



## Antibiotic Resistance of Bacteria of the *Bacillus cereus* Group Isolated from Attiéké, A Local Dish Sold in the Streets of the Town of Daloa (Côte d'Ivoire)

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### Authors' contributions

This work was carried out in collaboration between all the authors. Author KKA designed the study, wrote the protocol, carried out the sampling and carried out the various analyzes in collaboration with author AMCED. Author KKA wrote the first draft of the manuscript. The statistical analysis was carried out by author KKC. Authors CI and KI were supervisors. They proceeded to read the manuscript, to perfect it while managing the documentary research. All authors have read and approved the final manuscript.

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### ABSTRACT

*Bacillus cereus* often causes problems in the food industry. It deteriorates the marketable quality of food. *Bacillus cereus* spores can withstand high temperatures and persist in finished or semi-finished products. The objective of this work was to assess the level of resistance to antibiotics in strains of *Bacillus cereus* isolated from attiéké, a local delicacy sold in the streets of Daloa. To do this, around sixty (60) suspected strains of *Bacillus cereus* isolated from attiéké were identified using standard biochemical tests and thirty-five (35) were confirmed by the API 20E gallery. These thirty-

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five confirmed strains were subjected to an antibiogram according to the recommendations of the Antibiotic Committee of the French Society of Microbiology (CASFM). The results obtained after analysis show that the strains of *Bacillus cereus* are subdivided into four profiles. No resistant strain has been demonstrated for imipenem, gentamycin and vancomycin. No strain produced broad spectrum beta-lactamases. The least active molecule was trimethoprim-sulfametoxazole with a level of 40%. This study showed the existence of multi-resistant strains of *Bacillus cereus*, which would require monitoring of resistance in street foods, particularly attiéké

**Keywords:** *Bacillus cereus*; antibiotic; attiéke, resistance; multi-resistant.

## 1. INTRODUCTION

*Bacillus* is a particularly heterogeneous bacterial genus. They are Gram-positive, facultative anaerobic bacilli [1]. The *Bacillus cereus* group comprises six genetically very close species forming endospores [2,3] corresponding to *Bacillus anthracis*, *Bacillus cereus sensu stricto*, *Bacillus mycoides*, *Bacillus pseudomycoïdes*, *Bacillus thuringiensis* and *Bacillus weihenstephanensis*. [1]. The species of the *Bacillus cereus* group belong to the genus *Bacillus*. The *Bacillus cereus* group comprises six genetically very close species forming endospores [2,3] corresponding to *Bacillus anthracis*, *Bacillus cereus sensu stricto*, *Bacillus mycoides*, *Bacillus pseudomycoïdes*, *Bacillus thuringiensis* and *Bacillus weihenstephanensis*. They are quite large bacilli, measuring 1 to 1.8 µm in diameter by 4 to 8 µm long, usually producing short chains. Very widely distributed in nature, the *B. cereus* group behaves as an opportunistic pathogen responsible for systemic and local infections. It is also responsible for food poisoning. Pathogenic strains of the *B. cereus* group are responsible for food poisoning in two forms including a diarrheal form, causing abdominal cramps and profuse diarrhea, and an emetic form, causing severe nausea and vomiting [4]. The species of the *B. cereus* group are ubiquitous in the environment (soil, water, etc.). Therefore, their presence in food is inevitable. Heat treatments, with the exception of canning, do not eliminate spores in food. Spores are present in almost all food categories prior to storage, usually in too high a number to cause food poisoning. The development of *B. cereus* in attiéké contributes to its rapid deterioration while limiting shelf life. Consumption of attiéké contaminated with species of the *B. cereus* group could represent a risk to the health of the consumer. *B. cereus* and related genera are frequently identified as the cause of human foodborne illness of moderate severity worldwide despite a few reported fatal cases [5]. In addition, the strains can resist certain antibiotics such as

β-lactams and third generation cephalosporins, thus making it difficult to treat the poisonous infections for which they are responsible. However, they are sensitive to Chloramphenicol, Clindamycin, Vancomycin, Ciprofloxacin, Erythromycin, Gentamycin, Tetracycline and Streptomycin [6,7,8]. The objective of this work was to assess the level of antibiotic resistance of *Bacillus cereus* strains isolated from the attiéké sold in the streets of Daloa.

