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Microbiological Assessment of Ready-to-Eat Food Sold in Urban Primary Schools, Douala, Littoral Region, Cameroon

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Abstract: Ready-to-eat (RTE) food sold to school going children at school premises can not only provide essential nutrient and energy but also a route for foodborne diseases (FBD). However, there is a paucity of data on microbiological assessment of RTE food sold in primary schools in Douala. The aims of this study was to investigate the microbiological quality of food sold in primary schools and to evaluated the food safety knowledge and practice of vendors to promote a safer school-based food for a better health and well-being of our children. Socio-demographic characteristics and the hygienic practices of 60 RTE food vendors were collected using structured questionnaire. A total of 60 food samples from three different food items (beans, spaghetti and meat) were analyzed for the presence of bacterial and fungal pathogens. Twenty-five grams of each food sample was transferred in to 225 ml of buffered peptone water and homogenized. The homogenates were serially dilute and a volume of 0.1 ml dilution was spread on solid media and incubated at 35-37°C for 24 hours and 5 days. Antibiotic susceptibility testing was done for isolated species using Muller Hinton agar and data was entered in excel and exported to SPSS version 20.0. For analysis. The overall prevalence of bacterial pathogens was 23.1% (26/60) and the total mean enterobacteriaceae count (MEC) was $8.203333x10^4$ CFU/ml in which the value ranged from $3.4\ x10^4$–$2.06\ x\ 10^5$ CFU/ml. The total mean fungal count (MFC) was $1.0341x10^5$ CFU/g which varied from 0 –$2.8x10^5$ CFU/ml. Of the total of 60 samples examined, 93.3% (56/60) were found positive for S. aureus of which 15% (09/60) of isolates were contaminated. Citrobacter freundii was isolated in 23.4% (14/60) of RTE food. The greatest number of S. aureus was found in beans and the lowest number in spaghetti. These findings indicated that, the ready-to-eat food sold to primary school children in Douala metropolis represent an important potential health risk to school going children. There is a need for Public Health authority to establish guidelines and standards in order to safeguard the wellbeing of the school going children.

Keywords: Ready-to-eat, Microbiological Assessment, Food Vendors, Antibiotic Susceptibility, Douala

1. Introduction

Globally, billions of people are at risk of FBD with an estimated 582 million cases, resulting to 420,000 deaths annually. The global burden of foodborne disease was 33 million Disability Adjusted Life Years (DALYs) and the highest estimated burden was observed in Africa [1]. These statistics represent only the tip of iceberg because only a small fraction of those that got ill from infected food actually seek medical care. Food is said to be any substance in a liquid or solid form that can be taken through ingestion to supply essential nutrients which provides energy for growth and

development of the human body [2]. However, food also served as nutrients for microorganism's growth. These microorganisms consist of bacteria, viruses, parasites and fungi. Microbiological quality of food is an indicator the safety of the food. Recent estimates indicated that there are more than 400 sources of FBD that have been recognized [3]. RTE is food intended by the producer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to an acceptable level microorganisms of concern [4]. In most primary schools in tropical Africa, RTE are produced unsupervised and without compliance to any food safety guidelines if they exist. These foods are sold in unhygienic surroundings with houseflies, fruit flies and air dust serving as potential sources of contamination.

RTE sold in primary schools are of public health because they have become one of the most common risks associated with the increase in outbreaks of FBD. Several food poisoning outbreaks due to street foods have been documented. For instance, in Brazil between 2000 and 2018, 13,163 FBD outbreaks were reported in the country during this period, involving 247,570 cases and 195 deaths [6]. Likewise, in South Africa, from January 2013 to December 2017, 327 FBDs outbreaks were reported causing illness in 11 155 individuals, with 78% seeking medical care, 4% required hospital admissions and 0.4% deaths [7]. The Food and Agriculture Organization of the United Nations (FAO) through the School, Food and Nutrition Framework is promoting a healthy school food environment, an adequate and safe meals, an integrated and effective food and nutrition education throughout the whole school system [8].

Cameroon is an African country known as 'Africa in miniature' largely renowned for its delicious, spicy and wide variety of dishes. The Cameroon Ministry of Public Health has no specific regulations of RTE food [9]. Food vendors in Cameroon usually have very low knowledge of hygienic food handling practices in basic food safety measures and personal hygiene. In Buea, South-West Region Cameroon, the prevalence of feco-oral parasites among street-food vendors was 56.7% [10]. In Fako Division, Cameroon, Akoachere [11] indicated that hygiene and vegetable preservation practices by vendors were poor and could aggravate contamination with pathogenic bacteria and parasites.

