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Prevalence of *Bacillus cereus* in ready-to-eat boiled and fried rice in the Tamale Metropolis of Ghana

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ABSTRACT

The most popular street vended foods in Ghana; fried rice, Jollof and Waakye were examined in the Tamale Metropolis from April to May 2015 to determine the prevalence of *B. cereus*. Street vended Fried rice, Jollof rice and Waakye were purchased, transported aseptically and Bacteriological Analytical Manual guidelines were used to isolate and enumerate *B. cereus.* The samples were classified based on the microbial loads of $<10^3$ cfu/g, $10^3 \leq 10^4$ cfu/g and $>10^5$ cfu/g for satisfactory, borderline and unsatisfactory respectively. Fried rice recorded the highest number of unsatisfactory samples 18/30 (60.0%), followed by Waakye 8/30 (26.7%) and the least was Jollof 7/30 (23.3%). There is the need for regular training for vendors and further work to determine toxins produced by *B. cereus.*

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1. Introduction

Bacillus cereus is a spore forming and ubiquitous environmental bacterium that is found to contaminate food often times due to their presence in soil and atmosphere. *Bacillus cereus* is a heat resistant bacterium capable of surviving most cooking procedures due to the production of highly thermo-resistant spores. Food poisoning is usually resulted from surviving spores after heat treatment. *B. cereus* produces two types of toxin, emetic and diarrhoeal causing two types of illnesses.

There have been several food-related outbreaks and prevalence studies of this organism recorded in foods worldwide (1,2,3). There have also been prevalence studies of *B. cereus* from commercially vended foods in Africa (4,5,6). Street foods, which are mainly ready-to-eat-foods are integral source of food for many people in the developing countries especially in Africa. Most of the street foods or ready-to-eat foods sold in Africa are prone to contamination since they are easily exposed to the outside environment especially during services to consumers. This is compounded by the number of hours the food stay by the street before the last person buys it. More so, temperature is known to have an effect on growth and toxin production of *Bacillus cereus* in cooked rice. The poor hand hygiene exhibited among street food vendors couple with low level of education in Africa also aggravates the safety of street foods. However, high awareness level among consumers of fast foods about foodborne diseases has been reported in Ghana (7). There is no report on the prevalence of *Bacillus cereus* in street vended rice and waakye in the Tamale Metropolis of Ghana. The most popular street vended foods are fried rice, Jollof (rice cooked with stew together) and Waakye (rice and beans cooked together). The objective of the study is to enumerate *B. cereus* in street vended fried rice, Jollof and Waakye in the Tamale Metropolis and determine whether they are satisfactory, borderline or unsatisfactory.

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2. Materials and methods

2.1. Study area

The research was carried out in the Tamale Metropolis in the Northern region of Ghana. It is the only Metropolis and the most populous in the Northern half of the country. There are many street food vendors of fried rice, Jollof and Waakye in the Metropolis. Geographically, the Metropolis lies between latitude 09°16 and 09°34 North and longitude 00°57 West.

2.2. Sampling method and sample collection

A survey was conducted employing transect walk as a tool and two hundred and forty (240) food vendors selling Jollof rice, Waakye and fried rice were counted in the Tamale Metropolis. The food vendors were grouped into three groups and identified as opened, semi-closed and closed. The open group samples were taken from vendors who opened their food directly to the environment without any sheds. The semi-closed group represented vendors that sell under sheds and have barriers in front that will prevents the customer from having direct access to the food. The enclosed group of vendors sell their foods under enclosed sheds or rooms that allows only the vendor to serve the food. Thirty (30) samples each of fried rice, Jollof (rice cooked with stew together) and Waakye (rice and beans cooked together) were purchased randomly from street venders in the Tamale Metropolis. Ninety samples were taken in total. Samples were served by vendors in tight polyethylene bags, placed on ice cubes in an ice chest and transported to the laboratory within two hours. Samples were taken from April to May 2015.

3.3. Sample preparation, media preparation and bacterial culture

Using aseptic technique, 25 g of each sample was weighed and homogenized with 225 ml of phosphate-buffered saline (PBS, 4 g NaCl, 0.1 g of KCl, 0.72 g of Na_2HPO_4 and 0.12 g of KH_2PO_4) using stomacher bags (Classic 400, Seward limited, UK). The homogenized samples were serially diluted in 9 ml of PBS until 10^{-4}. Manitol egg-yolk polymyxin agar (MYP agar) (Oxoid, Basingstoke, UK) plates were prepared according to instructions of the manufacturer. The MYP agar plates were inoculated with 0.1 ml of the different serial dilutions (10^0, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) per plate per sample and incubated for 18-40 h at 30°C (8).

