Abstract: Food borne illness caused by microbial contamination of foods is an important international public health problem and is known to be a major cause of diarrhea, especially in developing countries. Safety of food is a basic requirement of food quality. The study was aimed at isolating and enumerating the microorganisms from ready to eat food sold at different food outlets within Benin City. Traditional culture method was adopted for the isolation of Microorganisms via pour plate method and pure cultures were isolated by streak plate method. Identification of microorganisms were by colonial morphology, Gram staining, microscopy, and further biochemical analysis for confirmation of isolates. All the screened food samples had varying levels of bacterial and fungal growth ranging from (2.6±1.732 - 5.0±2.08 $\times 10^3$) Cfu/g for heterotrophic bacterial count, (2.0±1.00- 9.0±1.950$\times 10^2$) Cfu/g for coliform count and (5.0±3.00 - 9.0±2.081) Cfu/g for fungal count. Microorganisms isolated ranges from spoilage groups such as *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Pediococcus* species, *Pseudomonas* species, *Aspergillus niger, Candida* species, *Fusarium* species and *Rhizopus* species. The presence of these microbes suggests significant public health hazards. Therefore stringent enforcement of standard and food safety measures is advised to curtail future outbreak of food borne diseases.

Keywords: Microorganisms, Ready-to-eat foods, Coliform, Contamination, Heterotrophic, Out-break.

INTRODUCTION:

Ready to eat food (RTE) is the food intended by the producer or the manufacturer for direct human consumption with the need for cooking or other processing effective to eliminate or reduce to an acceptable level microorganisms of concern (Preston, 2004). According to Merve and Barry, (2020), these foods do not require further preparation prior to consumption except washing, thawing or moderate reheating. Consumption of these product has increased

dramatically over recent decades and this trend is expected to continue, as they are designed to fit into busy lifestyles by requiring minimal preparation time.

However, despite their advantages, ready to eat food are associated with increased concerns over their safety. Improper handling of these foods can result in foodborne illness. Safety of food is a basic requirement of food quality (WHO, 2015)

Unsafe food containing harmful viruses, bacteria, fungi, parasites or chemical substances cause more than 200 diseases ranging from diarrhea to cancers. An estimated 600 million that is almost 1 in 10 people in the world fall ill after eating contaminated food and about 420, 000 die every year. Children under 5 years of age carry 40 % of the foodborne disease burden, with 125,000 deaths every year (WHO, 2015: Dimowo and Omonigho, 2017).

Foodborne pathogens commonly associated with these foods are *Salmonella, Staphylococcus aureus* and *Escherichia coli* 0157:H7. *Listeria monocytogenes* is known as the deadliest foodborne pathogen because it causes the most food-related death (USDA, 2002)

Rice is a monocotyledonous plant (*Oryza sativa*) it is one of the most important food in the world (Boyce *et al*., 1996). After maize, rice is the grain with the second highest worldwide production. It is however one of the most important grains with regards to human nutrition and calorie intake. In Nigeria, Rice is mostly used in the improvement of the nutritional quality, increase in food security, and support sustainable land care (Chomvarin *et al.,* 2006). It has been the most prevalent food in more commercial food outlets because it can easily be prepared and can also be made into different varieties. The patronage of consumers of these commercial food outlets within Benin City has increased with time as a result of the busy schedule of parents and guardians. Due to the increase in the consumption of rice in most part of the country, there is a need to determine the safety, especially when it is been prepared and sold at commercial food outlets. In this study, the microbial assessment of ready to eat food sold in some outlet in Benin City, Edo sate, Nigeria was carried out.

Materials and Methods:

Description of Study area:

Benin City is the capital of Edo state, this state is located in southern part of Nigeria. It consists of different senatorial districts, Edo North, Edo South, Edo East, Edo West and Edo Central. The City consist of different local government area (Dimowo and Omonigho, 2017)

Collection of Samples:

The samples were randomly purchased from fast food centers within Ugbowo and Ekosodin in Egor local government, Benin City, Edo state, Nigeria. The food samples were collected and placed in a well labelled polyethylene sterile bag. The sample were immediately transported to the laboratory for microbiological analysis. These samples were used for enumeration, isolation and identification of bacteria and fungi.

Isolation and Enumeration of Bacteria and Fungi Isolates

Total aerobic bacterial and fungal counts were carried out by pour plate method on Nutrient agar and potato dextrose agar respectively. Enumeration of coliform was carried out on macConkey agar. Serial dilution was carried out by weighing 25 g of the sample (cooked rice) into a sterile conical flask containing 225ml sterile distilled water, 10 fold serial dilutions were carried out using sterile test tubes (Dimowo and Omonigho, 2017). 1 ml aliquot from the 10^{-2}, 10^{-5} and 10^{-8} dilutions were inoculated in duplicates respectively in sterile petri dishes. Sterilized medium were poured into the plates and they were incubated by inversion. The plate containing nutrient and MacConkey agar were incubated at 37 ^{0}C and that of Potato dextrose agar incubated at 28 ± 2^{0}C. Enumeration of colony forming unit was calculated using the formula according to Dimowo and Omoregie, (2023).

