Microbiological analysis of ready to eat food (cooked rice and beans) sold among different restaurant in University of Port Harcourt, Port Harcourt, Nigeria

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Abstract: This study reports on the microbiological analysis of ready to eat food (cooked rice and beans) sold in University of Port Harcourt, Port Harcourt, Nigeria. The total colony count of ready to eat (cooked rice) ranged from $2.45x10^5$cfu/g to $17.8x10^5$cfu/g and $3.5x10^4$cfu/g to $17.1x10^4$cfu/g for ready to eat beans samples, for bacterial. The data's revealed that bacteria isolated from both food samples collected from the restaurants in University of Port Harcourt are *Bacillus cereus, Staphylococcus aureus, Escherichia coli*, and *Klebsiella pneumoniae* which is mainly associated with food poisoning because of its ability to produce toxins. From the result gotten, it was indicated that these ready to eat food samples that were analyzed, did not meet the bacteriological quality standard. The presence of pathogenic bacteria in ready-to-eat foods should receive particular attention, because their presence indicates public health hazard and give warning signal for the possible occurrence of food borne intoxication. More closely supervision should be made on these restaurants around the university of Port Harcourt community by relevant authorities, and more analysis should be carried out on other food samples sold in the University of Port Harcourt community, to ensure proper food quality standard.
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1. Introduction

Ready-to-eat food is not a nominated food or class of food within Standard. This Product group is defined as: Food that is ordinarily consumed in the same state as that in which it is sold and does not Include nuts in the shell and whole, raw fruits and vegetables that are intended for hulling, peeling or washing by the consumer (NSW, 2009). Although it is extremely difficult to pinpoint the precise beginning of human awareness of the presence and role of microorganisms in foods, the available evidence indicates that this knowledge preceded the establishment of bacteriology or microbiology as a science (Jay *et al.* 2005).

Some ready-to-eat foods also are regarded as 'potentially hazardous'. Such foods can support the growth of pathogenic (food poisoning) bacteria and must be kept at certain temperatures to minimize the growth of any pathogens that may be present in the food or to prevent the formation of toxins in the food (NSW, 2009)

There is a wide variety of ready-to-eat foods. Examples include, but are not limited to, Sandwiches, kebabs, sushi, takeaway foods and bakery products (NSW, 2009). Ready-to-eat foods usually include a number of ingredients which may or may not be cooked. Due to the variety of ready-to-eat foods, the interpretation of microbiological results obtained from testing must account for the method of processing and the individual components of the food (NSW, 2009). To assist with interpreting the microbiological analyses of such foods as part of our monitoring and surveillance program (i.e. surveys), the NSW Food Authority uses criteria that are based on interpretive guides published by the United Kingdom's Health Protection Agency and by Food Standards of Australia, New Zealand (FSANZ, 2001; NSW, 2009)

Because human food sources are of plant and animal origin, it is important to understand the biological principles of the microbial biota associated with plants and animals in their natural habitats and respective roles (Jay *et al*., 2005). Although it sometimes appears that microorganisms are trying to ruin our food sources by infecting and destroying plants and animals, including humans, this is by no means their primary role in nature (Jay *et al*., 2005). In our present view of life on this planet, the primary function of microorganisms in nature is self-perpetuation.

The microbial spoilage of foods may be viewed simply as an attempt by the food biota to carry out what appears to be their primary role in nature (Jay *et al*., 2005). Food borne illness is defined as diseases, usually either infectious or toxic in nature, caused by agents that enter the body though the ingestion of food (WHO, 2007). Governments all over the world are intensifying their efforts to improve food safety in response to an increasing number of food

safety problems and rising consumer concerns (WHO, 2007). "Food borne illnesses account for about one of every 100 U.S. hospitalizations and one of every 500 deaths" (Buzby *et al*., 2001).

Food borne diseases are known to contribute to both human morbidity and mortality as well as to health care costs (Campbell *et al.,* 1998). Most food-related illnesses have historically been attributed to one of five major groups of pathogenic bacteria (Mboto et al., 2012). These five groups are *Salmonella, Shigella, Clostridium botulinum, Clostridium perfringens, Bacillus cereus, and Staphylococcus aureus*. These have been joined by the emerging pathogens such as *Yersinia enterocolitica, Escherichia coli, Listeria monocytogens,* and *Campylobacter jejuni* (Mboto et al., 2012).

The aim of this study is to determine the microbiological quality of ready to eat food (cooked rice and beans) sold in University of Port Harcourt, Port Harcourt, Nigeria.

