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Bacterial Assessment of Selected Ready-To-Eat Foods Sold at Some Restaurants within Federal Polytechnic Offa, Kwara State

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Abstract

Foodborne illness can occur following the intake of food containing high number of viable bacteria and their toxins, although highly common and of public health concern, it is a very preventable occurrence. The research aimed at isolating and identifying the microorganisms that are present in ready to eat foods sold at the Federal Polytechnic, Offa, Kwara State, Nigeria and to study their antibiotics sensitivity patterns. A total of three (3) samples of ready to eat food (White Rice, Beans and Moin moin) were collected from three different canteens within the school premises. Standard methods were used for the total aerobic bacterial counts, coliform counts, Staphylococcus count and Salmonella-Shigella counts. Identification of isolates were performed using various biochemical tests and the antimicrobial sensitivity of bacterial isolates obtained from the food samples was also performed using disc diffusion assay. Beans sample has the highest total aerobic bacteria, Staphylococcus, Salmonella and Shigella counts of $26.67 \pm 8.32 \times 10^5$ cfu/mL, $3.00 \pm 1.00 \times 10^5$ cfu/mL and $4.67 \pm 3.06 \times 10^5$ cfu/mL respectively and Moin moin sample has the highest total coliform count of $6.67 \pm 4.93 \times 10^5$ cfu/mL. The bacteria isolated from the samples were identified as *Enterococcus aerogenes*, *Proteus vulgaris*, *Citrobacter freundii*, *Escherichia coli*, *Bacillus* species, *Staphylococcus aureus*, *Micrococcus luteus* and *Salmonella enterica*. *Proteus vulgaris* produced the highest zone of inhibition of 15.00 mm with ciprofloxacin while none of the isolates produced a zone of inhibition with rifampicin. It can be concluded from this study that some of the foods sold have considerable number of bacteria which can pose a health risk to the consumers.

Keywords: Bacterial assessment, Foodborne illness, Pathogenic bacteria, Food, Antibiotics susceptibility.

INTRODUCTION

Safe food is pertinent for a healthy and quality life. Inaccessibility to safe food can lead to a number of diseases, mostly affecting people with health impairment, children and the elderly (Azounwu *et al.*, 2018). According to the World Health Organization, '550 million people become ill and 230,000 die yearly due to diarrheal diseases associated with the ingestion of foods already contaminated by microbial pathogens' (WHO, 2020). In another report by World Bank (2018), the overall reduction in productivity associated with food-borne diseases in developing countries is estimated to cost \$95.2 billion annually and the total expenditure in treating food-borne illnesses per year is estimated at \$15 billion. Food-borne diseases have been a major challenge for all nations and people of the world since time immemorial (WHO, 2020). Further, Asia and sub-Saharan Africa records the highest occurrence of food-borne diseases compared to other continents. Microbial pathogens that are food borne related and also of public health concern such as *E. coli*

O157:H7, *Salmonella*, *Vibrio*, *Shigella*, *Clostridium* specie and *Staphylococcus aureus* have been isolated from fresh-cut fruits, vegetables and ready-to-eat foods sold on the streets, markets, bukateria, cafeterias, schools, major cities and fast-food restaurants in Nigeria (Oje *et al.*, 2018; Abebe *et al.*, 2020). Nigeria, the most populous nation in sub-saharan Africa, is currently dealing with issues related to foodborne pathogens (FBP), which are typically only discussed during outbreaks. botulism outbreak connected to food consumption in Abuja was reported in January 2018 to the Nigerian Centre for Disease Control (NCDC) (Okunromade *et al.*, 2020).

Food handlers and vendors have been implicated in the contamination of foods by microorganisms.

Because of the widespread poverty in this region as well as scarcity and ignorance, many people consume any available food that satisfies their hunger or quenches their thirst (Akther *et al.*, 2021).

Ready-to-eat foods consumption is a common practice among students on campus across the globe (Azounwu *et al.*, 2018). The huge profit made from the daily sales of food by campus fast food vendors provides employment opportunities to the growing teeming population in our societies.

Most Polytechnics and Universities are frequently located far from home, and most hostels throughout the world forbid students from cooking in order to lower the chance of fire outbreak and other potential home accidents. As a result, the bulk of students rely on and frequent the ready-to-eat food outlets dispersed throughout campus for their daily needs.

It is firmly held that many students on campus are frequently more concerned with their convenience than with the quality, safety, and hygienic aspects of their food. Thus, it is of utmost importance that the standard of these ready-to-eat foods be uncompromised, considering the health implications that could be incurred (Abebe *et al.*, 2020).