In the recent years, there has been anarchical creation of primary schools in the Douala metropolis, and many without administrative authorizations. Various types of food with objectionable microbiological quality are sold to pupils and teachers during school break periods. Data on microbial quality of food sold in primary schools in Cameroon is lacking, despite the fact that the number of primary schools in cities has skyrocketed. Therefore, the study was aimed at investigating the microbiological quality of RTE food sold in primary schools and to evaluated the food safety knowledge and practice of RTE food vendors to promote a safer school-based meal for a better health and well-being of our children.

2. Materials and Methods

2.1. Study Area and Setting

The study was carried out in 20 randomly selected primary schools in Douala metropolis. The schools were made up of 10 privates and 10 government owned, distributed in the 5 subdivisions (Administrative units) of the Littoral Region. Douala is a cosmopolitan city and the economic capital of Cameroon.

2.2. Study Design and Period

Cross-sectional study was used to assess microbiological quality of food sold to children in selected primary schools in Douala city and assess the knowledge and practices of food vendors on the hygienic food handling practices in basic food safety using a pre-tested questionnaire from March to April 2017.

2.3. Sample and Data Collection

The study consisted of 60 RTE food vendors for data collection. A total of 60 (10 from each food item) samples were collected from 3 RTE food items namely: beans, spaghetti and cow meat. The food samples were collected in sterile conical flask and covered with aluminum foil to prevent contamination and transported to the laboratory Pasteur Plus (Nylon, Douala) in a chilled cooler bag. The food samples were processed within 2 hours. The food preparation is shown in table 1.

2.4. Evaluation of Hygiene and Preservation Practices of Food Vendors

A face to face interview using pre-tested structured questionnaire was used to collect the demographic characteristics and personal hygiene practices of food vendors. The questionnaire was prepared in English version, translated into French and administered in both languages.

2.5. Sample Collection and Processing

Twenty–five grams (25g) of each sample was aseptically weighed and transferred to a stomacher bag to which 225 mL of sterile 0.1% buffered peptone water was added. The sample was blended for two minutes at 230 RPM in a stomacher to produce a homogeneous sample. The homogenate was serially diluted (10^{-1} to 10^{-2}) and 0.1 ml of each of the homogenates was then inoculated into corresponding labelled plates of nutrient agar for mesophilic aerobic bacteria, violet Red Bile Lactose (RBL) agar for fecal coliform bacteria, Baird Parker agar for pathogenic *staphylococcus aureus,* and potato dextrose (LAB M) agar medium for fungi.

The plates for mesophilic aerobic bacteria counts were incubated at 30ºC for 72 hours, those of fecal coliform incubated at 44°C for 48 hours, Baird Parker agar plates incubated at 37°C for 48 hours while those for fungal counts were incubated at room temperature (27±1°C) for 5 days. In

addition, selective and differential media (Eosin methylene blue agar, MacConkey agar, Saboureaud agar, Chapman agar, Shighella Salmonella (SS) agar and peptone water, Rappaport-Vassiliadis Soya (RVS) broth, Xylose Lysine Deoxycholate (XLD) agar) were also inoculated and incubated at 35ºC for 18-24 hours accordingly [12].

2.6. Cultural, Morphological and Biochemical Characterization of the Isolate

The isolates were identified using Bergey's manual of determinative bacteriology [13]. Colony appearance was observed and recorded while Gram's reaction and spore staining were carried out to assess the morphology of the bacterial isolates. In addition, biochemical tests such as indole, citrate utilization, catalase, and coagulase tests were carried out. Lactophenol cotton blue reagent was used for the fungal identification used.

2.7. Description of Bacteriological Loads of the Sample

Colonies on each plate was counted by using colony counter after 24 hours and described as colony forming unity per milliliter (CFU/ml) by using the following formula [14].:

$$\text{CFU/ml}=\frac{\text{Number of colonies x Dilution factor}}{\text{Volume of the sample}}$$

2.8. Antibiotic Susceptibility Pattern

The isolated bacterial species were tested for their sensitivity pattern against the following antibiotic: cefuroxime, ceftriaxone, oxacilline, and penicilline G using the Kirby-Bauer agar disc diffusion method on Mueller-Hinton agar (MHA). Three to five colonies of bacteria were taken and suspended in nutrient broth and incubated for 4 hours at 37°C. Turbidity of broth culture was compared against 0.5 McFarland turbidity standard. By using sterile cotton swab, the organism in the broth was swabbed over the entire surface of MBA. Antibiotic discs were applied within fifteen minutes using sterile forceps on the surface of medium and incubated at 35-37°C for 18–24 hours.

