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MICROBIAL EXAMINATION OF DIFFERENT COOKED RICE SOLD AT DIFFERENT RESTAURANTS IN UNIVERSITY OF MAIDUGURI

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ABSTRACT

This study focused on examination of rice served in university of Maiduguri restaurants for microorganisms. Three different rice samples which include fried rice, jollof rice and white rice were collected aseptically from different restaurants in University of Maiduguri campus. Different media which include MacConkay agar, Nutrient agar**,** Malt extract agar**,** Sabouraud dextrose agar**,** Corn meal agar, Lacto phenol cotton blue was prepared and used for culturing the organisms. After the culturing of the organisms macroscopic, microscopic and biochemical tests were used for the isolation and identification of the organisms. Three types of bacteria and fungi were identified. The bacteria isolated include*Staphylococcus aureus* from the fried rice, *Escherichia coli* isolated from Jollof rice and *Klebsiella pneumonia* were isolated from white rice with average load of $2.0x10^5$, 5.0×10^7 and $2.0x\ 10^7$respectively. While the fungi isolated from fried rice was *Aspergillus niger, Aspergillus flavus* from jollof rice and *Fusarium* species from white rice with average load of 3.0×10^5, $6.8 \times 10^{7,}$ 3.4×10^7cfu/g. This report showed that ready-to-eat food samplesdid 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 foodborne poisoning More closely supervision should be made on these restaurants around the University of Maiduguri by relevant authorities, and more analysis should be carried out on other food samples sold in the University of Maiduguri environment, to ensure proper food quality and safety standards.

Keywords: Microbiological analysis, Rice, Aspergillus

INTRODUCTION

The recent status of increasing incidences of food borne illnesses is alarming; this led to an increased concern regarding food handling practices and the assessment of food safety measures taken during processing and preparation of food items. Microbiological quality of food indicates the number of microbial contaminants it has, a high level of contamination indicates low quality of food storage and its handling more likely to transmit diseases (Oranusi *et al*., 2013). Bacterial count in prepared food and water is a key factor in assessing the quality and safety of food. It also reveals the level of hygiene adopted by food handlers in the course of preparation of such foods. In a study by Scallan *et al*. (2011), approximately 48 million occurrences of food borne illness occur annually across the globe, among which 128,000 instances lead to hospitalization, resulting in 3000 deaths. Disease outbreaks that are due to infection transmitted through contaminated foods that unsurprisingly include the cooked rice also known as ready-to-eat rice (Berger *et al*., 2010; Painter *et al*., 2013).

Such incidence of food related outbreaks mostly results from improper food handling and poor practices. These mishandlings are adopted at various stages of food supply chain such as harvesting, storing, preparation,

processing, transportation, display, and serving. Furthermore, outbreaks can have detrimental effects on country's national economy, either directly or indirectly. World Health Organization (WHO) has stated that foodborne outbreaks and illnesses have seriously affected the health and economic aspects of developed and developing countries and are regarded as a constant threat (WHO, 2007). With increasing population, the affinity for dine out places has been increased among peoples of all age groups and background likewise the food consumption in restaurants recently have delete greatly increased (Soriano *et al*.,2000); his is due to the increase in demands for ready to eat food (RTE). Ready-to-eat foods can be described as foods and beverages that can be bought directly from street vendors or hawkers and are consumed at the point of sale or at later time in what is referred to as take-away without further processing. It could be raw or cooked, hot or chilled, baked or fried and can be consumed without further treatment (Tsang, 2002).

Examples of such ready-to-eat foods include pizza, cooked noodles, rice, meat pie, burger, coleslaw and fried chicken.The reasons for increase in demands for these kind of take-away foods processed in restaurants includes (but not limited to); studying, majority of staff and students on campus don't prepare food themselves or take it along with them to the campus and this led to an increase in demand for food which gives opportunity to cafeterias and canteens to serve as the major vending sites where both staff and students purchase food daily and in most cases these foods are not adequately processed, protected from flies and usually refrigeration is unavailable. Also travelling is another reason for patronizing ready to eat food from restaurants in which travelers that are away from home have no option but to buy ready to eat food. Streetfood can be a good vehicle for the transmission of foodborne microorganisms. Safety of food is highly dependent on the way food is handled. Statistics reveal that major food borne diseases have been associated with dining in restaurants (CDC, 2011). In addition, it is stated that restaurants are more likely to be the cause for food borne outbreaks as they cater for a larger number of consumers and, as a result, the chances of errors in processing food are wideranging (Jones and Angulo, 2006). Therefore, it is essential for people involved in the food handling to be aware of food safety measures and proper food handling practices (Tonder *et al*., 2007). The aim of this study is to examine the microbiological quality of rice served in university of Maiduguri restaurants with view to ascertaining possible solutions for effective preparation and handling of food by the vendors.

