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Prevalence and characterisation of *Bacillus cereus* in cooked rice retailed in Ilara-Mokin, Nigeria

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ABSTRACT

Bacillus cereus is widely distributed in different food products and causes a variety of symptoms associated with food poisoning. Rice has been suggested as a vehicle for contamination and being involved in *B. cereus* intoxication. Based on the aforementioned risks, studies of *Bacillus cereus* incidence along with its isolation and characterization are essential to establishing the safety of cooked rice. Using the selective culture technique, pure isolates of *Bacillus cereus* strains were characterized and identified based on cultural, and biochemical features. Characterization was done on the ability of the bacterial strains to hydrolyse casein and starch, ferment lactose, and lyse red blood cells (haemolysis). Out of the 47 samples screened, 45 isolates were detected in 14 samples. Antibiotic susceptibility testing revealed that all isolates showed resistance to Ampicillin (10 µg), but were susceptible to Erythromycin (15 µg), Vancomycin (30 µg), Tetracycline (30 µg), Ampicillin (10 µg), Gentamicin (10 µg), and Chloramphenicol (30 µg). 62.2%, 64.4%, 0% and 46.7% were capable of producing amylase, protease, ferment lactose and lyse red blood cells respectively. This study shows that 14 samples out of 47 samples of cooked rice in this work have the possible risk of foodborne infections/ intoxication that occurs as a result of the possibility of the development of *B. cereus* in favourable conditions and consumption of these products. Basically, prevention is by proper handling of raw materials, controlling the temperature of cooking and storing rice, and personal hygiene of food handlers.

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1. Introduction

Rice, as a consequence of its cultivation, harvesting, and handling, is often contaminated with spores of *Bacillus cereus*, a ubiquitous microorganism found mainly in the soil.

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B. cereus can multiply under temperature conditions as low as 4ºC in foods that contain rice and have been cooked or subjected to treatments that do not produce commercial sterility. Albeit, cooking rice above 55ºC can kill *B. cereus* cells, spores can survive cooking and then germinate and grow bacteria when growth conditions are suitable (1).

B. cereus is a rod-shaped spore-forming bacterium that belongs to the Bacillus genus. It is gram-positive and motile due to flagella.

B. cereus is a habitual saprophyte, which is resistant to low humidity, high temperatures, dehydration, radiation and acidity (2). This microorganism is of interest in public health as it is considered an opportunistic pathogen that produces food toxins. *B. cereus* produces two types of illness, the emetic and diarrhoeal syndrome, depending on the context in which it grows. The diarrheal syndrome is an example of a toxic infection by enterotoxin produced as a consequence of ingestion of a large number of vegetative cells or spores that pass the stomach barrier, during their growth in the small intestine (3). The emetic toxin "cereulide" is a cyclic peptide produced during the growth of *B. cereus* in the food itself (4).

Rice is the grain of herbaceous plants of the genus *Oryza* cultivated for more than 8000 years and of which about 750 million tons are produced per year mainly for human consumption (5). Two–third of the world population relies on rice due to its important nutritive property and energy value. Persons in the Asia Pacific region, parts of the Caribbean and Latin America largely depend on rice for caloric supply and nutrition. Consumption of rice is increasing in Africa (6). Rice is a principal source of fibre, energy, minerals, proteins, vitamins, antioxidants and other biomolecules which may act in synergy and exerted an advantageous effect on health (5-6). This cereal is presented to the consumer in different ways: whole, husked, or white depending on the treatment to which the grain is subjected.

The harvested rice kernel, known as paddy, or rough rice, is enclosed by the hull, or husk. Milling usually removes both the hull and bran layers of the kernel, and a coating of glucose and talc is sometimes applied to give the kernel a glossy finish. Rice that is processed to remove only the husks, called brown rice and contained about 8% protein and small amounts of fat and is a source of thiamine, niacin, riboflavin iron and calcium. Rice that is milled to remove the bran as well is called white rice and is greatly diminished in nutrients (5-7). Rice also accept any forms of industrial cooking and processing, steaming, parboiling, instant, and ground (7). Each of them represents a different risk for the consumer depending on the subsequent treatment that consumer applies prior to consumption.

