Microbiological Safety of Ready-To-Eat Foods and Hand Hygiene Assessment of Food Handlers in a Nigerian Private University

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Abstract: Ready-to-eat foods are widely available in public places including tertiary institutions. The safety of the foods is often compromised by poor hygiene and inadequate sanitary facilities and can have debilitating effects on human health. Unfortunately, the microbiological quality of foods sold at Nigerian universities is rarely routinely assessed. Food samples from the cafeteria of a Nigerian private University, the surfaces on which they are prepared and served and stool samples from the food handlers were assessed for microbiological quality using standard microbiological procedures. The obtained data were analyzed using descriptive statistics. Microbial counts of foods that were sampled ranged from 0.1 x 10^6 cfu/g for hot dog to 4.13 x 10^6 cfu/g for rice. The highest number of isolates (19/51, 37.3%) was from the hands of food handlers while the least number (6/51, 11.8%) was from swabbed surfaces. The presence of *Burkholderia cepacia, Raoultella ornithinolytica* and *Klebsiella pneumoniae* from the samples is indicative of poor microbiological safety of ready-to-eat food and suggests unhygienic practices by the food handlers. Active surveillance of ready-to-eat foods is required to ensure food safety.

Key word: Food safety, microbiological quality, poor hygiene, sanitation, ready-to-eat foods.

INTRODUCTION

Food is essential to all living things hence its safety is not negotiable to ensure the well-being of the consumer is not adversely affected. Food safety is the assurance that upon consumption of food, there is no harm or injury to the consumer (Feed The Future, 2020). The safety of food for consumption cannot be overemphasized. Food safety is however often compromised through contamination of food by various agents which can be living or non-living and can lead to food poisoning. Food poisoning is a situation where someone becomes sick upon consumption of food and is often characterized by vomiting, stomach ache or abdominal pain as well as diarrhoea (Kumar, 2020). Other symptoms such as fever, headache nausea and discomfort exist with no definite time limit for victims of food poisoning to feel the impact of the poisoning evidenced by the symptoms. The living organisms that are responsible for food poisoning include bacteria, parasites and viruses. Bacteria such as *Salmonella enterica* serovar Typhimurium, *Vibrio vulnificus, Escherichia coli,* among others have been reported to cause food poisoning (Kumar, 2019). According to Fung *et al.* (2018), about 90% of food-poisoning episodes are caused by species of *Salmonella, Campylobacter, Listeria, Bacillus, Vibrio, Staphylococcus* and *Escherichia. E. coli* O157:H7, a notorious pathogenic strain of *E. coli,* has been found in plant products such as vegetables and fruits as well as animal products like chicken, pork and even milk resulting in severe symptoms (including kidney failure) in victims who ingested it in contaminated food (Fonseca *et al.,* 2011; Alum *et al*., 2016). Parasites such as *Toxoplasma* as well as viruses such as rotavirus and astrovirus have also been implicated as agents of food poisoning albeit to a lesser degree than bacterial agents (Kumar, 2020). Non-living agents of food poisoning are toxins and chemicals some of which also serve as allergens in the body. Kassahun and Wongiel (2019) reported 35 food poisoning cases in Ethiopia in 2018 with an attack rate of 25.58 per 10,000 persons and identified risk factors associated with the food-poisoning cases.

In Nigeria, the most populous African country, food-borne illnesses are considered to have high health (resulting in over 200,000 deaths annually) (Ezirigwe, 2018)

and economic impacts with productivity losses estimated at over $6 million by the World Bank (Jaffee *et al.,* 2019). Unfortunately, the standards of hygiene and sanitation in the country are low (as open defecation and lack of access to potable water are common) disproportionately affecting the majority of the populace who live below the poverty line and are unsurprisingly prone to food-borne illnesses via contamination.

The respite however in recent times is that there have been more interests in assessing the food safety situation in Nigeria with a view to proffering solutions in form of relevant interventions (Feed The Future, 2020). Even though research on food safety has increased in Nigeria over the past three decades (National Bureau of Statistics, 2019), the safety of food vended in the country's educational institutions particularly Universities is largely understudied. This study assessed the microbiological safety of ready-to-eat foods sold to students in a Nigerian private University and the level of hygiene maintained by the food handlers.