2.9. Data Analysis

All statistical analyses were conducted using SPSS for Windows (version 20.0). The Chi-square test was applied to compare proportions between public and private schools. Analysis of variance (ANOVA).) was used to determine differences in the means bacterial load. Statistical significance was set at $p < 0.05$.

2.10. Ethical Consideration

The study was approved by the University of Douala Institutional Review Board. Informed consent was sought and obtained before the collection from the interested food handlers within the selected schools. The identities of the participants involved in the study were kept anonymous and they were free to withdraw anytime during the study.

3. Results

3.1. Socio-characteristics of the RTE Food Vendors in the Primary Schools

The table 2. shows the characteristics of RTE food vendors in the primary schools in Douala city. In the selected schools, sixty food vendors were assessed and the majority 95.0% (57/60) were females aged between 30 and 39 years (40%). The mean age was 39.20 (SD=7.961) while the minimum and maximum ages were 24 and 55 years respectively. Concerning the length of years in service, 33.3% (20/60) of study subjects had between 5-9 years of work experience.

3.2. Hygienic Practice of the Study Participants

In the study, 93.3% (56/60) of the food vendors possessed a medical certificate delivered by the Littoral Regional Delegation of Public Health (Table 3). Shockingly, 60.0% (36/60) of them have not heard about FBD and 95.0% (57/60) had not undertaken any formal training on food safety hygiene practice. There was a significant difference between vendors of private and public schools with regards to awareness of FBD (P=0.015) and job stability (P=0.029). None of the food vendors wore uniforms, and all handled money while serving food to pupils with bare hands.

3.3. Bacterial Load of Isolated Bacterial Species from RTE Food Primary School in Douala

The total mean aerobic mesophilic bacteria count (AMBC) was 9.471667×10^4 CFU/ml which varied between 3.0×10^4 – 2.0×10^5 CFU/ml. The total mean enterobacteriaceae count (MEC) was 8.203333×10^4 CFU/ml in which the value ranged from 3.4×10^4–2.06×10^5 CFU/ml and the total mean staphylococcal count (MSC) was 7.198333×10^4 CFU/ ml varying from $0 – 1.95000 \times 10^5$ CFU/ ml (Table 4). A total of 60 of samples from RTE food sold in the primary schools were analyzed, and Enterobacteriacae were present in all.

Staphilococcus aureaus was the most frequent isolate 93.3% (56/60) followed by *salmonella* 60.7% (37/60) and *Shigella* 57.4% (35/60) (Table 5). *Citrobacter freundii* was present in 23.4% (14/60) of sampled food, *Escherichia coli* 18.0% (11/60), *Enterobacter aeruginosa* 5.2% (5/60) and *Klebsiella pneumoniae 6.6*% (4/60).

When data were compared, no significant different was observed in the mesophilic aerobic bacteria count (P=0.358), Enterobacteriacae count (P=0.705) and Staphylococcal count (P=0.696) between public and private schools. The greater number of *S. aureus* was found in beans and a lower number in spaghetti. Of the total of 60 samples examined, 93.3% (56/60) were found positive for *S. aureus* of which 15% (09/60) of isolates were beyond acceptable levels of microorganisms.

Fungal profile and isolated species in selected foods

The total mean fungal count (MFC) was 1.0341×10^5 CFU/g which varied from $0 – 2.8 \times 10^5$ CFU/ml. *Candida species* were the most frequent isolate 36.1% (22/60) followed by *Candida albicans* 34.4% (21/60), and *Candida dubliniensis* 13.1% (8/60). The level of fungal contamination from the total food

sampled was 33.3% (20/60). *Candida species* were the predominant fungal species found in beans and meat 8 (13.3%) (Figure 1) while *candida albicans* occurred more in spaghetti 8 (13.3%).

