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Assessment of the Microbial Quality of Food Sold in Some Restaurants within Katsina Metropolis

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Abstract

Foods are substances that when consumed provide the body with nutrients for growth and development. Foods for consumption should be within the acceptable limit of microbial load, exceeding it may lead to food-borne intoxication and illness. This study aimed to determine the microbial quality of commonly sold foods in some restaurants within Katsina metropolis. Physiochemical properties of the food namely; pH, temperature, colour, and taste were determined using a pH meter, thermometer, and organoleptic approach respectively. Conventional methods of identification were used to identify the isolates and a modified method by Kirby Bauer was employed to determine antimicrobial assays of the identified isolates. The results obtained showed that the physiochemical parameters of pH and temperature range between 5.50 to 7.40, and 30.0 ^{o}C to 32.0 ^{o}C respectively with a normal taste and colour. Total bacterial (1.04 x10^{7} CFU/g) and fungal (4.8 x10^{6} CFU/g) counts of the samples fall within the unsatisfactory limits set by the International Commission on Microbiological Specifications for Food (ICMSF). A total of nine (9) bacteria and five (5) fungi were identified from fifteen (15) samples examined. E. coli (26.31%) was most prevalent followed by Enterobacter aerogens and Staphylococcus epidermis (15.78%), Bacillus subtilis, and Salmonella spp (10.52%) while Proteus mirabilis, Micrococcus luteus, Pseudomonas aeroginosa, and Clostridium spp. have the least occurrence (5.26%). Aspergillus flavus (45.45%) was most prevalent among the fungal isolates followed by Mucor (27.27%) and Botrytis, Alternaria, and Fusarium chlamydosporum (9.09%) have the least occurrence. The antimicrobial assay shows that Gentamycin, levofloxacin, Erythromycin, and Ciprofloxacin were effective against Micrococcus luteus, Staphylococcus spp., Clostridium spp., and Bacillus subtilis. Norfloxacin and Amoxicillin were resisted by Micrococcus luteus and Staphylococcus spp., Ketoconazole and Fluconazole inhibited Mucor and Aspergillus flavus but were resisted by Alternaria, Botrytis, and Fusarium chlamydosporum. It was concluded that foods sold in some restaurants within the Katsina metropolis fall within the unsatisfactory limits of ICMSF standards, thus, improved strict hygiene compliance is recommended.

Keywords: Antibiotic, Assay, Satisfactory, Specification, ICMSF.

INTRODUCTION

Food is any substance that is edible and free from contaminants or dangerous substances, regarded as safe and hygienic originating from plants and animals (David *et al.*, 2012). Contamination of food with pathogenic microorganisms or their toxins as well as other environmental contaminants makes it a vehicle of disease transmission (WHO, 2022). Poor hygiene in handling and processing is the main cause of microbial food contamination. Several authorities (Regulatory bodies like NAFDAC) are responsible for the provision of standards for food producers to ensure the production of foods free of pathogenic and opportunistic microorganisms, thus, providing limits above which food is considered unsafe for human consumption. Foods are commonly associated with microorganisms, which can produce toxins or cause food poisoning (Akpoka *et al.*, 2019). Microbial quality is necessary to ensure a safe product for consumers, but total prevention is nearly impossible. Food safety issues are of major importance to world health (Sabuj *et al.*, 2020), with reported cases of 1 death out of 10 people making a total of 420, 000 deaths yearly due to contaminated food (WHO, 2022).

Microorganisms use food as a source of nutrients and are affected by various factors, such as nutrient content, hydrogen ion concentration (pH), temperature, relative humidity, and water activity. These factors are characterized as intrinsic and extrinsic. Intrinsic factors affect the number and types of Microorganisms that

