Antimicrobial Resistance Pattern of Some Microorganisms Isolated from Packaged Food Samples

ABSTRACT

Aims: The aim of this study was to determine the presence of microorganisms in packaged foods which are very popular among the elites, who may assume that these foods will be totally save, especially for their children; and determine theresistance pattern of microorganisms isolated from these selected packaged food samples.
Study design: The study employed an experimental study design.
Place and Duration of Study: Samples were collected between March and May, 2021 and the study spanned from January 2021 to April 2022. The research was carried out in the Microbiology Laboratory of Pure and Applied Biology Departement, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.
Methodology: Isolation of microorganisms were done by standard microbiological methods. The abilities of bacteria isolates to produce biofilm were determined and quantified at 492 nm with HALOMPR-96 visible microplate reader. Bacteria and fungi isolates were tested for their sensitivity to antimicrobials with different antibacterial and antifungal disc and results compared with guidelines from Clinical and Laboratory Standard Institute (CLSI, 2018). Selected bacteria and fungi isolates were characterized molecularly by 16S rRNA and ITS respectively.
Results:A total of 17 bacteria and 13 fungi isolated from sampled packaged food belonging to 6 bacteria genera and 4 fungi genera respectively were obtained. All bacteria isolates were either moderate or weak biofilm producers. A 100 % sensitivity to Gentamicin and Ofloxacin was observed among Gram positive bacteria. All fungi isolates were sensitive to one or more of the antifungal used. Molecular identity of selected bacteria and fungi isolates revealed their closest isolates from available isolates in GenBank.
Conclusion: Packaged foods are important source of microorganisms that can be of public health importance. Ingestion of contaminated food products can lead to food poisoning. The resistance of these microbes to antibiotics makes such a difficult situation to handle.

Keywords:*packaged food, food spoilage, antimicrobial resistance, biofilm, fungi resistance*

1. INTRODUCTION

Many packaged and quick-serviced food products have overwhelmed retail outlets, with spaghetti, noodles, and flakes among the most popular food products. This results from their convenience, short time of preparation and affordability (Young et al., 2020). Most packaged foods are made from flour and grains and are either steamed or deep-fried in oil. In Nigeria, demand for fast or packaged food and consumption continue to rise. Nigeria is Africa's largest consumer of packaged foods, with instant noodles rising from 1.1 billion to 1.44 billion packets (WINA, 2014). Packaged foods typically have a longer shelf life in the northern hemisphere and tropical regions due to their low moisture content and low water activity. Proper food packaging is vital in preserving food quality throughout transportation, distribution, and storage (Kontominas, 2016; Abalkhail, 2023).

Spoilage in packaged foods is due to bacteria and fungi (mycotoxins) or their metabolites that contaminate it during processing and handling(Afshari et al., 2022). The ability of

microorganisms to persist on food surfaces thus spreads foodborne pathogens and influences their safety (Young and Waddell, 2016). Microbial cross-contamination refers to the transfer (either direct or indirect) of microorganisms (such as bacteria, viruses, parasites, or fungi) from a contaminated item to a non-contaminated one (Minnesota Department of Health, 2007). Cross-contamination by foodborne pathogens is a significant concern due to the increased health risks posed by consuming contaminated food (SafarpoorDehkordi et al., 2017). The transfer of foodborne pathogens from inert surfaces to food has been extensively documented (Erickson et al., 2015). Several studies have shown that various foodborne pathogens, including Escherichia coli and Listeria monocytogenes, can survive on utensils and equipment surfaces for hours or even days (Martinon et al., 2012).

Microorganisms enter foods, including packaged food, during their preparation, handling and packaging, causing illnesses when ingested (Havelaar et al., 2015). Food contamination and its consumption is one major threat to humans(Pesavento et al., 2010). These microorganisms form biofilm and cause associated infections over time due to the transfer of resistance genes through horizontal gene transfer (Bridier et al., 2015). Among the most relevant bacterial pathogens that cause foodborne disease are Brucella spp., Campylobacter sp., Salmonella spp., Yersinia sp., Listeria sp., and Escherichia coli spp. (Scallan et al., 2011) Moreover, bacteria in the order Bacillus, Clostridium, Sporolactobacillus, Soporosarcin, and Desulfotomaculum are involved in most foodborne outbreaks in Europe in 2015.

The availability of antimicrobial agents to treat infections caused by food spoilage has significantly improved humans' and animals' health and life expectancy. However, antibiotic use has led to antimicrobial resistance in bacteria, which has become a global issue affecting both public and animal health (O'Neill, 2016; Parvin et al., 2020).

Biofilms protect pathogenic and spoilage microorganisms, allowing them to survive longer and contribute to cross-contamination from packaging to food (Valeriano et al., 2012). Biofilms are structured communities of bacterial cells encased in a self-produced polymeric matrix, adhering to surfaces (Bhutia et al., 2021). Modern industrial production processes provide an ideal environment for biofilm development due to extended production times, high-volume output, large growth areas, and the structure of manufacturing plants, all of which contribute to their antibiotic resistance (Makovcova et al., 2017). Given the increasing prevalence of antibiotic resistance in both primary and processed food products, it is crucial to understand the resistance patterns of microbes found in packaged food samples to antimicrobial agents and to identify such with multiple antibiotic resistances.

