermo Fisher Scientific); 0.25 $\mu$L of the DreamTaq polymerase (5U, Thermo Fisher Scientific); 1 $\mu$L of template DNA; and water for molecular biology tests (Bio-Rad). Reaction conditions were as follows: 95°C–15 min, 30× (95°C–30 s, 49°C–30 s, 72°C–1 min), 72°C–2 min. Amplified PCR products were analysed on 1.5% (*w/v*) agarose gel (Prona) in a TBE buffer (1×) containing 0.15 $\mu$g/mL of Midori Green Advance DNA Stain (Genetics). A GeneRuler™ 1 kb DNA Ladder (Thermo Fisher Scientific) and GeneRuler™ 100 bp DNA Ladder (Thermo Fisher Scientific) were used as molecular weight markers. Gels were run at 120 V for 1 h and photographed with a digital camera. The expected PCR products size was: *ces* 1271 bp, *hbl* 1091 bp, *nhe* 766 bp, and *cytK* 421 bp (Table 1).

### Antimicrobial susceptibility testing

Antibiotic susceptibility was assessed using the disc diffusion method. The test inoculum was prepared from colonies grown on PCA plates (Biomerieux, Marcy-l'Étoile, France) that had been incubated at 35°C for 18 h. Colonies were suspended in a 0.9% saline solution (Polpharma, Starogard Gdański, Poland) to obtain a suspension equivalent to the turbidity of a 0.5 McFarland standard. The cell suspension was used to swab the surface of a Mueller-Hinton agar plate (GRASO, Starogard Gdański, Poland). Discs with the following concentration of antibiotics (Oxoid) were used: penicillin G (10U), ampicillin (10 $\mu$g), cephalothin (30 $\mu$g), imipenem (10 $\mu$g), gentamicin (10 $\mu$g), chloramphenicol (30 $\mu$g), erythromycin (15 $\mu$g), tetracycline (30 $\mu$g), ciprofloxacin (5 $\mu$g), clindamycin (2 $\mu$g), sulfamethoxazole-trimethoprim (25 $\mu$g), rifampin (5 $\mu$g), ceftriaxone (30 $\mu$g), teicoplanin (30 $\mu$g), amoxicillin-clavulanic acid (30 $\mu$g), and quinupristin/dalfopristin (15 $\mu$g). The inhibition zones were measured after 20 h of incubation at 35°C. All strains were classified as sensitive, of intermediate susceptibility and resistant, following the recommendations for *Staphylococcus* spp. in the Clinical and Laboratory Standards Institute guideline M100-S22–2 [CLSI, 2012]. *Staphylococcus aureus* ATCC 25923 was used for quality control.

## RESULTS AND DISCUSSION

*B. cereus* is mainly isolated from dairy-based and flour-based products. In Poland, the presence of the presumptive *B. cereus* group is officially monitored mainly in 2 categories of food products: (a) confectionery products and products with uncooked cream, and (b) confectionery products and products with heat-treated cream which are a milk-flour-based product. The remaining reporting cases concern food indicated in consumer notifications and potential sources related to the occurrence of food poisoning.

In the years 2017–2018, the microbiological laboratory of Provincial Sanitary and Epidemiological Stations tested a total of 21,200 food samples as part of a monitoring scheme and isolated 598 presumptive *B. cereus* strains. The percentage of samples in which presumptive *B. cereus* occurred was found to be low: 2.57% in the year 2017 and 3.07% in 2018. A high level of milk-based desserts contamination with *B. cereus* of was determined in a study by Amin *et al*. [2018], *i.e.* 45% of the 150 samples tested (pudding, custard, rice with milk). What is more, research conducted by Organji *et al*. [2015] showed a high percentage of contamination of raw milk. A total of 110 samples was screened for the presence of *B. cereus* and 31.8% of the samples yielded *Bacillus*-like growth. Among them, 54.28% of the samples were *B. cereus*-positive. In recent years, other research has been conducted in Poland on *B. cereus* prevalence in other food products. Berthold-Pluta