MATERIALS AND METHODS

***Sample collection*:** The following samples were randomly collected:

a. Food samples – rice, fried rice, jollof rice, porridge, spaghetti, and hot dog (three samples each from which a composite was obtained).
b. Swabs from hands of six food handlers (one swab sample from each food handler)
c. Swabs from preparation table and serving counters (one swab sample each)
d. Stool samples from food handlers (one stool sample from each food handler).

Food samples were collected from the cafeteria of the University into sterile containers and were immediately transported to the laboratory (within 100 metre radius) for processing. To obtain swab samples from food handlers, a sterile swab stick was immersed in sterile normal saline to moisten the swab and used to rub the palms and digits of each food handler radially and in an inside-out fashion. The swab was immediately transferred into a pre-labeled container containing 5 ml of sterile normal saline. Swabs of food preparation and serving surfaces were also obtained by rubbing the surfaces with sterile normal saline-moistened swab sticks and transferred into pre-labeled containers containing 5 ml of normal saline as previously described. Finally, a stool sample was obtained from each of six food handlers whose hands were swabbed to screen for carriage of enteric pathogens.

***Sample processing*:** The samples were processed in the laboratory on the same day as the laboratory of collection.

***Sample processing for microbial isolation*:** The food samples, swabs and food handlers' stool samples were processed in the laboratory for microbial isolation as outlined below.

***Processing of food samples*:** One gram each of rice, fried rice, jollof rice, porridge, spaghetti, and hot dog collected at the point of sale was first enriched in 10 mL of sterile peptone water and incubated at 37^{o}C for 24 hours. The enriched culture after incubation was evenly mixed for homogenization and then sub-cultured using pour plate method (Greenwood *et al.,* 1984) on mannitol salt agar (MSA) (Oxoid, United Kingdom), ethylene methylene blue (EMB) agar (Oxoid, United Kingdom), *Salmonella-Shigella* agar (SSA) (Oxoid, United Kingdom), MacConkey agar (Oxoid, United Kingdom) for bacteria isolation and Potato Dextrose Agar (PDA) (Oxoid, United Kingdom). To estimate the number of heterotrophic bacteria, a thousandth (10^{-3}) and a hundred thousandth (10^{-5}) dilution of each food sample was plated on plate count agar (PCA) (Oxoid, United Kingdom) using pour-plate method and incubated at 37 ^{o}C for 24 hours.

***Processing of swabs*:** The swab samples obtained from six food handlers were labelled FHH1 - FHH6, and preserved

temporarily in sterile normal saline solution until when needed. With a sterile Pasteur pippete, 0.1 mL of each homogenized swab suspension was sub-cultured onto MSA (Oxoid, United Kingdom), EMB (Oxoid, United Kingdom), SSA (Oxoid, United Kingdom), MacConkey agar and PDA (Oxoid, United Kingdom) (Oxoid, United Kingdom) using pour-plate method. Food serving and food preparation surfaces were processed in similar way as the swabs from the hands of the food handlers.

***Processing of stool samples*:** Food handlers' stool samples were screened for the possible carriage of pathogenic microorganisms by the handlers. The samples were cultured on the respective aforementioned media for microbial isolation. Isolated microorganisms were sub-cultured to ensure purity and subsequently maintained on agar slants at 4^oC till further work.

***Isolate identification*:** In addition to morphological characterization and Gram staining of bacterial isolates, the isolates were identified using the Vitek 2 system (Biomerieux Inc., Hazelwood, MO, USA). Briefly, colonies of a pure culture of an isolate were transferred into 3 mL of sterile saline in a clear plastic tube using a sterile pipette. The suspension was adjusted to turbidity equivalent to McFarland standard of 0.5-0.63 using a turbidometer. The plastic tube containing the suspension was placed on the Vitek 2 tray and a suitable cassette (a 64-well reagent cassette) fitted with a transfer tube was inserted to enhance the loading of the cassette with the suspension via the transfer tube in the vacuum chamber of the Vitek 2 system. Upon filling the wells, the cassette was removed and placed into the card-sealing chamber where the card was sealed with an accompanying cutting off of the transfer tubes. Then the cards were automatically taken into the incubator chamber with identity of the isolate displayed on the monitor after the incubation period (Barnett *et al.*, 2000).