2. MATERIAL AND METHODS

Sample Collection: Six (6) different types of packaged food samples that include noodles, spaghetti, tomato paste, sugar, corn flakes and whole corn meal were purchased from different supermarkets in Ogbomoso and transported to the microbiology laboratory of LAUTECH, Ogbomoso, for analysis. A total of 24 samples were obtained, along with four samples for a type of packaged food.

Procedure for Isolation: The food samples were aseptically transferred into sterilized peptone water (500 mL) each. The broth was then incubated in a shaker incubator (120 rpm, 30oC) for 24 hours. The broth was then serially diluted and plated on Nutrient agar and Potato Dextrose agar for bacteria and fungi isolation, respectively. Bacteria culture plates were incubated at 37oC for 24 hours, and PDA was incubated at 25oC for 48 hours. Bacteria and fungi culture plates were further sub-cultured until pure cultures were obtained. Pure isolated microorganisms were then maintained on NA and PDA at 4oC for bacteria and fungi, respectively, for further studies.

Identification of Bacterial and Fungi Isolates: The bacterial isolates were subjected to biochemical tests and morphological characteristics following Bergey's Manual of Systematic Bacteriology (Bergey, 2000). The fungal isolates were identified based on their macroscopic and microscopic features, as Gaddeyya et al. (2012) described.

Biofilm Determination by Isolated Bacteria: The abilities of the bacterial isolates to produce biofilm were determined according to the method described by Amao et al. (2019). The biofilm production ability was quantified at 492 nm using a HALOMPR-96 visible microplate reader, after introducing 125 µl of 30% acetic acid solution and incubating at 28°C for 15 minutes. The result of biofilm formation was interpreted as reported by Singh (2017):

Non-biofilm formers = OD cut
Weak biofilm formers = OD
Moderate biofilm formers = 2 cut cut
Strong biofilm formers = OD cut

Antimicrobial Susceptibility Testing of Bacteria and Fungi Isolates: Mueller-Hinton agar (Lab M, UK) was used for antimicrobial susceptibility testing. Bacterial inoculum (0.5 McFarland) was swabbed onto the surface of sterile Muller Hinton agar plates, and the antibiotic disc was placed on it after allowing the plates to rest for 10 minutes. The antibacterial disc used (product of Rapid Laboratories, UK) contains Augmentin (30µg), Erythromycin (5µg), Cloxicillin (5µg), Cefuroxime (30 µg), Gentamicin (10µg), Ceftazidime (30 µg), Ofloxacin (5µg) and Ceftriaxone (30 µg). The plates containing antibiotic discs were then incubated at 37oC overnight, and the zone of inhibition was measured and interpreted as described in Clinical and Laboratory Standard Institute guidelines (CLSI, 2018).

Fungal inoculums were swabbed on the Mueller-Hinton agar and allowed to be set for 10 minutes before antifungal discs were placed. Antifungal disc (product of Rapid Laboratories, UK) used were Amphotericin B (100µg), Ketoconazole (50µg), Miconazole (50 µg), Econazole (50 µg) and Clotrimazole (50µg). It was then incubated at 25oC for 72 hours. The inhibition zones were measured and interpreted for all antifungal discs as described in Clinical and Laboratory Standard Institutes guidelines (CLSI, 2018).

Molecular Characterization of Selected Isolates: Molecular identification of bacteria found to have 50 % and above resistant to antibiotics were performed using 16S rRNA sequencing, and fungal isolates with > 50 % resistance were identified through sequencing of the ITS1 and ITS4 regions.

Statistical Analysis: The data obtained were analyzed using an average of three independent replicates. Statistical analyses were performed using one-way analysis of variance (ANOVA) on IBM SPSS version 24 software at a 95% confidence interval.

3. RESULTS AND DISCUSSION

A total of seventeen (17) bacteria and thirteen (13) fungi isolates were isolated from the collected packaged foods. Tables 1 and 2 show their morphological, microscopic, and biochemical identification. The bacteria were 13 Gram-positive and 4 Gram-negative bacteria belonging to 6 genera: Bacillus*, Parapusillimonas, Burkholderia, Pseudomonas, Azotobacter*and*Paenibacillus*(Table 1). Also, fungi isolated from these samples belong to 4 genera: Fusarium*, Aspergillus, Colleotrichum*and *Meyerozym*(Table 2). Considerable bacteria and fungi contamination levels in all the packaged foods samples were observed. The low microbial load in various packaged food samples may be attributed to the bioactive secondary metabolites produced by the microorganisms with antimicrobial properties. It may also be due to the preservatives used in the production chain (Costa *et al.,* 2020). The percentage occurrence of bacteria genera revealed that the genus *Bacillus* was most prevalent at 70.58%, while others have a percentage occurrence of 5.88 % each. A similar research conducted by Akhigbemidu*et al.* (2015), also reported the isolation of bacteria and fungal species; the microbial analysis showed the presence of *Bacillus*, *Pseudomonas* and other genera of bacteria found in packaged noodles. This finding is consistent with the report of Asoso et al. (2022), who isolated *Bacillus subtilis*, *Lactobacillus fermenti*, *Pseudomonas stutzeri*, *Rothia* sp., *Penicilliumnotatum*, *Saccharomyces* sp., *Aspergillusniger*, *Mucormucedo*, *Aspergillusflavus*, *Aspergillusfumigatus*, and *Rhizopusstolonifer* from packaged tomato paste. Akintobiet al. (2018) identified *A. niger*, *R. stolonifer*, *Mucor* spp.,

and *A*. *flavus* has been associated with tomato deterioration at Umuahia market, Abia State, Nigeria. This contamination could be linked to poor post-processing and storage conditions, creating favourable environments for spoilage microbes. The fungi's ability to produce spores, their ubiquitous nature, and intrinsic factors such as temperature, relative humidity, and pH contribute to their proliferation.