There is a rising concern worldwide on the increased resistance of microbes isolated from RTE (Akther *et al.*, 2021) due to the lack of adequate treatment options, bacterial-related resistant illnesses have become a significant challenge in the management of infectious diseases in health care delivery systems. Therefore, this work is aimed at detecting the presence of some enteric pathogens from some ready-to-eat foods sold within the campus of Federal Polytechnic Offa, Kwara State.

MATERIALS AND METHODS

Collection of Samples

Three (3) samples of ready to eat foods (Rice, Beans and Moin moin) were collected from three different restaurants (labelled with location X, Y and Z respectively) within the Federal Polytechnic Offa, the samples were collected randomly; thrice within the space of one month. Generally, samples were collected between 10:00 am - 12:00 pm. The samples were collected in sterile plastic containers and transported to Microbiology laboratory of the Department of Biological Sciences for further analysis.

Isolation of Microorganisms

Each food sample was macerated using a sterile marble mortar. One (1) gram of each food sample was homogenized in sterile water and the volume of the homogenate was made up to 10 mL to obtain a 1:10 suspension. Serial dilution was then performed up to 10^{-5} dilution, 0.1mL from the 10^{-3} dilution was then transferred unto Nutrient agar plates and incubated at 37°C for

24 hours, same volume was spread plated on MacConkey agar plates and incubated for 48 hours at 37°C, Mannitol Salt Agar and *Salmonella-Shigella* Agar plates were incubated at 37°C for 24 hours.

Enumeration and Identification of Bacterial Isolates

After 24 hours of incubation, the colonies of bacteria were counted using a colony counter and the number of colonies on a plate was multiplied by the dilution factor to give a plate count per gram of the samples.

Identification of isolates were performed by subjecting pure cultures of each isolate to Gram staining and biochemical tests such as coagulase, catalase, indole, oxidase, methyl red, urease, glucose and motility test for proper identification. Macroscopic and microscopic examination of the bacterial isolates were also done (Cheesbrough, 2000).

Preparation of McFarland Standard

McFarland 0.05 % standard was prepared by introducing 0.05 ml of 1% BaCl in 9.95 ml of 1% H₂SO₄, the solution was shaken well (Cheesbrough, 2000).

Antibiotic Susceptibility Test of Isolates

A tincture of each bacteria isolate was picked with a loop from freshly sub-cultured medium and inoculated into 0.9% saline solution and mixed well by vortex mixture and the turbidity of the saline solution was compared with that of a McFarland standard. A cotton swab was then dipped into the turbid saline solution and was used to make a lawn on Mueller Hinton agar (MHA). With the aid of sterile forceps, Specific antibiotic disks namely; Pefloxacin (10µg), Gentamycin (10 µg), Ampliclox (30 µg), Zinnacef (20 µg), Amoxacillin (30 µg), Rocephin (25 µg), Ciprofloxacin (10 µg), Streptomycin (30µg), Septrin (30 µg) and Erythromycin (10 µg) were placed on the inoculated Mueller Hinton agar (MHA) media and the disks were slightly pressed on the agar plate and incubated at 37°C for 24 hours (Graham *et al.*, (1985)

RESULTS

Aerobic Bacterial Plate Count from Food Samples

The total aerobic bacteria count (TBC) obtained from the samples at location X, Y and Z ranged from $1.33 \pm 0.58 \times 10^5$ to $26.67 \pm 8.32 \times 10^5$ cfu/g (Table 1). Beans sample had the highest total aerobic bacteria count (TBC) of $26.67 \pm 8.32 \times 10^5$ cfu/g while Rice sample has the lowest TBC of $1.33 \pm 0.58 \times 10^5$ cfu/g. The total coliform count (TCC) obtained from the samples of location X, Y and Z ranged from $6.67 \pm 4.93 \times 10^5$ to $1.23 \pm 0.58 \times 10^5$ cfu/g. Moin moin

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 sample has the highest total coliform count (TCC) of $6.67 \pm 4.93 \times 10^5$ cfu/g while Rice sample has the lowest TCC of $1.23 \pm 0.58 \times 10^5$ cfu/g. The total *Staphylococcus* count (TSC) obtained from the samples from all locations ranged from $1.00 \pm 1.00 \times 10^5$ to $3.00 \pm 1.00 \times 10^5$ cfu/g. Beans sample has the highest total *Staphylococcus* count (TSC) of $3.00 \pm 1.00 \times 10^5$

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cfu/g while Rice samples have the lowest TSC of $1.00 \pm 1.00 \times 10^5$ cfu/g. The total *Salmonella-Shigella* count (TSSC) obtained from the samples from all locations ranged from $1.33 \pm 0.58 \times 10^5$ to $4.67 \pm 3.06 \times 10^5$ cfu/g. Bean samples has the highest TSSC of $4.67 \pm 3.06 \times 10^5$ cfu/g while Moin moin samples has the lowest TSSC of $1.33 \pm 0.58 \times 10^5$ cfu/g.

