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Full Length Research Paper

Comparative study of ready-to-eat foods from road-side and eateries in Benin City, Nigeria

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The microbiological qualities of ready to eat foods sold in road-side and eateries were studied using the standard microbiological methods. The mean viable bacterial counts in food samples obtained from eateries ranged from 2.4 $\times 10^3 \pm 0.23$ to 4.8 $\times 10^4 \pm 0.23$ cfu/g, while the mean viable bacterial counts in food samples obtained from road side canteen ranged from 9.0 $\times 10^4 \pm 0.43$ to 2.20 $\times 10^5 \pm 0.40$ cfu/g. The fungal counts in the food samples obtained from eateries ranged from 3.0 $\times 10^3 \pm 0.15$ to 3.5 $\times 10^4 \pm 0.18$ cfu/g while the fungal counts in food samples obtained from road side ranged from 2.5 $\times 10^4 \pm 0.33$ to 5.3 $\times 10^4 \pm 0.22$ cfu/g. The microorganisms isolated were identified based on their cultural characteristics, Gram staining and biochemical tests. A total of eight bacterial isolates were obtained from the salad samples which included *Staphylococcus aureus*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Micrococcus* sp., *Escherichia coli*, *Bacillus licheniformis* and *Klebsiella* sp. The fungi isolated were: *Saccharomyces cerevisiae*, *Mucor mucedo*, *Aspergillus flavus*, *Fusarium* sp., *Aspergillus niger*, *Penicillium* sp. and *Rhizopus* sp. Therefore, good personal hygiene, proper sanitation practice and the use of clean utensils during the preparation of ready to eat foods are recommended to avoid food poisoning and spoilage associated with the isolated microorganisms.

Key words: Ready-to-eat foods, hygiene, microbial analysis, food poisoning, canteen.

INTRODUCTION

Ready-to-eat foods can be described as foods ready for immediate consumption at the point of sale. They could be raw or cooked, hot or chilled and can be consumed without further heat treatment (Clarence et al., 2009; Tsang, 2002; Oranusi and Braide, 2012; Mahakarnchanakul et al., 2010). There are certain factors that make street foods popular. These include familiarity, taste, low cost and convenience (Mahakarnchanakul et al., 2010). Our society shows a social pattern characterized by increased mobility, large number of itinerary workers and less family or home centered activities. This situation, however, has resulted in more ready-to-eat foods taken outside the home. Thus, food vendor services are on the increase and the responsibility of good manufacturing practices of food including good sanitary measures and proper handling have been transferred from individuals/families

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to the food vendors who rarely enforce such practices (Musa and Akande, 2002; Clarence et al., 2009).

Food consumption is an important pathway for bacteria to infect humans, hence the presence of antimicrobial resistant bacteria in foods warrants particular attention. Antimicrobial resistant bacteria have been recovered from both healthy humans (Okeke et al., 2000) and a wide variety of foods, which include vegetables (Osterblad et al., 1999), confectionary (Pinegar and Cooke, 1985) meat and meat products and poultry (Schoeder et al., 2004). Hence food contaminated by faecal material from healthy humans may be an important source of antibiotic resistant organisms that later cause human infections (Teuber, 1999; Schoeder et al., 2004).

Contamination of food may occur during and after processing of such food. Contamination of ready-to-eat food is of primary concern because such organisms may be pathogenic thereby leading to outbreak of food-borne illness. Food-borne illnesses may occur when food sources that contain pathogenic microorganisms are consumed raw or improperly cooked (Sangoyomi et al., 2012). The health implications cannot be over-emphasized. E. coli for instance can induce gastroenteritis (Olowe et al., 2008); Staphylococcus aureus isolates have been implicated in a number of clinical cases (Adegoke and Komolafe, 2009; Serrano et al., 2004; Adegoke et al., 2010). Ready-to-eat foods have been reported to be easily available, affordable, provide diverse/variable food source, employment and with a potential for improving food security and nutritional status and general social security (Draper, 1996). It is, however, a variable source of food-borne pathogen (Abdussalam and Kaferstein, 1993; Arambulo et al., 1994; Mensah, 2002; Patricia and Azanza, 2005). Moreover, non-pathogenic organisms that may contaminate man's food chain from time to time may serve as reservoir of genes for antimicrobial resistance in organisms. These genes are encoded by integrons that occur on plasmids or that are integrated into the bacterial chromosome. Antimicrobial resistant strains of animal or human commensals that do not produce disease may transmit their resistance genes to pathogenic organisms whenever they occur in humans.