3.4. Observation, counting of colonies and enumeration of results

Rough and dry colonies with bright pink background surrounded by egg yolk precipitates of the incubated MYP agar (per the advice of the manufacturer) were identified as *Bacillus cereus* and counted with the colony counter. The results were calculated per gram (cfu/g) of the initial samples taken for each plate that was within 30 to 300 colonies. Colonies above 300 and uncountable with the 10^{-4} dilution were considered too numerous to count (TNTC) hence were deemed to be above $>10^5$.

3.5. Data analysis and interpretation of results

Results were entered into Microsoft excel, presented with descriptive statistics and represented with tables. Based on the guideline of the Center for food safety (9), the samples were classified to be satisfactory, borderline or unsatisfactory per the guidelines set for the interpretation of results for specific foodborne pathogens in ready-to-eat food. For *B. cereus*, $<10^3$ cfu/g is considered satisfactory hence no action is required, $10^3 \leq 10^4$ cfu/g is considered as borderline hence vendors will be advised to find out the sources and adopt measures to improve the situation, and $>10^5$ cfu/g is considered unsatisfactory hence vendors must be stopped from selling food items to the general public. The prevalence was calculated as the percentage of positive samples by the total samples multiplied by hundred percent.

3. Results

3.1. General classification of various samples

Among the samples taken from the various food categories, fried rice recorded the highest number of unsatisfactory samples, followed by waakye and the least is Jollof (Table 1). The number of the different categories of vendors and the unsatisfactory samples obtained are also represented in Table 2.

Table 1. Summary of the various food samples taken and their classifications

Sample	Classification		
	Satisfactory ($<10^3$ cfu/g)	**Borderline** ($10^3 \leq 10^4$ cfu/g)	**Unsatisfactory** ($>10^5$ cfu/g)
Fried rice (30)	8	4	18
Jollof (30)	6	17	7
Wakye (30)	10	12	8
Total (90)	24 **(27.7%)**	33 **(36.7%)**	33 **(36.7%)**

3.2. Fried rice samples

Of the thirty (30) samples of fried rice, 25 (83.3%) were taken from the enclosed vendors while 5 (16.7%) were taken from semi-enclosed vendors. There was no

Table 2. Categories of vendors and percentages of unsatisfactory samples

Type of vendors	% of vendors	% of unsatisfactory samples
Enclosed	46 (51.1%)	20/46 (43.5%)
Semi-enclosed	29 (32.2%)	10/29 (34.5%)
Opened	15 (16.7%)	3/15 (20.0%)
Total	90 (100%)	33/90 (33.6%)

fried rice taken from vendors selling from opened vendors. Majority of vendors who sell fried rice do it in an enclosed place, hence it was not surprising that there was no fried rice sold in the open. Table 3 shows the percentage of fried rice samples classified as satisfactory, borderline and unsatisfactory. Fried rice from the enclosed vendors had the highest number of unsatisfactory samples. The prevalence rate of *B. cereus* in fried rice was 66.7%.

Table 3. The percentage of fried rice samples based on classification

Classification	% of samples
Satisfactory	8 (26.7%)
Borderline	4 (13.3%)
Unsatisfactory	18 (60.0%)
Total	30 (100%)

3.3. Jollof samples

Table 4. The categories of jollof vendors sampled

Type of vendors	% of vendors
Enclosed	13 (43.3%)
Semi-enclosed	13 (43.3%)
Opened	4 (13.3%)
Total	30 (100%)

Table 4 above represents the categories of Jollof vendors sampled within the period of the research. In total, 6 (20%) samples were classified as satisfactory, 7 (23.3%) as unsatisfactory and 17 (56.7%) samples were on the borderline. Of the 6 satisfactory samples, 3 (50%) were from the enclosed vendors, 2 (33.3%) were from semi-enclosed and only 1 (16.7%) was from an opened vendor. For the 7 unsatisfactory samples, 1 (14.3%) was taken from an enclosed vendor and 6 (85.7%) were from semi-enclosed vendors. With the 17 samples on the borderline, 9 (53.0%) were taken from enclosed vendors, 5 (29.4%) from semi-enclosed and 3 (17.6%) from opened vendors. The most unsatisfactory samples were taken from semi-enclosed vendors. The prevalence rate of *B. cereus* in Jollof was 80%.