***Data analysis*:** Data analysis was done using descriptive statistics to understand trends and observe differences among variables.

RESULTS AND DISCUSSION

Enumeration of microbial loads of food samples

The plate counts of the microorganisms isolated from food samples are presented on Figure 1. Particularly notable are the high plate counts observed in rice (4.13×10^6cfu/g) and spaghetti (3.46×10^6cfu/g) indicating a high microbial loads. Odu and Peter (2013) also reported high microbial load averaging 6.14×10^5cfu/g in cooked rice vended in University of Port Harcourt, Nigeria. Even though the microbial loads of egg and hot dog were lower than other foods assessed (0.26×10^6cfu/g and 0.1×10^6cfu/g respectively), the counts were still worryingly high and beyond the threshold for bacteriological quality of food as indicated by World Health Organization (WHO, 2007). The high microbial counts of these foods are indicative of poor hygienic practices and sanitary conditions under which food preparation takes place. To underscore this assertion, all the ready-to-eat foods in this study have been cooked which should have appreciably reduced microbial load (if any still remains after heating). Therefore, high microbial counts as seen in this study suggests post-cooking contamination of the food and are most likely to be from poor hand hygiene and appalling sanitary conditions. Techer *et al.* (2013) and Englmaierova *et al.* (2014) reported that eggshell surface contamination is often as a result of poor sanitary conditions and can become exacerbated by transport and packaging processes. Spaghetti has the second highest microbial count of all the food samples (3.46×10^6cfu/g) in this study. This is unsurprising because spaghetti is often dished out with fork and in some instances, vendors support with their hands for easy grip while dishing (selling) spaghetti. Therefore, the high microbial load of spaghetti may have been from handling of the food.