MATERIALS AND METHOD

Samples of ready to eat cooked rice samples were collected aseptically from restaurants in University of Maiduguri campus located at Bama road Maiduguri, Borno state. Samples were collected in sterile plastic containers. The samples were then taken to the department of Microbiology laboratory, University of Maiduguri for analysis.

Preparation of Media

The media used for bacteria isolation were Nutrient Agar, MacConkey Agar and Malt extract Agar while Sabouraud dextrose agar and Corn meal agar for fungal isolation. All media used were prepared according to the manufacturers' instructions. After preparation they were sterilized by autoclaving at 121^{O}C for 15 minutes after which they were allowed to cool and 15mlsaliquots were poured on sterile Petri dishes. About 0.1ml of suspensions (a mixture of sample and normal saline) was deposited onto the surface of the

solid media and incubated at 30°C for 24 hours for bacteria while 72 hours for fungal isolates.(Fouzia and Amir, 2011).

Isolation of Bacteria

Ten grams of each ready-to-eat sample 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 rice. The homogenized food was aseptically transferred into a sterile beaker. One ml of the homogenized food sample was aseptically transferred using a 1ml sterile pipette into a test tube containing nine ml sterile distilled water and tenfold serial dilution was carried out.

Identification of Bacterial Isolates

After incubation, bacteria were identified based on the cultural, morphological and reaction to biochemical tests which includes Catalase, Coagulase, Indole, Citrate and Methyl red(Cheesebrough, 2010).

Macroscopic Identification of Fungal Isolates

Identification was based on morphological characteristics such as color of the colonies on the surface and on the reverse side of the plates and growth characteristics. (Cappuccino and Sherman, 1996)

Microscopic Identification

Using a sterile inoculating needle, a small portion from the growing edge of each colony was picked and placed on a clean grease free slide. A drop of lacto phenol in cotton blue was added to the slide, the organism was teased out and covered with a cover slip and then viewed under the microscope using x10 and x40 objective lens (Cappuccino and Sherman, 1996).

RESULTS

Table 1 shows the results obtained after assessment of bacteria in some selected rice sample served in the University of Maiduguri Restaurants. One type of Bacteria was isolated from each rice sample in which *Staphylococcus aureus* was the bacteria isolated from the fried rice sample, *Escherichia coli* was isolated from Jollof rice and *Klebsiella pneumonia* was contained in the white rice preparation. It shows that the bacterial load in jollof rice is more than that of other two sample 5.0 x 10^7cfu/g as against 2.0x10^5cfu/g for fried rice and 2.0x 10^7cfu/g for white rice.

Table 1: Bacterial Assessment of Ready to Eat Rice Served in University of Maiduguri Restaurants

	RICE SAMPLE		
	FR	**JR**	**WR**
Morphological characteristics	Smooth, cream, opaque colonies with entire edge	Smooth, pinkish circular colonies lactose fermenters	Large, shiny, dark pinkish, lactose fermenters
Dilution factor	10^2	10^5	10^5
Number of colonies cfu/g	200	50	20
N.A(1/D. f X Nc)	1000 x 200 = 2.0x10^5	1000,000 x 50 = 5.0 x 10^7	1000,000 x 20 = 2.0x 10^7
Gram stain	+ Cocci	- Rod	- Rod
Catalase	+	-	-
Coagulase	+	-	-
Indole	-	+	-
Citrate	-	-	+
Methyl red	+	_	_
Probable organism	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumonia*

Key; FR = Fried rice, JR = Jollof rice. WR = white rice, N.A = Nutrient agar, D.f = Dilution factor, Nc = Number of colonies

It can be seen from Table 2 that the fungal load in jollof rice sample was higher than that of other two samples 6.8 x 10^7 as against 3.4 x 10^7 for white rice and 3.0 x 10^5 for fried rice.

Table 2: Mean values of fungal colony count (cfu/g) from Ready to Eat Rice.

