American Journal of Microbiological Research, 2025, Vol. 13, No. 3, 52-57
Available online at http://pubs.sciepub.com/ajmr/13/3/3
Published by Science and Education Publishing
DOI:10.12691/ajmr-13-3-3

Epidemiology and Phenotypic Profile of *Bacillus cereus* Strains Isolated from Diarrhoeal Stools of Children Aged 0 to 5 Years in Port-Bouët, Abidjan

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Received May 12, 2025; Revised June 14, 2025; Accepted June 22, 2025

Abstract **Background:** Diarrhoeal diseases represent a major cause of morbidity and mortality among children under five years, particularly in sub-Saharan Africa. Although diarrhoea caused by Bacillus cereus is infrequent and typically mild, confirming its presence remains challenging. This study aimed to characterise the phenotypic features of Bacillus cereus strains isolated from diarrhoeal stools of children aged 0–5 years at Port-Bouët General Hospital. document gives formatting instructions. **Methods:** A prospective cross-sectional study was conducted from February to September 2020 at the Clinical Bacteriology Unit of the Pasteur Institute in Côte d'Ivoire. Faecal samples from children aged 0 to 5 presenting with diarrhoea were collected using rectal swabs and transported in Cary-Blair medium. Presumptive Bacillus cereus strains were isolated using brain-heart infusion broth for pre-enrichment, Mossel (MYP) agar for primary culture, and sheep blood agar for confirmation. Biochemical identification was conducted using the API 50CH gallery, and antibiotic susceptibility was determined by Kirby-Bauer disk diffusion according to EUCAST requirements. **Results:** Of 186 stool samples analysed, 16 Bacillus cereus isolates were obtained. Half of these patients were under 12 months, with a sex ratio of 1.28. All isolates exhibited peritrichous motility and complete resistance to benzylpenicillin, ampicillin, amoxicillin, and trimethoprim. Low resistance rates were observed for clindamycin (6.25%), vancomycin (12.5%), and imipenem sensitivity reached 93.75%. Clinically, abdominal pain (87.5%), vomiting (56.25%), and fever (87.5%) were the predominant symptoms observed. **Conclusion:** Bacillus cereus should be routinely included in stool culture protocols due to its distinctive phenotypic profile and potential pathogenicity.

Keywords: *Antibiotic resistance, Bacillus cereus, Diarrhoea, Port-Bouët, Stool culture*

Cite This Article: Adompo Jaurès Cédric Yapi, Kalpy Julien Coulibaly, Syndou Meité, Alice Britoh-Mlan, Flore Zaba, Ivanne Alexia Dechy Yapi, Gninissemet Armel Bahan, Sodji Emilie Karen N'goran, Sally Otolorin Diakité, and Mireille Dosso, "Epidemiology and Phenotypic Profile of *Bacillus cereus* Strains Isolated from Diarrhoeal Stools of Children Aged 0 to 5 Years in Port-Bouët, Abidjan." *American Journal of Microbiological Research*, vol. 13, no. 3 (2025): 52-57. doi: 10.12691/ajmr-13-3-3.

1. Introduction

Foodborne diseases remain a major but underreported public health challenge globally, particularly in low-resource settings where diagnostic capacities and surveillance infrastructure are insufficient [1]. Among the numerous bacterial agents implicated, *Bacillus cereus* is frequently isolated from contaminated food products and is recognized as a significant cause of toxin-mediated foodborne illness worldwide [2,3,4] .Diarrhoeal diseases remain a leading cause of morbidity and mortality in children under five, particularly in sub-Saharan Africa. Despite improvements in water, sanitation, and hygiene, the burden remains substantial [5]. Diarrhoea is clinically defined as the passage of three or more loose stools per day, often exceeding 300 grams in daily stool output [6]. Children under five are especially vulnerable to such infections due to their immature immune systems and limited metabolic reserve, which often predispose them to more severe outcomes [1,7,8].

