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Antimicrobial characterization of *Bacillus cereus* Group Strains Isolated from Different Food Sources in Apulia and Basilicata Regions

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Abstract: *Bacillus cereus* is a gram-positive bacterium widespread in the environment, especially in soil and dust. It produces two types of toxins causing vomiting and diarrhea. Nowadays, food borne outbreaks due to *Bacillus cereus* group bacteria (especially *Bacillus cereus* sensu stricto) are increased, as reported by European Food Safety Authority, representing a very huge problem in agri-food chain. In this work, we analyzed 118 strains belonging to *Bacillus cereus* group, isolated from several food sources (fruit and vegetables, dairy products, bakery products) concerning their susceptibility to antibiotics currently most used to treat infections. Many strains showing an intermediate susceptibility to clindamycin, erythromycin, and tetracycline were detected, suggesting an evolving acquisition of resistance against these antibiotics. Moreover, one strain showed intermediate resistance to meropenem, another antibiotic currently used to treat infections caused by *Bacillus cereus*. Beside the antimicrobial characterization, all strains were studied comparing their antimicrobic phenotype with the presence/absence of antimicrobial genes in their genome. The analysis showed a not complete correlation between genes carried by the strains and their phenotype, demonstrating that the antibiotic resistance is due not only to genetic factors, but also to other factors such as the inappropriate use of antibiotics that can determine an acquired resistance for bacteria.

Keywords: antibiotic resistance; *Bacillus cereus* group; food poisoning; minimum inhibitory concentration; WGS

1. Introduction

The *Bacillus cereus* group, also named *Bacillus cereus* sensu lato, is an heterogenous group of aerobic or facultative anaerobes bacteria, consisting in several species phylogenetically correlated [1,2]. They are Gram positive, ubiquitous in the environment and can grow up at optimal temperature ranging from 30 to 40°C and in the range pH between 5 and 8.8 [3]. Most strains are catalase positive and mobile [1]. One of the peculiar characteristics of these microorganisms is their ability to form spores, metabolically dormant cell types, resistant to extreme conditions including heat, freezing, drying and radiation (commonly used in food industry) [4,5].

In the environment, they populate all kinds of soils and waters, sediments, plants in the spore form, but they are also detected in animals [6,7]. Because of its widespread in environment, *Bacillus* spores could contaminate raw food ingredients (vegetables, milk, fruit, spices, cereals) employed during food processing. Thus, a wide variety of finished food products might contain these bacteria and germination/outgrowth during storage is also possible, causing foodstuff spoilage [8].

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The B. cereus group consists of several species, including Bacillus cereus sensu scricto, Bacillus thuringensis, Bacillus weihenstephanensis, Bacillus wiedmanni, and Bacillus toyonensis [2,9,10].

B. cereus sensu scricto (*B. cereus* s.s.) is the model species of the *Bacillus cereus* group. It can complete a full saprophytic life cycle, but also be an opportunistic human pathogen [11] that causes gastrointestinal illness, bacteremia, endocarditis, respiratory and urinary tract infections, endophthalmitis and meningitis [2,10,12]. Moreover, *B. cereus* s.s. is one of the most common pathogens in food poisoning [13]. It causes two type of food poisoning: the diarrhea and the emetic syndrome. The diarrhea syndrome is caused by a complex of three toxins (hemolysin BL, nonhemolytic enterotoxin and cytotoxic protein), while the emetic syndrome is due to cereulide, a thermostable protein [11].

B. thuringiensis is an entomopathogen affecting many orders of insect and for this reason used as biopesticides [10]. More recently, however, *B. thuringiensis* was found involved in various infectious diseases in humans, such as food-poisoning-associated diarrheas and wound infections [14].

B. weihenstephanensis generally is non-pathogenic saprophyte or rarely associated with diseases in humans or other animals. Some strains of *B. weihenstephanensis* could produce the emetic toxin called cereulide (like *B. cereus* s.s.) responsible of food intoxication accompanied by vomiting [15].