TABLE 1. Primers used in the multiplex PCR for the detection of virulence genes in *Bacillus cereus* group.

| Targeted gene | Primer name | Sequence (5'–3') | Product size (bp) |
|---|---|---|---|
| *hbl* | hblF | GTA AAT TAI GAT GAI CAA TTTC | 1091 |
| | hblR | AGA ATA GGC ATT CAT AGA TT | |
| *nhe* | nheF | AAG CIG CTC TTC GIA TTC | 766 |
| | nheR | ITI GTT GAA ATA AGC TGT GG | |
| *cytK* | cytKF | ACA GAT ATC GGI CAA AAT GC | 421 |
| | cytKR | CAA GTI ACT TGA CCI GTT GC | |
| *ces* | cesF | GGT GAC ACA TTA TCA TAT AAG GTG | 1271 |
| | cesR | GTA AGC GAA CCT GTC RGR AAC AAC A | |

*et al.* [2019] tested samples of herbs and spices, pasta, rice, breakfast cereals, infant formulas, pasteurized milk, fresh acid and acid/rennet cheeses, mould-ripened cheeses and ripening rennet cheeses. Test results of 585 samples showed that 38.8% were contaminated by *B. cereus*. Moreover, the study carried out in Tunisia revealed a high level of food contamination by *B. cereus* [Gdoura-Ben *et al.,* 2018], where 27.8% of the 687 food samples tested (spices, cereals, cooked food, fresh-cut vegetables, canned, seafood, raw and cooked poultry meats, pastry and dairy products) were found to be contaminated. When analysing 515 samples of dairy products, Proroga *et al.* [2019] found 26.8% of them to be contaminated with *B. cereus.* Similar results were obtained by Kong *et al*. [2021]. In this study, the contamination rate in the collected samples of meat and meat products was 26.37% (159/603). Our results indicate a lower frequency of contamination of samples collected as part of monitoring studies than the results obtained by other authors. Monitoring studies in this area conducted in Poland, due to the relatively high number of samples, are one of the few studies conducted in the field of detecting presumptive *B. cereus* at such a large scale in the world. Data on the occurrence of *B. cereus* in these areas is extremely limited.

The toxin gene profiles established with the use of a multiplex PCR were presented in Table 2. The *nhe* genes were present in 244 of the 267 (91.39%) isolates. The *hbl* genes were detected in 53.56% of the tested *B. cereus* strains, while the presence of the *cytK* gene was demonstrated in 44.19%. The lowest occurrence was found for the *ces* gene. Among the 267 isolates tested, the *ces* gene was found in 14 isolates only (2.62%). In 4.49% of the analysed *B. cereus* strains, no *hbl*, *nhe*, *cytK*, *ces* genes were found. Among the 267 tested strains, all groups were present (A–G) and the following profiles were identified: A (31.09%), B (0.37%), C (19.85%), D (8.99%), E (2.25%), F (28.84%), and G (1.5%). In 2.62% of the analysed isolates, toxin profiles consistent with the above-mentioned classification [Ehling-Schultz *et al.,* 2006] were not detected. With regard to the identified occurrence of two additional new patterns, their classification was proposed as group H (*hbl*) and group I (*hbl*, *cytK*). The new profile H was identified for 1 strain isolated from pastries, while the I profile was detected in 4 strains isolated from pastries and 1 strain from vegetables.