Sample	*Aspergillus niger*	*Aspergillus flavus*	*Fusarium spp*
F. R	3.0 x 10^5	ND	ND
J. R	ND	6.8 x10^7	ND
W. R	ND	ND	3.4 x10^7

Key: N.D=Not detected, F.R= Fried Rice, W.R= White Rice and J.R= Jollof Rice

For the fungal assessment of the rice samples table 3 shows the results obtained. It shows that at least one species of fungi was isolated from each rice sample. *Aspergillus niger* was the fungal specie isolated from fried rice sample, *Aspergillus flavus* was isolated in Jollof rice sample while *Fusarium species* was identified in the white rice sample.

Table 3: Fungal Assessment of Ready to Eat Rice Served in University of Maiduguri Restaurants

	RICE SAMPLE		
	MACROSCOPIC OBSERVATION	**MICROSCOPIC OBSERVATION**	**ISOLATED ORGANISM**
FR	Cottony appearance, initially white to yellow and then turning black.	Slightly brown stipes color, smooth walled surface, glubose shape, very rough and irregular conidia surface	*Aspergillus niger*
JR	Smooth white mycelia, Which grow and produce olive and dark green conidia	Pale brown and roughened stipescolor,quietlysphericalsurface,glubo seand ellipsoidin shape, smooth and finely roughened conidia surface	*Aspergillus flavus*
WR	Colonies are white to creamcolored, moderately curve and short	2 or more celled macroconidia, thick walled, smooth and cylindrical phialides, long microconidia	*Fusarium* species

FR = Fried rice, JR = Jollof rice. WR = white rice.

The Morphological Appearances of *Aspergillusniger, Aspergillus flavus* and *Fusarium species* were show in Figure 1, 2 and 3 respectively.

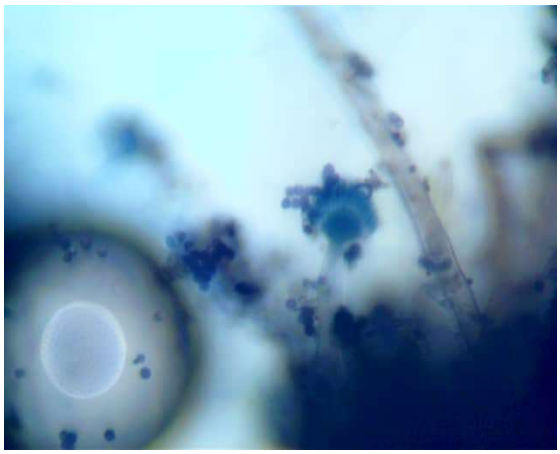

Figure 1: Microscopic view of *Aspergillus niger* from fried rice sample(using× 10, ×40 obejective lens)

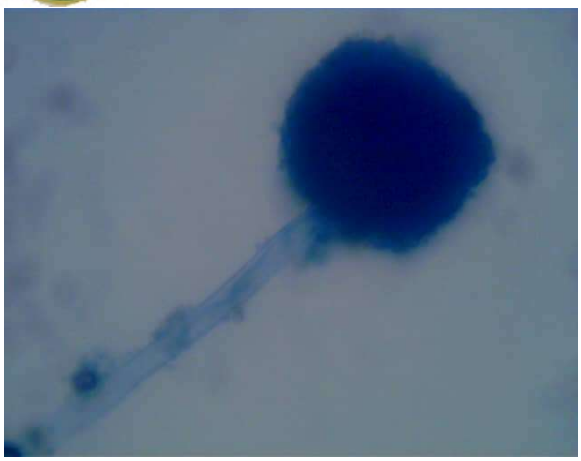

Figure 2: Microscopic view of *Aspergillus flavus* from jollof rice sample (using× 10, × 40 objective lens)

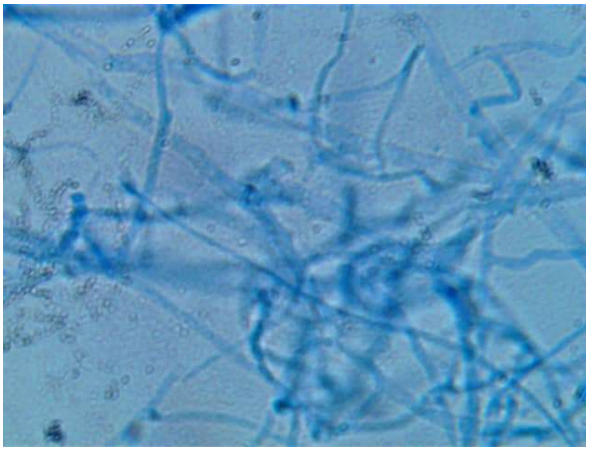

Figure 3: Microscopic view of *Fusarium* species from white rice sample (using × 10, × 40 objective lens)