Concerning *B. wiedmanni*, it is not considered a human pathogen; however, Miller and colleagues [16] characterized the strain FSL W8-0169T, isolated from dairy foods and dairy environments as a potential pathogen being capable of expressing the toxins HBL and the non-haemolytic toxin NHE.

At last, *B. toyonensis* has been isolated from pathogenic intraocular specimen, demonstrating its ability to cause endophthalmitis [17].

B. cereus s.s. and related bacteria are considered manly responsible of foodborne diseases. According to EFSA, *B. cereus* originates an important number of food-borne illnesses in humans [18] and the real number of cases is underestimated, at the date. In particular, *B. cereus* s.s. has been incriminated as a cause of toxin-induced emetic and diarrheagenic syndromes after ingestion [1] which represent the foremost worries for the public Health service [19], considering the different spectrum of diseases provoked (from gastrointestinal forms that require short time of recovery to most serious systemic diseases like bacteremia and septicemia that could have a fatal outcome). Generally, *B. cereus* related infections symptoms start from 0.5 to 16 hours after ingestions of contaminated food and disappear within 24 hours and do not require drug treatments [20]. Severe *B. cereus* infections were treated with antibiotics, but an excessive and wrong use of these molecules could lead to antibiotic resistance phenomenon [21]. Nowadays, antibiotic resistance is a huge problem in public health and a global priority because the infections caused by resistant bacteria are harder to treat than those caused by non-resistant ones, leading to higher medical costs, prolonged hospital stays, and increased mortality [22].

Hence, active surveillance of food contamination is necessary. Moreover, an antibiotic resistance surveillance plan is also important, not only concerning clinically relevant bacteria but also for other pathogens, like *B. cereus* group members, in order to prevent new infections and to know how dealing with them in presence of resistant bacteria.

In this study we analyzed different food sources regarding the presence of bacteria belonging to *Bacillus cereus* group to investigate the presence of these in different food sources and we evaluated *in vitro* their sensitivity to antimicrobials, also correlating these results with genetic analyses.

2. Results

2.1. Isolation of B. cereus Group Strains

In the analyzed samples, the isolation of *B. cereus* colonies was evidenced by the presence of typical color morphology, being dull gray and opaque, with a rough matted surface and irregular perimeters, with zones of hemolysis surrounding colonies.

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2.2. MALDI-TOF Mass Spectrometry (MS) Analysis

By MALDI-TOF MS, all the analyzed strains were identified as *B. cereus* using the commercial BDAL library (MBT Compass library v 7.0.0.0) with a log(score) generally between 1.7 and 2.0, accompanied by the following comment: "*Bacillus anthracis, cereus, mycoides, pseudomycoides, thuringiensis* and *weihenstephanensis* are closely related and members of the *Bacillus cereus* group. In particular, *Bacillus cereus* spectra are very similar to spectra from *Bacillus anthracis*. *Bacillus anthracis* is not included in the MALDI Biotyper database. For differentiation, an adequate identification method must be selected by an experienced professional. The quality of spectra (score) depends on the degree of sporulation: Use fresh material".

2.3. WGS Analysis

In detail, 68 *B. cereus* sensu stricto, 23 *B.thuringensis*, 19 *B. wiedmannii*, 7 B. *toyonensis* and 1 *B.weihenstephanensis* were identified by WGS. As regards the food matrices from which strains were isolated, 42 *B. cereus* sensu stricto were detected in milk and dairy products (including ice cream), 11 in fruit and vegetables, 7 in bakery products, 3 in fish-based products, 2 in meat and 3 in mixed plates (russian salad, focaccia with scamorza, sandwich with cured meat).