colonize food (Rolfe and Daryaei, 2020). pH is an indicator of the amount of acid or base present in a food (Varghese *et al.*, 2015), and can be measured using a pH meter. The pH range of growth for moulds is 1.5 to 9.0 (Moral *et al.*, 2017), and microorganisms can be grouped into three groups: Neutrophiles, Acidophiles, and Alkaliphiles. Water activity is the measure of the amount of water available for microbial growth, and can be reduced by drying and other methods of preservation (Moral *et al.*, 2017 & Rolfe and Daryaei 2020). The environment can affect both the microorganisms and the food itself during processing and storage, such as temperature, relative humidity, and oxygen (Rolfe and Daryaei, 2020). Microorganisms can be affected by temperature and can be grouped as psychrotrophs, mesophiles, and thermophiles (Magar, 2022). Relative humidity is a measure of water activity in gaseous form, and dry conditions are considered better for food storage than moist conditions (Nester *et al.*, 2004). Microorganisms differ in their requirement for oxygen, which can lead to spoilage of food stored in the presence of oxygen (Moral *et al.*, 2017). Food contamination is the presence of contaminants such as toxins, microorganisms, heavy metals, and environmental contaminants (Canadian Institute of Food Safety, C.I.F.S., 2022). Thus, this study aimed to assess the microbiological quality of ready-to-eat food sold within Katsina metropolis.

MATERIALS AND METHODS

Sampling Area and Sample Collection

Random sampling was done within Katsina metropolis and five major restaurants were chosen and coded (R1, R2, R3, R4 and R5). Three varieties of Ready-to-eat (RTE) food (Jollof rice (JR), fried rice (FR) and Tuwo with vegetable soup (TVS)) were collected from each of the five restaurants in takeaway packs provided by the restaurants and were transported aseptically to Umaru Musa Yar'adua University microbiology laboratory for analyses.

Physiochemical Analysis of food samples

The physicochemical properties including; Temperature, pH, colour, taste and texture of the food samples were analysed following the methodology of Varghese *et al.* (2015).

Hydrogen ion concentration (pH) was measured using a pH meter, 50g of food sample was measured and placed into a beaker, the pH electrode was dipped into buffer solution for 1 minute, it was removed and dipped in the food sample, and the meter was allowed to settle and the result was taken and recorded. The temperature of the food samples was taken using a Mercury thermometer, the thermometer was wiped with cotton wool soaked in alcohol, 50g of the food was weighed and placed in a beaker and the thermometer was dipped into the food sample, it was allowed to stand for 1 minute and the reading was taken and recorded. Organoleptic properties were determined using senses of taste, sight and touch.

Sample Preparation

Ten (10g) of each sample of the food was homogenized with sterile distilled water using sterile mortar and pestle as the stock solution

Mean Bacterial and Fungal Count

The prepared food samples (Stock solution) were serially diluted to 10^{-5} dilution fold by transferring 1mL of the stock sample into a test tube containing 9mL of distilled water as the second dilution and the process were repeated up to the fifth dilution aseptically. The samples were inoculated in a culture media (Nutrient agar and Potato dextrose agar) which were prepared according to the manufacturer's instructions by pour plate method, NA media plates were incubated at 37°C for 24 hrs while PDA was incubated for 5 days at room temperature. Colonies of bacteria that developed on NA after incubation were counted using colony counters, the colony forming unit per gram of food was calculated and the colony morphology was observed and recorded. The colonies appeared as a mixed culture and were further sub-cultured. Pure culture was obtained and used for subsequent tests and identification. Fungal colonies that developed after 5 days of incubation at room temperature were observed and counted. The colony-forming unit was calculated per gram of food and the colony morphology was observed and recorded. The colonies were sub-cultured and a pure culture was obtained and used for subsequent tests.

Identification of Isolates

Following the incubation period of each isolate, pure cultures obtained for bacteria were observed microscopically and macroscopically, and biochemical tests including catalase, oxidase, citrate, indole, urease, Voges-Prauskeur test (VP) and Methyl-red test (MR) were carried out in accordance to the procedure used by Varghese and Joy (2014) to confirm the isolates. Pure cultures obtained for fungi were compared to the standard Atlas reported by Lina (2013) and identified using their macroscopic and microscopic structures.

Standardization of Test Organisms

The test organisms were standardized by preparing 0.5 McFarland standard, 0.05mL of 1% of Barium chloride ($BaCl_2$) and 9.95mL of 1% sulphuric acid (H_2SO_4) solutions were mixed, an overnight growth of the test organisms were inoculated into a test tube containing 10mL of normal saline until its turbidity matched that of the prepared 0.5 McFarland standard (Murray *et al.*, 2016).