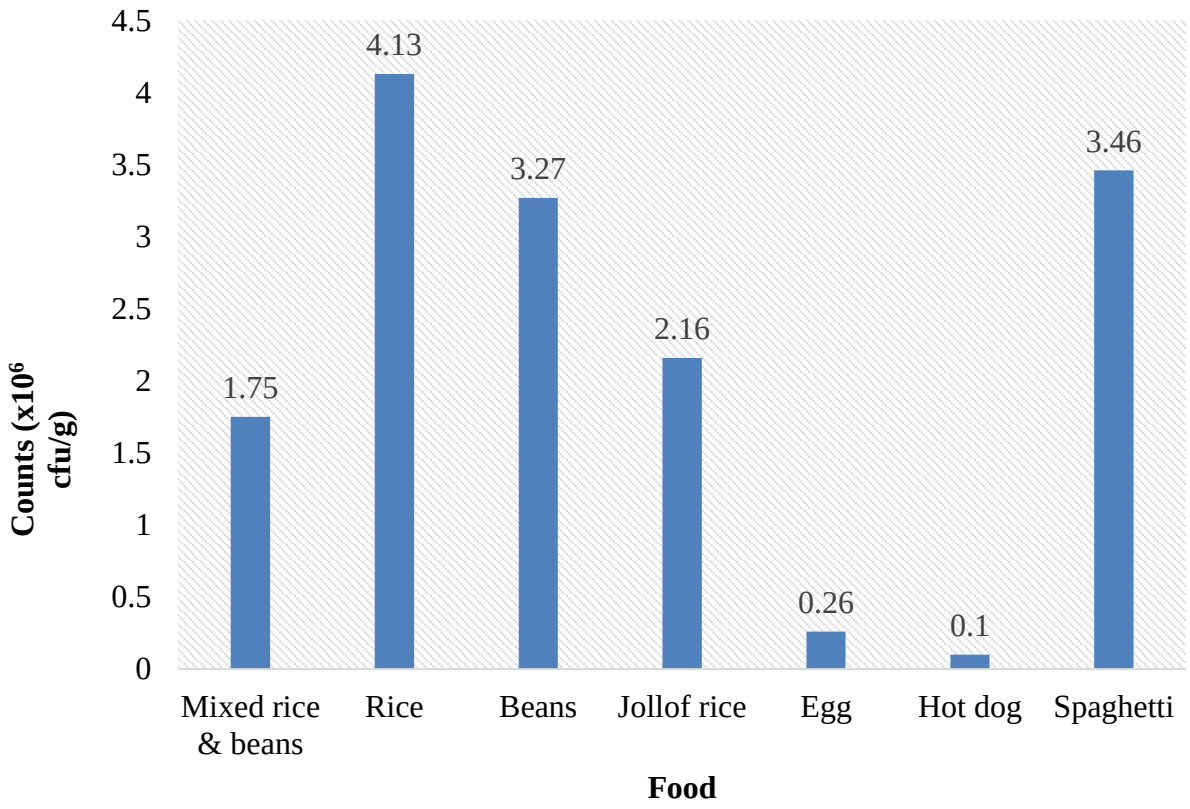

Figure 1: Plate counts of food samples

Microbial isolation

Following observation of colonial morphology on the respective media plates, microorganisms were isolated from the respective samples as shown in Table 1. The highest number of isolates was from the hands of food handlers (19/51, 37.3%) while the least was from swabbed surfaces (food preparation and serving surfaces) (6/51, 11.8%).

The isolation of microorganisms from the hands of food handlers and the surfaces on which foods are prepared and served probably explains why there were recoveries of microorganisms from the ready-to-eat foods which is a huge threat to the health of the consumers of the foods. Odu and Peter (2013) asserted that the presence of microorganisms in ready-to-eat foods is suggestive of poor handling, processing and packaging of such foods. The presence of faecal coliforms like *Escherichia coli* in foods, for instance, is indicative of faecal contamination which renders the food unfit for consumption. The isolation of microorganisms from the stools of the food handlers in this study was not unexpected as stool is hub of many microorganisms, both pathogenic and non-pathogenic (Santosh *et al.*, 2012). Sala *et al.* (2005) reported food poisoning outbreak in a hospital that was traced back to a vendor who did not maintain hand hygiene. Research has also shown the possibility of cross-contaminating surfaces with hands that are not free of germs (Barker *et al.*, 2004). This necessitates maintaining proper hand hygiene by all food handlers as improperly washed hands are very likely to still carry pathogenic organisms which can find their way into food during preparation or serving.

Table 1: Microorganisms isolated from sample sources

Sample source	Number of isolates	Percentage (%)
Food	10	19.6
Swabbed surfaces	6	11.8
Food handlers' hands	19	37.3
Food handlers' stools	16	31.4
Total	51	100

Table 2: Identified isolates and their sources

Isolate Identity	Frequency	Source (n)
Burkholderia cepacia	10	Food (6)
		Food handlers' hand (4)
Raoultella ornithinolytica	3	Food (1)
		Food handlers' stool (2)
Klebsiella pneumoniae	1	Food (1)
Unidentified	4	Food handlers' hand (1)
		Food handlers' stool (3)
Total	**18**	

Identification of isolated microorganisms

Based on the morphological features and Gram reactions, 18 of the 51 isolated microorganisms (representing 35.3%) were systematically selected putting into cognizance their sources and the observed trends. The identities are presented in Table 2. Interestingly, 2 of the 3 isolates identified as *Raoultella ornithinolytica* were from the stool samples of two different food handlers while the third was isolated from fried rice. *Raoultella ornithinolytica* is a rare, encapsulated, Gram-negative organism known to have the capacity to convert histidine to histamine in fish resulting in poisoning when such fish is consumed and can cause diarrhoea, vomiting, and urinary tract infection in affected persons (Silva *et al.,* 2016). Finding this organism in stools of 2 of the 6 food handlers indicates that there is some level of carriage in the individuals. The recovery of the microorganism in the stools of the food handlers could potentially explain its recovery from fried rice and suggests faecal contamination of the food. This is very worrisome as it directly compromises the microbiological safety of the food.

