

Antibiotics Resistance Profile of *Bacillus cereus* Strains Isolated from Soil and Pepper in Brazzaville

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Abstract

Introduction: *Bacillus cereus* and spores produced in various ecological niches are responsible for toxic infections in humans. This study is conducted to determine the antibiotics resistance profile of *B. cereus* strains isolated from soil and pepper consummated in Brazzaville. **Methodology:** An antimicrobial susceptibility test of 16 *B. cereus* strains from soil and peppers was performed using 11 antibiotics by the Kirby-Bauer's diffusion on disc method. **Results:** Results revealed 100% (16/16) of resistance in penicillin G, amoxicillin, ceftazidime, rifampicin, and colistin, also 18.75% (3/16), 11.76% (2/16), and 18.75% (3/16) of resistance in doripenem, vancomycin and chloramphenicol respectively. In addition, we have observed 100% (16/16), 81.25% (13/16), 76.47% (13/16), 35.29% (5/16), 35.50% (6/16), and 12.5% (2/16) of sensitivity to linezolid, tigecycline, ciprofloxacin, vancomycin, doripenem and chloramphenicol respectively. However, all strains have been multidrug resistant (MDR) to betalactams, polypeptides, and ansamycins. Moreover, 7 strains (43.75%) have been variably multiresistant. One strain, Ri10 has been resistant to beta-lactams, polypeptides, ansamycins, cyclins and glycopeptides. No strain was ultrasensitive (XDR) or largely insensitive (PDR) to different antibiotics. **Conclusion:** This study reveals that 51% of strains have been resistant to antibiotics, 32% are sensitive, and 17% have intermediate resistance. These results partly explain the high rate of gastroenteritis observed in Brazzaville due to food poisoning.

Keywords

Antibiotics Resistance, *Bacillus cereus*, Soil, Pepper

1. Introduction

Infectious diseases are nowadays responsible for high morbidity. These diseases are mostly linked to food contamination of microbial origin and result in gastrointestinal symptoms occurring after the consumption of a meal [1]. Many studies have reported a high level of contamination of food products by microorganisms and several consummated foods around the world have been identified as vectors of microorganism transmission [2] [3].

B. cereus is one of the bacteria with the highest rate of food contamination. It forms an independent group belonging to the *Bacillus* genus, currently comprising eight species: *B. cereus* (*sensu stricto*), *B. anthracis*, *B. thuringiensis*, *B. mycoides*, *B. pseudomycoides*, *B. weihenstephanensis*, *B. cytotoxicus* and *B. toyonensis* [4]. More recently, 13 species have been proposed as new: *B. gaemokensis*, *B. manliponensis*, *B. bingmayongensis*, *B. wiedmanni*, *B. paranthracis*, *B. pacificus*, *B. tropicus*, *B. albus*, *B. mobilis*, *B. luti*, *B. proteolyticus*, and *B. nitirireducens* [5].

Mainly known to cause mild or severe food poisoning, sometimes leading to death, *Bacillus cereus* is a germ with many characteristics such as the omnipresence in different environments, the formation of spores, the adaptation in variable conditions, the production of virulence factors and harmful toxins in food, making it an opportunistic human pathogen even at very low loads [6]. Most of the studies affirmed that the emetic and diarrheal syndrome caused by *Bacillus cereus* could occur whenever bacterial load in a food reaches 5 to 8×10^4 CFU/g [7]. A recent study showed its presence in pepper (*Capsicum*), a food widely consummated around the world during meals, in sauce or in powder form, inside condiment dishes [8].

However, some studies have shown that *B. cereus* group strains could serve as a transferable source of antibiotic resistance genes in the food chain. Moreover, the emergence of antibiotic resistance in *B. cereus* currently poses a real problem in the treatment of patients. Most *B. cereus* strains produce β -lactamase-like enzymes and are considered resistant to β -lactam antimicrobial agents. The treatment of other *Bacillus cereus* infections is complicated because those *Bacillus* have rapid and progressive evolution and a high incidence of multidrug resistance [9].

Consequently, the presence of transposons and plasmids in *B. cereus* helps them in the acquisition and transmission of resistance genes characterized by an increasing level of resistance, particularly against several drugs [9]. Antibiotic resistance in *B. cereus* currently poses a real public health problem and remains a serious problem in searching for resolutions. Thus, the emergence and spread of antibiotic resistance is the subject of several studies and is reported by the WHO as one of the major health issues of the 21st century [1]. This work is initiated in order to determine the antimicrobial resistance profile of *B. cereus* group strains isolated from soil used and pepper consummated in Brazzaville.