Rice; with pH close to 7, consisting of 79% of carbohydrates, 7% protein and 2% fat plus vitamin and minerals, can act as an excellent growth medium for *B. cereus* once it has been cooked because it is in that moment when the humidity of the substrate reaches water activity values suitable for the growth of microorganism. *B. cereus* can be found in soil, plants and even intestinal tract of insects and mammals. Bacteria can move from the soil to paddy fields, their spores can persist for years and these can hide in raw rice, even surviving during cooking due to their resistance to extreme temperatures (8-9). Even if the vegetative cells of *B. cereus* do not grow, they can survive 48 weeks on fresh and dry storage without loss of viability. Nevertheless, the viability of the pathogens is reduced in 16 weeks, if the storage occurs at temperatures above 45^{o}C with water activity around 0.78.

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The main problem posed by the contamination of foods with *B. cereus* is the presence of heat-resistant spores that survive normal cooking temperatures for rice (9). The aim of this study is to determine the prevalence of *B. cereus* in different forms of cooked rice in Nigeria including fried rice, jolof rice, white rice and ofada rice. *B. cereus* isolates were further assayed for their ability to hydrolyse casein and starch, lyse red blood cells, ferment lactose and their susceptibility to antibiotics as evidence of pathogenicity potential to consumers.

2. Materials and Methods

2.1. Collection of sample

The various food samples were obtained from food vendors within Ilara-Mokin. The varieties include: 'Jollof rice', which is a cooked long grain rice with tomatoes, onions, spices and meat as a concoction; 'Fried rice' is a dish of cooked rice that was stir-fried with cooking oil before mixing with other ingredients including vegetables, seafood and meat; 'White rice' is a dish of boiled long or short grain rice without the addition of ingredients but served with desired sauce; 'Ofada rice' is a locally grown rice in Nigeria, it is known as unpolished rice in its natural state without genetic modification. It is boiled and served with an indigenous sauce in plantain leaves for a characteristic aroma and flavour. They were transported to Elizade University laboratory properly for analysis. Altogether, 47 samples of food were examined. These number includes 10 samples of fried rice, 15 samples of jollof rice, 14 samples of white rice and 8 samples of ofada rice.

2.2. Enumeration of *B. cereus*

B. cereus counts were assayed in the examined products according to the colony count technique. The samples were collected under sterile conditions in the amount 10 g and homogenized using mortar and pestle with some diluent (90 ml sterile water) for 5 min. One millilitre of the homogenates was spread on chromogenic *B. cereus* agar plates. Five typical colonies were picked from each plate (large, yellow and surrounded by turbidity) and streaked on chromogenic *B. cereus* agar to obtain distinct colonies for further test. The examination of the isolates was performed using the ISO 7932 procedure taking into account properties regarded as characteristic of *B. cereus*. The isolates were examined for their ability to incorporate the chromogenic substrate 5-chromo-4-chloro-3-indole-β-glucoyranoside, which is cleaved by the enzyme β-glucosidase present in *B. cereus* resulting in the yellow colonies. These isolates identified and confirmed by such examination were stored on *B. cereus* chromogenic agar slant for further analysis.

2.3. Antimicrobial susceptibility testing

The sensitivity of *B. cereus* to 6 antimicrobial agents was tested using the standards Kirby-Bauer disk diffusion method (10 – 14). The *B. cereus* isolates were streaked on the nutrient agar plate and grown 16-18 h at 37°C. The colony was then picked and suspended using 0.85% physiological saline to 0.5 McFarland standard and spread on the surface of a Mueller-Hilton agar plate. After the inoculum was dried, the antimicrobial disks were put on the surface of the plates. The Mueller-Hilton agar plates were incubated 16-18 h at 35±2°C, and the inhibition zone was measured.

The isolates were classed as susceptible (S), intermediate (I) or resistant (R) according to CLSI guidelines and the inhibition zone diameter interpretation (13-14).

2.4. Characterization of *B. cereus* isolates with respect to their capacity for casein hydrolysis, starch hydrolysis and lactose fermentation

Altogether, 45 isolates were gotten from 14 samples out of 47 samples that were tested including 7 isolates from fried rice, 10 isolates from jollof rice, 7 isolates from white rice and 21 isolates from ofada rice. Apart from identity confirmation carried out according to ISO 7932 procedure, all isolates were tested for their abilities to: Hydrolysed casein, starch and lactose fermentation.

2.4.1. Starch Hydrolysis

The ability to hydrolyse starch was determined by streaking cultures of the examined isolates onto nutrient agar pre-dried on petri plates to which 0.25% soluble starch had been added and incubated at 37^{o}C for 24 h. The presence of the zone of inhibition indicates the hydrolysis of starch. A drop of iodine solution was placed on the edge of the colonies. The amylolytic properties (hydrolysis of starch) were confirmed by the bluish-black coloration formed (15).