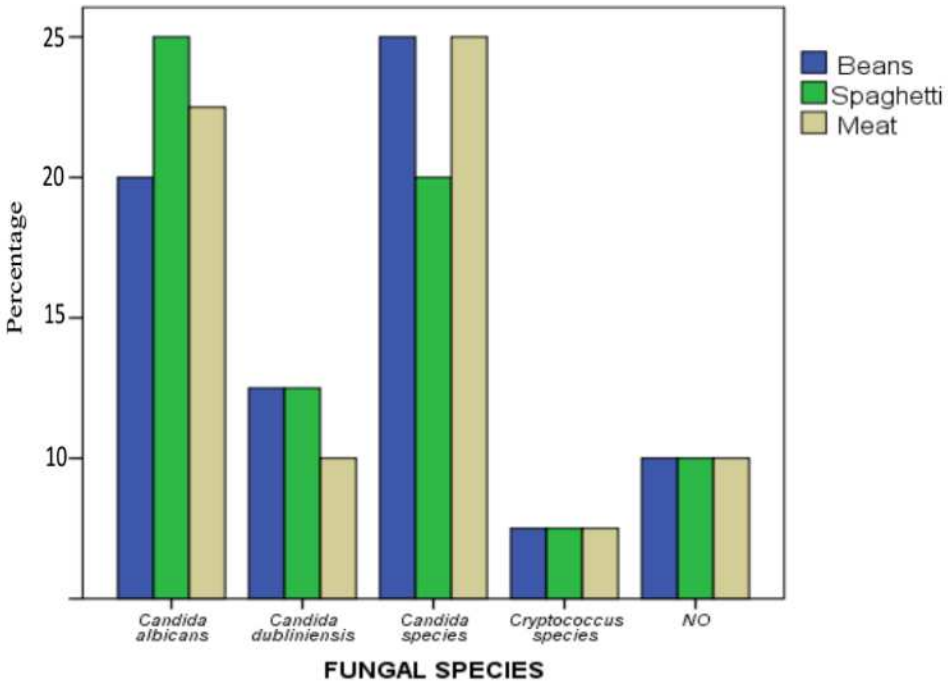

Figure 1. *The distribution of fungal isolates with regard to type food.*

Antimicrobial susceptibility test

In the present study, *Escherichia coli, Salmonella*, *Klebsiella pneumoniae*, and *S. aureus* showed 100% sensitivity to ciprofloxacin whereas *Escherichia coli.* showed 100% resistance to Erythromycin. *S. aureus* showed 66.7% resistance to Penicillin G and 100% sensitivity to amoxicillin. *C feundii* was highly resistant to Ceftriaxone (92.8%).

4. Discussion

Health, education, and food security continue to be main targets of United Nations Sustainable Development Goals Initiative. Schools can make a substantial and long-term impact on the above mentioned issues through several entry points and one of them being the provision of safe and nutritious meals to school going children [8]. Owing to the globalization of our food supply and the growing urbanization of the population, ensuring food safety has become an important public health issue. In its efforts to improve food safety, the WHO has introduced the concept of "From farm to plate, make food safe" in 2015 [15]. Thus, the survey was carried out to determine the microbiological quality of RTE sold in 20 primary schools in the metropolis of Douala, in Littoral Region, Cameroon.

In the study, 52.5% (32/60) of food vendors were aware of the existence of the quality control measures to verify and ensure production of safe food. Majority of food vendors 55.7% (34/60) sold uncovered food thus exposing them to flies and airborne pathogens. Fifty-nine percent (36/60) were ignorant that bacteria cause FBD. However, this is in contrast with results obtained in Sri Lanka [16], which found that 53.7% of food handlers knew that bacteria cause FBD. With regard to factors affecting the development of bacteria, only 27.9% (17/60) were aware. Shockingly, only 18.8% (11/60) of respondents mentioned that contaminated water will cause diarrhoea while 24.6% (15/60) attributed diarrhoea to eating with dirty hands. Nearly all food vendors 98.1% showed the medical certificates as evidence to have undertaken medical examination that exempted them from being a source of any infectious diseases. The Cameroon

Ministry of Public Health emphasized medical examination as a sine qua none condition for anybody to be involved in food trade. However, regular medical check of the vendors was lacking. Once the medical certificate is delivered, the food vendors assume it covers the whole period of an academic year.

The overall prevalence of 23.1% (26/60) bacterial pathogens (above the reference value of $>10^5$ CFU/ml) was detected in RTE food sold to primary school children in Douala metropolis. These results are lower than 65% recorded in Gondar, Ethiopia [17] and 100% in Kathmandu, India [18]. In the present study, 19 bacteria species namely; *Acinetobacter, Citrobacter freundii, Escherichia coli, Enterobacter spp, Enterobacter aerogenes, Enterobacter cloacea, Hafnia alvei, Klebsiella otoxica, Klebsiella pneumonia, Morganella morganii, Proteus mirabilis, Proteus. penneri, Providencia rettgeri, Pseudomonas aeruginosa, Serratia marcescens, Yersinia enterocolitica, Staphilococcus aureus, Salmonella,* and *Shighella* were isolated from the beans, spaghetti and meat. Similar organisms were isolated from RET food sold in primary school children in Abeokuta, South-West Nigeria [19].

