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Research Paper

CHARACTERIZATION AND ANTIMICROBIAL RESISTANCE OF *Bacillus cereus* STRAINS ISOLATED FROM *Attiéké garba* SOLD IN DALOA (CÔTE D'IVOIRE)

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Abstract

ABSTRACT

Bacillus cereus is known to cause problems in the food industry because it affects the quality and safety of food. *Bacillus cereus* spores can withstand certain heat treatments and therefore can persist in finished or semi-finished products. The objective of this work was to estimate the level of antibiotic resistance on *Bacillus cereus* strains isolated from *Attiéké garba* sold in Daloa.

Approximately thirty-two (32) suspected strains of *Bacillus cereus* isolated from *Attiéké garba* underwent identification using standard biochemical tests and the API 20E gallery. Then 20 selected strains were subjected to antibiograms according to the recommendations of the CASFM. There are five antibiotic profiles called P1 to P5 and their prevalence has been known. No resistance strain has been demonstrated for imipenème, gentamycin and vancomycin. No strain produced broad-spectrum beta-lactamases. The least active molecule was trimethoprim-Sulfametoxazole with a rate of 40%.This study showed the existence of strains of multi-resistant *Bacillus cereus*, which would require monitoring of resistance in street foods, particularly *Attiéké garba*..

Key words: *Bacillus*, antibiotic, *Attiéké*, antibiogram, strains.

INTRODUCTION

The genus *Bacillus* belongs to the family of Bacillaceae. It is a particularly heterogeneous bacterial genus. These are Gram positive, or variable Gram stained bacilli, aerobic but

optionally aero-anaerobic [1]. The *Bacillus cereus* species belong to the genus *Bacillus*. The *Bacillus cereus* group includes six genetically very similar endospore-forming species [2,3] corresponding to *Bacillus* anthracis, *Bacillus cereus* sensu stricto, *Bacillus* mycoides, *Bacillus* pseudomycoides, *Bacillus* thuringiensis and *Bacillus* weihenstephanensis. These are large bacilli, measuring 1 to 1.8 µm in diameter by 4 to 8 µm long, usually producing short chains. Very widespread in nature, the B. cereus group behaves as an opportunistic pathogen responsible for systemic and local infections. It is also responsible for foodborne illness. Pathogenic strains of the B. cereus group are responsible for foodborne illness in two forms: a diarrheal form, causing abdominal cramps and profuse diarrhea, and an emetic form, causing severe nausea and vomiting [4].

Species in the B. cereus group are ubiquitous in soil and the environment. Therefore, their presence in food is inevitable. Heat treatments, with the exception of canning, do not kill spores in food. Spores are present in almost all food categories before they are stored, usually in too small a number to cause foodborne illness (TIA). The development of B. cereus in *Attiéké* contributes to its rapid deterioration while limiting the shelf life [4].

Consumption of *Attiéké* contaminated with species of group B. cereus could represent a risk to the health of the consumer. B. cereus and related genera were frequently identified as the cause of moderate serious human foodborne illnesses worldwide despite some fatal cases reported [5]. In addition, the strains can resist certain antibiotics such as β-lactams and third generation cephalosporins, thus making it difficult to treat the toxi-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 estimate the level of antibiotic resistance on *Bacillus cereus* strains isolated from *Attiéké garba* sold in Daloa. The information collected will make it possible to make a good medical prescription in the event of toxiinfection caused by *Bacillus cereus*.

MATERIALS AND METHODS

Sampling

The strains that were used to carry out this work come from *Attiéké garba*. About thirty-two (32) suspected strains of *Bacillus cereus* isolated from *Attiéké garba* were introduced into brain heart broth (Biorad, Paris, France) for further work.

Identification of strains

The strains were seeded on TSA medium (Tryptone-Soy agar) (Biomérieux, Paris, France) added with emulsified egg yolk for reading lecithinase according to the method of [9] and the work of [10]. Then the strains were cultured on nutrient agar to perform Gram staining and other conventional biochemical tests. It is first of all the demonstration of respiratory enzymes (catalase and oxidase) according to the work of [11]. and [12].growth on the mannitol-mobility medium. [12,13] then the use of citrate as the only carbon source on the citrate medium of Simmons according to the work of [13] and finally the tests TSI on the environment Kligler Hajna (Biorad, Paris, France). This test also makes it possible to observe the production of H2S and of gas [13].

The presumptive isolates of *Bacillus cereus* were confirmed by the API 20E gallery.