Table 1: Mean Total Aerobic Bacterial Counts Isolated from Food Samples

Location	Food Sample	Microbial Counts ($\times 10^5$ cfu/g)			
		TBC	TCC	TSC	TSSC
X	A	26.67±8.32 ^b	2.33±1.53 ^a	0.00±0.00 ^a	0.00±0.00 ^a
	B	5.33±3.05 ^a	1.33±0.58 ^a	0.00±0.00 ^a	0.00±0.00 ^a
	C	13.67±3.27 ^a	6.56±1.53 ^b	1.33±1.53 ^a	0.00±0.00 ^a
Y	A	3.00±1.00 ^a	6.67±4.93 ^a	0.00±0.00 ^a	4.67±3.06 ^b
	B	1.33±0.58 ^a	1.23±0.58 ^a	1.00±1.00 ^a	0.00±0.00 ^a
	C	8.00±4.00 ^b	4.33±3.21 ^a	0.00±0.00 ^a	1.33±0.58 ^a
Z	A	11.00±3.61 ^a	4.33±2.52 ^a	3.00±1.00 ^b	0.00±0.00 ^a
	B	9.00±3.00 ^a	3.67±1.15 ^a	0.00±0.00 ^a	0.00±0.00 ^a
	C	9.67±4.73 ^a	1.33±0.57 ^a	0.00±0.00 ^a	2.67±2.89 ^a
FAO STANDARD		10 ⁴ cfu/g	10 cfu/g		
FAO - Food and Agricultural Organization					

Values are means \pm SD of replicate (n = 4) of microbial count of food. Values with different superscripts on the same column are significantly different at (p <0.05).

Key: THC: - Total bacteria count, TCC: - Total coliform count, TSC: - Total *Staphylococcus* count, TSSC: - Total *Salmonella shigella* count.

A = Beans, B = White Rice, C = Moin moin

Identification of Bacteria Isolated from the Selected Food Samples

Based on cultural, staining and biochemical characteristics, the isolated bacteria from food samples from location X were found to be *Enterobacter aerogenes*, *Proteus vulgaris*, *Citrobacter freundii*, *Escherichia coli*, *Bacillus* specie, *Staphylococcus aureus* and *Micrococcus luteus*, while that of location Y

were *Bacillus* specie, *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus* and *Citrobacter freundii* and that of location Z were *Proteus vulgaris*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus* specie, *Enterobacter aerogenes*, *Citrobacter* specie and *Salmonella enterica* as presented in [Tables 2 and Figure 1](#).

Table 2: Biochemical Characterization of Bacteria Isolated from Samples at Location X, Y and Z

Location/biochemical tests	Catalase	Oxidase	Indole	Motility	Methyl Red	Urease	Coagulase	Glucose	Probable Organism
X, Z	+	-	-	+	-	-	-	+	<i>Enterobacter aerogenes</i>
X, Y, Z	+	+	-	+	-	-	-	+	<i>Bacillus sp</i>
X, Z	+	-	-	+	+	+	+	-	<i>Proteus vulgaris</i>
X, Z	+	-	-	+	+	+	-	+	<i>Citrobacter freundii</i>
X, Y	+	-	+	+	-	-	-	+	<i>Escherichia coli</i>
X, Y, Z	+	-	-	-	+	+	+	+	<i>Staphylococcus aureus</i>
X, Y	+	-	-	+	+	-	-	+	<i>Micrococcus luteus</i>
Y,Z	+	-	-	+	+	-	-	+	<i>Salmonella enterica</i>
Z	+	-	-	+	+	+	-	+	<i>Citrobacter sp</i>