Figures 1 and 2 showed the prevalence of different genera of bacteria and fungi isolated from the packaged food products. The genus *Bacillus* was the most prevalent among the bacterial genera, with a percentage occurrence of 70.58 % (Figure 1). *Aspergillus* was the most prevalent fungi genus, with 73.08 % occurrence (Figure 2). Similar work carried out by Amina *et al.* (2023), reported the isolation of more Gram-positive bacteria than Gram-negative ones in their study. This might be because some Gram-positive bacteria, such as *Bacillus,* have spores-forming abilities that ensure survival during harsh environmental conditions.

Table 1: Morphology, Microscopic and Biochemical characterization of isolated bacteria

SAMPLE CODE	Gram stain	VP	Pigment	Motility	Nitrate reduction	Spore	Catalase	Indole	Citrate	H_2S	Casein hydrolysis	Urease	Oxidase	Starch hydrolysis	Sugar fermentation: Maltose	Mannitol	Fructose	Sucrose	Probable identity
Mix T2	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	*Bacillus licheniformis*
Mix T1	+	+	-	+	-	+	+	-	+	+	+	-	+	-	+	+	-	+	*B. pumilus*
CarP 1b	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	-	+	-	*B. thuringiensis*
CarP 1b1	+	+	+	+	+	+	+	-	+	-	+	-	-	+	+	-	+	+	*B. cereus*
CarP 1	-	-	+	+	-	-	-	-	+	-	-	+	+	-	-	-	-	+	*Parapusillimonasgranuli*
GolN1	-	-	+	+	+	-	+	-	+	-	-	+	+	+	+	+	+	+	*Azotobactervinelandii*
GolN2	+	-	-	+	-	+	-	+	+	-	+	-	-	+	+	+	+	+	*B. megaterium*
Gol N1b	-	+	+	+	-	-	+	-	+	-	-	-	-	-	-	+	+	+	*Burkholderiapseudomalle*
Inf C2	+	-	+	+	+	+	+	-	+	-	-	+	-	+	-	+	+	+	*B. megaterium*
Inf C1	+	+	+	+	-	+	-	+	+	-	+	-	-	-	+	+	+	+	*B. toquilensis*
Inf C3	+	+	-	+	-	+	-	-	+	-	+	-	-	-	-	+	+	+	*B. pumilus*
Ind N1	+	+	-	+	-	+	-	-	+	+	+	-	-	-	-	-	+	+	*B. pumilus*
Che N1	+	-	+	+	-	+	-	-	+	-	+	-	+	+	+	+	+	+	*B.utropicus*
Che N2	+	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	*B. cereus*
DanS2	+	-	+	+	+	+	+	+	-	-	-	-	+	+	+	-	+	+	*Paenibacillusthiaminolyticus*
VitaT2	+	+	-	+	-	+	+	-	+	-	+	-	+	-	+	+	+	+	*B. aeolius*
Nas C2	-	-	+	+	-	-	-	+	+	-	-	-	-	-	+	-	-	+	*Pseudomonas syringae*

Keys: - (negative test); + (positive test); VP (Voges-Praskauer)

Table 2: Morphological and Microscopic Identification of Isolated fungi

Lab Code	Pigmentation	Spore	Surface	Form	Elevation	Margin	Probable Organism
TomT1f	White	chlamydospores	Smooth	Circular	Raised	Entire	*Fusarum*sp.
GolS2f	Brown	Acospore	Dull	Circular	Flat	Lobate	*Aspergillus fumigatus*
CarP1f	Greyish White	Conidiospore	Rough	Circular	Raised	Lobate	*Colletotricumtruncatum*
MixT2f	Brown	Conidiospore	Rough	Circular	Raised	Entire	*A. japonicas*
MixT3f	Whitish Brown	Acospore	Rough	Circular	Raised	Lobate	*A. brasiliensis*
CarP2f	White-greyish	chlamydospores	Smooth	Circular	Raised	Entire	*Fusarumoxysporum*
VitaT2f	Outer Margin White with Inner Margin Green	Zygospore	Rough	Circular	Raised	Entire	*Aspergillus aculeatus*
InfC2f	Black	Zygospore	Rough	Circular	Raised	Lobate	*A. niger*
GolS1f	Pale Green	Conidiospore	Dull	Irregular	Raised	Entire	*A. flavus*
GolS3f	Black	Zygospore	Rough	Circular	Raised	Lobate	*A. niger*
NasC1f	Black	Zygospore	Rough	Circular	Raised	Lobate	*A. niger*
GolS2f	Brown	Acospore	Dull	Circular	Flat	Lobate	*A. fumigates*
DanS1f	White-brownish	chlamydospores	Smooth	Circular	Raised	Entire	*F. solani*
DanS2f	Outer Margin White with Inner Margin Green	Zygospore	Rough	Circular	Raised	Entire	*A. aculeatus*
GolN2f	Outer Margin White with Inner Margin Green	Zygospore	Rough	Circular	Raised	Entire	*A. aculeatus*
GolN1f	Cream	ascospores	Smooth	circular	Flat	Entire	*Meyerozymguilliermondii*
InfC1f	Outer Margin White with Inner Margin Green	Zygospore	Rough	Circular	Raised	Entire	*A. aculeatus*