In Nigeria, most of these products are stored under unhygienic conditions. They are often displayed in open trays or container in the market or are hawked along the street and major runways. Contamination of food can occur at any point in the production chain (from the raw materials, processing, packing, transportation, storage or marketing) to consumption. Because of improper processing, handling and storage of these foods could be subject to contamination by microorganism (Musa and Akande, 2002).

In Nigeria, there is little or no knowledge of transmission of foodborne diseases among food handlers working in ready-to-eat food centers. Most proprietors of ready-toeat food centers are not duly licensed and their staff properly selected. Therefore, this work was designed to examine the microbiological quality of ready-to-eat foods from road-side and eateries in Benin City, to reduce the risk of food poisoning.

MATERIALS AND METHODS

Sample collection

A total of 18 ready-to-eat food samples were purchased from roadside local food centers and high class eateries within Benin metropolis. All samples were collected in sterile containers and were taken under aseptic condition to the laboratory for microbiology analysis.

Sample preparation

Ten grams of each sample were weighed and mashed in a sterile laboratory mortar and pestle and aseptically introduced into 90 ml of sterile distilled water, properly shaken before a 10 fold serial dilution was prepared.

Preparation of culture media

All media were prepared accordingly to manufacturer's instruction. The media used in this study were nutrient agar (used for heterotrophic bacterial count) and potato dextrose agar (used for fungal count).

Isolation and enumeration of microorganisms

One milliliter from appropriate dilutions was plated out by pour plate method on nutrient agar and potato dextrose agar. The nutrient agar plates were incubated at 37°C for 24 h while the potato dextrose agar plates were incubated at room temperature 28°C for 72 h. After incubation, discrete colonies of culture on nutrient agar and potato dextrose agar plates were counted and expressed in cfu/ml.

Characterization and identification of bacterial isolates

Bacterial isolates were identified on the basis of cultural morphological and biochemical tests according to Jolt et al. (1994) and Cheesbrough (2006). The fungal colonies were identified as described by Harrigan (1998).

Total coliform count

From the homogenate, 1 ml of serially diluted (1:10, 1:100 and 1:1000) sample was transferred into 9 ml sterilized lauryl sulphate tryptose (LST) broth in triplicate. For each dilution, the tubes were incubated at 35° C for 48 ± 2 h to evaluate tryptose broth used as a pre enrichment medium. After primary incubation, one (0.3 mm) loopful of positive tube (gas formation by the action of chloroform bacterial in fermenting lactose medium tube) was transferred to Brilliant Green Lactose Bile (BGLB) broth, further incubated at 35° C for 48 ± 2 h for total coliforms count. Inverted Durham fermentation tubes were added into test tubes before the addition of BLGB broth to allow easy identification of gas production. Then, the number of tubes with positive gas production was counted. Most probable number (MPN) of coliform bacteria per gram sample was calculated from MPN table based on number of tubes of BGLB broth producing

Samples	Eateries	Road side canteen		
Samples	Mean±SD	Mean ±SD		
Egusi soup	8.0 x10 ³ ±0.10	7.2 x10 ⁵ ±2.92		
Salad	4.9 x10 ⁴ ±1.37	1.22 x10 ⁵ ±0.40		
Pounded yam	0.0	7.4 x10 ⁴ ±0.63		
Vegetable soup	1.29 x10 ⁴ ±0.16	2.20 x10 ⁵ ±0.40		
Moimoi	6.1 x10 ³ ±0.39	1.58 x10 ³ ±0.70		
Beans	8.2 x10 ³ ±0.13	2.13 x10 ⁵ ±0.63		
Porridge yam	4.8 x10 ⁴ ±0.23	1.43 x10 ⁵ ±0.23		
Jollof rice	2.4 x10 ³ ±0.23	1.03 x10 ⁵ ±0.43		
Amala	3.1 x10 ³ ±0.40	9.0 x10 ⁴ ±0.43		

 Table 1. Total viable bacterial counts (cfu/g) in the ready-toeat food samples.

 Table 2. Total fungal counts (cfu/g) in the ready-to-eat food samples.