2. Material and Methods

2.1. Study Design

The study was conducted in the bacteriology department of the national public health laboratory in Brazzaville and in the cellular and molecular biology laboratory of the Faculty of Sciences and Technics of Marien NGOUABI University between March and April 2021.

2.2. Sampling

The *Bacillus cereus* strains were isolated from two types of samples: (a) an environmental dry soil taken at 30 cm depth at the Faculty of Sciences and Technics, using a sterile tube. (b) The second sample was a fermented pepper, close to the pepper's characteristics of *Capscicum annum* species, purchased at a market in Brazzaville.

2.3. Isolation and Identification of Bacterial Strains

Using each sample, decimal dilutions have been performed for *Bacillus cereus* isolation on Mossel agar medium. Petri dishes were incubated at 37°C for 24 hour. A total of 16 strains were obtained, notably 10 and 6 from pepper and soil, respectively. These different strains were phenotypically characterized using conventional methods. After a morphological reading, a typical pink colony purification was realized by successive culture on agar medium. Strains were kept from a culture contained in LB broth supplemented with 40% of glycerol and stored at 4°C.

2.4. Bacterial Strains and Culture Conditions

Sixteen (16) strains of *Bacillus cereus* pre-isolated from soil and pepper samples according to the ISO method are subcultured on specific Mossel agar. Of these 16 strains, six were isolated from soil, namely: two strains of *Bacillus cereus* (sensu stricto: Ri7 and Ri9) and each one strain of *Bacillus weidmani* (Ri8), *Bacillus thuringiensis* (Ri10), *Bacillus anthracis* (Ri4), and *Bacillus albus* (Ri1). In addition, 10 strains were isolated from pepper: 6 strains of *Bacillus cereus* (sensu stricto: Ri14, Ri19, Ri20, Ri21, Ri25a, and Ri11), 1 *Bacillus* sp. (strain Ri25b), 1 *Bacillus albus* (strain Ri22), and 2 *Bacillus thuringiensis* (strains Ri23, Ri17).

2.5. Antibiotic Sensitivity Test

A sensitivity test of strains to different antibiotics was conducted using the Kirby and Bauer's standard disc diffusion (susceptibility) method [10] [11]. Antibiotics tested are penicillin G (10 U), Amoxicillin AML (10 µg), Ceftazidime CAZ (10 µg), Doripenem DOR (10 µg), Ciprofloxacin CIP (5 µg), chloramphenicol C (30 µg), Tigercycline TGC (15 µg), Rifampicin RD (5 µg), Vancomycin VAN (30 IU), Colistin CT (50 µg) and Linezolid LNZ (10 µg). Reading of inhibition diameters and their interpretations were made according to the guidelines of the Clinical Laboratory Standards Institute [12].

The results obtained were notified on a form and recorded on a computer file. These values were transcribed into sensitive (S), resistant (R), and intermediate (I) categories after comparison with the critical diameters according to the recommendations [12].

The presence or absence of resistance phenotypes was indicated by the signs (+) and (–) respectively, and a numeric coding was used to indicate the number of multidrug resistant (MDR) phenotypes.

2.6. Statistical Analyzes

A Microsoft Excel program was used for statistical analysis. The diameters have been analyzed, and percentages calculated statically. Experimental values were represented as the mean and standard deviation.

3. Results

3.1. Sensitivity Study

B. cereus strains studied showed different levels of resistance to antibiotics tested (Figure 1). All strains tested showed resistance against β -lactams (penicillin, amoxicillin, and ceftazidime), Ansamycins and polypeptides with a resistance rate of 100% (Table 1). Doripenem was the most active antibiotic of the β -lactam family with a rate of 18.75%. This same resistance was observed with chloramphenicol and tigecycline (Table 1). However, linezolid remained the most active antibiotic on all strains tested with a rate of 100%.

3.2. Antibiotic Resistance Profile

Bacillus cereus strains showed varying levels of resistance depending on the antibiotic tested (Table 2). Strain Ri10 showed resistance against 5 antibiotics of different families. 6 strains showed resistance to 4 antibiotics from different families while all other strains were resistant to at least 3 antibiotics (Figure 2).

