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Isolation and identification of microorganisms associated with Jollof rice sold at Bukateria in Obafemi Awolowo University, Ile -Ife, Osun State, Nigeria

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Abstract

This study assessed the microbiological quality of Jollof rice by isolating, identifying and characterizing the microorganisms associated with jollof rice. Six replicate samples were collected from different locations in Obafemi Awolowo University campus, Ile-Ife, Osun-State, Nigeria. Maconkey, Nutrient and Potatoes Dextrose agars were used for the isolation and determination of microbial load. Standard morphological and biochemical tests were carried out for the identification and characterization of isolates. A total of 10 bacteria and 10 fungal species were isolated. The total bacterial count ranged from 3.6×10^{3}cfu/g to 1.54×10^{5}cfu/g, while the total fungal count ranged from 1.04×10^{4}sfu/g to 3.0×10^{5}sfu/g. The organisms presumptively identified with the percentage occurrence include; *Staphylococcus saprophiticus* (20%), *Proteus vulgaris* (10%), *Bacillus subtlis* (10%), *Proteus mirabilis* (10%), *Micrococcus varians* (10%), *Bacillus licheniformis* (10%), *Bacillus cereus* (10%), *Bacillus polymyxa* (10%), *Micrococcus luteus* (10%) for bacteria isolates and *Aspergillus niger* (30%), *Aspergillus oryzae* (30%) and *Aspergillus candidus* (40%) for fungi isolates. Good personal hygiene, proper sanitation practice and the use of clean utensils during food preparation are recommended to avoid food poisoning and spoilage.

Keywords: Food Safety and Hygiene; Foodborne organisms; Contamination; Jollof Rice

1. Introduction

Food hygiene means the conditions and measures that are necessary to ensure the safety of food from production to consumption. Food safety is very critical and necessary but there are some dangers associated with it when it is overlooked [1].

Food contamination may occur during and after processing and the contamination of such food is of primary concern because such organisms may be pathogenic thereby leading to outbreak of food-borne illness. Food-borne illness occurs when food sources that may contain pathogenic microorganisms are consumed raw or improperly cooked [2].

Rice is a grain of a monocotyledonous plant *Oryza sativa* and is the most important staple food for a large part of the world, especially in Africa, South Asia, the Middle East, Latin America, and the West Indices. Rice is the grain with the second highest worldwide production, after maize [3].

Rice is one of the important source of calorie, it is a staple cereal to be consumed in large quantity throughout the world thus it is been called queen among the cereals [4]. Rice has been considered as one of the major crops after wheat and cotton throughout the world and it has been cultivated on area of 2590 hectares with a production of 5720 thousand ton [5].

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It provides half of total dietary carbohydrate, especially in Asian countries and it is a suitable diet for more than three billion people, supplying 50-80% of their daily calorie intake [6]. The genus *Oryza* is distributed in tropical and sub-tropical regions of the world.

Research has shown that the nutritional value of rice is given by its constituent in carbohydrate, sugar, fibre, energy, protein, water, iron, magnesium and zinc. Rice is used for making a lot of products and beverages because of its nutritional value, providing more than one fifth of the total calories consumed worldwide by humans [3].

Also, rice is used for product such as amazake, horchatee, rice milk and sake. Rice flousr has also been recommended for people on gluten-free diet because of the absence of gluten in the grain [7].

Safe food is a basic human right despite the fact that many foods are frequently contaminated with naturally occurring pathogenic microorganisms. These organisms cannot be detected organoleptically (seen, smelled or tested) [8].

Microbiological quality of food is an indicative of the amount of microbial contaminants. It contains a high level of contamination as an indication of low quality of food storage and its handling more likely to cause diseases [9].

The number of microorganisms in prepared food and water is a key factor in assessing the quality and safety of food and also shows the level of hygienic processes that food handlers use in preparing such foods. Food and water in particular have been described as the route for the transmission of microbial disease among is those caused by coliforms [10].

Majority of students in Obefemi Awolowo University Campus do not prepare food themselves or take it along with them to the University. This demand for food gives opportunity to the bukateria and canteens to serve as major vending sites where students can purchase food daily [11]. Therefore, this work was designed to examine the microbiological quality of Jollof rice in Obafemi Awolowo University, Ile-Ife Campus so as to determine whether this food meets the acceptable microbiological standards and specifications for foods.