Pure culture Isolation

Streak plate method was used for the determination of pure culture of bacterial isolates, while the pure culture isolation of mould was by picking the spores of young mycelium using sterile wire loop needle to potato dextrose agar plates. Pure culture isolates of bacteria were stored on nutrient agar slant and refrigerated. Pure culture of moulds were later stored on PDA slants at 4 ^{0}C (Dimowo and Omonigho, 2017).

Characterization and Identification of Microorganisms:

The identification of bacterial and fungal colonies was done according to the Standard Operation Procedure. The colonies of the different culture media were examined microscopically for their characteristics appearance which includes colonies size, shape, color, consistency, pigmentation and texture of the colonies. Gram staining and Lacto phenol Cotton blue staining was done to reveal the morphology, characteristics group and arrangement of the cells of both bacteria and fungi respectively. Biochemical tests were done for the identification of the organisms which include catalase test, coagulase, indole and oxidase test (Lodder, 1970; Barnett and hunter, 1972).

Results:

The results of the microbiological analysis carried out on ready-to-eat foods (Fried rice, jollof rice and white rice) from ten different outlets are as follows:

Table l depicts, the total heterotrophic bacteria, coliform and fungal count (cfu/g) from the food samples sold at different locations around Ugbowo and Ekosodin all in Benin City. Samples collected from area I (physical science complex) had the lowest total heterotrophic bacterial count, while samples collected from area E (Buka) had the highest heterotrophic bacterial count of $5.0 \pm 2.08 \times 10^3$ cfu/g. Samples collected from area D (Osasogie) also had bacterial count of $4.9 \pm 2.081 \times 10^3$ cfu/g.

Highest coliform count was recorded from samples collected from area J (Life science complex) having a count of $9.0 \pm 1.950 \times 10^2$ cfu/g and lowest coliform count was recorded from samples collected from area G (basement) this had a count of $2.0 \pm 1.00 \times 10^2$ cfu/g. samples collected from area D (Osasogie) and G (Basement) had the same value of fungal count of 5.0 ± 3.00, while samples from area H (Ekosodin) had the highest fungal count of 9.0 ± 2.081. In all, there was more heterotrophic bacterial count compared to coliform and fungal count.

Table 2; depicts the results of the biochemical test carried out on the bacterial isolates. The isolates were *Escherichia coli*, *Bacillus cereus*, *Pseudomonas* species and *Pedicoccus* species

and *Staphylococcus aureus*. All the isolates were catalase positive except *Pediococcus* species also the isolates were all oxidase negative except *Pseudomonas* species.

Table 3; explains the morphological characteristic of the fungal isolates. The isolates were *Rhizopus* species, *Aspergillus niger*, *Fasarium* species and *Candida* species. They measured between 10 – 32 mm in diameter. *Rhizopus* species appeared white with conidia, while *Aspergillus niger* and *Fasarium* species appeared black and pink respectively.

TABLE l: Total Heterotrophic bacterial, coliform and fungal count (Cfu/g)

Sample area	Total Heterotrophic Bacterial Count Mean ± S.D	Total Coliform Count Mean ± S.D	Total Fungal Count Mean ± S.D
A	$3.6 \pm 1.00\times10^3$	$5.0 \pm 0.05\times10^2$	6.0 ± 2.081
B	$3.0 \pm 1.154\times10^3$	$4.0 \pm 1.044\times10^2$	8.0 ± 1.00
C	$3.0 \pm 1.732\times10^3$	$6.0 \pm 0.10\times10^2$	8.0 ± 1.00
D	$4.9 \pm 2.081\times10^3$	$5.0 \pm 0.50\times10^2$	5.0 ± 3.00
E	$5.0 \pm 2.08\times10^3$	$6.0 \pm 0.818\times10^2$	6.0 ± 0.50
F	$3.6 \pm 1.154\times10^3$	$5.0\pm1.755\times10^2$	7.0 ± 1.258
G	$3.6 \pm 0.577\times10^3$	$2.0 \pm 1.00\times10^2$	5.0 ± 3.00
H	$4.6 \pm 2.309\times10^3$	$3.0 \pm 0.819\times10^2$	9.0 ± 2.081
I	$2.6 \pm 1.732\times10^3$	$5.0 \pm 1.114\times10^2$	6.0 ± 0.50
J	$2.9 \pm 0.577\times10^3$	$9.0 \pm 1.950\times10^2$	7.0 ± 1.527

Key:
A ---- UBTH, B ---- Main gate C ---- Faculty of Art Science Complex D ----- Osasogie E----- Buka, F----- BDPA, G ---- Basement, H---- Ekosodin, I ---- Physical Science Complex, J ---- Life Science Complex.