A multiresistance profile has been observed in a variable proportion of strains depending on antibiotics classes. (16/16) 100% of strains were resistant to 3 antibiotic classes (betalactams, polypeptides and ansamycins), 43.75% (7/16) of strains resistant to 4 antibiotics classes and only one strain (Ri10) was resistant to 5 antibiotic classes (betalactam, polypeptide, ansamycins, cyclins and glycopeptides) (Figure 3). Table 2 describes the multiresistant (MDR), ultraresistant

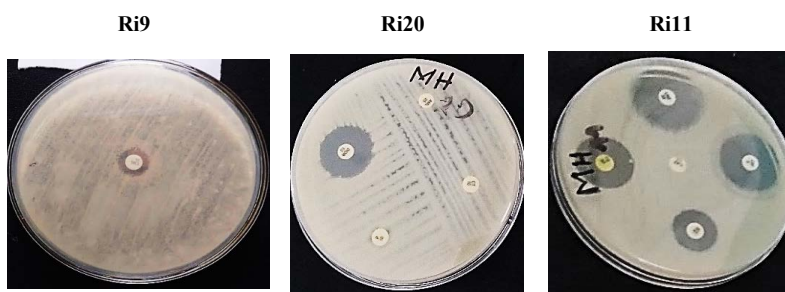


Figure 1. Antibiotics susceptibility profile of *B. cereus* strains (Ri9, Ri20, and Ri11).

Table 1. Antibiotics sensitivity of 16 *Bacillus cereus* strains isolated from soil and pepper samples consummated in Brazzaville.

Category	Antibiotic family	Antibiotics	<i>Bacillus cereus</i> group (n = 16)		
			R (%)	I (%)	S (%)
Beta-lactam	Penicillins	Penicillin G (P)7 15 µg	16 (100)	0 (00.00)	0 (00.00)
		Amoxicillin (AML) 25 µg	16 (100)	0 (00.00)	0 (00.00)
	3rd generation cephalosporins	Ceftazidime (CAZ) 10 µg	16 (100)	0 (00.00)	0 (00.00)
	Carbapenem	Doripenem (DOR) 10 µg	3 (18.75)	7 (38.88)	6 (35.50)
Other families	Glycopeptides	Vancomycin (VA) 30 IU	2 (11.76)	9 (52.94)	5 (35.29)
	Polypeptides	Colistine (CT) 50 µg	16 (100)	0 (00.00)	0 (00.00)
	Ansamycins	Rifampicin (RD) 5 µg	16 (100)	0 (00.00)	0 (00.00)
	Oxazolidines	Linezolid (LNZ) 10 µg	0 (00.00)	0 (00.00)	16 (100)
	Cyclines	Tigecycline (TGC) 15 µg	3 (18.75)	0 (00.00)	13 (81.25)
	Phenicolates	Chloramphenicol (C) 30 µg	3 (18.75)	11 (68.75)	2 (12.5)
	Fluroquinolones	Ciprofloxacin (CIP) 5 µg	0 (00.00)	3 (18.75)	13 (76.47)

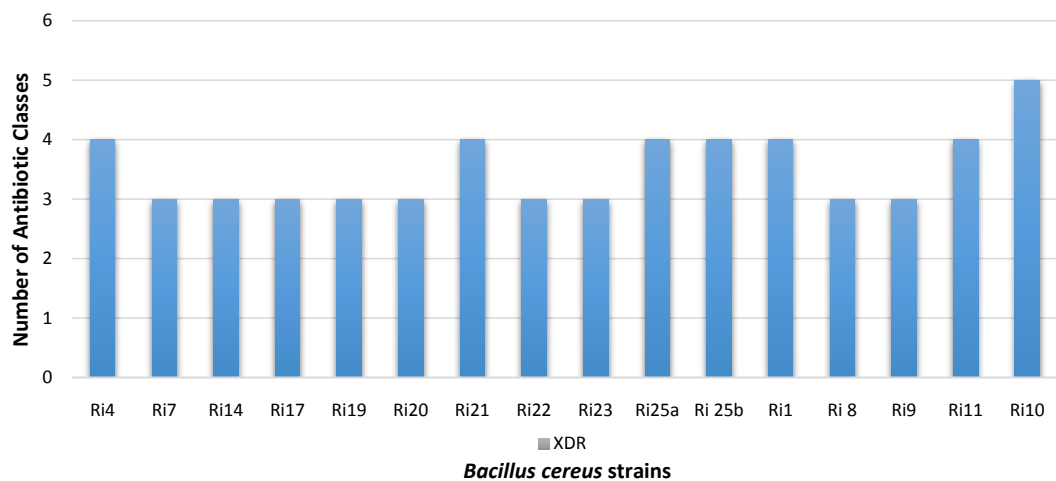


Figure 2. Antibiotics resistance profile of strains by class. Multidrug resistance (MDR), ultra resistance (XDR) and Broad insensitivity (PDR) proportions of *B. cereus* strains according to antibiotic classes.

(XDR), and largely insensitive (PDR) frequency of each strain according to antibiotics tested. (+) and (-) sign marks presence and absence of resistance respectively.

Table 2. Resistance level of variable strains depending on the antibiotic tested.