Complee	Eateries	Road side canteen		
Samples	Mean±SD	Mean±SD		
Egusi soup	8.0 x10 ³ ±0.01	5.3 x10 ⁴ ±0.22		
Salad	3.0 x10 ³ ±0.15	4.3 x10 ⁴ ±0.29		
Pounded yam	9.0 x10 ³ ±0.10	$2.5 \times 10^4 \pm 0.33$		
Vegetable soup	3.5 x10 ⁴ ±0.18	$9.5 \times 10^4 \pm 0.20$		
Moimoi	6.6 x10 ³ ±0.05	$3.2 \times 10^4 \pm 0.27$		
Beans	1.4 x10 ⁴ ±0.10	4.7 x10 ⁴ ±0.21		
Porridge yam	4.0 x10 ³ ±0.08	3.5 x10 ⁴ ±0.19		
Jollof rice	3.0 x10 ³ ±0.15	2.1 x10 ⁴ ±0.30		
Amala	1.5 x10 ³ ±0.04	7.0 x10 ⁴ ±0.15		

Bacterial isolates	Fungal isolates			
Staphylococcus aureus	Mucor mucedo			
Bacillus subtilis	Aspergillus flavus			
Enterobacter aerogenes	Saccharomyces			
Pseudomonas aeruginosa	Penicillium sp.			
<i>Micrococcus</i> sp.	Aspergillus niger			
Escherichia coli	Fusarium sp.			
Bacillus licheniformis	<i>Rhizopus</i> sp.			
Klebsiella sp.				

gas at the end of incubation period.

RESULTS AND DISCUSSION

The mean viable bacterial counts in food samples obtained from eateries ranged from 2.4 $\times 10^3 \pm 0.23$ to 4.8 $\times 10^4 \pm 0.23$ cfu/g while the mean viable bacterial counts in

food samples obtained from road side canteen ranged from 9.0 $\times 10^4 \pm 0.43$ to 2.20 $\times 10^5 \pm 0.40$ cfu/g (Table 1). The highest bacterial counts was recorded in vegetable soup obtained from road side canteen while the lowest bacterial counts was recorded in *jollof* rice obtained from eateries. The fungal counts in the food samples obtained from eateries ranged from 3.0 $\times 10^3 \pm 0.15$ to 3.5 $\times 10^4 \pm 0.18$ cfu/q while the fungal counts in food samples obtained from road side ranged from 2.5 x10⁴±0.33 to 5.3 $x10^4 \pm 0.22$ cfu/g (Table 2). The salad samples obtained from eateries had the lowest fungal counts while equsi soup obtained from road side canteen had the highest fungal counts. The microbial counts in the food samples obtained from road side were comparably higher than the microbial loads of food samples obtained from eateries. The microorganisms isolated from the ready to eat food samples analyzed included eight bacterial isolates and seven fungal isolates. The bacterial isolates were Staphylococcus aureus. Bacillus subtilis. Enterobacter aerogenes, Pseudomonas aeruginosa, Micrococcus sp., Escherichia coli, Bacillus licheniformis and Klebsiella sp. The fungal isolates include Saccharomyces cerevisiae, Mucor mucedo, Aspergillus flavus, Fusarium sp., Aspergillus niger and Rhizopus sp., this is seen in Table 3. The occurrence of the microorganisms isolated from the ready to eat food samples is presented in Tables 4 and 5.

Despite the high level of contamination of the ready-to eat foods in this study, most of the sampled foods contained total aerobic plate counts of 10^3 to 10^5 cfu/g. These foods are therefore considered fit for human consumption (ICMSF, 1974; FAO/WHO, 2005). The ICSMF (1974) identified foods with counts >10⁶ cfu/g as unacceptable for consumption. The high microbial load recorded for food samples obtained from road side canteen could be associated with the poor hygienic practices and handling of the product by the sellers. Lack of storage facilities could have heightened the chances of contamination (Baro et al., 2007). The ready-to-eat foods are maintained throughout the day under room temperature, this encourages proliferation of contaminants. The microorganisms isolated from the ready to eat food samples analyzed included eight bacterial isolates and seven fungal isolates. Our results revealed that the bacterial isolates were S. aureus, B. subtilis, E. aerogenes, P. aeruginosa, Micrococcus sp., E. coli, B. licheniformis, Klebsiella sp. This finding is similar to previous reports by Desmarchelier et al. (1994), Nichols et al. (1999) and Mensah et al. (2002). The fungal isolates include S. cerevisiae, M. mucedo, A. flavus, Fusarium sp., A. niger and Rhizopus sp. This result was in agreement to that reported by Wogu et al. (2011), who isolated similar fungi from ready-to-eat foods, although few data are available on the occurrence of fungi in ready-to-eat food. Some of the microorganisms isolated from the ready to eat have been reported to be associated with food spoilage and food poisoning (Jay, 1996). Organisms implicated in