2. Material and methods

2.1. Sample Collection

Jollof rice samples were collected over a period of six weeks in sterile containers and are transported to the laboratory for microbiological analysis. A total of 24 samples were collected during the research work from different locations within the campus like New-Bukateria, Awolowo Hall bukateria, Fajuyi Hall bukateria and Obafemi Awolowo University central market.

2.2. Microbiological Analysis

2.2.1. Isolation of bacterial and fungi Isolates

The samples were homogenized to allow a greater surface area; One gram was weighed and transferred into a test tube containing sterile distilled water for serial dilution. Fifteen ml (15 ml) of the prepared molten agar which was left to cool was poured aseptically into the dishes and gently swirled in clockwise and anticlockwise direction to allow for even distribution of the colonies on the surface of the agar. The agar was allowed to set. Nutrient and MacConkey agar were incubated in an inverted position at 37°C for 24 hours while the PDA plates were incubated without inverting at 25°C for 2-5 days [12].

2.3. Total Bacterial and Fungi Count

After the incubation period, different colonies were observed and plates within 30-300 colonies were counted and recorded, this was taken as the total viable count. Bacterial colonies were counted using CFU/g while fungi Isolates were counted using SFU/g [13].

2.4. Identification and Characterization

Bacterial isolates were characterized based on their colonial morphology, cellular morphology and biochemical characteristics [14]. Identification of bacterial isolates was done according to the Bergey's Manual of Determinative Bacteriology (Holt and Martinez, 1999. The fungi isolates were identified with reference to Compendium of Soil Fungi and Pictorial Atlas of Fungi [15].

2.5. Biochemical Identification of Isolates

Catalase, Oxidase, Methyl Red, Voges Proskeaur, Nitrate Reduction, Citrate Utilization, Motility spore staining, Indole, Gelatin, Casein, Starch hydrolysis, Growth at different temperature and pH and Sugar fermentation were some of the biochemical tests carried out to identify the organisms.

3. Results

The total aerobic plate count of bacterial isolates from Jollof rice sample is presented in table 1 and ranges from 3.60 x 10^4 cfu/g to 1.54 x 10^5 cfu/g. Also, the total fungi count for fungal isolates is presented in table 1 and ranges from 1.04x10^4sfu/g to 3.0x10^5 sfu/g. The colony morphology of the bacteria isolates is shown in table 2 while for fungi isolates is shown in table 3. Probable identity of fungal isolates is presented in table 3 and includes *Aspergillus niger, Aspergillus oryzae and Aspergillus candidus.*

Biochemical characteristics of bacterial isolates and probable identity of bacteria isolates are presented in table 4 and includes *Staphylococcus saptophiticus, Micrococcus luteus, Micrococcus varians, Bacillus subtilis, Proteus mirabilis, Bacillus cereus, Bacillus polymyxa, and Bacillus licheniformis*. The percentage occurrence of bacterial isolates is shown in Fig 1. *Staphylococcus saprophiticus* had the highest occurrence (20%) while, *Micrococcus varians, Bacillus subtilis, Proteus mirabilis, Bacillus cereus, Bacillus polymyxa, and Bacillus licheniformis. Micrococcus luteus* had the lowest occurrence (10% each).

The percentage occurrence for fungal isolates is shown in Fig 2. *Aspergillus candidus* had the highest occurrence (40%) while *Aspergilllus oryzae and Aspergillus niger* had the lowest occurrence (30% each).

Table 1 Total Aerobic Plate Count for Bacteria Isolates and Total Fungi Count for Fungi Isolates

Isolation	Total Aerobic Plate Count (TAPC) (cfu/g)	Total Fungi Count (TFC) (sfu/g)
First	1.20x10^4	2.56x10^4
Second	4.9x10^4	1.04x10^4
Third	2.5x10^3	1.25x10^4
Fourth	5.0x10^4	2.0x10^4
Fifth	1.3x10^4	3.0x10^5

Table 2 Colony Morphology of Bacterial Isolates

Isolate Code	Shape	Colour	Size	Elevation	Edge	Surface	Pigment
A	Irregular	Cream	Big	Flat	Entire	Wet	None
B	Circular	Cream	Small	Flat	Entire	Wet	None
N	Circular	Cream	Big	Flat	Entire	Wet	None
D	Circular	Cream	Small	Flat	Entire	Wet	None
E	Circular	Cream	Small	Flat	Entire	Wet	None
F	Circular	Cream	Big	Flat	Entire	Wet	None
G	Circular	Pink	Big	Flat	Entire	Wet	None
H	Circular	Pink	Big	Flat	Entire	Wet	None
I	Circular	Cream	Small	Flat	Entire	Wet	None
J	Rhizoid	Yellow	Big	Flat	Undulated	Wet	None