Antimicrobial susceptibility testing (AST)
The modified methods of Kirby Bauer (disc diffusion) were used for AST. In this method, Mueller Hinton agar was prepared according to the manufacturer's instruction, poured into a petri dish and left to solidify, 0.5mL of bacterial suspension was poured onto the surface of the prepared medium and was spread using a sterilized L-shape glass rod, sensitivity disc was placed onto the inoculated medium of both gram-positive and gram-negative plates respectively, the plates were inverted and incubated at 37°C for 24hrs. The zone of inhibition which shows the effectiveness of the antibiotic against the organism was measured using a measuring ruler by placing it horizontally and vertically across the cleared area close to the antibiotic disc, the values obtained were added and divided by 2 for the average value.

RESULTS

Physiochemical Properties of the Food Samples
The physicochemical properties of the food samples analysed fall within the acceptable limit set by ICMSF. pH ranges from 6.10-6.29, 5.90-7.40, 5.50-7.30 for Jollof rice (JR), Fried rice (FR) and Tuwo with vegetable soup (TVS) respectively and a temperature range of 30-32.5^{0}C across all food samples analysed. The taste, texture and colour of the food samples fall within acceptable limits Table 1.

Table 1: Physiochemical Properties of the Food Samples

Sample location	Sample ID	pH	Temperature (^{0}C)
R1	JR	6.10	30.0
	FR	5.90	32.0
	TVS	5.70	32.0
R2	JR	6.90	32.0
	FR	7.15	31.0
	TVS	7.30	32.5
R3	JR	6.20	32.0
	FR	6.00	32.0
	TVS	5.80	31.5
R4	JR	6.50	31.0
	FR	7.40	32.0
	TVS	5.50	31.0
R5	JR	6.30	30.0
	FR	5.90	30.0
	TVS	7.20	32.0

Key: R1-R5 =Restaurant one to five, JR= Jollof rice, FR= Fried rice, TVS= Tuwo with Vegetable soup.

Aerobic Mesophilic Bacterial Plate Count
The results in Table 2 showed that samples of food from restaurant one (R1) harbour the highest bacterial count of 1.04 x10^7, 9.3x10^6 and 8.1x10^6 for JR, FR and TVS respectively while R2 shows the least mean bacterial count of 2 x10^5 and 7x10^5 for FR and TVS respectively which are all below the acceptable limits set by ICMSF. Sample of JR from R2 is the only sample within the acceptable limits with bacterial load too few to count.

Table 2: Mean Aerobic Mesophilic Bacteria (CFU/mL)

S/n	Collection point	JR	FR	TVS
1	R1	1.04 x10^7	9.3 x10^6	8.1x10^6
2	R2	TFTC	2.0 x10^5	7.0 x10^5
3	R3	1.5 x10^6	6.0 x10^5	8.0 x10^5
4	R4	2.9 x10^6	3.2 x10^6	4.0 x10^6
5	R5	2.5x10^6	1.5 x10^6	2.9 x10^6

Key: R1-R5 =Restaurant one to five, JR= Jollof rice, FR= Fried rice, TVS= Tuwo with Vegetable soup, TFTC= Too Few to Count. (ICMSF Acceptable limits: 10^3 - satisfactory, <10^3 to ≤10^5 - borderline and ≥10^5 - unsatisfactory)

Aerobic Yeast and Mold Plate Count

The result of Yeast and Mold Plate Count showed that samples of food (JR, FR and TVS) from restaurant one (R1) have the highest fungal count of 4.8 x10^6, 4.0 x10^6 and 4.4 x10^6 for JR, FR and TVS respectively while (R2) showed the least of mean yeast and mould count in JR (1.0 x10^5) and TVS as TFTC. R3 shows the least in FR with 1.7 x 10^6 as shown in Table 3 below.