2.4.2. Extracellular protease production activity

The ability of the examined isolates to hydrolyse casein was tested by streaking cultures onto nutrient agar plates supplemented with 15% skimmed milk and incubating them at 37^{o}C for 24 h. The appearance of clear zones around the colonies indicated the degradation of casein (15).

2.4.3. Lactose Fermentation

The isolates were also tested for their ability to ferment lactose. In brief, a colony of the examined isolate was selected with a sterile loop to test tubes with a liquid medium containing 1.5 g peptone water, 0.5 g phenol red, 1 g of lactose in 200 mL distilled water. The mixtures were incubated at 37^{o}C for 72 h. The colour change of the medium from red to yellow indicates lactose fermentation (15).

2.5. Virulence test

2.5.1. Haemolysis Test

A Nutrient medium of 5% human blood was used and this medium is called Blood Agar. Haemolysis was determined by making a spot inoculation of the isolate on the blood agar plate. This was then incubated overnight. The culture showing a darkening or discolouration of the medium in the vicinity of growth demonstrates alpha-haemolysis. Cultures showing clear halos around colonies and under are exhibiting β-haemolysis (15).

3. Results

3.1. Prevalence of *B. cereus* in Rice

The prevalence *of B. cereus* in the various cooked rice samples was illustrated in Table 1. Out of the 47 samples examined, *B. cereus* bacterial cells were present in 14 samples (29.8%), of which *B. cereus* was most frequently found in Ofada rice (10.6%), despite the fact that it has the least number of samples examined.

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Table 1. The prevalence of *B. cereus* in the retailed cooked rice

Sample type	sample size	Number of positive Samples	Number of positive Samples (%)
Fried Rice	10	2	20
Jollof Rice	15	4	26.67
White Rice	14	3	21.42
Ofada Rice	8	5	62.5

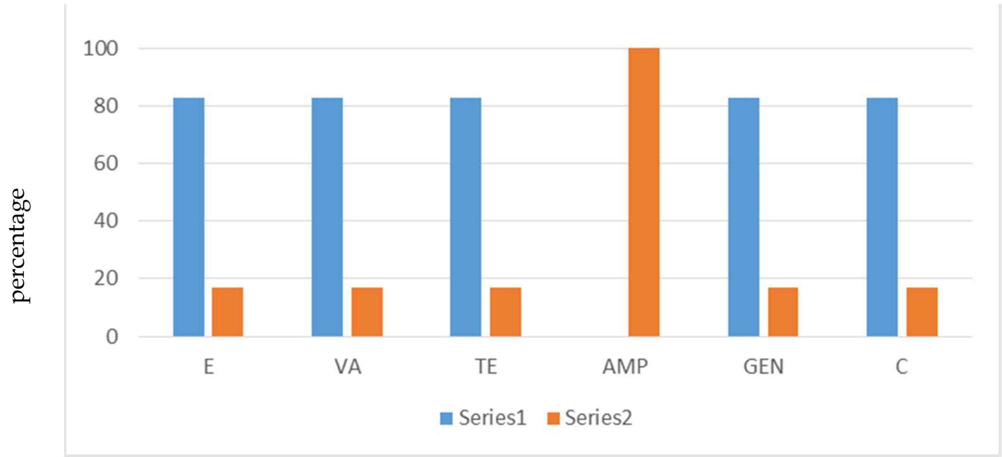

Figure 1. The frequency of antibiotic resistance among the bacteria strains isolated from cooked rice.

Erythromycin (15 µg)

VA: Vancomycin (30 μg)

TE: Tetracycline (30 μg)

AMP: Ampicillin (10 μg)

GEN: Gentamicin (10 μg),

C: Chloramphenicol (30 μg)

Table 2. Biochemical and physiological properties of presumptive *B. cereus*

Product	Number of tested isolates	Lactose fermentation	Starch hydrolysis	Casein hydrolysis	Heamolysis
White rice	7	0	6	7	6
Jollof rice	10	0	6	7	6
Fried rice	7	0	9	9	7
Ofada rice	21	o	7	6	2

3.2. Antibiotics sensitivity test

All isolates showed resistance to ampicillin and 93% of isolates were sensitive to erythromycin, vancomycin, tetracycline, gentamicin and chloramphenicol.