Strains	Antibiotic family										
	P	AML	CAZ	DOR	CT	RD	TGC	C	VA	CIP	LNZ
Ri4 <i>Bacillus anthracis</i>	+	+	+	-	+	+	-	+	-	-	-
Ri7 <i>Bacillus cereus</i>	+	+	+	-	+	+	-	-	-	-	-
Ri14 <i>Bacillus cereus</i>	+	+	+	+	+	+	-	-	-	-	-
Ri17 <i>Bacillus thuringiensis</i>	+	+	+	-	+	+	-	-	-	-	-
Ri19 <i>Bacillus cereus</i>	+	+	+	-	+	+	-	-	-	-	-
Ri20 <i>Bacillus cereus</i>	+	+	+	-	+	+	-	-	-	-	-
Ri21 <i>Bacillus cereus</i>	+	+	+	+	+	+	-	-	+	-	-
Ri22 <i>Bacillus albus</i>	+	+	+	+	+	+	-	-	-	-	-
Ri23 <i>Bacillus thuringiensis</i>	+	+	+	-	+	+	-	-	-	-	-
Ri25a <i>Bacillus cereus</i>	+	+	+	-	+	+	-	+	-	-	-
Ri 25b <i>Bacillus</i>	+	+	+	-	+	+	+	-	-	-	-
Ri1 <i>Bacillus albus</i>	+	+	+	-	+	+	+	-	-	-	-
Ri8 <i>Bacillus weidmani</i>	+	+	+	-	+	+	-	-	-	-	-
Ri9 <i>Bacillus cereus</i>	+	+	+	-	+	+	-	+	-	-	-
Ri11 <i>Bacillus cereus</i>	+	+	+	-	+	+	+	-	-	-	-
Ri10 <i>Bacillus thuringiensis</i>	+	+	+	-	+	+	+	-	+	-	-

Legend: Penicillin G (P), Amoxicillin (AML), Ceftazidime (CAZ), Doripenem (DOR), Vancomycin (VA), Colistin (CT), Rifampicin (RD), Linezolid (LNZ), Tigercycline (TGC), Chloramphenicol (C), (+): presence of resistance and (-): absence of resistance.

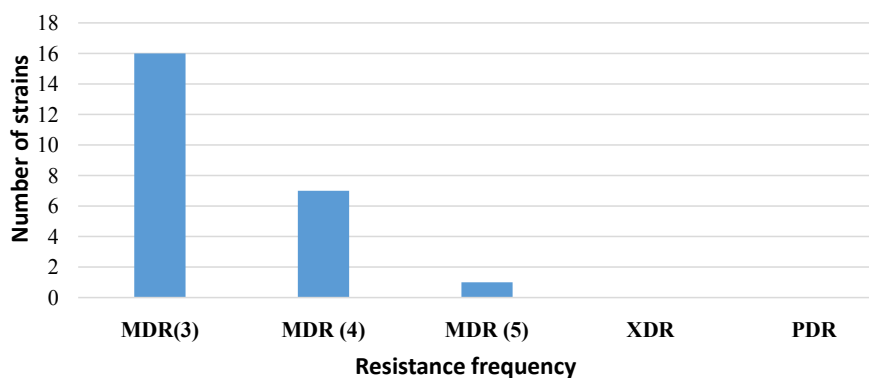


Figure 3. Multi resistance (MDR), Ultra resistance (XDR) and broad insensitivity (PDR) frequency of 16 *B. cereus* strains according to the number of antibiotic classes (multi resistant phenotype to 3, 4 and 5 classes of antibiotics).

3.3. Resistance Phenotypes

Resistance phenotype analysis of strains to antibiotics has mostly presented resistance phenotypes. **Figure 3** shows the multidrug resistance (MDR) phenotype, with a predominance of strains resistant to at least three different families of antibiotics (MDR 3).

4. Discussion

The increasing frequency of multiple antibiotic resistance is currently a concern for both food processors and public health. This study aimed to determine the resistance profile of *B. cereus* isolated from soil and pepper samples consummated in Brazzaville. The great adaptation of *B. cereus* in unfavourable growing conditions leads to increase its ability to resist antibiotic pressure. [13] reported that food contamination rates due to *B. cereus* in Switzerland were 78%, while, [14], reported only 8.2% contamination of samples of aquatic products sold in China.