Table 3: Mean Yeast and Mold Plate Count

S/n	Collection point	JR	FR	TVS
1	R1	4.8x10^6	4.0x10^6	4.4x10^6
2	R2	1.0x10^5	3.4x10^6	TFTC
3	R3	2.7x10^6	1.7x10^6	3.6x10^6
4	R4	3.5x10^6	3.3x10^6	1.4x10^6
5	R5	2.5x10^6	2.6x10^6	3.2x10^6

Key: R1-R5 =Restaurant one to five, JR= Jollof rice, FR= fried rice, TVS= Tuwo with Vegetable soup, TFTC= Too Few to Count. (ICMSF Acceptable limits: 10^3 as satisfactory, <10^3 to ≤10^5 as borderline and ≥10^5 as unsatisfactory)

Identification of bacterial Isolates

Bacterial Isolates were identified using morphological characteristics of both macroscopic and microscopic appearance on culture medium and under the microscope using the gram staining technique. The isolates were further confirmed using biochemical reactions; catalase, oxidase, citrate, methyl red, Voges-Proskauer, indole and urease as shown in Table 4 below.

Table 4: Biochemical Tests and Gram Reaction of the Bacterial Isolates

S/N	Gram Reaction	Catalase	Oxidase	Citrate	Mr	Vp	Indole	Urease	Suspected Microbes
1	-	+	-	+	+	-	-	+	*Proteus mirabilis*
2	+	+	+	+	-	-	-	-	*Micrococcus luteus*
3	-	+	-	+	-	+	-	-	*Enterobacter aerogens*
4	-	+	+	+	-	-	-	+	*Pseudomonas aeruginosa*
5	-	+	-	-	+	-	-	-	*Salmonella spp*
6	+	-	-	+	-	-	-	-	*Clostridium spp.*
7	+	+	-	-	-	+	-	+	*Staphylococcus epidermidis*
8	-	+	-	-	+	-	+	-	*Escherichia coli*
9	+	+	+	+	-	+	-	-	*Bacillus subtilis*

Key: MR= Methyl Red, VP= Voges-Prauskeur + = Positive, - = Negative

Frequency of Occurrence of Bacteria in the Food Samples

A total of 19 bacteria were isolated from the three samples of food analysed across five restaurants, *Escherichia coli* has the highest occurrence of 5 (26.31%) and *Proteus mirabilis*, *Micrococcus luteus*, *Pseudomonas aeruginosa* and *Clostridium* specie had the least occurrence of 1 (5.26%) each as shown in Table 5 below.

Table 5: Percentage Occurrence of Isolated Bacteria

Bacteria isolated	Frequency of Occurrence (%)
Proteus mirabilis	1 (5.26)
Micrococcus luteus	1 (5.26)
Enterobacter aerogens	3 (15.78)
Pseudomonas aeroginosa	1 (5.26)
Salmonella spp	2 (10.52)
Clostridium spp	1 (5.26)
Staphylococcus epidermidis	3 (15.78)
Escherichia coli	5 (26.31)
Bacillus subtilis	2 (5.26)
	19 (100)

Frequency of Occurrence of Fungi in the Food Samples

A total of 11 fungi were isolated across five restaurants, *Aspergillus flavus* has the highest occurrence of 5 (45.45%) while *Alternaria*, *Botrytis* and *Fusarium chlamydosporum* had the lowest occurrence of 1 (9.09) as shown in Table 6 below.

Table 6: Percentage Occurrence of Isolated Fungi

Organisms	Frequency of Occurrence (%)
Aspergillus flavus	5 (45.45)
Alternaria	1 (9.09)
Botrytis	1 (9.09)
Mucor	3 (27.27)
Fusarium chlamydosporum	1 (9.09)
	11(100)

Antibacterial Susceptibility Pattern of the Isolates

Tables 7 and 8 below show the sensitivity pattern of gram-positive and negative bacteria respectively. The result below shows levofloxacin, chloramphenicol, and ciprofloxacin to be the most effective inhibitors of microbial growth whereas amoxicillin and norfloxacin were the least effective, *Staphylococcus* spp and *Proteus mirabilis* were the most inhibited organism by the antibiotics used.