NesG1f	Cream	ascospores	Smooth	circular	Flat	Entire	*Meyerozymaguilliermondii*
NasC2f	Yellowish Green	Conidiospore	Dull	Irregular	Raised	Entire	*A. flavus*
TomT2f	Whitish Brown	Acospore	Rough	Circular	Raised	Lobate	*A. brasiliensis*
InfC3f	Yellowish Green	Conidiospore	Rough	Irregular	Raised	Entire	*A. oryzae*
CarP1f	Yellowish Green	Conidiospore	Rough	Irregular	Raised	Entire	*A. oryzae*
NasC2f	Outer Margin White with Inner Margin Green	Zygospore	Rough	Circular	Raised	Entire	*A. aculeatus*
GolS1f	Pale Green	Conidiospore	Dull	Irregular	Raised	Entire	*A. flavus*
CheN1f	White-greyish	chlamydospores	Smooth	Circular	Raised	Entire	*F. oxysporum*
GolN2f	Brown	Conidiospore	Dull	Irregular	Raised	Entire	*A. terreus*

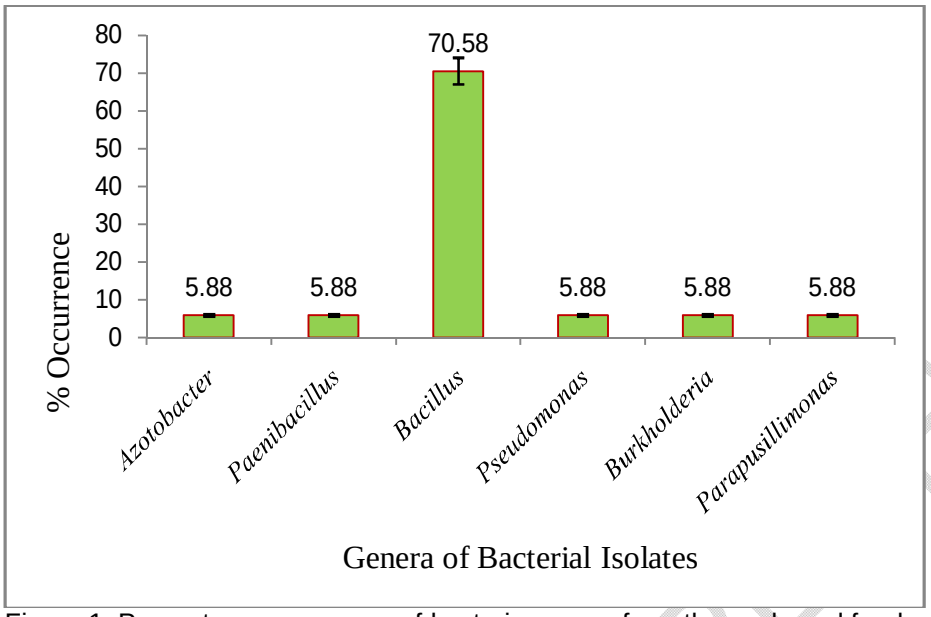

Figure 1: Percentage occurrence of bacteria genera from the packaged food samples