3.3. Characterization of *B. cereus* Isolates

Table 2 shows the biochemical characteristics of the 45 isolates from the chromogenic culture system. All 45 isolates exhibited the characteristic yellow colour. All the 45 isolates were negative for lactose fermentation. 64.28% of the isolates were able to degrade starch, 50% of the isolates were able to lyse red blood cells, and 57.14% were able to show extracellular protease production activity.

4. Discussion

4.1. Prevalence of *B. cereus* Isolates

Bacillus cereus is a Gram-positive foodborne pathogen that causes various symptoms and is found in multiple types of food. In this study, 14 of the 47 samples of cooked rice were positive for *B. cereus,* indicating that cooked rice is a potential risk to consumers. The prevalence of *B. cereus* in various cooked rice was shown in Table 1.

Among the examined products, ofada rice has the highest occurrence of *B. cereus*, this is contrary to the report in (17) where fried rice has the highest occurrence of *B. cereus* and this was attributed to the mode of preparation of fried rice because the rice is been parboiled before been stir-fried invariably parboiling will support sporulation of the *B. cereus* spores. Ofada rice is a locally grown rice in Nigeria, it is known as unpolished rice in its natural state without genetic modification. It is boiled and served with an indigenous sauce in plantain leaves for a characteristic aroma and flavour. Based on personal observation, the cleaning of ofada rice is not usually thorough. During milling of kernel after harvesting, only the husks is been removed and the bran is been retained which sometimes contain some soil particles, this may be one of the reasons for the high occurrence of *B. cereus* in it, this is in accordance with (5) which implies that soil is one of the natural reservoirs of the spore-forming *B.*

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cereus. Also, poor hygiene during cooking and selling such as the use of leaves that are contaminated to serve the rice to consumers could increase the level of contamination.

Research has shown that open-air stalls increase the opportunity for environmental pollution (18); for instance, cooked rice sold in open-air stalls can be exposed to dust-containing spores, increasing the chance of *B. cereus* contamination.

4.2. Characterization of *B. cereus* isolates

None of the isolates were able to ferment lactose. According to Berthold-Pluta *et al.* (15), *B. cereus isolates* isolated from milk and milk products show more ability to degrade lactose while those isolated from cereals, pasta and rice show little or no ability to degrade lactose. This finding shows a selection or adaptation of *B. cereus* strains during milk processing. Moreover, although some *B. cereus* isolates are unable to ferment lactose, they can grow in milk products upon hydrolysis of milk proteins or by glucose consumption following the fermentation of lactose by a competitive microorganism, for example lactic acid bacteria. 64.28% of the *B. cereus* isolated from cooked rice were able to hydrolyse starch and this is in agreement with (15) where rice product shows 64.4% starch degradation. Starch is a major component of this cooked rice, thus the presence of amylase-positive strains of *B. cereus* can lead to potential spoilage of these products. One of the distinguishing traits of emetic-type strains from the remaining *B. cereus* strain is the ability to hydrolyse starch (19). Therefore, the ability of *B. cereus* to hydrolyse starch can be regarded as the tendency for pathogenicity.

4.3. Antimicrobial Susceptibility of *B. Cereus*

Beyond food poisoning, *B. cereus* is also associated with non-gastrointestinal infections.

Antibiotic susceptibility testing can provide a reference for the clinical treatment of food poisoning. This study shows the characteristic antibiotic susceptibility pattern of the isolates. All the isolates were resistant to Ampicillin (100%). Which is in accordance with Yu et al 2020 where *B. cereus* isolated from ready-to-eat food showed 99.73% resistance to ampicillin. One isolate was resistant to all the antibiotics used. Approximately 93% of the isolates showed various degrees of susceptibility to all antibiotics used except ampicillin. All of the isolates were resistant to the β-lactam antibiotic. This result is unsurprising since *B. cereus* can produce β-lactamase (20 – 21). According to the results of our antimicrobial sensitivity testing, suspected *B. cereus* infections should not be treated clinically with broad-spectrum ampicillin.

5. Conclusions

In this study, we evaluated the prevalence of *B. cereus* in cooked rice retailed in Ilara-Mokin. Our results indicated that the retailed cooked rice is highly contaminated with *B. cereus* which has the tendency for food spoilage and pathogenicity which may increase the potential risk of foodborne diseases. In addition, most of the strains exhibited some level of resistance to the antibiotic and this is an important implication for clinical treatment.

Conflict of Interest

Each of the authors has no competing moral or financial interest in relation to the work described.

Also, this work has neither been published nor considered for publication anywhere else. We attest that neither animals nor humans was used in any of the experiments. The manuscript has been approved by all authors.

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