2. Materials and methods

Samples of ready to eat foods (cooked rice and beans with stew) were randomly obtained from High class fast food centers within University of Port Harcourt, Port Harcourt, Nigeria. Samples were collected with sterile containers, and were taken under aseptic condition to the laboratory for microbiological analysis.

2.1. Isolation and Identification of Isolates

Ten grams of each ready-to-eat food samples were weighed using a weighing balance and placed into a sterile blender, 90ml of distilled water was also added and the mixture homogenized to obtain a thoroughly blended meat. The homogenized food was aseptically transferred into a sterile beaker. One ml of the homogenized food sample was aseptically transferred using a sterile one ml sterile pipette into a test tube containing nine ml sterile distilled water and tenfold serial dilution was carried out. All media used were prepared according to the manufacturer's instruction. After preparation it was sterilized byautoclaving at $121^{O}C$ for 15 minutes after which it was allowed to cool and 15mls aliquots was poured on sterile Petri dishes. About 0.1ml of suspensions (a mixture of sample and normal saline) was deposited into the surface of the solid media and incubated at $30^{o}C$ for 24 hours. After incubation, they were stored in a refrigerator at $10^{o}C$ (Fouzia and Amir, 2011).

3. Result analysis

A total of ten samples of ready to eat food (cooked Rice and Beans with stew) were analyzed microbiologically for bacteria and fungi count.

3.1. Enumeration of isolates

The total heterotrophic bacteria count of ready to eat rice ranged from $2.45x10^5$cfu/g to $17.8x10^5$cfu/g (Table 1). The total heterotrophic bacteria count of ready to eat beans ranged from $3.5x10^4$cfu/g to $17.1x10^3$cfu/g (Table 1). It was observed that ready to eat rice has the highest bacteria count. The total staphylococcus count ranges from $1.3x10^3$cfu/g to $2.2x10^3$cfu/g (Table 1) for ready to eat rice, and ranges from $2.2x10^3$cfu/g to $3.4x10^3$cfu/g (Table 1) for ready to eat beans (all from the restaurant sample). No Salmonella and Shigella count from any of the food samples.

Table 1: Total heterotrophic bacteria count of ready to eat foods

Samples	Total Heterotrophic bacteria count		Total Staphylococcus count	
	Cfu/g	Log cfu/g	cfu/g	Log cfu/g
Rice ($R_{1)}$	$3.1x10^5$	5.49	$1.6x10^3$	3.20
Rice ($R_{2)}$	$17.8x10^5$	6.25	$1.5x10^3$	3.19
Rice ($R_{3)}$	$3.2x10^5$	5.50	$1.7x10^3$	3.24
Rice ($R_{4)}$	$2.45x10^5$	5.38	$1.3x10^3$	3.11
Rice ($R_{5)}$	$4.15x10^5$	5.61	$2.2x10^3$	3.34
Beans ($B_{1)}$	$7.2x10^5$	5.85	$1.55x10^3$	3.19
Beans ($B_{2)}$	$17.1x10^3$	4.23	$1.6x10^3$	3.20
Beans ($B_{3)}$	$4.2x10^4$	5.38	$1.7x10^3$	3.24
Beans ($B_{4)}$	$3.5x10^4$	4.54	$1.6x10^3$	3.20
Beans ($B_{5)}$	$5.15x10^5$	5.71	$3.4x10^3$	3.58

3.2. Identification of isolates

The isolates obtained from the sampled ready-to-eat foods sold in restaurants in University of Port Harcourt were identified as *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae,* and *Staphylococcus aureus*.

3.3. Frequency of occurrence of Isolates

Table 2 shows the frequency of occurrence of isolates obtained from the sampled ready-to-eat foods. It showed that *Staphylococcus aureus* [8(42.1%)] is the most predominant organism obtained from the sampled ready-to-eat foods. This was followed by *Escherichia coli* [5(26.3%)] and *Bacillus cereus* [4(21.1%)]. *Klebsiella pneumoniae* [2(10.5%)] was the least predominant as shown in Table 2.