Consequently, results obtained in our study showed that most *B. cereus* strains are resistant to the betalactam classes (penicillin G, amoxicillin, and cef-tazidime). It confirms the hypothesis of the natural resistance of *B. cereus* to the majority of betalactam antibiotics, supporting the results obtained by [11] on the resistance of *B. cereus* isolated from ready fast foods in China. In fact, *B. cereus* strains are known as intrinsic producers of Metallo- β -Lactamases with chromosomal resistance to penicillin and cephalosporins. [15] reported strong betalactam resistance of *B. cereus* in studies focused on raw cow's milk sold in Ethiopia.

A strain resistance rate of 18.75% (3/16) to doripenem has been observed. Doripenem is degraded by dehydropeptidase I, a renal tubular dipeptidase enzyme. Thus, this resistance could be explained by the production of betalactamases capable of carbapenem hydrolysis. In fact, these enzymes inactivate the majority of betalactams and are encoded by genes carried in transposons, plasmids, or other mobile genetic elements that could be horizontally transferred towards other bacterial species [16]. Regarding the class of cyclins, 18.75% of the resistance rate has been observed. This rate is different to results reported by [17] on the resistance of *B. cereus* isolated from rice in the United States, which showed a resistance rate of 98% to tetracycline [17]. This difference could be explained by using tigecycline as the cyclin instead of tetracycline.

We report a resistance percentage of 11.76% (5/16) to vancomycin, lower than that obtained by [18], with a resistance rate of 87% for *B. cereus* isolated from pasteurised milk in China. Additionally, *B. cereus* can acquire resistance to commonly used antibiotics such as ciprofloxacin, cloxacillin, erythromycin, tetracycline, and streptomycin [19]. In contrast to the findings of [20] on the resistance of *B. cereus sensu lato* isolated in Ghanaian dairy farms and traditional products, these results have shown that the *B. cereus* strains show resistance to chloramphenicol, which may be the consequence of the acquisition and presence of chloramphenicol resistance genes.

Our results are similar to those obtained by [21] on the resistance of *B. cereus* isolated from environmental samples in the United States. Variation in antibiotic resistance extent and pattern of *B. cereus* could be impacted by factors such as sources of strain isolation, geographic areas, or frequency of antibiotic applications.

[22] reported that *B. cereus* strains from stool, food, and environmental sam-

ples in Serbia have higher resistance proportions than those from food and the environment. Results in our study shows that the 16 *B. cereus* strains are sensitive to linezolid, tigecycline, ciprofloxacin in significant proportions, similar results obtained by [23] during their work reported in dairy local products in China, [24] in Cameroon obtained the same in raw cows and processed milk.

All *B. cereus* strains tested here proved to be resistant to at least 3 different families of antibiotics. This result indicates that *B. cereus* strains tested during this study are multiresistant (MDR) as reported by [25]. As a result, the rate of increasing antibiotic resistance of *B. cereus* could result from the occurrence of high severity infections which could be fatal.

The prevalence of pepper consumption among the Congolese population makes pepper an unavoidable support for *Bacillus cereus* growth, owing to endospore production, which provides resistance despite adverse environmental conditions. *B. cereus* spores could potentially contaminate raw food material and be found in all stages of manufacture, particularly in the production as well as in the sale of pepper jars, with a high probability of favouring its deterioration and causing food poisoning [8].

In our study, antibiotic resistance proportion and multidrug resistance emergence attributed to *Bacillus cereus* strains therefore constitute a public health problem. In particular, the emergence of multi-resistant strains is a medical situation to be feared because antibiotic resistant genes could be transferred to other important pathogen agents on horizontal transfer and facilitate their dissemination within the bacterial population from the presence of mobile genetic elements such as plasmids and transposon present in *B. cereus* [26].

In addition, these strains could survive in the gastrointestinal tract and complicate treatment for younger or older users or for people with weakened immune function after infection [27]. All these complications set up a problem for infectious disease management caused by increased infection risk and treatment failures.

Thus, it remains to highlight the presence of genes responsible for multiresistance, as well as production of Metallo- β -Lactamases, in knowing *Bacillus cereus* strains' impacts on mechanisms of resistance.

5. Conclusion

The study shows the characteristic resistance to antibiotics of *B. cereus* strains. A strong resistance (100%) of the strains has been observed against antibiotics of the polypeptide classes and ansamycins. Linezolid and ciprofloxacin were the most active antibiotics against the strains tested. The strains isolated in this work are multiresistant. The multiresistance phenotype is observed in 100% of cases against 3 classes of antibiotics (betalactams, polypeptides, and ansamycins). These results indicate that antibiotic resistance such as tigecycline, ciprofloxacin, and chloramphenicols could occur. These data could lead to other in depth studies focused on resistance mechanisms of *B. cereus* strains isolated in the Re-

public of Congo. *B. cereus* therefore represents a potential source of transferable resistance genes in the food chain and to other medically important pathogens.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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