Although *B. cereus* is not commonly included in routine stool culture protocols, data from various countries suggest its involvement in paediatric diarrhoeal cases. This pathogen is ubiquitous in the environment and has been isolated from a wide range of food sources, including dairy products, cooked rice, meats, vegetables, and ready-to-eat meals [1,2,9,10,11].

Taxonomically, *B. cereus* is part of a genetically related group of seven species known as *B. cereus sensu lato*, which includes *B. anthracis*, *B. thuringiensis*, and *B. cytotoxicus* [12,13]. Among them, *B. cereus sensu stricto* is responsible for two distinct syndromes: an emetic form caused by the heat-stable cereulide toxin, and a diarrhoeal form linked to enterotoxins [14]. Although *B. cereus* is often overshadowed by better-known enteropathogens such as *Escherichia coli*, *Salmonella spp.*, and *Shigella spp.*, its role in gastroenteritis may be underestimated [12,15]. First identified as a foodborne pathogen in the 1950s , it continues to be underdiagnosed due to the typically mild and self-limiting nature of infections, as well as the absence of routine screening protocols in many African microbiology laboratories [14,16]. The lack of selective media and molecular diagnostic tools further complicates detection and epidemiological assessment.

In Côte d'Ivoire, Diarrhoea is the second leading cause of outpatient consultations among children, after malaria [17]. Structural factors such as overcrowding, poor sanitation, low income, and limited access to safe drinking water exacerbate this burden, especially in peri-urban areas like Port-Bouët, where informal settlements house more than 60% of the population [18]. Limited research has been dedicated to *B. cereus* infections, and current laboratory protocols rarely prioritise its detection during gastroenteritis outbreaks [19,20]. Given the absence of active surveillance, the epidemiology of *B. cereus* in this region remains poorly characterised**.**

In this context, the present study aimed to isolate and phenotypically characterise *B. cereus* strains from stool samples collected from children aged 0–5 years hospitalised with acute diarrhoea at the General Hospital of Port-Bouët. The findings are intended to inform diagnostic practices and contribute to future efforts in foodborne disease surveillance and control in Cote d'Ivoire.

2. Methodology

2.1. Sample Collection

Bacillus cereus strains were isolated during microbiological analysis of stool samples collected from children aged 0 to 5 years. These children were brought by their parents to the paediatrics consultation or emergency department of Port-Bouët General Hospital for evaluation of acute gastroenteritis, either febrile or afebrile. Stool samples were obtained via rectal swabs and promptly transported to the laboratory in Cary-Blair transport medium.

2.2. Bacterial Isolation and Identification

After pre-enrichment, samples were streaked onto MYP agar and incubated at 30°C for 18–24 hours, following incubation, plates were examined for colonies exhibiting the characteristic morphology of *B. cereus*—large, pink colonies surrounded by an opaque, whitish halo resembling a candle wax drop. These colonies were subcultured on nutrient agar for purification. Presumptive *B. cereus* colonies were further inoculated onto sheep blood agar and incubated overnight at 37°C to assess haemolytic activity, finally Isolates were subjected to biochemical identification using the API 50CH system (bioMérieux).

2.3. Antibiotic Susceptibility Testing

Antibiotic susceptibility was assessed using the standard disc diffusion method on Mueller-Hinton agar. A total of twelve antibiotic discs were tested against the *Bacillus cereus* isolates. The antibiotics included: Ampicillin (10 µg), Amoxicillin (10 µg), Penicillin G (10 U), Ciprofloxacin (5 µg), Clindamycin (2 µg), Vancomycin (30 µg), Gentamicin (10 µg), Imipenem (10 µg), Meropenem (10 µg), Tetracycline (30 µg), Trimethoprim-Sulfamethoxazole (1.25 µg/23.75 µg), and Erythromycin (15 µg). Due to the absence of specific EUCAST interpretative criteria for the *Bacillus* genus, zone diameter measurements were interpreted based on the critical breakpoints of the antibiotic discs used, in accordance with previously established non-species-specific guidelines. The reference strain *Bacillus cereus* ATCC 14579 were used for quality control.