Antibiotic resistance study

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 [14]. It was carried out on 20 formally identified *Bacillus cereus* isolates. Twelve (12) antibiotic-impregnated discs (Becton, Dickinson and Company, Switzerland) were tested. These were: Ampicillin (10 µg), Penicillin (10 U), Cefepime (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (5 µg), Lincomycin (2 µg), Vancomycin (30 µg), Gentamicin (10 µg), Imipenem (10 µg), Tetracycline (30 µg), Trimethoprim-Sulfametoxazole (1.25 µg / 23.75 µg) and Erythromycin (15 µg). 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 Microbiology Society [15].

RESULTS

Identification of isolates

Classical biochemical tests have identified approximately 20 of the 32 isolates as *Bacillus cereus*. The API 20 E gallery made it possible to conform its 20 *Bacillus cereus* isolates. These 20 isolates were used to produce the antibiogram.

Antibiotic resistance profile

Taking into account the antibiotic typing performed on the 20 isolates of the B. cereus group, there are five antibiotic profiles called P1 to P5. The prevalences of the different profiles are mentioned in the table below (Table 1).

Table 1: Prevalence of B. cereus isolates compared to resistance profiles

Résistance profiles	Antibiotics	Number of isolates	%
P1	Amp, Pen, Fep, Sxt	9	45,00
P2	Amp, Pen, Fep, Lcn, Sxt	5	25,00
P3	Amp, Pen, Fep, Lcn, Cip, Chl	3	15,00
P4	Amp, Pen, Fep, Cip, Tet, Sxt	2	10,00
P5	Amp, Pen, Fep, Cip,Chl, Tet, Sxt, Ery	1	05,00

Antibiotic resistance rates

The antibiotic resistance rates of the isolated strains are shown in FIGS. 1 and 2. No resistance strain has been demonstrated for imipeneme, gentamycin and vancomycin. No strain produced broad spectrum beta-lactamases. The least active molecule is trimethoprim-Sulfametoxazole with a rate of 40% resistance.

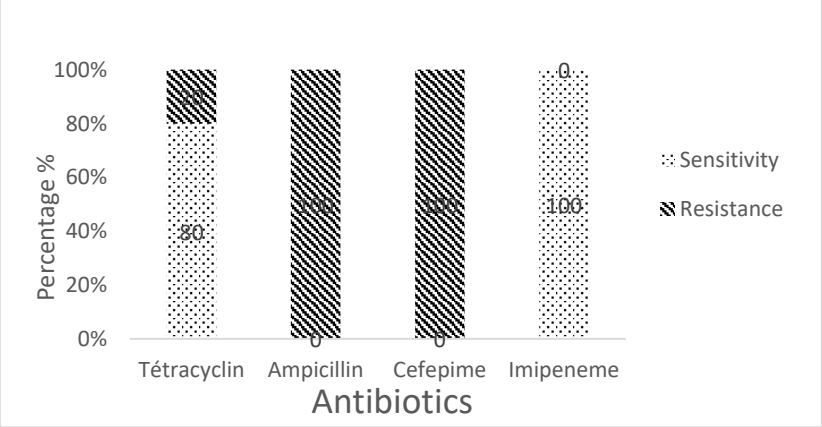

Figure 1: Resistance of B. cereus strains to beta-lactams

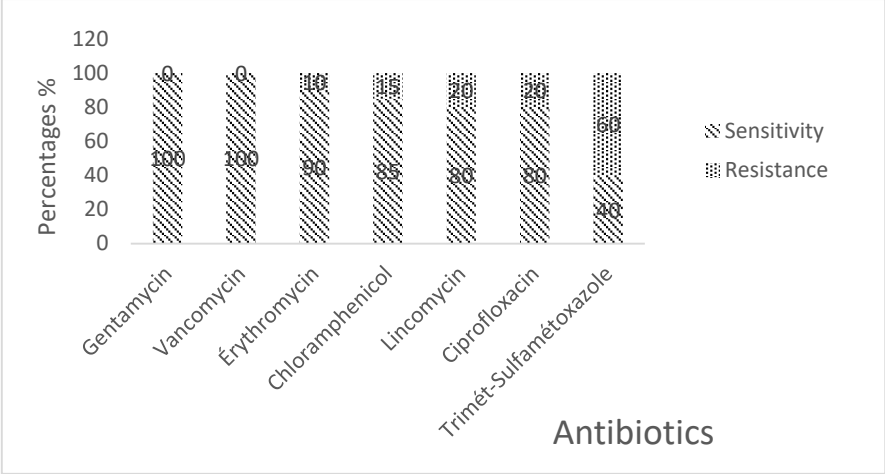

Figure 2: Resistance of B. cereus strains to other antibiotics

Multi-resistant strains

Most of the isolates studied were multi-resistant. Thus, the isolates obtained from *Attiéké* were resistant to between four and eight antibiotics. High resistance rates were obtained with isolates resistant to four and six antibiotics. (Figure 1).