3.4. Waakye samples

Table 5. The categories of waakye vendors sampled

Type of vendors	% of vendors
Enclosed	8 (26.6%)
Semi-enclosed	11 (36.6%)
Opened	11 (36.6%)
Total	30 (100%)

Table 5 above represents the categories of Waakye vendors sampled within the period of the research Among all the Waakye samples taken, 10 (33.3%) samples were classified as satisfactory, 12 (40%) were on the borderline and 8 (26.7%) were unsatisfactory. There was no enclosed sample that was satisfactory. Seven (70%) of the satisfactory samples came from the semi-enclosed vendors while 3 (30%) were from the opened vendors. With the 12 samples on the borderline, 4 (33.3%) came from the enclosed vendors, 3 (25%) from the semi-enclosed and 5 (41.7%) were from opened vendors. Concerning the 8 unsatisfactory samples, 4 (50%) were from enclosed vendors, 1 (12.5%) from semi-enclosed and 3 (37.5%) from opened vendors. Semi-enclosed recorded the highest number of unsatisfactory samples. The prevalence rate of *B. cereus* in Waakye was 70%.

4. Discussion

Street food vending and buying have been integral part of the populations of most developing countries. Street food vendors are largely not regulated by most Food agencies in Africa as more attention is being paid to imported food as they are perceived to be likely unwholesome than internally or locally produced foods. The hygienic conditions of the various categories of vendors observed during this study were similar, but for the exposure of the vended food to the environment. The prevalence of *B. cereus* obtained from this research work for all the food sampled was 72.2%. This is higher than the prevalence rates of *B. cereus* reported by Mensah *et al.* (5) in street foods in Accra (5.5%) and food contamination rates of 37.5% of *B. cereus* recorded by Isara *et al.* (4) among ready-to-eat food restaurants in Nigeria. The prevalence rate in our study is again higher than the 14.1% rates observed in salad with rice in Morocco (6). This study further buttresses the assertion of Saba and Gonzalez-Zorn (10) that microbiological contamination of street food in Ghana is alarming. Tamale is the third most populous Metropolis in Ghana. Due to the high unemployment levels amongst the youth and the increasing demands for street foods, many middle-aged women most of whom had no previous knowledge in food safety are

engaged in the vending of food on the streets. This might have accounted for the very high prevalence rate of *B. cereus* in the food samples examined. The optimum temperature for the growth of *B. cereus* in boiled rice was reported to be between 30°C and 37°C, but growth also occurred during storage at 15°C and 43°C (11). These temperature ranges are the ambient temperature ranges in most African countries hence *B. cereus* may grow in cooked or fried rice that are not properly provided with temperature above 63°C (11) on the streets of Africa. Most of the street vendors do not provide the adequate temperature range needed after the initial temperature of the food during cooking. This may be so because street food vendors may incur further cost for any form of energy used to keep the food warm and hence increase the cost of the food. Increasing the cost of street foods by any amount may lead to low patronage of the food despite the fact that the cost increment is to provide the adequate temperature to make the food safer. Our study revealed that the most unsatisfactory samples came from the enclosed vendors 20/46 (43.5%), followed by the semi-enclosed 10/29 (34.5%) and the least came from the opened vendors (20%). This can be attributed to the fact that most enclosed and some semi-enclosed food vendors prepare food in larger quantities which are normally kept for a longer period especially when the rate of purchase is low. Left-over food may probably be kept for the following day and reheated to sell. Unlike the enclosed and the semi-enclosed vendors, the opened vendors normally prepare their food in smaller quantity and freshly everyday, hence the low levels of *B. cereus* in those food samples.

5. Conclusion

This study has demonstrated that some of the most popular types of ready-to-eat foods like fried rice, Jollof and Waakye that are sold on the streets of the Tamale Metropolis recorded high prevalence rates of *B. cereus*. The study has also established that the most contaminated or unsatisfactory of the samples taken was fried rice, followed by Waakye and Jollof. We have also observed that there are more unsatisfactory food samples from the enclosed vendors, followed by the semi-enclosed vendors and opened vendors. Street vendors required regular education on how to handle their food to reduce microbial contamination. Further studies must be carried out to determine whether the *B. cereus* found in the street foods can produce enterotoxins strains that will lead to foodborne illnesses.

Conflict of interest

The authors declare that they have no conflict of interest.

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