

## *Bacillus cereus* risk assessment in raw milk consumed in the informal dairy sector in Côte d'Ivoire

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# Background (1)

- In Côte d'Ivoire as elsewhere in Africa, milk plays an important role in human nutrition
- **Formerly** = consumed by people in rural areas of production
- **Now** = milk is widely consumed in urban areas
- Milk production activities are **important in West Africa and generate significant income in rural households** (34% of total income)
- Unregulated informal sector accounts for 80% of the channels of production, distribution and marketing
- Côte d'Ivoire is no exception to this rule:
  - ✓ 92% dairy farmers own small subsistence farms and herders did not go to school and have no hygiene awareness

## Background (2)

Currently no methods are applied by the actors in the production systems to substantially reduce the contamination.

**Consequence:** Milk is contaminated with pathogens in general and in particular with *Bacillus cereus* considered as an emerging opportunistic pathogen and an indicator of environmental contamination.

*B. cereus* is one of the bacteria associated with food poisoning in humans and causes **two types of foodborne illnesses:**

**A diarrheal intoxication**

***B. cereus* emetic toxin syndrome**

The type of poisoning is determined by the kind of enterotoxin elaborated.

# Aims

**Therefore, what is the risk associated with the consumption of raw milk contaminated by *B. cereus* for the consumer in Côte d'Ivoire ?**

## Objectives

Assess the risk associated with *B. cereus* incurred to the consumer of raw milk traditionally produced in Abidjan.

# Material and Methods (1)

- 5 sites of milk production and sale of raw milk were selected.
- Surveys were conducted with 15 herders, 15 vendors and 188 clients to identify practices of breeders and vendors who have an influence on milk quality, mode of consumption of milk and risk to the consumer.
- 320 samples were sampled at all stages of the production chain of milk.

Sites	Farms <b>N = 15</b>	Cows <b>N = 119</b>	Vendors <b>N = 15</b>
Port-bouet	4	21	6
Lièvre rouge	2	33	2
Songon-té	1	9	-
Abobo derrière rail	-	-	7
Abobo N'dotré	8	56	-

## Samples to determine critical points

- 119 udder milk
- 14 herder's bulk milk
- 17 retailer's pooled milk

## Samples to identify sources of contamination

- 113 udder skin swabs
- 22 hand swabs of farmers
- 14 rinsing water for utensils
- 16 environment samples
- 5 samples of water used to clean material for milking in 5 farms



## Material and Methods (2)

- ✓ All the samples were analyzed to isolate and identify *B. cereus* strains by classical bacteriological methods and by PCR
- ✓ Virulence genes of these strains were determined by PCR
- ✓ The sensitivity of the virulence strains to antibiotics commonly used to treat food poisoning was determined

# Results (1)

**No hygienic practices before, during milking in between milking by milkers. No cooling, pasteurization of milk after milking and milking was done in the mud and dung.**

**Sources of contamination by decreasing order of importance: Udder, water, Environment, Milk vendor containers, Hands of the milker (s) and Utensils**

<b>Samples</b>	<b>Percentage of samples contaminated (%)</b>	<b>Mean count (ufc/ml)</b>
Udder milk	26.9	$4.2 \times 10^2$
Herder's bulk milk	28.6	$10^2$
Retailer pooled milk	41.2	$4.1 \times 10^3$
Udder skin swabs	64.6	$3 \times 10^3$
Hand swabs of farmers	40.9	$1.3 \times 10^2$
Rinsing water for utensils	28.6	$0.3 \times 10^2$
Environment samples (Air)	56.3	$0.7 \times 10^2$
Water used to clean material for milking	60	$6.7 \times 10^2$

## Results (2)

12.8% of consumers reported they had food poisoning after consuming milk and diarrhoea was the most symptom reported. Severe cases requiring a stay of 1 to 3 days in hospital were reported by 12.5% of those infected.