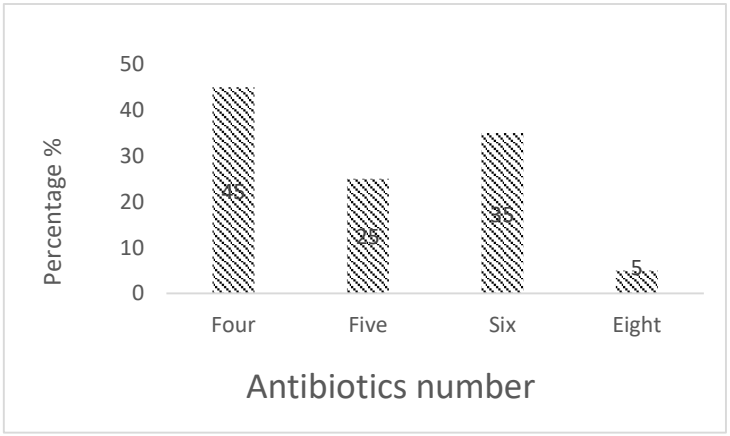

Figure 3: Distribution of rates of multi-resistant strains

DISCUSSION

Biochemical tests and the API 20E gallery made it possible to formally identify *Bacillus cereus* in samples of *Attiéké garba* sold on Daloa spaces and markets. Indeed according to the work of [16] several species *Bacillus* spores forming highly heat resistant (HRS) spores capable of surviving industrial ultra-high temperature (UHT) treatment have been isolated from food. Furthermore, among bacilli, B. cereus has been recognized as a

causative agent of food poisoning linked to emetic and diarrheal syndromes of food origin [11].The *Bacillus cereus* group includes gram-positive spore-forming bacteria that are found throughout the environment and have been isolated from a wide variety of foods [17,18]. In addition, *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 isolates from the B. cereus group are in agreement with those reported by the work of Guech (2015) with regard to their resistance to ampicillin and penicillin and their sensitivity to gentamycin and vancomycin. This resistance results from the production of inactivating enzymes by scrambling, 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 with respect to half of the strains tested and agrees with regard to the high resistance to trimethoprim-sulfametoxazole of most of the isolates tested.

All isolates are resistant to β-lactams including Ampicillin (100%), Penicillin (100%), cefepime (100%) and 80% to trimethoprim-sulfametoxazole. The results are consistent with several previous studies which have reported the resistance of the B. cereus group to trimethoprim-sulfametoxazole and to β-lactams such as ampicillin, penicillin, cefepime, and cephalosporins [8]. Indeed, the B. cereus group, with the exception of B. anthracis which is sensitive to penicillin, is for the most part resistant to trimethoprim and certain antibiotics of the β-lactam family, including ampicillin, cefepime, carbenicillin, cephalothin, cloxacillin and penicillin G [21]. In addition, B. cereus is generally sensitive to aminoglycosides, chloramphenicol, clindamycin, erythromycin, tetracycline and vancomycin [7,8]. However, various authors including [21] and [22] have shown that B. cereus species are multidrug resistant. These results have been confirmed by this study. In fact, the species of the B. cereus group studied showed variable resistance to ciprofloxacin (26.00%), tetracycline (26 %), chloramphenicol (20.00%) to erythromycin (13,33 %), and lincomycin (20 %). All these resistances observed are acquired resistances. Thus, the results reveal that there is an emergence of strains of the group B. cereus multidrug resistant to antibiotics. The high prevalence of strains resistant to antibiotics of the B. cereus group isolated from *Attiéké garba* suggests that the most commonly used antibiotic is ineffective in treating infections caused by the B. cereus group. The antimicrobial resistance patterns of group B. cereus

in food are useful in epidemiological studies. There is a higher proportion of multiple resistance patterns among group B. cereus isolates obtained from food [23]. The multiple resistance patterns observed reveal that these antimicrobial agents were used with abuse or at a sublethal dose in the environment.

CONCLUSION

This study showed the existence of strains of multi-resistant *Bacillus cereus* in *Attiéké garba* sold on the markets of Daloa. This would justify the establishment of a network to monitor the resistance of these strains and limit their spread. The isolates are multidrug resistant with high resistance to cefepime, ampicillin and trimethoprim-Sulfametoxazole. No resistance strain has been demonstrated for imipenem, gentamycin and vancomycin. The great variability in isolates shows their susceptibility to cause food poisoning. Therefore, they pose a public health problem. However, the number of enterotoxigenic bacteria consumed and the amount of toxin produced by a given strain will determine the outcome.

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