Table 3 Colony Morphology of Fungal Isolates

Isolate Code	Colour of Spores	Reverse Side of Agar	Nature of Aerial Hyphae	Pigmentation	Type of Aerial Hyphae
F1	Creamy	Creamy	Embedded	None	None
F2	Creamy	Creamy	Embedded	None	None
F3	White	Creamy	Embedded	Brown	Fluffy
E1	White	Creamy	Embedded	Black	Fluffy
E2	White	Creamy	Embedded	Brown	Fluffy
E3	Green	Creamy	Embedded	None	Velvet
LI	Black	Creamy	Embedded	None	Fluffy
L2	Creamy	Creamy	Embedded	None	Fluffy
L3	Green	Creamy	Embedded	None	Fluffy
L4	Green	Creamy	Embedded	None	Fluffy

Table 4 Biochemical Characteristics of Bacterial Isolate

	Gram reaction	Cellular	Catalase	Oxidase	Methyl red	Voges Proskauer	Indole	Nitrate Reduction	6.5% NaCl	Growth@4oC	Growth @20°C	Growth @70°C	pH 3.9	pH 9.4	Starch hydrolysis	Gelatin hydrolysis	Casein hydrolysis	Citrate utilization	Glucose	Fructose	Maltose	Mannitol	Xylose	Lactose	Sucrose	Dulcitol	Inositol	Trehalose	Raffinose	Galactose	Probable identity
A	+	C	+	-	+	-	-	+	+	-	+	-	-	+	-	-	-	+	+	+	+	-	-	+	+	-	+	+	-	+	**1**
B	+	C	+	-	+	-	-	+	+	-	+	-	-	+	-	-	-	+	+	+	+	-	-	+	+	-	+	+	-	+	**2**
N	-	R	+	-	+	+	+	-	-	-	+	-	-	+	+	-	-	-	+	+	+	-	-	-	+	-	-	+	-	+	**3**
D	+	R	+	+	-	-	-	-	+	-	+	-	+	-	+	+	+	+	+	+	-	+	-	+	+	-	+	-	-	+	**4**
E	+	R	+	+	+	+	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	-	-	-	+	-	-	-	-	-	**5**
F	+	C	+	-	-	-	-	-	-	-	+	-	-	+	-	-	-	+	+	+	+	-	+	-	+	-	-	+	+	+	**6**
G	+	R	+	+	+	-	-	+	+	-	+	-	-	+	-	+	+	-	+	+	+	-	+	+	+	-	-	+	+	+	**7**
H	+	R	+	+	+	+	-	+	+	-	+	-	+	-	-	+	+	-	+	+	+	-	-	+	+	+	-	-	-	+	**8**
I	+	R	+	+	+	+	-	-	-	-	+	-	-	-	-	+	+	+	+	-	+	-	+	+	+	-	-	-	-	+	**9**
J	+	C	+	-	+	-	-	+	+	-	+	-	-	+	-	-	-	+	+	+	-	-	-	+	+	-	-	-	+	+	**10**

KEYWORD: C = Cocci R= Rod + = Positive - = Negative; 1= *Staphylococcus saprophyticus* 2= *Staphylococcus saprophyticus* 3= *Proteus vulgaris* 4= *Bacillus subtilis* 5= *Proteus mirabilis* 6= *Micrococcus varians* 7= *Bacillus licheniformis* 8= *Bacillus cereus* 9= *Bacillus polymyxa* 10= *Micrococcus luteus*