Keys: + = Positive, - = Negative

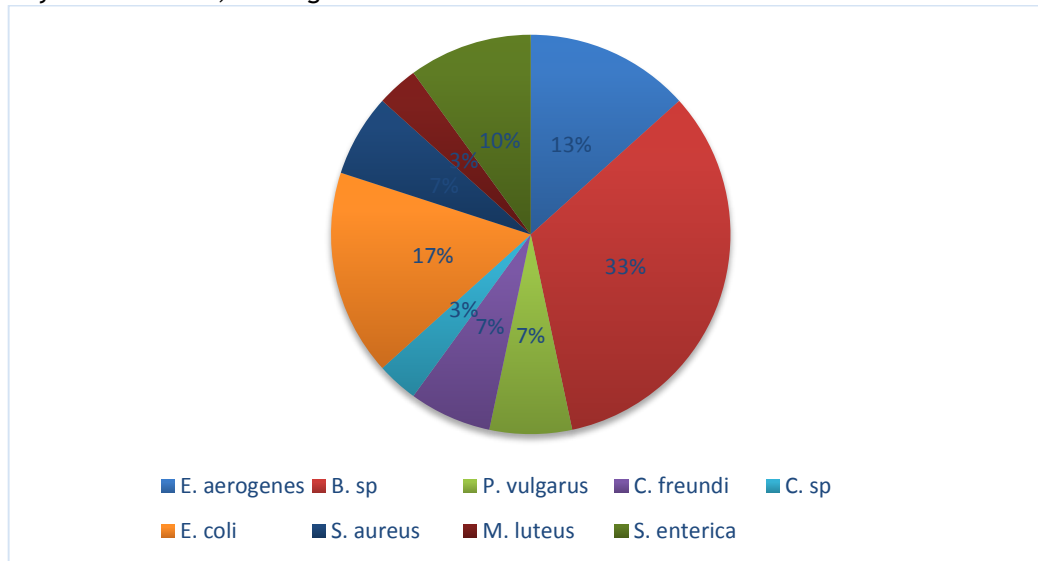


Figure 1: Frequency of Occurrence of Bacteria Isolated from the Food Samples

Antibiotic Susceptibility Test on Bacteria Isolated from Food Samples at Location X, Y and Z

The results of antibiotic susceptibility test on isolated bacteria from the location X, Y and Z is shown in Table 3. Most of the isolates demonstrated varying degree of susceptibility and resistance to the tested antibiotics. Pefloxacin exhibited antibacterial activity against *Escherichia coli*, *Enterobacter aerogenes* and *Salmonella enterica* with zone of inhibition of 5.00 mm, 4.00 mm and 7.00 mm respectively while *Proteus vulgaris*, *Staphylococcus aureus*, *Bacillus* specie and *Citrobacter freundii* are resistant to pefloxacin. Gentamycin demonstrated strong antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus* specie, *Enterobacter aerogenes*, *Citrobacter freundii*, *Salmonella enterica* and *Micrococcus luteus* with zone of inhibition of ranging between 3.00 - 6.00 mm, while *Proteus vulgaris* was resistant to gentamycin.

Ampiclox exhibited antibacterial activity against the isolates with zones of inhibition ranging from 4-00 - 11.00 mm except *Citrobacter freundii* which was resistant to ampiclox. *Proteus vulgaris*, *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter aerogenes*, *Salmonella enterica*, *Citrobacter freundii* and *M. luteus* were resistant to zinnacef. Ciprofloxacin demonstrated antibacterial activity against *Proteus vulgaris*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica*, *Bacillus* specie, *Citrobacter freundii* and *Micrococcus luteus* with zone of inhibition ranging from 4.00 - 15.00 mm. *Proteus vulgaris*, *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter aerogenes*, *Salmonella enterica*, *Citrobacter freundii* and *Micrococcus luteus* were resistant to rifampicin, streptomycin, trimethoprim-sulfamethoxazole and erythromycin as shown in Table 3.

Table 3: Antibiotic Sensitivity Test on Bacteria Isolated from Samples at Location X, Y and Z

Bacterial Isolates	Antibiotics/ Zone of Inhibition(mm)									
	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E
<i>Proteus vulgaris</i>	-	-	11.00	-	-	-	15.00	-	-	-
<i>Escherichia coli</i>	5.00	3.00	8.00	-	4.00	-	12.00	-	-	-
<i>Staphylococcus aureus</i>	-	4.00	6.00	-	7.00	-	6.00	-	-	4.00
<i>Bacillus</i> specie	-	7.00	4.00	2.00	4.00	-	8.00	2.00	-	-
<i>Enterobacter aerogenes</i>	4.00	6.00	6.00	-	6.00	-	-	-	6.00	-
<i>Bacillus</i> specie	-	-	4.00	-	2.00	-	-	-	-	-
<i>Citrobacter freundii</i>	-	4.00	-	-	4.00	-	4.00	-	2.00	-
<i>Salmonella enterica</i>	7.00	3.00	4.00	-	-	-	4.00	-	-	-
<i>Micrococcus luteus</i>	-	4.00	6.00	-	-	-	7.00	-	-	-