We also identified other members of *B. cereus* s.l.: *B. thuringensis, B. toyonensis, B. wiedmanni* and *B. weihestephanensis*. These species were identified mainly in milk and its derivates (16 *B. thuringensis*, 13 *B. wiedmanni*, 3 *B. toyonensis*, and 1 *B. weihestephanensis*) and vegetables (3 *B. thuringensis*, 1 *B. wiedmanni*, 3 *B. toyonensis*). Moreover, they were isolated also from meat (1 *B. thuringensis* and 1 *B. wiedmanni*), fish (1 *B. thuringensis* and 1 *B. wiedmanni*) and bakery products (2 B. *thuringensis*, 2 *B. wiedmanni* and 1 *B. toyonensis*).

In addition, 1 *B. wiedmanni* was found in a box of ravioli, a kind of Italian pasta, usually filled with meat, cheese, fish, vegetables or with a mix of them.

An overview of the identified strains, and of the food sources was provided in Table S1.

The bioinformatic analysis allowed us to predict genes responsible of antimicrobic resistance (Figure 1 and Table S2).

All strains possess at least two β-lactams resistance genes. In detail, we have found *bcI* in 68/118 (58%), *bcII* in 82/118 (69%), *bla* in 116/118 (98%), *bla1* in 116/118 (98%), *bla2* in 114/118 (97%), *blaP* in 32/118 (27%) and *blaZ* in 60/118 (51%).

2% of isolates (2/118) carried fluoroquinolone genes (*arlS*) and 2% (2/118) carried *catA* gene responsible for chloramphenicol resistance.

Macrolide resistance genes *Bacillus_cluster_A_intrinsic_mph*, *Bacillus_cluster_B_intrinsic_mph* and *mph(B)* were found in 52/118 (44%), 4/118 (3%) and 52/118 (44%) respectively.

Moreover, 25/118 (21%) carried tetracycline resistance gene (*tetL*) and 58/118 (49%) possess clindamycin resistance gene *(lsaB*)

Others AMR genes identified were vancomycin resistance genes: *vanR* (118/118 strains, 100%), *vanRA* (15/118 strains, 16%), *vanRB* (10/118 strains, 8%), *vanRF* (17/118 strains, 14%), *vanRM* (101/118 strains, 86%), *vanR-Pt* (15/118 strains, 13%), *vanS* (15/118 strains, 13%), *vanS-Pt2* (14/118 strains, 12%), vanW (0/118 strains, 0%), vanYA (7/118 strains, 6%), *vanYF* (10/118 strains, 8%), *vanY-Pt* (15/118 strains, 13%), *vanZF* (106/118 strains, 90%).