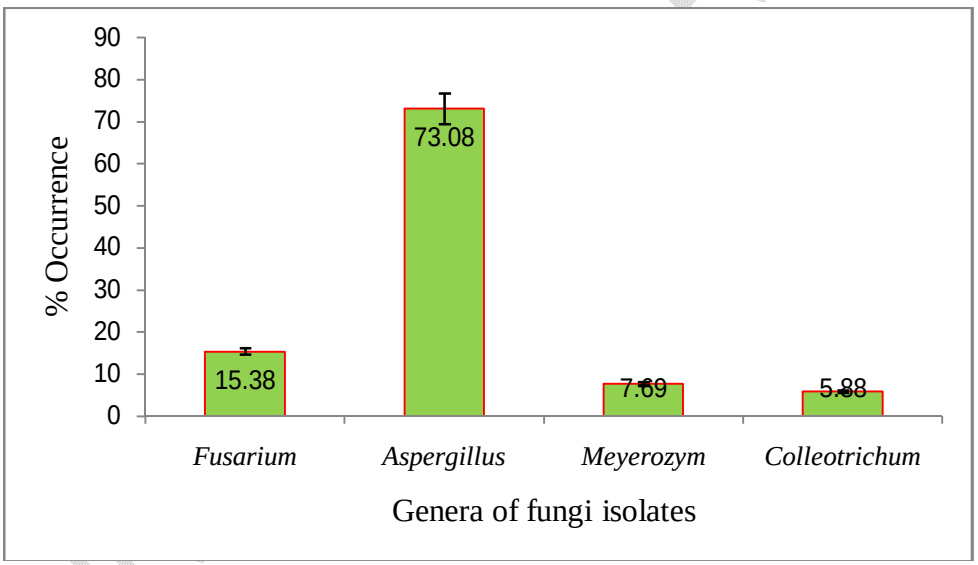

Figure 2: Percentage occurrence of fungi genera from the packaged food samples

Table 3 shows the abilities of bacteria isolates to produce biofilm. All isolated bacteria showed abilities to produce biofilm but at different levels. Six (6) bacteria isolates were moderate biofilm formers representing 35.29 %, eleven (11) isolates were weak biofilm formers representing 64.71 % (Figure 3). Biofilms can act as reservoirs for potentially pathogenic microorganisms that may contaminate food, posing a health threat when consumed (Jacksch*et al.,* 2021). The genus *Bacillus* was the dominant phylum, exhibiting moderate biofilm formation. This may be due to multiple species at a given site, which can enhance interspecies communication and cross-feeding, promoting biofilm biomass (Zupancic*et al.,* 2018).

The antimicrobial susceptibility test for all isolated bacteria and fungi was shown in Tables 4-6. From the result obtained, gentamicin and ofloxacin were active against the bacterial isolates at 100 %; erythromycin was active against the isolates at 92.31 % for all Gram-positive bacteria, while the least activities were observed for ceftazidime and ceftriaxone at 38.46 % and 46.15 % respectively (Table 4). Ciprofloxacin, gentamicin, oflaxacin and nitrofurantoin were active 100 % against all the Gram-negative bacteria, while *Burkholderiapseudomalle*(50 %) and *Parapusillimonasgranuli* (50 %) were sensitive to almost all the antibiotic used (Table 5). Also, from the four Gram-negative bacterial isolates, *Burkholderiapseudomalle* and *Parapusillimonasgranuli* were sensitive (100 %), followed by *Pseudomonas syringae*(75 %) to all antibiotics used (Table 5). The spread of antibiotic resistance in bacteria is a significant public health concern in various environments. Consuming foods without heat treatment, which may harbour multidrug-resistant bacteria even at low microbial loads, poses a danger, particularly to immunocompromised individuals. These resistant bacterial strains can survive the gastrointestinal tract, complicating treatment for those with weakened immune systems (Fiedler *et al.,* 2019).Agboola*et al*. (2021) mentioned that most bothersome concern is that resistance among microbes can surge with persistent and pervasive usage of antibiotics among the people.As noted by Jans*et al*. (2018), fermented dairy foods presented high antimicrobial resistant (AMR) bacteria with AMR against penicillins,fluoroquinolones, aminoglycosides,glycopeptides, sulfonamides, and tetracyclines.

All isolates were sensitive to one or more antifungal agents. Six (6) isolates were sensitive (100 %), while six (6) isolates were also resistant to all antifungal agents used (Table 6).In the work of Fusaro*et al*. (2024), AMR in street foods ranged between 5.2% and 70.8%;a stressfor the imperative need for all-inclusivemethodologies and coordinated efforts to challenge AMR under the “One Health” model.

Table 3: Biofilm production potentials of isolated bacteria

Probable microorganism	**Mean (CFU) ± STD**	**Biofilm Former Group**
Azotobactervinelandii	0.352±0.017	weak
Paenibacillusthiaminolyticus	0.755±0.533	moderate
Bacillus licheniformis	0.376±0.018	weak
B. thuringiensis	0.354±0.014	weak
B. cereus	0.469±0.090	moderate
B. pumilus	0.511±0.061	moderate
B. megaterium	0.295±0.013	weak
B. megaterium	0.398±0.031	weak
B. toquilensis	0.333±0.022	weak
B. pumilus	0.419±0.014	moderate
B. utropicus	0.410±0.055	moderate
B. aeolius	0.415±0.042	moderate
B. pumilus	0.180±0.009	weak
B. cereus	0.297±0.060	weak
Burkholderiapseudomalle	0.360±0.023	weak
Pseudomonas syringae	0.315±0.014	weak

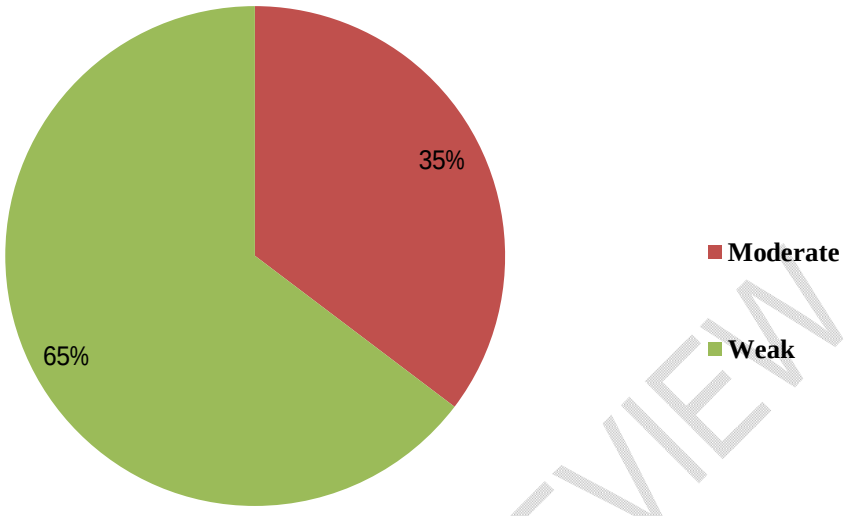

Figure 3: Percentage occurrence of biofilm producers among isolated bacteria

Table 4: Antibiotic Susceptibility Test of Gram Positive Bacteria to Antibiotic Disc