Table 7: Mean Antibiotic Susceptibility Pattern of Gram-Positive Bacterial Isolates

S/n	Antibiotics	Disc content(*ug*)	*Micrococcus luteus*	*Staphylococcus epidermidis*	*Clostridium perfringes*	*Bacillus subtilis*
			Zone of inhibition in Diameter (mm)			
1	Gentamycin (CN)	10	18 (S)	18 (S)	20 (S)	16 (S)
2	Levofloxacin (LEV)	20	20 (S)	20 (S)	20 (S)	19 (S)
3	Erythromycin (E)	30	17 (S)	20 (S)	20 (S)	20 (S)
4	Ciprofloxacin (CPX)	10	20 (S)	20 (S)	19 (S)	20 (S)
5	Chloramphenicol(CH)	30	14 (I)	17 (S)	18 (S)	13 (I)
6	Septrin (S)	30	5 (R)	13 (I)	20 (S)	16 (S)
7	Ripamficin (RD)	20	15 (I)	9 (R)	20 (S)	15 (I)
8	Norfloxacin (NB)	10	5 (R)	5 (R)	18 (S)	14 (I)
9	Ampiclox (APX)	20	15 (I)	7 (R)	20 (S)	18 (S)
10	Amoxicillin (AMX)	20	7 (R)	7 (R)	20 (S)	14 (I)

Key: 16-20mm and above as sensitive (S), 10-15mm as intermediate (I) and 0-9 mm as resistance (R)

Table 8: Mean Antibiotic Susceptibility Pattern of Gram-Negative Bacterial Isolates

S/n	Antibiotic	Disc content(*ug*)	*Proteus mirabilis*	*Enterobacter aerogens*	*Pseudomonas aeruginosa*	*Salmonella* spp	*E.coli*
			Zone of inhibition in Diameter (mm)				
1	Sparfloxacin (SP)	10	18 (S)	17 (S)	14 (I)	15 (I)	18 (S)
2	Ciprofloxacin(CPX)	10	16 (S)	20 (S)	20 (S)	18 (S)	20 (S)
3	Amoxicillin(AMX)	30	8 (R)	16 (S)	12 (I)	8 (R)	19 (S)
4	Augmentin (AU)	30	6 (R)	18 (S)	15 (I)	10 (I)	19 (S)
5	Gentamycin (CN)	10	16 (S)	20 (S)	18 (S)	20 (S)	16 (S)
6	Pefloxacin (PEF)	30	18 (S)	20 (S)	16 (S)	15 (I)	17 (S)
7	Tarivid (OFX)	10	15 (I)	20 (S)	20 (S)	20 (S)	20 (S)
8	Streptomycin (S)	30	8 (R)	20 (S)	14 (I)	17 (S)	16 (S)
9	Septrin(SXT)	30	19 (S)	20 (S)	17 (S)	14 (I)	20 (S)
10	Chloramphenicol(CH)	30	8 (R)	20 (S)	12 (I)	16 (S)	19 (S)

Key: 16-20 mm and above as sensitive (S), 10-15mm as intermediate (I) and 0-9 mm as resistance (R).