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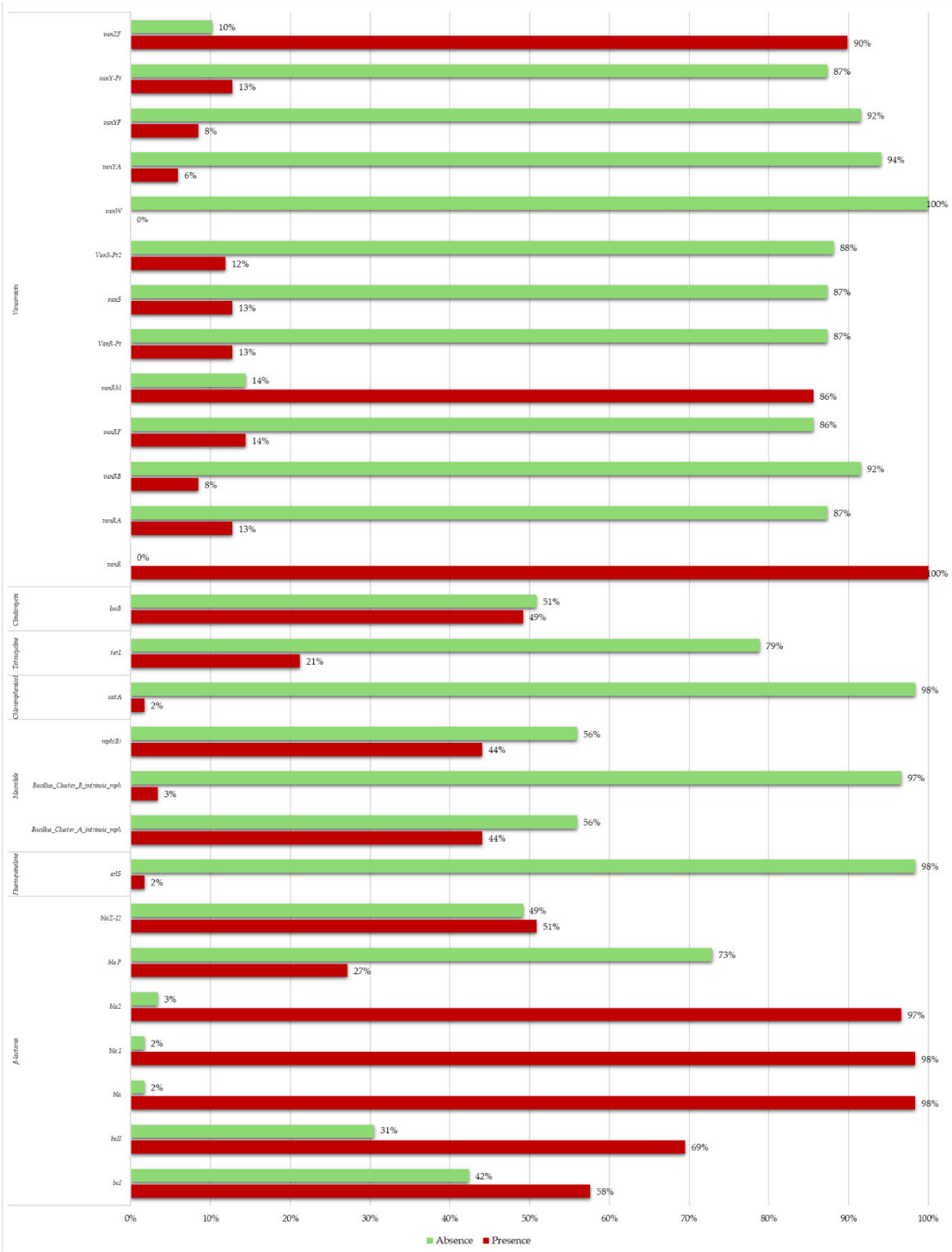

Figure 1. The histogram shows the percentages of presence (in red) or absence (in green) of AMR genes detected in *Bacillus cereus* group stains analyzed in this study.

2.4. Antibiotic Susceptibility Analysis

After 16h of incubation at 37°C, the 96 well plates have been read and MIC values checked. All the 118 strains (100%) showed resistance to penicillin G, according to literature [23] while were sensitive against gentamicin, doxycycline, ciprofloxacin, rifampicin, chloramphenicol, vancomycin, and linezolid. Concerning meropenem, all strains resulted to be susceptible to this antibiotic, except for one strain (0.9%) (BC399A) that showed an intermediate behavior.

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Moreover, 3/118 strains (2.5%) showed intermediate resistance to tetracycline (BC290A, BC329A, BC281A), 8/118 (6.8%) to erythromycin (BC2D, BC56B, BC93B, BC147A, BC166A, BC282A, BC398C, BC423A) and 23/118 (19.5%) to clindamycin (BC2A, BC4D, BC23B, BC25B, BC41A, BC56B, BC83B, BC85A, BC91C, BC156C, BC171A, BC187A, BC204A, BC223B, BC313A, BC329A, BC246A, BC266A, BC324A, BC335A, BC375A, BC391A, BC404A).

We observed some strains showing contemporary intermediate susceptibility towards two or more antibiotics. Specifically, the strain BC56B had intermediate resistance to erythromycin and clindamycin and the strain BC329A to both tetracycline and clindamycin.

An overview of MIC values and their interpretation is provided by Figure 2 and Table S3.