ISOLATES	CRX (30 µg)	GEN (10 µg)	CTR (30µg)	OFL (5 µg)	ERY (5 µg)	CXC (5 µg)	AUG (30 µg)	CAZ (30 µg)	No and % resistance
Paenibacillusthiaminolyticus	S	S	I	S	S	S	S	I	0
Bacillus licheniformis	S	S	R	S	S	S	S	R	25
B. thuringiensis	R	S	R	S	S	R	R	R	62.5
B. cereus	R	S	R	S	S	R	R	R	62.5
B. pumilus	S	S	S	S	S	S	S	R	25
B. megaterium	S	S	R	S	S	S	S	R	25
B. megaterium	R	S	S	S	S	S	S	R	25
B. toquilensis	I	S	S	S	S	S	S	S	0
B. pumilus	S	S	S	S	S	S	S	S	0
B. utropicus	R	S	R	S	S	R	R	R	62.5
B. aeolius	I	S	S	S	R	R	S	S	25
B. pumilus	S	S	S	S	S	R	S	S	12.5
B. cereus	R	S	I	S	S	R	R	R	50

Keys:CAZ: Ceftazidime, CRX: Cefuroxime, CTR: Ceftriaxone, ERY: Erythromycin, CXC: Cloxacillin, OFL: Ofloxacin, GEN: Gentamicin, AUG: Amoxycillin/Clavulanate, R: Resistant, S: Sensitive, I: Intermediate, V- Value reading, In- Interpretation, %- percentage

Table 5: Antibiotic Susceptibility Test of Gram Negative Bacteria to Antibiotic Disc

ISOLATES	CPR (5 µg)	CRX (30 µg)	GEN (10 µg)	OFL (5 µg)	AUG (30 µg)	CAZ (30 µg)	NIT (30 µg)	AMP (30 µg)	% resistance
Azotobactervinelandii	S	I	S	S	S	R	S	S	12.5
Burkholderiapseudomalle	S	S	S	S	S	S	S	I	0

Pseudomonas syringae	S	I	S	S	R	R	S	S	25
Parapusillimonasgranuli	S	S	S	S	S	S	S	I	0

Keys:CAZ: Ceftazidime, CRX: Cefuroxime, OFL: Ofloxacin, GEN: Gentamicin, CPR: Ciprofloxacin, AUG: Amoxycillin/Clavulanate, NIT: Nitrofurantoin, AMP: Ampicillin, R: Resistant, S: Sensitive, I: Intermediate, V- Value reading, In- Interpretation, %- percentage

Table 6:Antifungal Susceptibility Test for Isolated Fungi

SAMPLE CODE	**CTR50** µg	**5FC-1**	**KET50** µg	**MCZ50** µg	**EC50** µg	**NY100** µg	**AB100** µg	**% Resistance**
FPE 3-IC	R	R	S	S	S	S	R	42.86
Tom T 1f	S	S	S	S	S	S	S	0
GolS 2f	R	S	S	I	S	R	I	28.58
CarP 1f	S	R	S	S	S	S	I	14.29
Mix T 2f	R	R	I	R	I	R	R	71.43
Mix T 3f	I	R	S	S	S	R	R	42.86
Car P 2f	I	R	I	I	S	R	R	42.86
Vita T 2f	S	R	R	R	R	R	R	85.71
Infc 2f	R	R	R	R	R	R	R	100
Gols 1f	R	R	R	R	R	R	R	100
Gols 3f	S	R	S	S	S	R	S	28.58
Nasc 1f	S	S	S	S	S	S	S	0
Gols 2f	R	R	R	R	I	R	R	85.71
DanS 1f	S	R	S	S	S	R	S	28.58
DansS2f	R	R	R	R	R	R	R	100
Gold 2f	R	S	S	S	R	R	R	57.14
Gold 1f	R	R	S	R	S	R	S	57.14
Infc 1f	R	R	I	R	I	R	R	71.43
NasG 1f	S	S	S	I	S	S	R	14.29
Nasc 2f	S	S	S	S	S	S	S	0
TomT 2f	S	S	S	S	S	S	S	0
Infc 3f	R	R	R	R	R	R	R	100
CarP 1f	R	R	R	R	R	R	R	100
Nasc 2f	R	R	R	R	R	R	R	100
GolS 1f	S	S	S	S	S	S	S	0
Chen 1f	S	S	S	S	S	S	S	0
Gold 2f	R	R	R	S	S	S	S	42.86