Table 2: Frequency of occurrence of isolates obtained from the sampled ready-to-eat foods in University of Port Harcourt, Nigeria

Isolates	No. (%)
Bacillus cereus	4(21.1)
Staphylococcus aureus	8(42.1)
Klebsiella pneumoniae	2(10.5)
Escherichia coli	5(26.3)
Total	19(100.0)

3.3. Distribution of isolates

Table 3 shows the distribution of isolates obtained from the sampled ready-to-eat foods in University of Port Harcourt, Nigeria. From the result, *Staphylococcus aureus* was obtained from four rice samples (R_1 R_2 R_3 and R_5) and in four beans samples (B_1 B_2 B_3 and B_5). *Klebsiella pneumoniae* was obtained from two rice samples (R_1 and R_4). *Bacillus cereus* was obtained from two samples of rice (R_1 and R_3) and also from two beans samples (B_2 and B_5). Finally, *Escherichia coli* were obtained from two samples of rice (R_1 and R_5) and three samples of beans (B_1 B_3 and B_4) as shown in Table 3.

Table 3: Distribution of isolates obtained from the sampled ready-to-eat foods in University of Port Harcourt, Nigeria

Samples	Isolates
Rice (R_1)	*Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*
Rice (R_2)	*Staphylococcus aureus*
Rice (R_3)	*Bacillus cereus*, *Staphylococcus aureus*
Rice (R_4)	*Klebsiella pneumonia*
Rice (R_5)	*Escherichia coli, Staphylococcus aureus*
Beans (B_1)	*Escherichia coli, Staphylococcus aureus*
Beans (B_2)	*Bacillus cereus*, *Staphylococcus aureus*
Beans (B_3)	*Escherichia coli, Staphylococcus aureus*
Beans (B_4)	*Escherichia coli*
Beans (B_5)	*Bacillus cereus*, *Staphylococcus aureus*

4. Discussion

Ready to eat food do not need to be reheated before consumption. The data revealed that bacteria isolated from all food samples collected from the restaurants in University of Port Harcourt were *Bacillus cereus, Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia* by comparing their morphological and biochemical characteristics with standard reference organisms (Buchanan and Gribbons, 1974; Cheeshrough, 2003; Mboto et al., 2012).

The total heterotrophic count of bacteria ranged from $2.45x10^5$ cfu/g to $17.8x10^5$cfu/g for ready to eat rice and $3.5x10^4$cfu/g to $17.1x10^3$cfu/g for ready to eat beans samples. This is an indication of recontamination in food handling and hygiene techniques (Clarence et al., 2009; Mboto et al., 2012).

In this study, there was presence of *Staphylococcus aureus* in 4 rice samples (R_1 R_2 R_3 R_5) and as well as in 4 beans samples (B_1 B_2 B_3 B_5), collected from four different restaurants among the five that was analyzed. There was also the presence of *Klebsiella pneumoniae* in 2 Rice samples (R_1 and R_4) which is believed that it is a result of storage under a very cold temperature. Also there was presence of *Bacillus cereus* in 2 samples of Rice (R_1 and R_3) and also in 2 Beans samples (B_2 and B_5) of which produce toxins in the food. Also in 2 samples of Rice (R_1 and R_5) and in 3 samples of Beans (B_1 B_3 and B_4), the presence of *Escherichia coli* was found. Amongst all the samples analyzed, there were no fungi found in any of these samples. Microorganism isolated from ready-to-eat food samples in this study have been earlier found in foods, environment and other places and their pattern is similar to previous reports by Clarence et al. (2009) and Mboto et al. (2012). The presence of these organisms in ready-to-eat foods depicts a deplorable state of poor hygienic and sanitary practices employed in the food catering, food handling, processing and packaging of foods. Faecal coliforms such as *Escherichia coli* are generally considered as indisputable indicator of faecal contamination from warm blooded animals (Mboto et al., 2012).

The presence of *E. coli* (26.3%) in this ready-to-eat food samples is an indication of faecal contamination of the foods. This might be due to possible unhygienic handling of the foods during cooking and processing or due to possible contamination from the skin, mouth or nose of the handlers which might be introduced directly into the meat (Schroeder et al., 2005; Mboto et al., 2012). The isolation of *E. coli*, may be as a result of poor environmental conditions due to dust and contamination of the water used during cooking and processing (Talaro and Talaro, 2006; Mboto et al., 2012). *Klebsiella pneumoniae* (10.5%) another organism found in the meats is also a pathogenic organism of public health significance and concerns (Okonko et al., 2009).