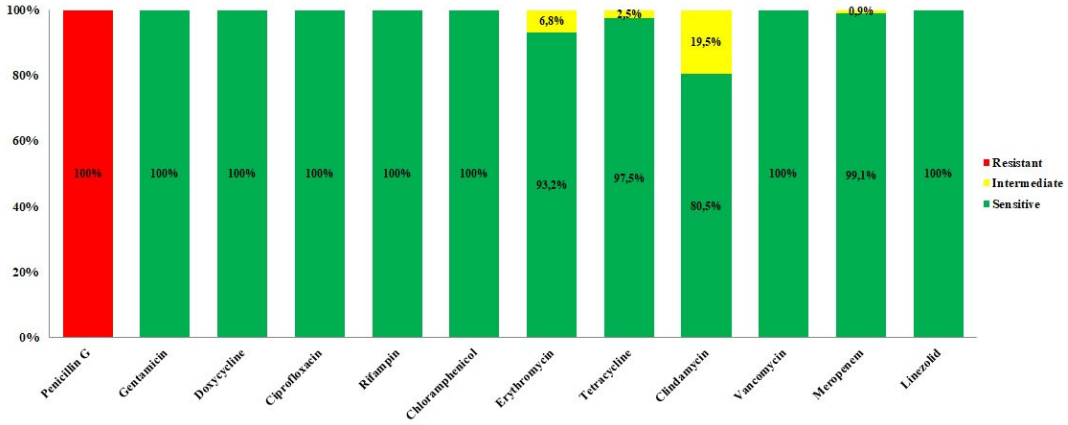

Figure 2. In this graphic are showed the percentages of sensitivity (in green), resistance (in red) and intermediate behavior (in yellow) of the *Bacillus cereus* group strains tested in this study against 12 clinically relevant antibiotics.

3. Discussion

B. cereus s.s. can easily contaminate various types of food. It has been frequently isolated in milk and dairy products, meat and derivatives and additionally in plant origin products (i.e. vegetables ready-to-eat or to be used as ingredient) as a consequence of soil contamination [4]. In our study, we identified *B. cereus* s.s. as the main member of *Bacillus cereus* s.l. involved in food contamination. Sixty-eight strains were identified as *B. cereus* s.s.; most of them (42 strains) were isolated from milk and dairy products (i.e. mozzarella, scamorza, ricotta), followed by vegetables and bakery products (flour, biscuits, sweets). Also, meat and fish resulted contaminated by *B. cereus* s.s., even if to a lesser extent.

Moreover, *B. cereus* s.s. was also found in mixed sources composed by mixed elements (sandwich with cured meats, russian salad and focaccia with scamorza) that makes it difficult to determine which single food is contaminated and at what level contamination may have occurred (at primary food level or during industry processing). Other members of *Bacillus cereus* group identified in food matrices in this study were *B. thuringensis, B. toyonensis, B. wiedmanni* and *B. weihestephanensis*.

Bacillus species overmentioned are not commonly human pathogens and useful particularly in the agricultural field [24–27]. These bacteria, even if are underestimated as risk for human health, can represent a serious risk in food safety. However, they could cause food poisoning, since some strains are able to produce toxins and virulence factors like *B. cereus* s.s. [14–16].

Bacteria can acquire ARGs through horizontal gene transfer (HGT) that involve different type of genetic elements (plasmids, integrons and transposons) but also different way of transfer (transformation, conjugation, transduction) [28]. Transfer could undergo among the same species or different ones, and it is also possible that residual DNA carrying ARGs could persist in environment for a long time even after the death of resistant strains, with the possibility to transfer them to other strains [29]. This is the reason why the investigation of antimicrobic susceptibility is interesting also for not-highly pathogenic and not pathogenic bacteria like *B. cereus* group members.

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Concerning antimicrobial susceptibility, all of 118 isolated strains were phenotypic sensitive to gentamicin, doxycycline, ciprofloxacin, rifampicin, chloramphenicol, vancomycin and linezolid according to data actually in literature [19,30–32]. Gentamicin, vancomycin and linezolid are commonly used in severe *B. cereus* infections [33] and our results confirmed the efficacy of these antibiotics.