Table 1. *The ingredients and description of food sold in primary schools in Douala city.*

Food items	Description	Cooking method	Ingredients	Preparation	Handling after cooking
Beans	Boiled beans	fried	Tomatoes, green spices (celery, leeks parsley), onion, red oil), pepper, seasoning (Maggi cube), ginger, salt, garlic, bush pepper, Penja white pepper.	-Wash and boil the beans –Fried the onions and tomatoes in red oil. Leave it for few minutes to cook - add ground green spices, garlic, pepper, bush pepper and white pepper. Allow to cool. -Add salt and seasoning – Pour in the boiled beans and fried for some 30 minutes	Spoon
Spaghetti	Boiled spaghetti	fried	Tomatoes, green spices, onions, pepper, garlic, salt seasoning, groundnut oil	-Boil spaghetti separately- fried onions and sliced tomatoes in groundnut oil for about 45 minutes and add garlic, pepper, green spices. add seasoning and salt. - pour in the boiled spaghetti and fried for 45 minutes	Fork assisted with hands
Meat	Stew	fried	Tomatoes, green spices garlic, pepper ginger, Penja white pepper, groundnut oil,	-slice, wash and boil the meat with salt and seasoning. separately – fried the onions and tomatoes in groundnut oil for 45minutes-add ground spices for few minutes add the boil meat and allowed to cook for some 30 minutes.	Fork

Table 2. *Characteristics of RTE food vendors in Primary Schools.*

Characteristics	n	%
Sex		
Male	3	5
Female	57	95
Age		
<29	6	10
30-39	24	40
40-49	22	36.7
>55	8	13.3
Point of Sales		
Public schools	40	66.7
Private schools	20	33.3
Source of water for cooking		
Municipal Water	20	33.3
Public tap	37	60.7
Well water	3	4.9
Length of Service		
< 4 Years	16	26.7
5-9 Years	20	33.3
10-15 Years	18	30.0
> 15 Years	6	10.0
Ownership of Medical Certificate		
Yes	59	98.3
No	1	1.7
Type of food sold		
Bean	20	33.3
Spaghetti	30	50.0
Meat	10	16.7
Ownership of medical certificate	59	96.7

The aerobic mesophilic bacteria were found in all RTE food sampled. The aerobic mesophilic bacteria count ranged between $3.5x10^4$ and $2.1x10^5$ CFU/ml hich was lower than those of a study conducted in Jigjiga City in Ethiopia [20]. The presence of saprophytic microorganisms such as aerobic mesophilic bacteria is an indication of poor quality and post heat-processing contamination of RTE food sold in the primary schools [4]. The mean aerobic mesophilic bacteria recorded for each food samples showed that beans ($1.03100x10^5$ CFU/ml) contains the highest amount of bacteria followed by meat ($1.01300x10^5$ CFU/ml), and spaghetti ($7.9750 x10^4$ CFU/ml). This is in contrast with study by Sileshi [21]. Though the meat may provide an optimum medium (nutrient abundance) for microbial growth than other food sampled, the present study provides a different scenery. Considering the maximum acceptable concentration of 10^5 CFU/ml for aerobic mesophilic bacteria [22] in RTE food for consumer protection, 27.3% (22/60) were contaminated.

The presence of *E. coli* is used as an indicator of possible faecal contamination of food or water to assess the hygiene status of a prepared food. In the study, E. coli was detested in 6.7% (4/60) of sampled RTE food sold to pupils at levels above 10^5 CFU/ml [23]. Most of the food vendors (60.7%) cover a relatively short distance to go and carry water for food preparation. The direct surrounding of the public tap water point, the utensils used to carry water and the personal hygiene of the carriers (Adolescents and older children) may also constitute a source of cross contamination. Water might represent one of the major source of contamination of RTE food sold in the primary schools. The present data indicated

that only 33.3% (20/60) of food vendors are connected to municipal water supply.

In the present study, the level of Enterobacteriacae contamination was lower (40%) when compared to the other studies conducted in Ethiopia (61.1%) [18], and 72% recorded in Jigjiga [20]. The presence of Enterobactericeae in the study might be attributed to water, poor hygienic practice of food handlers and cross contamination from raw meat, food handlers or food contact surfaces.