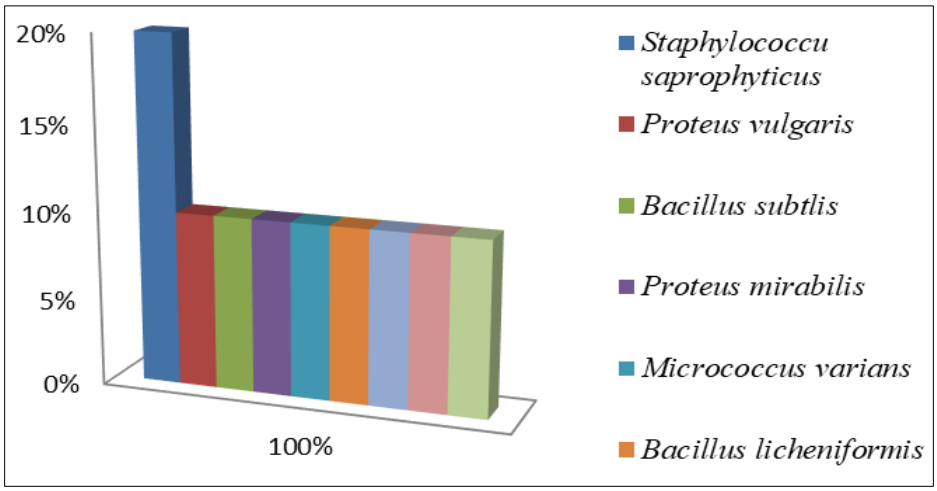

Figure 1 Percentage Occurrence of Bacterial Isolates

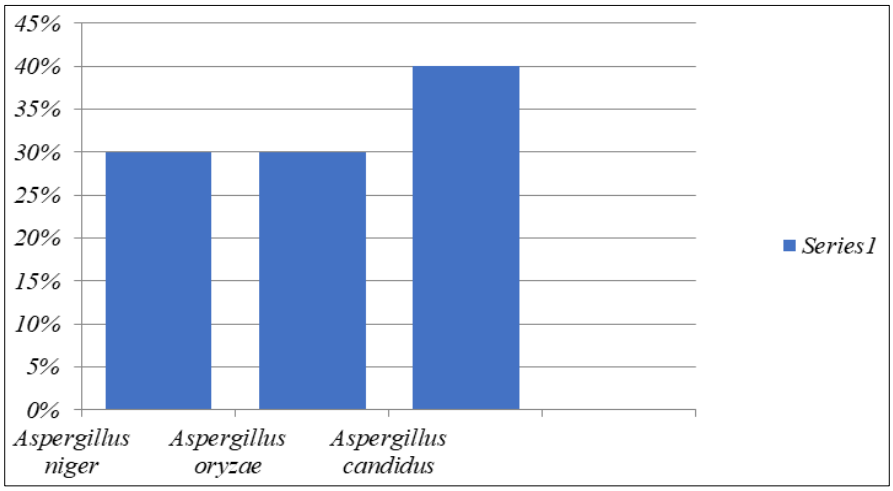

Figure 2 Percentage Occurrence of Fungi Isolates

4. Discussion

Pathogenic bacteria are the most common known causes of food contamination and food borne illness. Certain foods by vendors, e.g. white rice and Jollof rice, have been implicated in food poisoning outbreaks. Several types of microorganisms have been known to affect the quality of food, thereby constituting health hazards when foods contaminated with this organism are consumed [3].

The microbiological quality of ready-to-eat rice, e.g. Jollof rice, is said to be influenced by a number of factors such as handling, processing, storage and display may influence the microbiological load of Jollof rice at the point of sale which also have been implicated in food borne illness [16]. Studies have revealed the frequent contamination of street food in many developing world including Nigeria, India etc. [17].

In this study, the total viable count ranges from 1.28×10^{3}-1.55×10^{5}cfu/g. Earlier works by [9] also reported similar high bacterial population. The total fungal counts obtained from the Jollof rice samples ranges from 1.04×10^{4}-3.0×10^{5}sfu/g and this is also similar to the fungal counts reported by [11]. The implication of having a high microbial load in food is spoilage, high risk of contamination and food poisoning especially when the temperature of storage and pH is not within the optimum.

The International Commission for Microbiological Specification for Foods [18] states that ready-to-eat foods like Jollof rice with plate count between 0-10^{3} is acceptable, between 10^{4}-$\leq10^{5}$ is tolerable and 10^{6} and above is unacceptable.

Hence, the Jollof rice provided by bukateria in Obafemi Awolowo University, Ile-Ife campus to students is of acceptable and tolerable microbiological quality. The result presented heralded the fact that the ready-to-eat rice could be a source of diarrhoea or gastrointestinal disturbances if they exceed the acceptable limit [19].