Clindamycin is another antibiotic commonly used in treatment of *B. cereus* infections [33]. In our study, 23 strains (15 *B. cereus* s.s., 6 *B. thuringensis* and 2 *B. wiedmanni*) (20,3% of total strains) showed phenotypic intermediate susceptibility to clindamycin, suggesting an evolving acquisition of antibiotic resistance, in agreement with other studies [30,32,33].

Furthermore, 8 strains (4 *B. cereus* s.s., 2 *B. thuringensis*, 1 *B. wiedmanni* and 1 *B. toyonensis*), corresponding to 6,8% of strains, showed phenotypic intermediate susceptibility to erythromycin, according to current literature [9,23,32]; while a lower number of strains (3 isolates of *B. cereus* s.s., 2,5% of strains), showed an intermediate susceptibility to tetracycline, as previously reported [30,34,35].

We also observed an interesting intermediate behavior of only 1 *B. wiedmanni* strain against meropenem, an antibiotic often used in clinical practice. Meropenem belongs to carbapenems drug class, β-lactam antibiotics that are active against many aerobic and anaerobic gram-positive and gram-negative bacteria. It works against extended-spectrum β-lactamases [36], but may be more susceptible to metallo-β-lactamases. Probably, its intermediate behavior is due to the presence of the *bla2*, a chromosomal-encoded β-lactamase, which has penicillin, cephalosporin, and carbapenem-hydrolizing abilities [37].

Alongside these results, in this study we also demonstrated that often there is a not strict correlation between genes carried by bacterial strains (Table S2) and phenotypic characteristics (Table S3). For example, most *B. cereus* group strains isolated in this work carried vancomycin resistance genes, but all of them resulted susceptible to this antibiotic.

Concerning the 8 strains with intermediate resistance to erythromycin, all of them carried genes responsible for resistance to this molecule, but other strains did not show an intermediate or resistant phenotype even if they possess resistance gene to macrolide class.

Likewise, only 3 strains possess the gene responsible to tetracycline (*tetL*) and at the same time show an intermediate susceptibility to this antibiotic.

All these phenotypic and genetic data collected during this study are not surprising, considering the so called "silent resistome" [38–40]. The word resistome indicates all genes able to confer single or multiple antimicrobial resistance [41] and includes constitutively expressed genes, precursors and acquired AMR genes [40].

Particularly interesting is the concept of silent antimicrobial resistance genes, also called cryptic genes. These are plasmid or chromosomal DNA sequences, carried by bacteria, that are not normally expressed or expresses at very low level, without a non-correspondence to phenotypic resistance to the antibiotics [39,40]. Expression of AMR genes, usually, is not active because of the metabolic cost of this process and for this reason from the bacterial perspective silencing is very essential to preserve fitness. This phenomenon could explain why many strains of this study did not show alteration of antibiotic susceptibility even if they possess AMR genes.

In addition, we observe a very low percentage (1.7%) of strains showing an intermediate susceptibility against two different antibiotics simultaneously (2/118 strains), suggesting that also in *B. cereus* group bacteria a progressive acquisition of multidrug resistance could happen, leading to antibiotic treatment failure.

4. Materials and Methods

4.1. B. cereus Group Isolated Strains

A total of 118 *B. cereus* group strains analyzed in this study were isolated from different types of food (dairy products, vegetables and fruit, fish and meat, bakery products), collected in different production and distribution sites of different cities of Apulia and Basilicata regions, in Southern Italy.