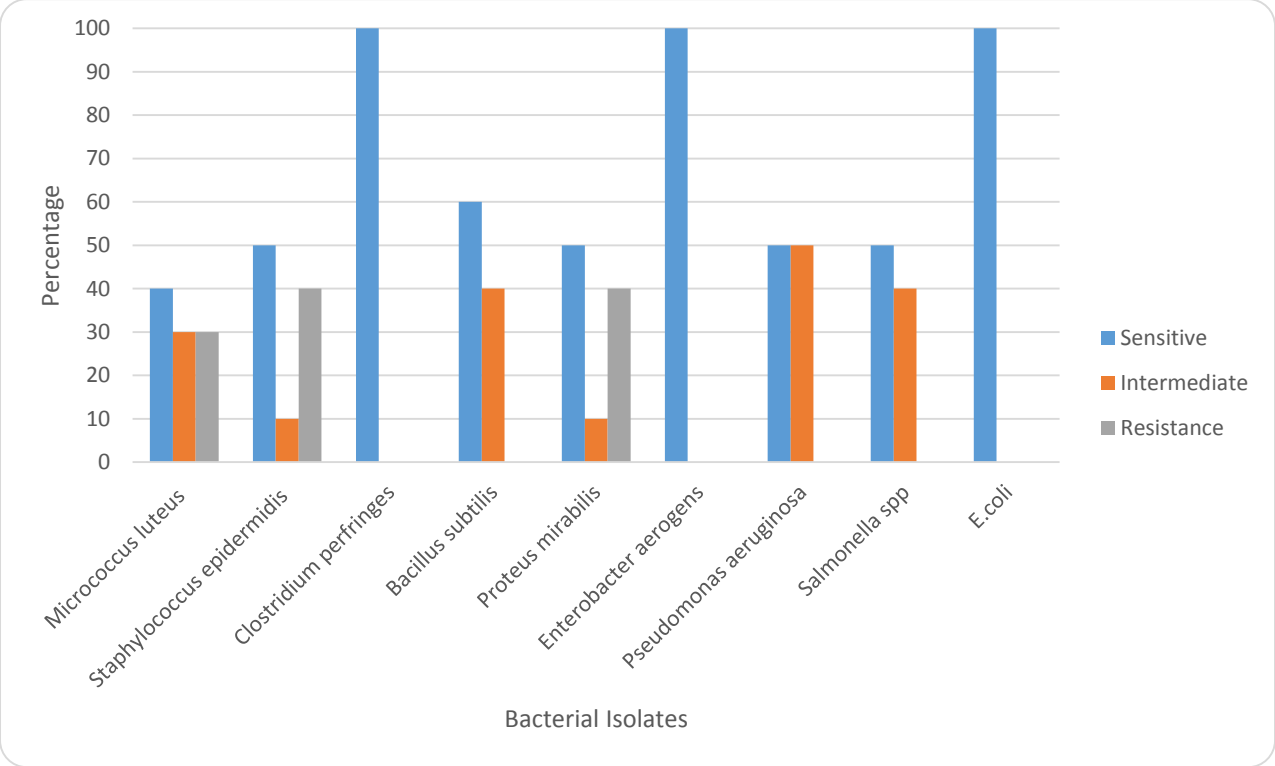

Figure 1: Percentage Susceptibility Pattern of the Bacterial Isolates to Antibiotics

Antifungal Susceptibility Pattern of Fungal Isolates

Table 9 below shows the sensitivity pattern of yeast and mould (fungi) against ketoconazole and fluconazole drugs, it shows the ability of the antifungal drugs to inhibit or stop the growth of fungi. Results were interpreted as susceptible (S), intermediate (I), or resistant (R) ranging from 16 to 20mm, 10 to 15mm and 0 to 9mm respectively, fluconazole was found to be the most effective in inhibiting *Mucor* spp, *Botrytis* and *Aspergillus flavus* than ketoconazole while *Alternaria* and *Fusarium chlamydosporum* where found to resist the antifungals used.

Table 9: Antifungal Profile of the Fungal Isolates to Ketoconazole and Fluconazole

S/n	Organism	Ketoconazole (50g)	Fluconazole (200mg)
1	*Mucor*	(14) I	(15) I
2	*Alternaria*	(7) R	(9) R
3	*Botrytis*	(8) R	(15) I
4	*A. flavus*	(12) I	(14) I
5	*Fusarium chlamydosporum*	(9) R	(7)R

DISCUSSION

The results of this research work show the bacterial Load (Total bacterial count) ranged from too few to count (TFTC) to 1.04 x10^7 expressed in colony-forming units per gram and the Fungal Load (yeast and mould count) ranged from too few to count (TFTC) to 4.8 x10^6 which are all within the unsatisfactory limits set by ICMSF (ICMSF: 10^3 as satisfactory, $<10^3 \leq 10^5$ as borderline and $\geq 10^5$ as unsatisfactory). These results Agrees with the results of Fowoyo and Baba-Ali (2015), as their result was above the acceptable microbiological limit of RTE foods. The high number of bacterial and fungal loads can be attributed to poor hygiene practices in the restaurants, lack of cooking at the appropriate temperature, lack of potable water and possible contamination from the workers which can lead to food poisoning due to the high count of microbes found in the food samples as the infectious dose requirement of microorganisms to cause infection can easily be reached (Alex *et al.*, 2017).