The microorganisms isolated from Jollof rice samples analysed include ten bacterial isolates and ten fungal isolates. The bacteria isolated from Jollof rice with their occurrence are: *Staphylococcus saprophyticus* (20%), *Proteus vulgaris* (10%), *Bacillus subtlis*(10%), *Proteus mirabilis* (10%), *Micrococcus varians*(10%), *Bacillus licheniformis*(10%), *Bacillus cereus* (10%), *Bacillus polymyxa* (10%) and *Micrococcus luteus* (10%) which is similar to that reported by [3, 11]. These organisms are mainly associated with food poisoning because of their ability to produce toxins and are always implicated in foodborne illness due to ingestion of contaminated foods.

In addition, the varieties of microorganisms obtained from Jollof rice in this study may be attributed to several factors which include the primary contamination of the raw materials to the handling of the finished products. The utensils used in the preparation of Jollof rice and sanitary condition of the processing environment especially air and dust-in-air may have contributed to increase in the level of contaminants. Also, the pH, environments, temperature, prolonged storage could multiply the potential hazardous levels in the foods product which directly can be implicated in illness especially when consumed [20].

The presence of pathogens in some foods is an indicator of food safety. Although little numbers of pathogens present in food represent a low risk, their existence can suggest fault in the production and subsequent handling which, if not monitored, could lead to serious health risks and implications. *Bacillus* sp. in foods indicate the probability that some food aroma such as spices (pepper) have been included after the main cooking processing [21].

Staphylococcal species isolated from Jollof rice sample which is *Staphylococcus saprophyticus* can induce food related illness. Staphylococcal foodborne disease is a major cause of foodborne disease globally due to endotoxins pre-formed by *Staphylococcus aureus* [22].

Micrococcus sp. are mostly soil borne bacteria that can proliferate and contaminate food and water adversely and affecting the quality portability of the food. They can cause many infections like meningitis, endocarditis, septic arthistis, septic shock and bacteremia [23].

Micrococcus luteus can be linked with human skin particularly the heads, arms and legs and it can possibly find its way into the sample when poor personal hygiene is been practiced by food handlers and this is similar to the result reported by [24].

Proteus mirabilis which is isolated is usually found in the intestinal tract of humans and clinically, it is most frequent pathogen of the urinary tract. Its occurrence in the Jollof rice sample is due to unhygienic processes adopted by food handlers [25]. It is from the family Enterobacteriaecae which is a gram negative rod which can as well ferment glucose. Also, *Proteus mirabilis* shows a haemolytic activity on blood agar. It is motile with peritrichous flagella and often fimbriae [26].

Moreover, fungi isolated from the samples with their incidence rate include; *Aspergillus niger* (30%), *Aspergillus oryzae* (30%) and *Aspergillus candidus* (40%). This result was in agreement with the report by [3]. Some of the microorganisms isolated from Jollof rice have been reported to be associated with food spoilage and food poisoning. The organisms that cause food borne diseases are numerous and include bacteria, fungi, viruses and parasites.

Some fungi are the most common environmental contaminants due to their ability to produce spores and this could be related to their existence in food samples. They have been implicated in many ready to eat food like Jollof rice. *Aspergillus* sp. are known to deleterious mycotoxins under favourable conditions. So, their existence in food must be treated with caution [27, 28].

However, *Apergillus candidus* has been associated with variety of diseases such as hypersensitivity diseases, and infectious diseases including aspergillosis, otomycosis, and onychomycosis. The dry conidia produced by *Aspergillus candidus* are easily dispersed in air, leading to the inhalation by humans and animals [29].

The occurrence of *Aspergillus* sp. in the foods could be due to the fact they are spore formers. Contamination of foods could have resulted from inappropriate processing, incomplete heating, or secondary contamination via contact with contaminated equipment and utensils [9].

Finally, according to report [30], mishandling and disregard to hygienic measures on the part of the food vendors have been reported to introduce contaminants and pathogens that can survive and proliferate in sufficient numbers to cause illness in the consumers. This critical situation calls for stringent public health regulation and implementation of food sanitation practices regarding the sale of cooked foods by vendors [20].

5. Conclusion and Recommendation

From this study, it has been concluded that most Jollof rice sold in Obafemi Awolowo University Ile-Ife, Osun State and its environ are contaminated with one or more microorganism because of the unhygienic practices surrounding their processing, storage and packaging which can pose health risk to the consumers. However, if preventive measures are not taken, outbreak of foodborne disease is imminent.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare that there is no conflict of interest.

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