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Cultivation was performed according to ISO 21871:2006 which specifies the method for the detection of *Bacillus cereus* from products intended for human consumption. Briefly, 5 g of food matrix were added to 45 mL of Buffered Peptone Water (BPW) (Biolife Italiana, Milan, Italy) and homogenized using a stomacher (PBI International, Milan, Italy) at 230 rpm for 30s. Then, 45 mL of double-strength Tryptone Soy Polymyxin Broth (TSPB) (Biolife Italiana, Milan, Italy) were added and samples were incubated at 30°C for 48 h. After incubation, 10 mL of enrichment broth were streaked onto the surface of solid selective medium Mannitol Egg Yolk Polymyxin Agar (MYP) (Biolife Italiana, Milan, Italy) and the plates were incubated at 30°C for 24-48h under aerobic condition. Then, typical presumptive *Bacillus cereus* group colonies for each sample were picked and subcultured on Columbia Agar with 5% sheep blood. After incubation step at 37°C for 18–24 h, the bacterial isolates were subjected to Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) [42] and subsequently to DNA extraction using the DNAeasy Blood and Tissue kit (Qiagen, Hilden, Germany) following the manufacturer's protocol for Gram-positive bacteria. Identification and typing of all strains were performed using Whole Genome Sequencing (WGS) method [30].

4.2. MALDI-TOF Mass Spectrometry Analysis

As first screening, MALDI-TOF MS analysis was performed for a fast identification of the *Bacillus cereus* group bacteria. The bacterial colony was directly transfer using a toothpick on the 96-well target plate (Bruker Daltonics, Germany), covered with 1 µL of 70% formic acid, dried at room temperature, and then overlaid with 1 µL of an α-cyano-4-hydroxycinnamic acid (CHCA) matrix solution (a saturated solution of α-cyano-4-hydroxycinnamic acid in in 50/50 [v/v] of acetonitrile/H2O containing 2.5% trifluoroacetic acid) and allowed to dry. The mass spectra were acquired using Microflex LT/SH™ mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany), operating in linear positive mode covering a mass range between 2 and 20kDa. For validation of runs, the instrument was calibrated using Bruker Bacterial Test Standard (BTS, Bruker Daltonik GmbH, Bremen, Germany), an extract of the *Escherichia coli* DH5α strain spiked with two additional pure proteins (RNase A of 13,683.2 Da and myoglobin of 16,952.3 Da).

4.3. WGS Sequencing and Bioinformatic Analyses

Typing analysis of the 118 strains was performed by WGS, since MALDI-TOF MS is not definitively exhaustive for the identification of the bacteria belonging to *Bacillus cereus* group. Extracted DNA quality and concentrations were estimated by Qubit 3.0 using Qubit dsDNA HS Assay (Thermo Fisher Scientific). Libraries were prepared using the DNA Prep Illumina (Illumina, San Diego, CA, United States) and sequencing was performed. For each isolate, paired-end genomic libraries were prepared using DNA Prep Illumina. Sequencing was performed on Illumina MiSeq platform with 500 cycle chemistry (2 x 250 pair-end reads). The raw data were trimmed using Trimmomatic (Galaxy Version 0.36.6) [43] and then the draft genomes were generated by SPAdes 3.12.0 [44] de novo assembler, with default parameters. Draft genomes were submitted to BTyper 2.3.2 [45] as previously described in Bianco and colleagues [30]. The many functions of the tool include the definition of species using ANI blasts and the prediction of the major virulence genes of the studied species. Additionally, with the aim to identify the antibiotic resistance genes, the draft genomes were also analyzed using the software ABRicate (Galaxy Version 0.8), which includes different predownloaded databases: ARG-ANNOT [46]; NCBI AMRFinderPlus [47]; CARD [48]; ResFinder [49]; PlasmidFinder [50]. The draft genomes of *B. cereus* identified have been deposited in GenBank as BioProject PRJNA826696.