## 2. MATERIALS AND METHODS

### 2.1 Sampling

The isolates used for this study were isolated from the attiéké sold on the streets of Daloa. Sixty (60) putative strains of *Bacillus cereus* isolated from attiéké were grown in tubes containing brain heart broth (BCC broth)(Biorad, Paris, France). These tubes were kept for further work.

### 2.2. Identification of Strains

All the strains stored in the tubes were inoculated one by one on TSA medium (Tryptone-Soy agar) (Biomérieux, Paris, France) supplemented with emulsified egg yolk. They were incubated at 37°C / 24 hours. First, the lecithinase was read according to the method of [9] and the work of [10]. Emulsified egg yolk is added to the Mossel medium. the inoculated isolates are incubated at 37°C. /24 h. the appearance of an opaque zone around the colony reflects a positivity of lecithinase.. Then the strains were cultured on nutrient agar to perform Gram staining and other standard biochemical tests. This is firstly the demonstration of respiratory enzymes (catalase and oxidase) according to the work of [11] and [12], growth on the mannitol-mobility medium [11,13], the use of citrate as the sole source of carbon on the citrate medium of Simmons according to the work of [13] and finally the TSI tests on the Kligler Hajna medium (Biorad, Paris, France). The colonies are inoculated onto the

sloped and pelletized Kliger Hajna medium. This test also makes it possible to observe the production of H<sub>2</sub>S and gas [13]. The presumptive isolates of *Bacillus cereus* were confirmed by the API 20E gallery according to the work of [14]. An API (Identification Apparatus and Process) gallery is a set of ready-to-use wells for the identification of microorganisms by quickly and easily performing miniaturized biochemical tests

### 2.3 Antibiotic Resistance of *B. cereus* Strains

An antibiogram of the bacteria of the *B. cereus* group was carried out on Mueller-Hinton agar (Biorad, Paris, France) according to the conventional method of diffusion in agar medium [15]. It was performed on 35 formally identified *Bacillus cereus* isolates. To perform the antibiogram, twelve (11) discs impregnated with antibiotic (Becton, Dickinson and Company, Switzerland) were used. The zones of inhibition, including the diameter of the antibiotic, were measured using a caliper and the diameters were interpreted according to the recommendations of the Antibiogram Committee

of the French Society of Microbiology. Antibiotics are delivered with the concentrations set by the Antibiogram Committee of the French Society of Microbiology [16] Table 1.

## 3. RESULTS

### 3.1 Identification of Isolates

The classical biochemical tests carried out made it possible to identify thirty-five (35) presumptive isolates of *Bacillus cereus* from the 60 strains isolated from attiéké sold in the streets. The realization of the API 20 E gallery made it possible to confirm its 35 isolates of *Bacillus cereus*. In addition, these 35 isolates were the subject of the study for antibiotic resistance. 3.2. Beta-lactam resistant rate The resistance rates of the isolates to the beta-lactams tested are variable. Thus all the isolates tested are resistant to cefepime and ampicillin. In addition, 20% of the isolates are resistant to tetracycline. Furthermore, no resistant strain has been demonstrated for imipeneme. No strain produced broad spectrum beta-lactamases Fig. 1.

Table 1. Discs impregnated with antibiotics used for work

N°	Antibiotic discs	Charges
1	Ampicillin	10 µg
2	Cefepime	30 µg
3	Chloramphenicol	30 µg
4	Ciprofloxacin	5 µg
5	Lincomycin	2 µg
6	Vancomycin	30 µg
7	Gentamicin	10 µg
8	Imipeneme	10 µg
9	Tetracycline	30 µg
10	Trimethoprim-Sulfametoxazole	1.25 µg/23.75 µg
11	Erythromycin	15 µg