Almost all the strains isolated from food and food-poisoning samples carried the *nhe* genes, as was observed in previous works, which is consistent with the results obtained in our study [Hansen & Hendriksen, 2001]. The enterotoxigenic profiles of 51 *B. cereus* strains isolated from food prove that the *cytK* gene and *hbl-nhe-cytK* enterotoxin genes (group A according to Ehling-Schultz *et al.* [2006] classification) were isolated among foodborne samples in 37% of the strains [Guinebretière & Broussolle, 2002]. A study carried out in Germany also presented similar results – 91.2, 83.0, and 37.4% of the isolates were positive for the *hbl*, *nhe,* and *cytK* toxins genes, respectively. The *ces* gene was not detected [Fiedler *et al.*, 2019]. The *hbl*ACD gene cluster was found in 39% of the *B. cereus* strains isolated from ready-to-eat food in China, the *nhe* (A, B, C) genes were found in 89, 99, and 94% of the isolates, respectively, the *cytK* gene – in 68% of strains, while only 7% of them were identified as carrying the *cesB* gene [Yu *et al.*, 2020]. According to the toxin gene profile classification by Ehling-Schulz *et al.* [2006], the majority of strains isolated from cucumbers, carrots, herbs, salad leaves, and ready-to-eat mixed salad leaves (79 of 147; 53.7%) belong to the toxin gene profile C [Fiedler *et al.*, 2019]. In addition, about 25% were not defined as a toxin gene profile. The occurrence of other toxin gene profiles F and G in the study was, respectively at 9%, and 3%. According to the results for *B. cereus* strains isolated from milk powder (130 isolates) and Ras-cheese (70 isolates), the *nhe* gene was detected in all strains (both tested products) [Abdeen *et al.*, 2020]. For milk, *cytK*, *hbl*, *ces* genes were prevalent in 55.5%, 33.3%, and 22.2% of the strains, respectively. Whereas, to

TABLE 2. Toxin profile of *Bacillus cereus* isolated from retail food in Poland.

| Profile | Gene | | | | Source (number of isolates) | | | |
|---|---|---|---|---|---|---|---|---|
| | *hbl* | *nhe* | *cytK* | *ces* | Pastries (240) | Delicatessen products (24) | Vegetables (2) | Spices (1) |
| A | ■ | ■ | ■ | | 74 | 8 | - | 1 |
| B | | ■ | ■ | ■ | 1 | - | - | - |
| C | ■ | ■ | | | 45 | 8 | - | - |
| D | | ■ | ■ | | 24 | - | - | - |
| E | | ■ | | ■ | 5 | 1 | - | - |
| F | | ■ | | | 70 | 7 | - | - |
| G | | | ■ | | 4 | - | - | - |
| Unclassified H* | ■ | | | | 1 | - | - | - |
| Unclassified I* | ■ | | ■ | | 4 | - | 2 | - |
| None detected | | | | | 12 | - | - | - |

*New classification groups; black boxes – gene presence. The source of strain isolation from food products and detailed results are described in Supplementary Table 1.