4.4. Antimicrobial Susceptibility Tests

Microdilution method was used to verify the behavior of each isolate towards the main antibiotic classes (β – lactam, aminoglycosides, tetracycline, fluoroquinolones, rifamycin, chloramphenicol, macrolides, lincosamides, glycopeptides, carbapenim and oxazolidinones), according to Clinical and

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Laboratory Standard Institute guidelines [51,52]. Antibiotics and related concentrations used for microdilution experiments were: penicillin G (0.031-4 µg/mL), gentamicin (0.125-16µg/mL), doxycycline (0.25-32 µg/mL), ciprofloxacin (0.25– 32 µg/mL), rifampin (0.25– 32 µg/mL), chloramphenicol (1-128 µg/mL), erythromycin (0.125-16 µg/mL), tetracycline (0.125-16 µg/mL), clindamycin (0.125-16 µg/mL), vancomycin (0.125-16 µg/mL), meropenem (0.125 - 16µg/mL) and linezolid (0.125 - 16µg/mL). Antibiotic powders were resuspended in appropriate solvent and then 100 µl of solution was pipetting into 96 well plates. Scalar dilutions of each antibiotic were set up, in a range of values included in the reference breakpoints.

After overnight incubation on Columbia Agar with 5% sheep blood, 1-2 bacterial colonies were suspended in sterile saline solution at 0.5 McFarland standard. The bacterial suspensions were further diluted 1:100 in CAMHB (cationic adjusted Muller-Hinton broth) and then inoculated into 96 well plates containing specific antibiotic. The bacterial growth was detected after 16 h of incubation at 37°C. Minimum Inhibiting Concentration (MIC) was detected for each antibiotic. It corresponds to the lowest antibiotic concentration able to inhibit bacterial growth. The obtained MIC values were interpreted using CLSI breakpoints [51,52].

The CLSI breakpoints (mg/mL) for penicillin G, meropenem, vancomycin, gentamicin, erythromycin, tetracycline, ciprofloxacin, clindamycin, chloramphenicol, and rifampin were those suggested for *Bacillus* spp. (not *Bacillus anthracis*), according to CLSI document M45 [52] whereas for linezolid and doxycycline interpretative criteria for *Staphylococcus* spp. were used according to CLSI M100 [51]. *Staphylococcus aureus* ATCC 29213 and *E. coli* ATCC 25922 were used as control strains, to check the experimental validity.

5. Conclusions

B. cereus infection is much more frequent than expected, and new data suggest its involvement in food contamination, even if the incidence of these foodborne diseases is also likely to be underreported because associated illness is usually self-limiting and not severe and hence, they usually remain undiagnosed [53,54]. *Bacillus cereus* group bacteria can be detected in food, prevalently in milk and its derivative, but also in other products (like vegetable, meat, and fish), causing food poisoning. Food contamination could happen at different stage of processing and is not possible to completely control this phenomenon. However, the management of the infections caused by these bacteria should involve also the analysis of antibiotic susceptibility of strains and their genetic makeup. Usually, in clinical practice, the most used antibiotics are meropenem, ciprofloxacin, vancomycin, erythromycin, clindamycin and linezolid. Monitoring the susceptibility of the circulating strains against these and/or other antibiotics, through minimal inhibitory concentration (MIC), could lead to an improvement of medical treatment strategies, in the selection of the most appropriate antibiotics, in order to avoid antibiotic therapy failure and the possible development of the antimicrobial resistance phenomenon. Moreover, molecular methods like WGS analysis, should be simultaneously performed to detect strains carrying "silent antimicrobial resistance genes". These strains represent a very huge problem for public health because they represent a reservoir of ARGs that could be transmitted via HGT among species and genera and they can become active in cell host, determining a new resistance phenotype [38–40].

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Table S1: ID strains, species of *B. cereus* group and source of isolation; Table S2: Presence (●) or absence of AMR genes detected in *Bacillus cereus* group stains analyzed in this study; Table S3: MIC value and their interpretation of the *Bacillus cereus* group strains tested in this study.

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Data Availability Statement: The data presented in this study are openly available in GenBank (BioProject PRJNA826696). [GenBank] [https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA826696] [BioProject PRJNA826696]

Conflicts of Interest: The authors declare no conflicts of interest.

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