Keys:MCZ: Miconazole, EC: Econazole, KET: Ketoconazole, CTR: Clotrimazole, AB: Amphotericin B, 5FC-1 :Carbofunzin, S: Sensitive, R: Resistant, I: Intermediate, V: Value reading, In: Interpretation, %: percentage

The BLAST result showed that the isolates Chen1, CarP1b1 and CarP1b were *Bacillus utropicus, B. cereus* and *B. thuringiensis,* respectively (Table 7). Characterized fungal isolates belong to the genera of *Aspergillus, Colleotrichum* and *Meyerozym*(Table 8). Figure 4a-b shows the phylogenetic relatedness of selected bacteria and fungi. In Figure 4a, the tree was divided into two clades; the isolates CarP1b1 and CarP1b were in the same clade with *Bacillus cereus* (NR113266.1). In Figure 4b, the tree was divided into four clades, with isolate InfC3f in the same clade as *Aspergillus oryzae*(EU680477.1) and GolN2f in the same clade with *Meyerozymaguilliermondii* (LC422370.1).

Table 7: Molecularly Characterized Bacteria Isolates with Their Accession Numbers

Isolate Code	**Accession number**	**Isolate identity**

Chen 1	OR400570	*Bacillus utropicus*
Chen 2	OR400571	*B. thuringiensis*
CarP1b	OR400572	*B. thuringiensis*
Carp1b1	OR400573	*B. cereus*

Table 8: Molecularly Characterized Fungi Isolates with Their Accession Numbers

Isolate Code	**Accession number**	**Isolate identity**
CarP 1f	OR400765	*Colleotrichumtruncatum*
InfC2f	OR400764	*Aspergillus niger*
GolS1f	OR400763	*A. fumigatus*
InfC1f	OR416115	*A. aculeatus*
NasC2f	OR416114	*A. aculeatus*
GolN1f	OR416113	*Meyerozymaguilliermondii*
GolN2f	OR416112	*A. terreus*
InfC2f	OR416111	*A. aculeatus*
VitaT2f	OR416110	*A. aculeatus*
MixT2f	OR416109	*A. japonicas*
InfC3f	OR416108	*A. oryzae*
DanS2f	OR416107	*A. aculeatus*

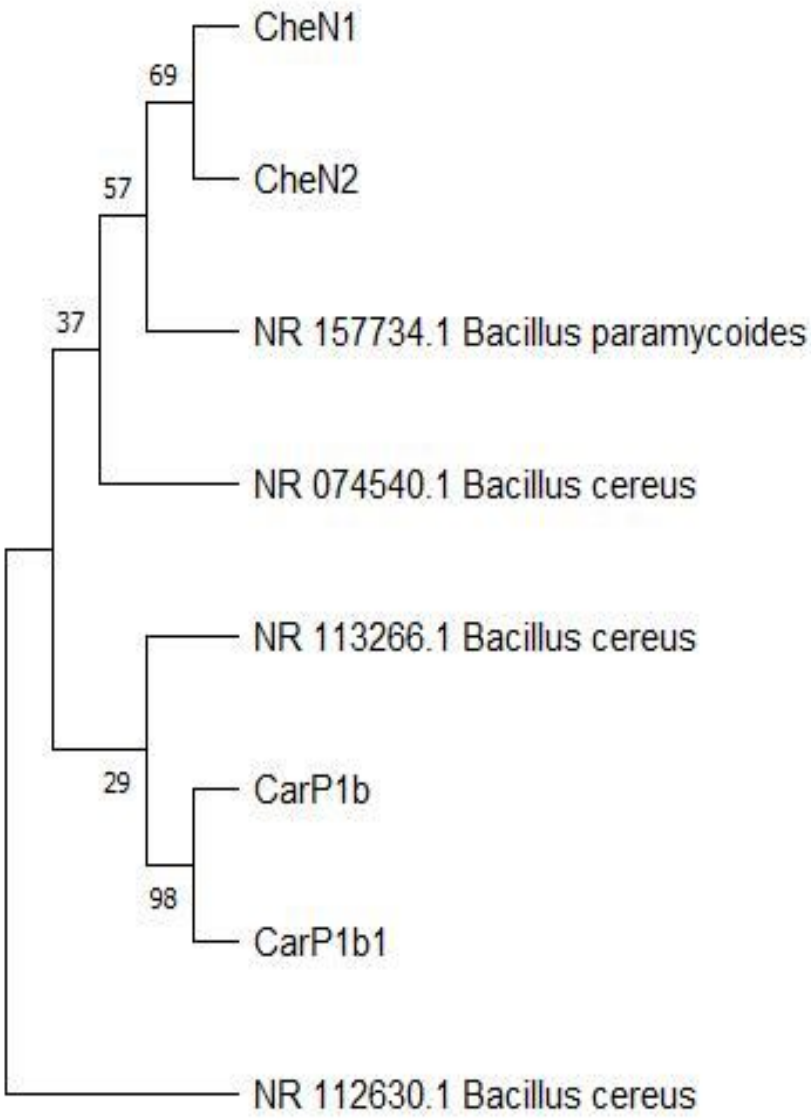

Figure 4a: Phylogenetic relatedness of selected isolated bacteria from packaged food samples