A total of nine (9) different bacterial species (*Salmonella spp.*, *Escherichia coli*, *Staphylococcus epidermis*, *Clostridium spp.*, *Enterobacter aerogens*, *Pseudomonas aeruginosa*, *Proteus spp.*, and *Bacillus spp.*) were identified from the restaurants with *E. coli* as the most prevalent (26.31%), similar to the result of Fowoyo and Baba-Ali, (2015) and Oranusi *et al.*, (2013).

These results are also in agreement with the findings of Nyenge *et al.*, (2012) who isolated *E. coli, Enterobacter* spp., *Proteus mirabilis, Micrococcus* spp., and *Staphylococcus spp*. The high number of *E. coli* indicates faecal contamination of the food as they form part of the human microbiota and animal gastrointestinal tract, thereby easily transmitted through food and water (Garcia *et al.*, 2010; Croxen *et al.*, 2014). *Staphylococcus* spp. are naturally found on human skin, suggesting contamination from food handlers during production and serving of the foods. Although, these organisms are opportunistic microorganisms, they have the potential to cause foodborne infection/intoxication or toxins mediated infection when consumed in large quantities (Bintsis, 2017). *E. coli*, *Staphylococcus and Bacillus spp*. demonstrate a potential health risk hazard as these organisms are pathogenic and have been implicated in foodborne diseases (Oranusi *et al.*, 2013).

Furthermore, a total of five (5) different fungal species were isolated from this research (*Aspergillus flavus, mucor, Botrytis, Alternaria* and *Fusarium chlamydosporum*) With *Aspergillus flavus* being the most prevalent (45.45%). Species of fungi like *Aspergillus spp.*, can produce a toxin known as mycotoxins which are carcinogenic (able to cause cancer) and can be deadly when consumed. The occurrence of mold (*Aspergillus* and *Mucor* species) in the food could result from their ability to produce spores that are heat resistant and may have survived the heat during processing. *Aspergillus* and *Mucor* are common environmental contaminants found in air and packaging materials warranting their easy exposure to processes (Lutpiatina, 2021). *Mucor* and *Aspergillus flavus* are similar isolates reported by Wogu *et al.*, (2010) and Fowoyo and Baba-Ali (2015).

The microorganisms isolated from this research were tested against antimicrobial drugs including levofloxacin, gentamycin and ciprofloxacin were found to be susceptible against all bacteria isolated from this research, chloramphenicol was as well susceptible to all bacterial isolates except *Proteus mirabilis,* septrin, amoxicillin, norfloxacin and ampiclox were resisted by *Micrococcus luteus, Staphylococcus* spp. The lack of activity of some antibiotics might be a result of misuse of drugs by individuals resulting in multidrug resistance (WHO 2015), Microorganisms initiate ways to prevent themselves from the activity of the drug after it has been introduced to it, the mechanism can be inherited by subsequent generation leading to ineffectiveness of the drug. Antifungal drugs ketoconazole and fluconazole were found to inhibit the growth of *Mucor* spp., *Aspergillus flavus,* while resisted by *Fusarium chlamydosporum* and *Alternaria. Botrytis* was found to be resistant to ketoconazole and susceptible to fluconazole as obtained from this research.

CONCLUSION

This study found out that food sold within Katsina metropolis harbours high bacterial and fungal loads (above acceptable limit, thus, Unsatisfactory) that are of potential health hazard and can cause health problems to individuals if proper measures to prevent contamination are not taken. It was also observed that most of the antibiotics tested were sensitive to the isolates and fluconazole was more effective against fungal isolates than ketoconazole.

RECOMMENDATION

It is recommended that the Government should enforce food Safety regulations like re-introduction of food inspectors to warrant good hygienic practices by food and restaurants handlers to reduce contamination. More awareness to the public on the importance of food safety is paramount to reduce the risk of food poisoning and foodborne diseases due to the consumption of contaminated food.

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