2.4. Statistical Analysis

Data were initially entered into a Microsoft Excel spreadsheet and subsequently imported into Epi Info software, version 7.2.3.1, for statistical analysis. Categorical variables were expressed as proportions.

2.5. Ethical Considerations

The study received formal approval from the Pasteur Institute of Côte d'Ivoire. Patient anonymity and the confidentiality of all collected data were strictly maintained throughout the research process, in accordance with ethical standards for biomedical research involving human participants. Each participant (or their legal guardian) was informed about the study's objectives, data collection procedures, potential benefits, and associated risks. Informed consent was obtained from all participants prior to inclusion in the study.

3. Results

3.1. Sample Collection and Bacterial Isolation

A total of 186 stool samples from children aged 0 to 5 years were collected and analysed between February and September 2020. From these, 16 bacterial strains belonging to the *Bacillus cereus* group were isolated and identified.

3.2. Epidemiological Characteristics

Among the children from whom *Bacillus cereus* was isolated, 50% were under 12 months of age, with a sex ratio of 1.28 (male to female). Almost all participants lived in shared housing compounds with access to potable water. In terms of food hygiene practices, the majority of parents (81.25%) reported inadequate food storage methods, typically involving reheating followed by storage at ambient temperature. Clinically, the most frequently reported symptoms included abdominal pain (87.5%), vomiting episodes (56.25%), and fever (87.5%), while general physical condition remained stable in nearly all cases (Table 1).

Table 1. Demographic, Environmental and Clinical Characteristics of Children with *Bacillus cereus* Isolates (n = 16)

Variable	Category	Frequency (n)	Percentage (%)
Age	≤ 12 months	8	50.0
	> 12 months	8	50.0
Sex	Male	9	56.3
	Female	7	43.8
Type of housing	Shared courtyard housing	13	81.3
	Other	3	18.8
Access to potable water	Yes	14	87.5
	No	2	12.5
Food storage practices	Adequate	3	18.8
	Inadequate	13	81.3
Reported ingestion of unsafe food	Yes	12	75.0
	No	4	25.0
Abdominal pain	Present	14	87.5
	Absent	2	12.5
Vomiting	Present	9	56.3
	Absent	7	43.8
General physical condition	Preserved	14	87.5
	Altered	2	12.5
Signs of dehydration	Present	1	6.3
	Absent	15	93.8
Fever	Present	14	87.5
	Absent	2	12.5

Table 2. Morphological, cultural and biochemical characteristics of *Bacillus cereus* isolates (n = 16)

Characteristic	Positive n (%)	Negative n (%)
Morphological Features		
Rod-shaped bacilli	16 (100)	-
Gram-positive staining	16 (100)	-
Arrangement in short chains	14 (87.5)	2 (12.5)
Central, non-deforming spore	14 (87.5)	2 (12.5)
Capsule	-	16 (100)
Motility (peritrichous)	16 (100)	-
Cultural Features		
Fastidious nutritional requirement	-	16 (100)
Anaerobic growth capability	16 (100)	-
Haemolysis on fresh sheep blood agar	16 (100)	-
Biochemical features		
Catalase production	2 (12.5)	14 (87.5)
Cytochrome oxidase activity	11(68.75)	5 (31.25)
Lecithinase production	16 (100)	-
Mannitol fermentation	-	16 (100)
Glucose fermentation	16 (100)	-
Indole production	-	16 (100)
Hydrogen sulfide (H_2S)	-	16 (100)

3.3. Bacteriological and Biochemical Characteristics

Macroscopically, the isolated *Bacillus cereus* strains were predominantly associated with stool samples exhibiting soft or liquid consistency, each representing 43.75% of positive cases. Mucous and bloody stools were less common, observed in 12.5% of the *B. cereus*-positive samples.

The presumptive bacterial isolates obtained from stool samples displayed distinctive morphological and cultural characteristics. Microscopically, fresh stool examination revealed an absence of leukocytes in 87.5% of the positive cases, and erythrocytes were absent in 93.75% of the analysed samples. The bacterial flora was considered balanced in 87.5% of the cases. All presumptive *B. cereus* colonies exhibited characteristic peritrichous polar motility. They were identified as Gram-positive bacilli, predominantly arranged in chains, with centrally located spores that did not deform the bacterial cell body. These non-encapsulated isolates exhibited peritrichous flagellar motility and were capable of facultative aerobic and anaerobic growth. On sheep blood agar, they produced clear zones of haemolysis, indicating strong haemolytic activity.