The study indicated that 93.3% of food sampled were found positive for *S. aureus* of which 15% isolates were beyond the threshold of the International Commission for Microbiological Specification for Food [22]. This is consistent with study conducted in Gondar [17] that revealed a high amount of *Staphylococcus aureus* in RTE food samples probably due to the fact that food handlers mostly use their hands with poor hand washing habit.

Table 3. *Hygienic practice of the RTE food vendors.*

	Where to store balance food		**Have you heard about foodborne illness**		**Source of water for cooking**			**Training program for food vendors**		**Existence quality control measures**	
Sex	**Fridge**	**Warming**	**Yes**	**No**	**Municipal Water Supply**	**Public Tap**	**Well Water**	**Yes**	**No**	**Yes**	**No**
Male	3 (5.0%)	0 (0.0)	0 (0.0)	3 (8.3%)	0 (0.0)	3 (5.0%)	0 (0.0)	0 (0.0)	3 (8.3%)	2 (6.2%)	1 (3.6%)
Female	53 (88.3%)	4 (6.7%)	24 (40.0%)	33 (55.0%)	20 (33.3%)	34 (56.7%)	3 (5.0%)	3 (8.3%)	54 (94.7%)	30 (50.0%)	27 (45.0%)
Total	56 (93.3%)	4 (6.7%)	24 (40.0%)	36 (60.0%)	20 (33.3%)	37 (61.7%)	3 (5.0%)	3 (5.0%)	57 (95.0%)	32 (53.3%)	28 (46.7%)
Point Of Sales											
Public Schools	37 (61.7%)	3 (5.0%)	11 (18.3%)	29 (48.3%)	13 (21.7%)	22 (36.7%)	2 (3.3%)	3 (5.0%)	34 (56.7%)	25 (41.7%)	15 (25.0%)
Private Schools	19 (31.7%)	1 (1.7%)	13 (20.0%)	7 (21.9%)	7 (11.7%)	15 (25.0%)	1 (1.7%)	0 (0.0)	23 (38.3%)	7 (11.7%)	13 (21.7%)
Total	56 (93.3%)	4 (6.7%)	24 (40%)	36 (60%)	20 (33.3%)	37 (61.7%)	3 (5.0%)	3 (5.0%)	57 (95.0%)	32 (53.3%)	18 (46.7%)
Length of Service											
< 4 Years	14 (23.3%)	2 (3.3%)	5 (8.3%)	11 (18.3%)	6 (10.0%)	9 (15.0%)	1 (1.7%)	1 (1.7%)	15 (25.0%)	7 (21.9%)	9 (32.1%)
5-9 Years	19 (31.7%)	1 (1.7%)	10 (16.7%)	10 (16.7%)	3 (5.0%)	15 (25.0%)	2 (3.3%)	0 (0.0)	20 (33.3%)	11 (34.4%)	9 (32.1%)
10-15 Years	28 (28.3%)	(1 (1.7%)	7 (11.7%)	11 (18.3%)	9 (15.0%)	9 (15.0%)	0 (0.0)	1 (1.7%)	17 (28.3%)	11 (34.4%)	7 (21.9%)
> 15 Years	6 (10.0%)	0 (0.0)	2 (3.3%)	4 (6.7%)	2 (3.3%)	4 (6.7%)	0 (0.0)	1 (1.7%)	5 (8.8%)	7 (21.9%)	7 (21.9%)
Total	56 (93.3%)	4 (6.7%)	24 (40.0%)	36 (60.0%)	20 (33.3%)	372 (3.3%))	3 (5.0%)	3 (5.0%)	57 (95.0%)	32 (53.3%)	28 (46.7%)

Table 4. *Mean bacterial and fungal count from food sampled in primary schools Douala city.*