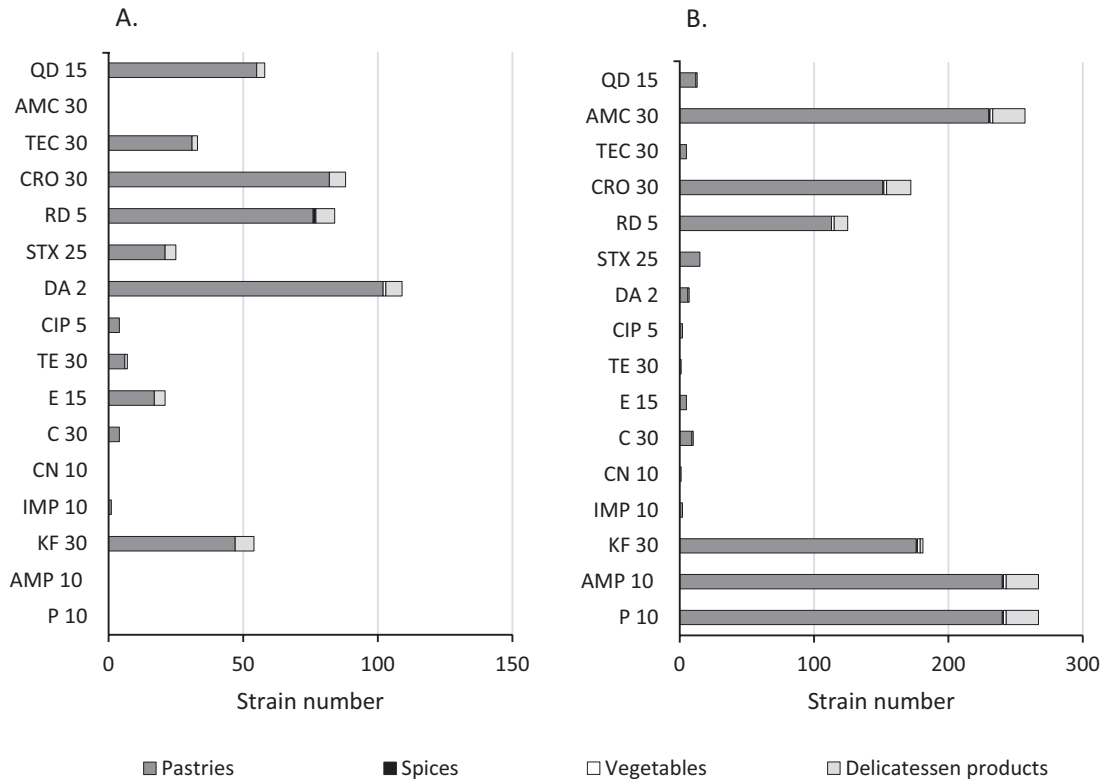


FIGURE 1. Susceptibility of *Bacillus cereus* stains isolated from food products to antimicrobials; A – number of intermediate strains, B – number of resistant strains; P 10 (penicillin G, 10U), AMP 10 (ampicillin, 10 $\mu$g), KF 30 (cephalothin, 30 $\mu$g), IMP 10 (imipenem,10 $\mu$g), CN 10 (gentamicin, 10 $\mu$g), C 30 (chloramphenicol, 30 $\mu$g), E 15 (erythromycin, 15 $\mu$g), TE 30 (tetracycline, 30 $\mu$g), CIP 5 (ciprofloxacin, 5 $\mu$g), DA 2 (clindamycin, 2 $\mu$g), STX 25 (trimethoprim-sulfamethoxazole, 25 $\mu$g), RD 5 (rifampicin, 5 $\mu$g), CRO 30 (ceftriaxone, 30 $\mu$g), TEC 30 (teicoplanin, 30 $\mu$g), AMC 30 (amoxicillin-clavulanic acid, 30 $\mu$g), QD 15 (quinupristin/dalfopristine, 15 $\mu$g).

the best of our knowledge, there is no data in the literature about *B. cereus* toxin profiles isolated from pastries or cakes.

The increase in drug resistance of strains isolated from food, including bacteria from the *B. cereus* group also gives rise to a serious problem. Antimicrobial resistance is a growing global threat that includes both human, animal and environmental issues. In our study, we investigated the susceptibility of *B. cereus* isolates to 16 antimicrobials. The antibiotic susceptibility of strains is presented in Figure 1. The conducted tests demonstrated the resistance of all analysed strains to penicillin G (100%) and ampicillin (100%). In addition, the tested isolates were resistant to: amoxicillin-clavulanic acid (96.25%), cephalothin (67.79%), ceftriaxone (64.42%), rifampicin (46.82%), trimethoprim-sulfamethoxazole (5.62%), quinupristin/dalfopristin (4.87%), chloramphenicol (3.75%), clindamycin (2.62%), teicoplanin (1.87%), erythromycin (1.87%), ciprofloxacin (0.75%), imipenem (0.75%), tetracycline (0.37%), and gentamicin (0.37%). The study confirmed intermediate susceptibility of the strains to: clindamycin (40.82%), ceftriaxone (32.96%), rifampicin (31.46%), quinupristin/dalfopristin (21.72%), cephalothin (20.22%), teicoplanin (12.36%), trimethoprim-sulfamethoxazole (9.36%), erythromycin (7.87%), tetracycline (2.62%), chloramphenicol (1.5%), ciprofloxacin (1.5%), and imipenem (0.37%).