Keys: PEF: Pefloxacin (10µg), CN: Gentamycin (10µg), APX: Ampiclox (30µg), Z: Zinnacef (20µg), AM: Amoxicillin (30µg), R: Rocephin (25µg), CPX: Ciprofloxacin, (10µg) S: Streptomycin (30µg), SXT: Trimethoprim-sulfamethoxazole (30µg), E: Erythromycin (10µg) Location X, Y, Z are the 3 canteens where the samples were obtained. Zones of inhibition ≤ 15 : Resistant

DISCUSSION

Ready-to-eat

food may be contaminated for a number of reasons. According to Azounwu *et al.* (2018), pathogenic bacteria, environmental pollutants, and a disdain for appropriate manufacturing and hygiene practises all contribute to the persisting relationship between food and diseases. Furthermore, Peters *et al.* (2017) noted that food sellers frequently have low levels of education, are not licenced, are not trained in food hygiene procedures, work in squalid surroundings, and have little to no awareness of the causes of food-borne illnesses.

The population of bacteria in food at any particular time depends on how it was handled, stored, the temperature it was kept and how long it was kept (Peters *et al.*, 2017).

The total bacterial counts obtained for all three samples from location X, Y and Z. The study revealed that the total aerobic bacteria count were within unsatisfactory limits according to the Food and Agriculture Organization (FAO, 2000) of the United Nations specification (1×10^4 cfu/g and coliform bacteria must not be more than 10) but zero colony forming unit per gram for *Escherichia coli* and *Staphylococcus* that should not be present in foods for it to be safe for consumption, which is not the case for the total counts obtained in this study as some of the foods sampled harbored large amount of these microorganisms. This result is similar to the work of Lambu *et al.* (2022), who reported high bacterial counts of 7.6×10^4 cfu/mL for the Rice and stew sample and 1.7×10^3 cfu/mL in the jollof Rice sample for some of the foods sampled at Kano University of Science and Technology, Wudil Campus.

The isolated bacteria from the food samples comprise mainly members from the family Enterobacteriaceae known to be associated with food borne illness. This agrees with the findings of Mengistu *et al.* (2022) who isolated *Escherichia coli*, *Staphylococcus aureus*, *Bacillus* specie, *Micrococcus luteus*, *Salmonella enterica* and *Citrobacter freundii*, from jollof Rice, Beans and Moin moin samples in Kumasi Ghana and with the work of Amadi *et al.* (2018) who isolated *Bacillus cereus*, *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus aureus* and *Citrobacter freundii* from the different Rice dishes analyzed. This is also in line with the work of Peters *et al.* (2017) whose study revealed the presence of *Staphylococcus aureus*, *Bacillus*

cereus, *Escherichia coli*, *Bacillus subtilis* and *Salmonella enterica* in ready-to-eat food (Moin moin, Rice and Beans).

The results of antibiotic susceptibility test on isolated bacteria from location X, Y and Z. According to standards by CSLI (2015) based on the zones of inhibition, all the bacteria isolates showed resistance to the various antibiotics analyzed. There was high resistance for rifampicin by the microorganism and all the Gram-positive bacteria also displayed resistance to ciprofloxacin, streptomycin, erythromycin and zinnacef amongst others.

Resistance of *Enterobacter* species to erythromycin, zinnacef and rifampicin has previously been reported by Eromo *et al.* (2016), this may be mediated by the production of β -Lactamses (Akther *et al.*, 2021). Also, as *E. coli* is known to produce Extended Spectrum β -Lactamses; these enzymes inactivate the potencies of antibiotics, which explains its exceptional insensitivity to classes of antibiotics as seen in this study. Eromo *et al.* (2016), reported that *E. coli* isolated from ready to eat food showed resistance to a number of antibiotics. The resistance to zinnacef, rifampicin, streptomycin, septrin and erythromycin recorded in this study disagrees with findings of Oje *et al.* (2018) who reported the sensitivity of *E. coli* to rifampicin, zinnacef and erythromycin respectively. Findings from this study agrees with some of the findings of Peters *et al.* (2017) but disagrees with the 100% susceptibility to amoxacillin by the isolated bacteria reported by Manasa and Thomas (2022). The multiple antibiotic-resistant ability of the bacterial isolates is of great concern to public health (Igbinsosa *et al.*, 2020), therefore food safety for ready-to-eat foods should be prioritized.

CONCLUSION

It can be concluded that the sampled ready-to-eat foods had some bacterial pathogens whose presence in the food samples pose a serious public health hazard to students and staff as these microorganisms have been implicated in food borne illnesses and diarrheal diseases. The bacterial counts were also above the limits stipulated by food agencies. It is also worthy of note that all the bacteria isolated from these foods were resistant to the antibiotics used. The importance of food safety and hygienic practices should therefore be emphasized to food vendors by relevant agencies.

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