Variables	**Aerobic mesophilic bacteria count**			**Enterobacteriacae count**			**Staphylococcal Count**			**Fungal count**		
Type of school	**Min**	**Max**	**Average**	**Min**	**Max**	**Average**	**Min**	**Max**	**Average**	**Min**	**Max**	**Average**
Public	$3.5x10^4$	$2.1x10^5$	$1.083x10^5$	$3.4x10^4$	$2.06x10^5$	$8.775x10^4$	0	$1.6x10^5$	$6.9361x10^4$	0	$2.8x10^5$	$9.5777x10^4$
Private	$3x10^4$	$1.8x10^5$	$7.4291x10^4$	$3.9x10^4$	$8x10^4$	$7.3458x10^4$	0	$1.95x10^5$	$7.5916x10^4$	0	$8.6x10^5$	$11.4875x10^4$
	Total MAMBC=$9.4716x10^4$			TMEC=$8.2033x10^4$			TMSC=$7.1983x10^4$			TFC=$1.034x10^5$		
Type of food												
Beans	$3.5x10^4$	$2.1x10^5$	$1.031x10^5$	$3.9x10^5$	$1.80x10^5$	$8.55x10^5$	0	$1.95x10^5$	$8.47 x10^4$	0	$8.6 x10^5$	$1.264 x10^5$
Spaghetti	$3.5x10^5$	$1.9x10^5$	$7.975x10^4$	$3.4x10^5$	$2.06x10^5$	$7.82 x10^5$	0	$1.6 x10^5$	$6.42x10^4$	0	$2.01 x10^5$	$8.74 x10^4$
Meat	$3x10^5$	$1.9x10^5$	$1.013x10^5$	$4.2x10^5$	$1.63x10^5$	$8.24 x10^5$	0	$1.11x10^5$	$6.705 x10^4$	0	$2.8 x10^5$	$9.645 x10^4$
	Total AMBC=$9.4716 x10^5$			TMEC=$8.2033 x10^5$			TMSC=$7.1983 x10^5$			TFC=$1.034 x10^4$		

Table 5. *Distribution of bacterial isolates from food samples by type of schools.*

		Private Schools			**Public schools**			**Total**
		Beans	**Spaghetti**	**Meat**	**Beans**	**Spaghetti**	**Meat**	
S/n	Isolated bacteria	n	n	n	n	n	n	
1	*Acinetobacter*	0	1	0	0	0	0	1
2	*Citrobacte freundii*	3	1	4	2	2	2	14
3	*Escherichia coli*	4	1	2	2	0	2	11
4	*Enterobacter spp*	1	1	0	0	0	0	2
5	*Enterobacter aerogenes*	1	1	0	1	0	2	5
6	*Enterobacter cloacea*	1	3	0	0	0	0	4
7	*Hafnia alvei*	0	1	0	0	1	0	2
8	*Klebsiella otoxica*	0	0	1	1	1	0	3
9	*Klebsiella pneumoniae*	0	0	2	0	1	1	4

		Private Schools			Public schools			Total
		Beans	Spaghetti	Meat	Beans	Spaghetti	Meat	
10	*Morganella morganii*	0	0	0	0	1	0	1
11	*Proteus mirabilis*	0	1	1	0	1	0	3
12	*Proteus. penneri*	1	0	0	1	0	0	2
13	*Providencia rettgeri*	0	0	0	0	1	0	1
14	*Pseudomonas aeruginosa*	1	0	2	1	0	0	4
15	*Serratia marcescens*	0	0	0	0	0	1	1
16	*Yersinia enterocolitica*	0	2	0	0	0	0	2
17	*Staphilococcus aureus*	12	10	11	7	8	8	56
18	*Salmonella*	4	8	8	6	5	6	37
19	*Shighella*	9	8	10	1	4	3	35

The presence of a large number of *S. aureus* in the study also indicate poor handling or sanitation of the food vendors. Thus, the consumption of these contaminated food by school going children constitutes a serious health risk. Though, the ICMSF standard for bacteria count is limited at 10^5 cfu/ml, the probability of production of enterotoxins by *S, aureus* increases when the count goes above 10^3 CFU/ml and thus, become resistant to boiling/cooking [23]. Considering this, the prevalence of pathogenic *S, aureus* of food sampled went up to 93.3%. When the levels of *S. aureus* count exceed 10^5 cfu/ml in the course of shelf life of a food, there is a risk of sufficient enterotoxin to cause illness [4]. The symptoms of FBD may last a few hours or several days. Typical symptoms include diarrhea, vomiting, abdominal cramps, headaches, nausea, dry mouth, and difficulty swallowing and flu-like symptoms (such as fever, chills, backache [5].

Enterobacter and *Citrobacter species* were also isolated from the food samples. The presence of theses microorganisms in RTE food is in agreement with other studies in Kenya [24] and Nigeria [25]. *Salmonella species* contaminated 62.0% (37/60) of food sampled. This contrast with others in Gondar [17] and Harare [26]. where no salmonella was detected in RTE food samples. The presence of *Salmonella* in the study could be ascribed to inadequate refrigeration due to erratic power supply, insufficient warming or reheating of food. The detection of *Shigella* in 17.2% of the RTE food sold to pupils may be potentially be injurious to their health. *Shigella* is easily destroyed by heat, adequate warming or heating of left-over food is an important control steps for shigellosis.