According to the antibiotic susceptibility tests, most of the *B. cereus* group strains isolated from food in Poland were resistant to β-lactam antibiotics including penicillin ampicillin, and cephalothin, which is consistent with previous studies on the antibiotic resistance of *B. cereus* in food products [Gao *et al*., 2018; György *et al*., 2021; Yibar *et al*., 2017]. Yu *et al.* [2020] found that most isolates were resistant to penicillin (99.7%), ampicillin (99.7%), and amoxicillin-clavulanic (97.6%), which is compliant with our results. Additionally, Yu *et al.* [2020] revealed that strains were resistant to rifampicin (83%), cephalothin (86.7%), quinupristin/dalfopristin (19.57%), tetracycline (15.49%), and trimethoprim-sulfamethoxazole (12.5%), while the results of our study indicate lower rates of resistance to these antibiotics (rifampicin (46.82%), cephalothin (67.79%), quinupristin/dalfopristin (4.87%), tetracycline (0.37%), and trimethoprim-sulfamethoxazole (5.62%). Most isolates were sensitive to: gentamycin (97.6%), imipenem (99.7%), ciprofloxacin (92.9%), chloramphenicol (94.6%), and teicoplanin (81%). Isolates also showed intermediate resistance to quinupristin (61.9%) and clindamycin (74.8%). In this study, *B. cereus* strains showed similar susceptibility to the above-mentioned antibiotics. Owusu-Kwarteng *et al.* [2017] reported resistance to penicillin (100%), amoxicillin (100%), ampicillin (98%), trimethoprim/sulfamethoxazole (80% with 20% intermediate resistant strains), and susceptibility to other antimicrobials such as chloramphenicol (99%), ciprofloxacin (100%), clindamycin (100%), erythromycin (92%), gentamicin (100%), quinupristin/dalfopristin (100%), rifampin (100%), tetracycline (97%), and vancomycin (100%) for *B. cereus* isolated from soil, milk or milk-based products. Similarly to the previous study and our results, the isolates

demonstrated 100% resistance to penicillin and were mostly sensitive to gentamycin, imipenem, ciprofloxacin, erythromycin, and chloramphenicol [Park *et al.*, 2020]. The presented result is consistent with findings of other authors demonstrating that *B. cereus* is susceptible to chloramphenicol, ciprofloxacin, erythromycin, gentamicin, and imipenem [Al-Khatib *et al.*, 2007; Zhao *et al.*, 2020].

Bacteria from the *B. cereus* group, in addition to food poisoning, are sometimes associated with infections, including among others, those of the central nervous system, bacteraemia, respiratory tract infections, and endocarditis [Bianco *et al.,* 2021; De Medts *et al*., 2018; Ribeiro *et al.,* 2022]. The incidence for this type of infection is low, although the mortality rate is high. The spread of antimicrobial-resistant bacteria in the environment is a major public health concern and is associated with increasing mortality and costs of treatment. Therefore, it is important to assess the antimicrobial susceptibility of bacteria isolated from food.

## CONCLUSIONS

The obtained results contribute to characterizing the diversity of *B. cereus* isolated from various products and their impact on food safety and public health. Our study revealed that 95.51% of *B. cereus* strains isolated from food products in Poland were positive for the presence of at least one or more toxin genes, with the highest occurrence of the *nhe* gene. Additionally, the tested strains were resistant to a wide spectrum of antibiotics tested. Due to the high prevalence of toxin genes and the occurrence of antibiotic resistance among the isolates, continuous monitoring of presumptive *B. cereus* is strongly recommended based on the 'One Health' approach in order to evaluate the risk posed to human health by food consumption.

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## CONFLICTS OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## SUPPLEMENTARY MATERIALS

The following are available online at http://journal.pan.olsztyn.pl/Characteristic-and-Antimicrobial-Resistance-of-Bacillus-cereus-Group-Isolated-from,152677,0,2.html; Detailed information about strains and antimicrobial susceptibility.

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