TABLE 2: Biochemical test results obtained on Isolates

S/N	Catalase	Oxidase	Motile	Gram	Indole	Probable Isolates

				stain		
1	+	-	+	-	+	*Escherichia coli*
2	+	-	+	+	-	*Bacillus cereus*
3	+	+	+	-	-	*Pseudomonas* sp
4	-	-	-	+	-	*Pediococcus* sp
5	+	-	-	+	-	*Staphylococcus aureus*

Table 3: The morphological characteristics of fungal Isolates

S/N	Conidia	Elevation	Colour	Size in diameter (mm)	Probable isolates
1	+	raised	White	10	*Rhizopus* sp
2	+	raised	Black	10	*Aspergillus niger*
3	+	raised	Pink	32	*Fusarium* sp
4	-	Flat	White	15	*Candida* sp

DISCUSSION:

The food (Rice) sample obtained from ten known location in Benin City shows a predominance of bacterial and fungal contaminants. In this study, the total heterotrophic bacterial count ranged from ($2.6 \pm 1.732 – 5.0 \pm 2.08 \times 10^3$) Cfu/g which were within the satisfactory level of $\leq 10^3$ for ready to eat food as specified by Center for Food Safety (Kigigha *et al.*, 2017). Bacteria

identified include *Escherichia coli, Bacillus cereus* and *Staphylococcus aureus, Pediococcus* and *Pseudomonas* species (Wogu *et al*., 2010). *Escherichia coli, Bacillus cereus* and *Staphylococcus aureus* were the prevalent heterotrophic bacterial isolate with bacterial count similar to the previous report by Nyoyoko *et al.*,(2020). Microorganisms isolated indicates that the foods may have been exposed to different contaminants and favorable conditions (pH, temperature, water, oxygen) for the multiplication of the microorganisms (Dimowo and Omonigho, 2017). Kigigha, (2017) reported that most of the bacterial isolates that occurs in ready to eat foods consumed in Nigeria are microbes of medical importance and that the isolates usually arises from the environment and poor hygienic status of the handlers. As such needs to practice improved hygiene in food. Coliforms are commonly used to indicate sanitary quality of food and water. The presence of coliforms be as a result of using cow dung as manure and contaminated water for irrigation (Dimowo and Omonigho, 2017). In this study, *Escherichia coli* was more significant in the food samples collected in UBTH, BDPA, Life Science Complex. Basement has the lowest microbial load for coliform count ($2.0\pm1.00 \times10^2$) Cfu/g. The total coliform count from this study ranges from (2.0 ± 1.00- $9.0\pm1.950 \times10^2$) Cfu/g which met the satisfactory level of 10^2 for ready to eat food as specified by Center for Food Safety (Kigigha *et al.*, 2017). These food borne bacterial pathogens are usually responsible for food borne diseases in humans such as traveller's diarrhea (Dimowo and Omonigho, 2017).

Food borne fungal causes serious spoilage of cooked food leading to enormous economic losses. In this study, Basement and Osasogie has the lowest fungal count of (5.0 ± 3.00) Cfu/g. According to International Commission on Microbiological Specification for Food (ICMSF), the maximum limit of mould and yeast is $\leq10^3$Cfu/g. In this study, the overall fungal count is 9.0 ± 2.081which met the satisfactory level as specified by ICMSF. Mould can produce mycotoxins that are associated with several acute and chronic diseases in humans.

Carry (1996) reported that the International commission on Microbiological Specification for Foods limits for total heterotrophic bacterial and fungal counts in the order of $\leq10^3$ is regarded acceptable for ready to eat foods. This basically implies that counts beyond these specified limits are unacceptable.

CONCLUSION:

In conclusion, our results indicated that most of the ready-to-eat rice samples examined met bacteriological quality standards. In the course of our investigation, we observed that most fast food centers cooked food overnight and then store it in the refrigerator for a subsequent use. This practice may be responsible for the microbial proliferation which occurs when the bulk rice is stored overnight, waiting for its final cooking many hours later. Hence, we suggest improving the hygienic measures and the conservation practices in order to minimize the microbial contamination of food so largely consumed. Moreover, it is recommended that a more close supervision of ready-to-eat food should be carried out by appropriate authorities.

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