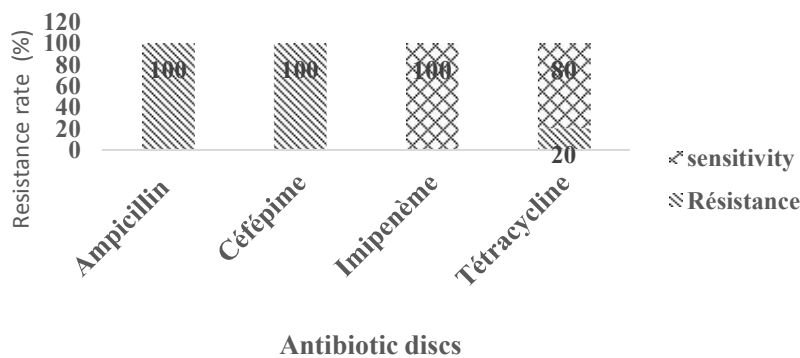


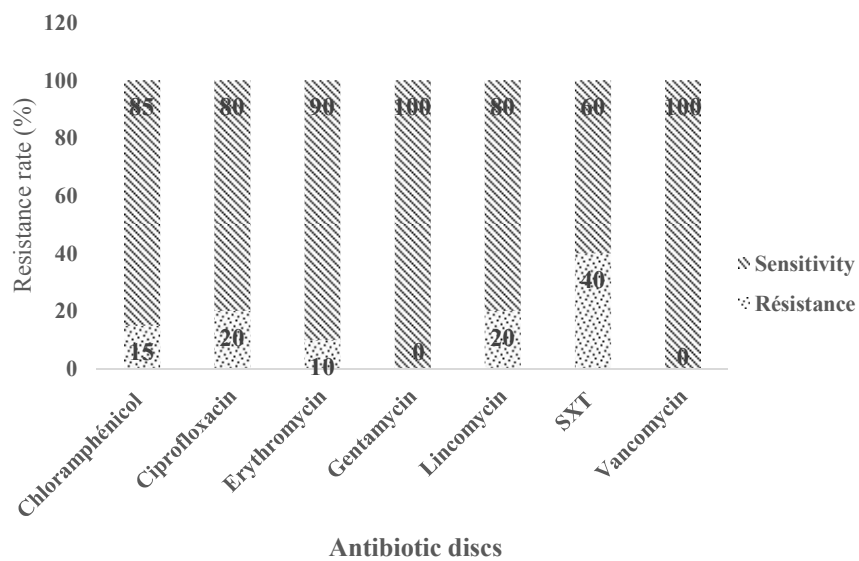
Fig. 1. Resistance of *B. cereus* strains to beta-lactams

### 3.2 Resistance Rate of *B. cereus* Strains to Other Antibiotics

As for other antibiotics, there is variable resistance ranging from 10% for erythromycin, 15% for chloramphenicol, 20% for lincomycin and ciprofloxacin, 60% for trimethoprim-Sulfametoxazole. Furthermore, no resistance was demonstrated for gentamycin and vancomycin in the different strains. However, the least active molecule is trimethoprim-sulfametoxazole with a resistance rate of 40% Fig. 2.

### 3.3 Multi-resistant Strains

The isolates studied were for the most part multidrug-resistant. Thus, the obtained attieke isolates were resistant to between four and eight antibiotics. Four profiles have therefore been identified. These are strains resistant to 4, 5, 6 and 8 antibiotics, respectively, with the corresponding resistance rates. The highest resistance rate was observed with isolates that are resistant to four antibiotics with 45.8% Fig. 3.