In the present study, *Citrobacter freundii* is emerging as an important biological contaminant of RTE food sold in the primary schools in the study area. The bacterium inhabits the environment (soil, water, sewage), food, and the intestinal tracts of animals and humans [27]. *C. freundii* is also a hospital-acquired pathogen, which caused diarrhea, urinary tract infection, peritonitis, bacteremia, brain abscess, meningitis and highly resistant to antibiotic [28]. Food borne outbreaks causing severe diarrhea ascribed to *C. freundii,* have been reported in China's Henan Province. [29]. The high findings of *C. freundii* in the RTE food sold in the present study could have been as a results of improper washings of various fresh green spices (parsley, leeks and celery) used for the preparation of beans and meat. Once harvested, these spices are transported in trucks without any forms of treatment and stock in open air on bared-floor in markets. Considering the fact that most of the RTE food vendors get their water supply from public tap, theses spices are washed in a basin rather than from the flowing tap water.

The mean fungal count of the food sampled have exceeded the maximum recommended standards set by the ICMSF [22]. *Candida species* have been reported as one of the most prominent molds occurring in RTE foods. *Candida albicans* is thought to be the major fungal pathogen of humans. Severe *Candida* infections are a serious health problem, particularly among immunocompromised individuals [30]. No toxigenic fungi such as *Aspergillus*, *Fusarium*, and *Penicillium* were isolated from the food sold to school going children.

Staphilococcus. aureus, Escherichia coli and *Klebsiella pneumoniae* isolates showed a higher degree of susceptibility towards ceftriaxone, oxacillin and lesser to penicillin G and amoxicillin. These findings are consistent with data obtained in Indian [14] and Ethiopia [18]. *Salmonella species* isolates were all sensitive to ciprofloxacin, and ceftriaxone which are in agreement with the study conducted in Gondar [31]. *C. freundii* was resistant to ceftriaxone. This is consistent with studies in India [32] and China [33]. The recognition of *C. freundii* to cause various infection is paralleled to the finding that *C. freundii* has become resistant to multiple classes of antibiotics. Thus, as suggested by Liu [34], clinical and environmental strains may be the reservoir of antimicrobial resistance determinants.

Three major limitations can be deduced from the cross-sectional survey. First, the associated risk factors were not ascertained, considering our objectives, Secondly, molecular investigations were not done for the identification of bacteria. Thirdly, owing to the small sample sizes, the results of the study could not be extrapolated to other metropolis in Cameroon.

5. Conclusion

These findings indicated that the RTE food sold to primary school children in Douala metropolis represent an important potential health risk to school going children. The high microbial counts recorded from beans, spaghetti and meat are indicative of improper handling of food by the food vendors and overall poor general hygiene.

Table 6. *Antimicrobial susceptibility pattern of bacterial isolates from RTE food sold in primary schools in Douala city.*

Antimicrobials tested		*Escherichia coli* (n=11)	*Salmonella* (n=37)	*Citrobacter freundii* (n=14)	*Enterobacter aerogenes* (n=5)	*Klebsiella pneumoniae* (n=4)	*S. aureus* n=9
Ceftriaxone	S	11 (100%)	37 (100%)	1 (7.2%)	3 (60.0%)	4 (100%)	9 (100%)
	R	0 (0.0%)	0 (0.0%)	13 (92.8)	2 (40.0%)	0 (0.0%)	0 (0.0%)
Oxacillin	S	10 (90.9%)	-	-	-	-	9 (100%)
	R	1 (9.1%)	-	-	-	-	0 (0.0%)
Pénicillin G	S	-	-	-	-	2 (50.0%)	3 (33.3%)
	R	-	-	-	-	2 (50.0%)	6 (66.7%)
Amoxicillin	S	-	-	-	-	2 (50.0%)	9 (100%)
	R	-	-	-	-	2 (50.0%)	0 (0.0%)
Erythromycin	S	0 (0.0%)					9 (100%)
	R	11 (100%)					0 (0.0%)
Ciprofloxacin			37 (100%)				9 (100%)
			0 (0.0%)				
Tetracycline	S	11 (100%)	32 (86.5)				
	R	00 (0.0)	5 (13.5)				

There is a need for Public Health authority to step into the issue in order to safeguard the well-being of the school going children.

Author Contributions

ALWN, designed the study and collected the data. BT, designed the study, analyzed the data and drafted the primary manuscript. SA, carried out field activities and analyzed the data. GBT collected the data and review the manuscript. HLFK designed the study and coordinated the study. All authors read and approved the submitted version.

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Conflicts of Interest

The authors declare that they have no competing interests.

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