E. coli is a normal flora of the human and animal intestine and has been identified as a leading cause of food borne illness all over the world (Hussein, 2007; Mboto et al., 2012). *E. coil* 0157.H7 strain was not detected in any of the ready-to-eat food samples examined. However, diarrhea caused by enterotoxigenic *E. coli* (ETEC) is highly prevalent in young children in developing countries as well as travelers (Duffy, 2006; Mboto et al., 2012). Ready-to-eat foods sold to the public in a restaurant are grossly contaminated with coliform bacteria as well as other bacterial forms.

In this study, the isolation of *Escherichia coli* was 26.3%, *Staphylococcus aureus* 42.1%, *Bacillus cereus* 21.1% and Klebsiella 10.5%. In a study by Gandham (2012), the isolation of *Escherichia coli* was 82.0% and Klebsiella 8.0%. In another study by Joshi et al. (1980), 73.0% isolation of *Escherichia coli* and 4.6% Klebsiella was reported which correlates well with the present study. Another study by Khanna et al. (1977) showed an isolation of *Escherichia coli* 21.1% and Klebsiella (2.8 %). *Echerichia coli* isolation rate in the present study was 26.3%. This is in contrast to the isolation rate seen in the study by Kanduja et al. (1969), Gowal et al. (1984) and Gandham (2012).

The presence of *Staphylococcus auerus*, a

pathogenic organism of public health concern and significance in these ready-to-eat food products might have contaminated the processed food products from source as a result of handling by processors. Improper handling and improper hygiene might lead to the contamination of food and this might eventually affects the health of the consumers (Dunn *et al*., 1995; Adebolu and Ifesan, 2001; Li-Cohen, 2002; Omemu and Bankole, 2005; Lando, 2006; Okonko *et al*., 2008 a,b,c).

Most researchers have looked at the consumer handling practices of individuals of different geographical area to potentially explain differences in foodborne illness rates in different populations. Research findings showed that individuals with higher levels of education, which have a strong positive correlation with high income (Younus et al., 2007), are more likely to eat raw clams, raw oysters, raw fish, raw sprouts and pink hamburger, besides of having unsafe hand and cutting board washing practices (Lando, 2006). Patil *et al.* (2005) combined findings from 20 studies using meta-analysis methods to estimate percentages of consumers engaging in risky behaviors, such as consumption of raw food, poor hygiene, and cross-contamination. They found that high income individuals reported greater consumption of raw food, less knowledge of hygiene, and poorer cross-contamination practices. Redmond and Griffith (2003) reviewed several studies regarding domestic food handling practices, with the majority of the studies placed in the United Kingdom, Northern Ireland and in the United States. They reported that compared with women, men are less knowledgeable about food safety and have riskier hygiene and cooking practices. Li-Cohen and Bruhn (2002) found that women, lower-income households, people 65 years and older, and non-college graduates practice safer food handling methods than men, higher-income households, people younger than 65 years and post-16 college graduates.

It is therefore suggested that ready-to-eat food processors and consumers should be educated on the adverse effect of using untreated or polluted water for processing as these could serve as sources of faecal contamination. However, food processors and consumers should observe strict hygienic measures so that they will not serve as source of chance inoculation of microorganisms and contamination of these processed ready-to-eat foods.

The presence of indicator and other organisms examined in this study is of special concern and perhaps the greatest danger associated with water used for food processing, drinking purposes and for human consumption is contamination by human excrement (Edema *et al*., 2001; Okonko *et al*., 2008a,b,c). The need for microbial assessment of water for production of food and food drinks should also be emphasized to reduce possible contamination (Fagade *et al*., 2005).

5. Conclusion

The findings of this study revealed that ready-to-eat foods sold at University of Port Harcourt, Port Harcourt, Nigeria are contaminated with pathogenic gram negative and gram positive bacteria. The possible sources of these contaminants are due to the unhygienic manner of handling food in the restaurant. This implies that these ready-to-eat foods are viable source of various diseases. Some of these diseases could spread and acquire epidemic status which poses serious health hazards. Irrespective of the presence of these gram negative and gram positive bacteria in ready-to-eat foods analyzed, it is believed that cooking processes and hygiene could greatly reduce the microbial load to harmless level (Agnes, 1995; Mboto et al., 2012).

Conclusively, the presence of these microorganisms in food courses food spoilage and food poisoning. Food should not only be nutritionally balanced, but should be microbiologically safe as well. From the result gotten, it was indicated that these ready to eat food samples did not meet the bacteriological quality standard (WHO, 2007). More closely, supervision should be made on these restaurants around the University of Port Harcourt community by relevant authorities, and further studies should be carried out on other food samples sold in the community, to ensure proper food quality standard.

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