SXT: Trimethoprim-Sulfametoxazole

Fig. 2. Resistance of *B. cereus* strains to other antibiotics

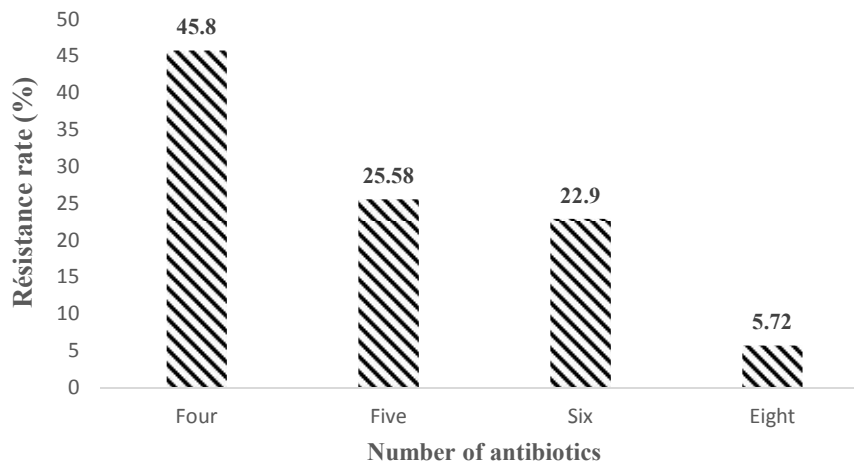


Fig. 3. Distribution of the levels of multi-resistant *Bacillus cereus* strains

#### 4. DISCUSSION

Biochemical tests and the API 20E gallery have identified *Bacillus cereus* in samples of attiéké sold in the streets of Daloa. Indeed, according to the work of, several species of *Bacillus* with spores resistant to high heat and capable of surviving industrial ultra-high temperature (UHT) treatment have been isolated in certain foods. Among the bacilli, *B. cereus* has been recognized as a causative agent of food poisoning linked to emetic and diarrheal syndromes of food origin [11]. In addition, bacteria of the *Bacillus cereus* group which form spores which are disseminated in the environment have been isolated from a wide variety of foods [17,18]. *Bacillus licheniformis* has also been associated with a variety of clinical syndromes, such as enteric disease, sepsis, peritonitis, ophthalmia, and food poisoning in humans in addition to bovine toxemia and abortions [19]. The resistance profiles of the isolates of the *B. cereus* group are in agreement with those reported by the work of [20] regarding their resistance to ampicillin and their sensitivity to gentamycin and vancomycin. This resistance results from the production of inactivating enzymes by jamming, which is the most common resistance mechanism in nature [20]. Also the resistance profile is opposite with those of [20] with regard to Erythromycin where they have an intermediate resistance towards half of the strains tested and agrees with regard to the high resistance to trimethoprim. sulfametoxazole from most isolates tested. The majority of isolates are resistant to  $\beta$ -lactams including ampicillin (100%), cefepime (100%) and 80% to trimethoprim-sulfametoxazole. The results are consistent with several previous studies that reported resistance of the *B. cereus* group to trimethoprim-sulfametoxazole and  $\beta$ -lactams such as ampicillin, cefepime, and cephalosporins [8]. In fact, the *B. cereus* group, with the exception of *B. anthracis* which is sensitive to penicillin, is for the most part resistant to trimethoprim and to certain antibiotics of the  $\beta$ -lactam family, including ampicillin, cefepime, carbenicillin, cephalothin and cloxacillin [21]. Thus *B. cereus* is generally sensitive to aminoglycosides, chloramphenicol, clindamycin, erythromycin, tetracycline and vancomycin [7,8]. However, various authors including [21] and [22] showed that *B. cereus* species are multidrug resistant. These results were confirmed by those of our study. Indeed, the *B. cereus* group species studied showed variable resistance to ciprofloxacin, tetracycline, chloramphenicol,

erythromycin, and lincomycin. All these observed resistances are acquired resistances. Thus, the results reveal that there is an emergence of strains of the *B. cereus* group that are multi-resistant to antibiotics. The high prevalence of multi-antibiotic resistant strains of the *B. cereus* group isolated from attiéké suggests that the most commonly used antibiotic is ineffective in treating infections caused by this bacteria. Resistance patterns to *B. cereus* antimicrobials in foods are useful in epidemiological studies. The multiple resistance patterns observed indicate that these antimicrobial agents are used with abuse or at a sublethal dose in the environment.

#### 5. CONCLUSION

The study revealed the presence of multi-resistant *Bacillus cereus* strains in attiéké sold in the streets of Daloa. This would justify setting up a resistance surveillance network for these strains and limiting their dissemination. The isolates are multidrug resistant with high resistance to cefepime, ampicillin and trimethoprim-sulfametoxazole. No strain resistance was observed for imipenem, gentamycin and vancomycin. The detection of *Bacillus cereus* in attiéké sold on the streets could cause a serious public health problem. The results of this work will allow the authorities to take rigorous measures when selling food on the outskirts of the streets, especially attiéké which is a delicacy much prized by the population.

#### DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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