The probability of consuming milk contaminated with *B. cereus* was 22.2%; Foodborne disease occurrence for the consumer was significantly related to the consumption of unheated milk. Chi square of Pearson = 5.01; Pc = 0.025; RR = 2.584; IC 95%: [1.074; 6.22]

Intensity	Diseases N = 24 (12.8)
Less serious (still working)	13 (54.2)
Moderately serious (stay at home)	8 (33.3)
<b>Serious (1 to 3 days in hospital)</b>	<b>3 (12.5)</b>

Exposure	Sick people after milk consumption	Healthy people after milk consumption	Total
Consumption of milk without thermal treatment	18	83	101
Consumption of milk after thermal treatment	6	81	87
<b>Total</b>	<b>24</b>	<b>164</b>	<b>188</b>

Symptoms	Case (N = 37)
<b>Diarrhea</b>	<b>19 (51.4)</b>
Fever	5 (13.5)
Stomach bloat	4 (10.8)
Stomach pains	3 (8.1)
Vomiting	3 (8.1)
Nausea	1 (2.7)
Headache	1 (2.7)
Cough	1 (2.7)





## Results (3)

**All *B. cereus* group strains (88 strains) isolated were virulent and harbored at least one of the virulence genes** by decreasing order *hblD* (79/88), *cytK-2* (79/88), *nheC* (65/88), *hblC* (60/88), *bceT* (34/88), *nheA* (32/88), *nheB* (22/88) and *hblA* (12/88), **all involved in the aetiology of diarrheal syndroms.**

7 strains (7.9%) contained the 3 components of the HBL complex and in 2 strains (2.3%), the 3 components of the NHE complex. Only 1 strain (1.1%) contained simultaneously the 3 components of HBL and NHE complex.

## Results (4)

They were resistant to several antibiotics including Tetracyclin (92%) and to a lesser extent to Ciprofloxacin (71.6%). The intermediately susceptible were detected with Vancomycin (52.3%), more and less Imipenem (47.7%).

All *Bacillus cereus* strains were susceptible to Clindamycin (100%) and to a lesser extent to Gentamicin (73.9%).

Antimicrobial agent	Number (%) of strains		
	Sensible	Intermediate	Resistant
Ampicillin (10 µg)	0 (0)	0 (0)	88 (100)
Penicillin (10 U)	0 (0)	0 (0)	88 (100)
Cefepime (30 µg)	0 (0)	0 (0)	88 (100)
Chloramphenicol (30 µg)	5 (5.7)	47 (53.4)	36 (40.9)
Ciprofloxacin (5 µg)	3 (3.4)	22 (25)	63 (71.6)
<b>Clindamycin (2 µg)</b>	<b>88 (100)</b>	<b>0 (0)</b>	<b>0 (0)</b>
Vancomycin (30 µg)	46 (52.3)	0 (0)	42 (47.7)
Gentamicin (10 µg)	65 (73.9)	20 (22.7)	3 (3.4)
Imipenem (10 µg)	42 (47.7)	36 (40.9)	10 (11.4)
<b>Tetracyclin (30 µg)</b>	<b>4 (4.5)</b>	<b>3 (3.4)</b>	<b>81 (92)</b>
Trimethoprim-sulfamethoxazole (1.255 µg/23.75 µg)	0 (0)	0 (0)	88 (100)
Erythromycin (15 µg)	32 (36.)	50 (56.8)	6 (6.8)



# Conclusion

- Some *Bacillus cereus* strains in milk and dairy products have beneficial effects and have been used as probiotics in the diets of immunocompromised people and against diarrhoea in young children.
- However, *Bacillus cereus* isolated in informal dairy sector and milk in Côte d'Ivoire were multi-toxigenic and multiresistant to antibiotics and **could be considered having a potential to cause food poisoning.**
- Multiresistance to antibiotics is not surprising considering how frequently these drugs have been used improperly in treating disease caused by pathogenic microorganisms at the farms and **indicates the presence of other pathogenic bacteria in milk.**
- These strains pose a significant health risk to the consumer, transmission of antibiotic resistance to other microorganisms in the gastrointestinal being possible.

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