Biochemically, all isolates showed a consistent absence of mannitol fermentation. Furthermore, all strains were catalase- and lecithinase-positive. However, cytochrome oxidase activity was variable among the isolates, with 68.75% testing positive for oxidase production (Table 2).

3.4. Antibiotic Susceptibility Profile

All *Bacillus cereus* isolates exhibited complete resistance (100%) to benzylpenicillin, ampicillin, amoxicillin, and trimethoprim (Table 3). Conversely, all isolates (100%) were fully susceptible to streptomycin, gentamicin, and meropenem.

Additionally, 93.75% of the strains were susceptible to clindamycin, 87.5% to vancomycin, and 93.75% to imipenem.

Table 3. Antibiotic susceptibility profile of Bacillus cereus isolates (n = 16)

Antibiotics	Susceptibility		
	Resistance Freq. (%)	Intermediary Freq. (%)	Sensibility Freq. (%)
Amoxicillin	16 (100)	-	-
Benzylpenicillin	16 (100)	-	-
Ampicillin	16 (100)	-	-
Erythromycin	2 (12,5)	4 (25)	10 (62,5)
Streptomycin	-	-	16 (100)
Gentamycin	-	-	16 (100)
Meropenem	-	-	16 (100)
Imipenem	1 (6,25)	-	15 (93,75)
Clindamycin	1 (6,25)	-	15 (93,75)
Tetracyclin	8 (50)	1 (6,25)	7 (43,75)
Vancomycin	2 (12,5)	-	14 (87,5)
Trimetroprime	16 (100)	-	-

4. Discussion

4.1. Study Limitations

The relatively small number of *Bacillus cereus* isolates analysed in this study limited the statistical power to establish strong associations between phenotypic characteristics and clinical outcomes. However, this work remains valuable as it was conducted under real-world field conditions in a resource-constrained setting, offering insights into local microbial ecology and health system capacities [2,11,19].

4.2. Epidemiological and Clinical Characteristics

In the present study, 50% of the paediatric patients were under 12 months old, with a male-to-female ratio of 1.28. The heightened vulnerability of infants to diarrhoeal diseases can be attributed to immature immune defences and the progressive loss of passive maternal antibodies. Additionally, weaning practices introduce dietary transitions that, when unhygienic or nutritionally imbalanced, may predispose children to enteric infections and malnutrition [21,22].

While most households in this cohort had access to potable water—indicative of recent public health progress in areas like Port-Bouët [18]. The majority (81.25%) employed suboptimal food preservation techniques, including ambient-temperature storage after reheating. These conditions are conducive to the proliferation of heat-tolerant spore-formers such as *B. cereus*, whose spores survive cooking and germinate upon cooling [13,16,19].

Clinically, children commonly presented with abdominal pain (87.5%), fever (87.5%), and vomiting (56.25%). These symptoms align with the diarrhoeal form of *B. cereus* gastroenteritis, often linked to enterotoxin production, particularly non-hemolytic enterotoxin (Nhe) and hemolysin BL (Hbl) [12,14,19]. Young children, especially those under two and school-aged, are more likely to develop severe symptoms **[1]**.

4.3. Bacteriological Characteristics

Macroscopic analysis of *B. cereus*-positive stool samples revealed predominantly loose or watery consistency. Fresh microscopy indicated a low prevalence of leukocytes and erythrocytes, supporting a non-invasive toxin-mediated pathology rather than inflammatory diarrhoea [23].