DISCUSSION

The microorganisms are ubiquitous that can live in air, water, inside soil, in food and even in living organisms. Each type of organisms has its specific conditions (environmental temperature, presence/absence of oxygen, pH, moisture, etc) for thriving, when they meet their required conditions they grow and reproduce. When they grow and reproduce in food, they release toxic chemicals which can cause various ailments when consumed. Bacterial count in prepared food and water is a key factor in accessing the quality and safety of food and also reveals the level of hygiene adopted by food handlers in the course of preparation of such foods (Christopher *et al.,* 2020).These findings are in agreement with that of (Christopher *et al.,* 2020) that conducted a research on the samples of fried rice and jollof rice from two different restaurants in Niger Delta region and reported the isolation of *Escherichia coli* and *Staphylococcus aureus* in both fried rice and jollof rice from one restaurant and *Escherichia coli and Bacillus cereus* from the other restaurant also the bacterial load in the jollof rice sample was higher than that of fried rice sample. Also, Oranusi *et al.* (2013) reported higher than what bacterial content in fried and jollof rice samples which is in accord with present study.

Similarly, Yeboah *et al.* (2010) reported the detection of *Klebsiella pneumonia* in a fried rice sample with load of 1.78 x 10^4cfu/g from some selected restaurants in Ghana which is lower than the count obtained in the present study (2.0x 10^7) cfu/g for *Klebsiella pneumonia.Staphylococcus aureus* is a normal flora of the skin and nasal passage. Its presence in food may be due to human contact and this is an indication of poor hygiene of the vendors (Nichols *et al*., 1999). *E. coli* is especially of faecal origin and have been in many cases of food borne diseases (Eni, *et al*., 2010). However, their presence is an indication of possible fecal contamination of food, water or food workers and poor hygienic processing practices (Little *et al*., 1998). Similarly, for fungal assessment in each of the 3 rice samples, one fungal specie has been identified and is an indicator that, apart from bacterial contamination there was co-existence of fungal contamination in each sample.

Aspergillus niger was identified in fried rice sample, *Aspergillus flavus* in jollof rice sample and *Fusarium* species was isolated from white rice sample. Similarly, the fungal

load in jollof rice sample was higher than that of other two samples. The existence of *Aspergillus niger* and *Aspergillus flavus* could be due to the fact that they are spore formers and their heat resistant spores may have survived processing while vegetative cells were eliminated.

Tahir *et al.* (2012) reported the fungal contamination of all rice samples collected from different local markets of Lahore to be *Rhizopus* spp., *Mucor* spp., *Aspergillus* spp. and *Penicillium* spp. with the highest fungal count of $11.73x10^5$cfu/ml. Also, in this study, *Aspergillus* species were isolated from the rice samples, having *Aspergillus niger* from fried rice sample and *Aspergillus flavus* from jollof rice respectively. *Aspergillus* spp. was the most spoilage fungi isolated from most type of foods and this result resembles the study conducted by Easa (2010) when he isolated different species of *Aspergillus* from traditional fast foods. The existence of these organisms in these rice samples may be attributed to several factors which may not be limited to the initial contamination of raw materials to the handling of the finished products. The utensils used in the preparation of cooked rice and sanitary condition of processing environment especially air and dust in air may have contributed to increase in extent of contaminationsEasa (2010).

According to the standards of International Commission for Microbiological Specification for Foods (ICMSF, 1996), ready-to-eat foods with plate count between 0-10^3cfu/g is Satisfactory, between $10^4 \leq 10^5$cfu/g is borderline and 10^6cfu/g and above is unsatisfactory. Most of the microbial loads in the samples of the present study are within the unsatisfactory range which implies some degree of unhygienic practices in the preparation of these foods.

CONCLUSION

The findings from this study shows that all the samples analyzed, were not free from microbial contaminations which are not safe for consumption. Therefore, it is of utmost importance to keep cooked rice fit and safe for consumption by taking necessary preventive measures so as to avoid contamination during and after processing.

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