Over half (10/18, 55.6%) of the isolates selected for identification were identified as *Burkholderia cepacia* complex, a Gram-negative non-fermenting betaproteobacterium (Depoorter *et al.,* 2016). This species is an environmental soil organism commonly found in the rhizosphere (root surfaces) of plants and is responsible for the "slippery rot" in onions as well as soft rot in certain vegetables (Moore *et al.,* 2001). Four *Burkholderia cepacia* strains were isolated from the hands of 3 of the 6 food handlers (two strains were isolated from an individual). The organism was also found in food samples including porridge, hot dog, and spaghetti. Having been associated with food spoilage, *B. cepacia* isolated from the hands of food handlers may indicate that the handlers may have handled spoilt food items like rotten

onions and vegetables or come in contact with surfaces where these food items were kept without properly washing their hands afterwards. The recovery of six *B. cepacia* strains from ready-to-eat foods and four *B. cepacia* strains from people who handled and sold the food to consumers calls for concern and again raises alarm about poor hand hygiene practices as the *B. cepacia* strains found in the foods may have come from the handlers carrying the microorganisms in their hands. Although *B. cepacia* constitutes "minor" threat to healthy people, those with certain underlying health issues and weakened immune systems are more susceptible to infections caused by *B. cepacia* (CDC, 2010). Also noteworthy is its resistance to many antibiotics due to its rapid mutation and survival in extreme conditions (Tavares *et al.*, 2020).

The isolation of *Klebsiella pneumoniae*, a Gram negative encapsulated gut bacterium, from fried rice also calls for concern due to its virulence which can lead to infection and multi-drug resistance when ingested. Mousa (2014) reported twenty percent recovery rate of *Klebsiella pneumoniae* from street vended, ready-to-eat foods in Cotonou, Benin Republic. An alarming sixty-five percent of the *K. pneumoniae* strains harbored bla$_{TEM}$ gene coding for resistance to beta-lactam drugs. In addition, twenty-seven percent of the stains were also resistant to imipenem underscoring the antimicrobial potential of the strain which threatens public health immensely. The occurrence of *K. pneumoniae* in ready-to-eat foods was also reported by Gandham (2012) as well as Odu and Peter (2013).

The assessment of microbiological safety of ready-to-eat foods and hygiene levels of food handlers in this study revealed that there is a need for active surveillance so as to protect consumers from food poisoning and other food-borne illnesses. As food safety is important to health, measures to ensure that foods are safe for consumption particularly among students of tertiary institutions who patronize food vendors a lot owing to insufficient time to cook (or in some institutions are not even allowed to cook in their hostels), should be adequately put in place. Food should not only be nutritious and tasty; it has to be microbiologically safe for consumption as well. This study is however limited by the inability to characterize all the bacterial isolates and any of the fungal isolates. Moreover, other microbial agents responsible for food poisoning and illnesses such as parasites and viruses were not sought in this study which may mean that the observations of food contamination in this study are a gross underrepresentation of the realities. Unfortunately, 4/18 (22.2%) of the isolates subjected to Vitek 2 identification system were not identifiable. It would have been wholesome to employ more discriminatory method like whole genome sequencing to reveal their identities as they may be very important microorganisms to the environment and to public health but such methods were not employed due to resource constraints. Furthermore, other factors like water quality, proximity to water source as well as level of education of food handlers that could influence microbiological quality of ready-to-eat foods were not assessed.

CONCLUSION

The microbiological analyses of food samples, surfaces for preparing and serving food as well as the stool samples from food handlers carried out in the study revealed poor hygienic practices among the food handlers which may have been responsible for the recovery of microorganisms the ready-eat-foods sold by the food handlers. A plausible means of transmission is via the use of contaminated hands for food processing and serving. There is therefore the need to sensitize food handlers on the importance of maintaining proper hand hygiene and survey the microbiological quality of ready-to-eat food to enhance food safety. From the evidence provided by this study, we recommend that food vendors

should ensure that food items such as vegetables, spices, and others showing any signs of spoilage or deterioration are gotten rid of and not cooked for consumption. Also, it is pertinent that food is properly cooked to reduce the chance of microbial survival and contamination. Moreover, there should be regular cleaning and sanitation of the environment including surfaces where food is processed, cooked, and served must be done. Furthermore, hand hygiene must be imperatively practiced by all food handlers and vendors. Finally, food servers and handlers should be properly kitted with their aprons and caps maintained clean.

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