Bacterial isolates	Egusi soup	Salad	Pounded yam	Vegetable soup	Moimoi	Beans	Porridge yam	Rice	Amala
S. aureus	-	+	+	+	+	+	-	+	+
B. subtilis	+	+	+	-	+	+	-	+	+
E. aerogenes	+	-	-	+	-	-	+	-	-
P. aeruginosa	+	+	-	+	-	-	+	-	-
Micrococcus sp	-	-	-	-	+	+	+	-	+
E. coli	-	+	-	-	-	-	-	-	-
B. licheniformis	-	-	+	-	+	+	-	+	-
Klebsiella sp	+	+	+	+	-	-	+	-	-

Table 4. Distribution of the bacterial isolates in the food samples.

+ = Present; - = Absent.

Table 5. Distribution of the fungal isolates in the food samples.

Fungal isolates	Egusi soup	Salad	Pounded yam	Vegetable soup	Moimoi	Beans	Porridge yam	Rice	Amala
Mucor mucedo	+	+	-	+	-	+	+	-	+
Aspergillus flavus	+	-	+	+	+	+	-	-	+
Saccharomyces cerevisiae	-	+	-	-	-	-	-	-	+
Penicillium sp	+	+	+	-	+	-	+	+	-
Aspergillus niger	+	+	+	+	+	+	-	+	-
Fusarium sp	+	+	+	+	-	-	+	-	-
<i>Rhizopus</i> sp	-	-	-	-	-	-	-	+	-

+ = Present; - = Absent.

food-borne diseases are numerous and diverse bacteria, fungi, viruses and parasites (Jay, 1996; Chukwu et al., 2010; De Rover, 1998). *E. coli, S. aureus, Bacillus* species and *Pseudomonas* sp. were isolated from the ready to eat foods indicating poor sanitary control and practices. These organisms are known food borne pathogens and opportunistic pathogens that have been implicated in food-borne disease outbreaks (Oranusi and Braide, 2012; Mudgil et al., 2004; Oranusi et al., 2006, 2007; Yadav et al., 2011; Tambeker et al., 2008).

Fungi are common environmental contaminants due to their ability to produce spores; this could explain their presence in ready to eat food. They have been implicated in ready to eat foods. Species of Aspergillus, Penicillium and Fusarium are known to produce deleterious mycotoxins under favourable conditions (Oranusi and Braide, 2012), their presence in ready to eat food must therefore be treated with caution. Aspergillus species which were the most predominant in this study are among the most abundant and widely distributed organisms on earth. Members of this genus are saprophytic moulds. Most live in the environment without causing disease. Virtually, all the common Aspergillus species have been recovered at some time from agricultural products. A. niger and A. flavus were isolated from the ready to eat food samples analyzed.

The occurrence of *S. cerevisiae* may be due to its role in fermentation of agricultural products. *S. cerevisiae* is

well known for converting carbohydrates into alcohol and other aroma compounds such as esters, organic acids and carbonyl compounds. Their presences in food sometimes represent assimilation of glucose, galactose and lactic acid by them. Examination for the presence of pathogens in ready-to-eat food contributes to food safety (Roberts and Greenwood, 2003; Elviss et al., 2009; Willis et al., 2009). Although low numbers of food pathogens probably represent a low risk, their presence can suggest fault(s) in the production and/or subsequent handling which, if not controlled, could lead to an unacceptable increase in risk (Health Protection Agency, 2009). The presence of Bacillus sp. in these foods indicate the possibility that spices such as pepper have been added after the main cooking process (Health Protection Agency, 2009). Evidence show that food can be a reservoir of extra-intestinal infections caused by epidemic strains of E. coli causing uncomplicated urinary tract infections (Manges et al., 2008) and other severe infections (Johnson et al., 2002; Manges et al., 2006).

Conclusion

The results of this present study indicated that the readyto-eat food samples sold in road side canteen and eateries had microbial contaminants. Hence, it is recommended that a more close supervision of ready-to-eat food should be carried out by relevant authorities.

Conflict of interests

The author(s) did not declare any conflict of interest.

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