Isolates exhibited characteristic traits of the *B. cereus* group: Gram-positive rod morphology, peritrichous motility, central non-deforming spores, and β-hemolysis on blood agar. These features, along with lecithinase and catalase positivity and mannitol fermentation negativity, are well-documented in the literature [12,16,24]. Similar findings were reported by Chon in Korea, where the majority of *Bacillus cereus* isolates tested positive for key biochemical activities, including salicin hydrolysis (86%), starch fermentation (89%), hemolysis (89%), motility (100%), and lecithinase production (89%) [25].

Oxidase positivity was noted in 68.75% of isolates, though this trait varies among strains. Given the biochemical overlap within the *B. cereus* group, advanced identification methods such as MALDI-TOF mass spectrometry or PCR-based assays targeting toxin genes (e.g., *nhe*, *cytK*, *hbl*) are increasingly recommended [26,27,28].

4.4. Antibiotic Resistance

All isolates demonstrated resistance to penicillin-class antibiotics (benzylpenicillin, ampicillin, and amoxicillin) and trimethoprim. This is consistent with the known intrinsic resistance of *B. cereus* to β-lactams, mediated by chromosomally encoded β-lactamases, including both serine β-lactamases and metallo-β-lactamases [16,29,30].

High susceptibility rates were observed for streptomycin, gentamicin, meropenem, clindamycin, vancomycin, and imipenem. These findings are consistent with previous studies and confirm the preserved efficacy of aminoglycosides and glycopeptides against *B. cereus* [31]. Resistance to trimethoprim, as noted in this study, has been previously reported and may be due to efflux pumps or enzymatic bypass mechanisms [32,33].

Fiedler [33] reported comparable antimicrobial susceptibility patterns, with the majority of *Bacillus cereus* strains showing high sensitivity to ciprofloxacin (99.3%), chloramphenicol (98.6%), amikacin (98.0%), imipenem (93.9%), erythromycin (91.8%), and gentamicin (88.4%), while moderate susceptibility was observed for tetracycline (76.2%) and trimethoprim-sulfamethoxazole (52.4%).

Colistin susceptibility was not evaluated, as this polymyxin is generally ineffective against Gram-positive bacteria due to the absence of an outer membrane [34]. Antibiotics susceptibility test show alarming rate of resistance to beta-lactam antibiotics specially ampicillin and penicillin [35].

The pattern of resistance observed underscores the importance of local antimicrobial stewardship and highlights the diagnostic relevance of including *B. cereus* in differential diagnoses of paediatric diarrhoea, especially in settings where refrigeration is limited.

5. Conclusion

In summary Half of the patients included in the study were under 12 months of age. Most children lived in shared courtyard-type housing with access to piped water. However, inadequate food storage practices were prevalent among households, particularly reheating followed by storage at ambient temperature; conditions known to favour the growth of thermotolerant organisms such as *B. cereus*.

The predominant clinical features among patients included abdominal pain (87.5%), vomiting (56.25%), and fever (87.5%), with preserved general condition in most cases.

Presumptive identification of *B. cereus* was based on colony morphology, Gram staining, and standard biochemical tests, with confirmation achieved via β-haemolysis on fresh sheep blood agar.

Antimicrobial susceptibility profiling revealed complete resistance of all *B. cereus* isolates to benzylpenicillin, ampicillin, amoxicillin, and trimethoprim. In contrast, all strains were susceptible to streptomycin, gentamicin, and meropenem. These findings reflect the known intrinsic resistance patterns of *B. cereus* and underscore the importance of accurate identification for clinical management and outbreak prevention.

Looking forward, further investigations employing advanced molecular techniques are planned to genotype the isolates and establish a more definitive link between strain characteristics and clinical outcomes.

Declaration of Conflicting Interests

The authors declare no potential conflicts of interest in the research, writing, and/or publication of this article.

Authors' Consent

After several readings and corrections of the manuscript, the authors approved the publication of this article.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to the Department of Bacteriology and Virology of the Institut Pasteur of Côte d'Ivoire for providing both the facilities and financial support necessary to conduct the microbiological analyses. Special thanks are also extended to the paediatrics consultation unit of the General Hospital of Port-Bouët for their collaboration in participant recruitment, and to the National Antibiotic Reference Center for their valuable technical assistance.

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