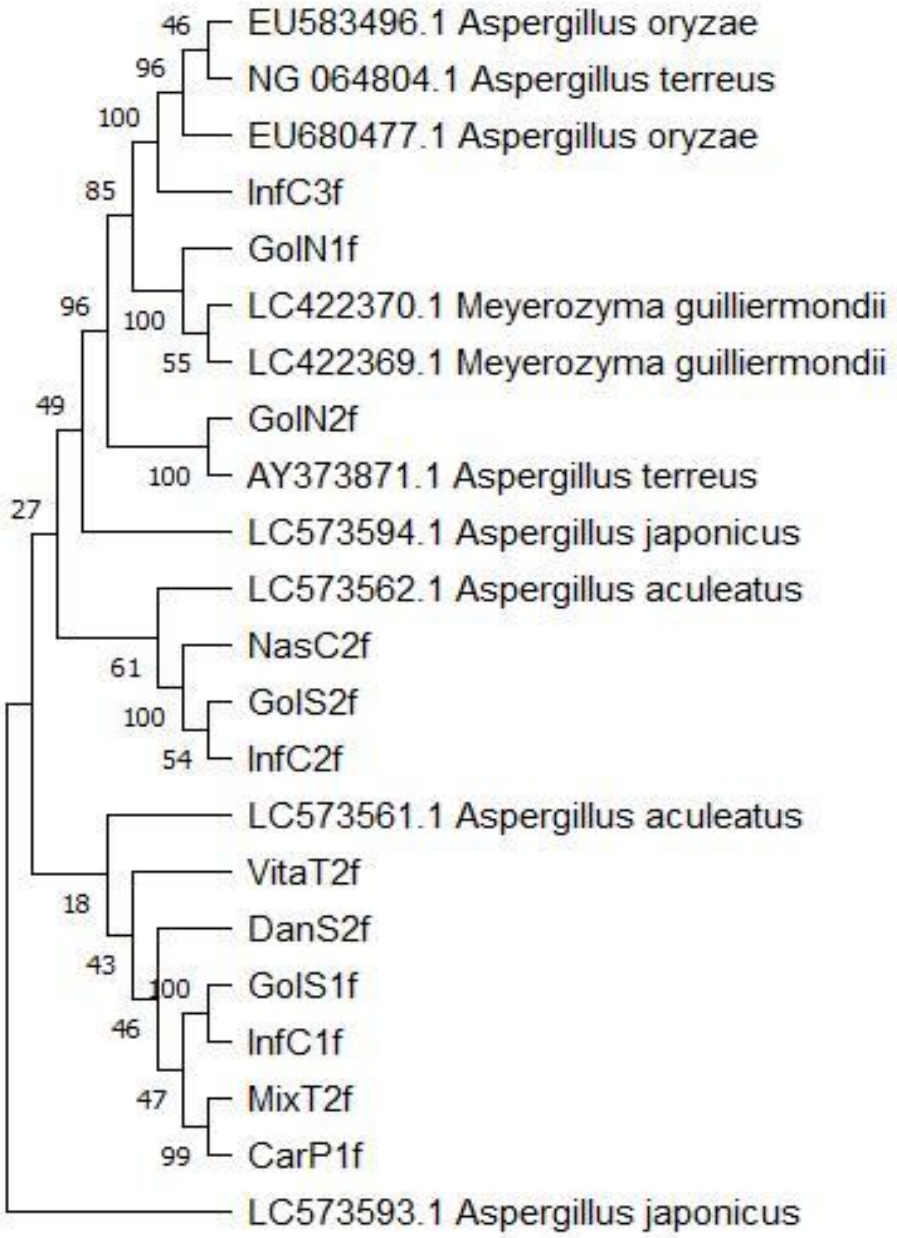

Figure 4b: Phylogenetic related of selected isolated fungi from packaged food samples

CONCLUSION

A total of 17 bacteria and 13 fungi were isolated from all the packaged food samples used in this study. Bacterial and fungal isolates present in the packaged food samples used in the study have been identified and the abilities of isolated microorganisms to produce biofilm at different quantities have been investigated. Isolated microorganisms have multiple antibiotic resistant patterns to various antibiotics. Packaged foods are important source that harbor different pathogenic microorganisms which may have implications on the health of the

public. Ingestion of contaminated food products can lead to food poisoning. The resistance of these microbes to antibiotics makes such a difficult situation to handle.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

REFERENCES

1. Young, L., Waddell, J., & Nelson, R. (2020). *Convenience and affordability driving the rise of packaged foods: A comprehensive review of global trends*. Food Science Journal, 45(3), 245–258. DOI: 10.1002/fsj.12345
2. WINA (World Instant Noodles Association). (2014). *Global demand for instant noodles: Statistical report 2014*. Retrieved from www.wina.org.
3. Kontominas, M. G. (2016). *Food packaging science and technology*. Journal of Food Quality, 39(4), 231–245.DOI: 10.1111/jfq.12123
4. Minnesota Department of Health. (2007). *Food safety and the risks of microbial cross-contamination*. St. Paul: MDH Publishing.
5. Erickson, M. C., Liao, J., Ma, L., & Doyle, M. P. (2015). *Foodborne pathogens and cross-contamination during processing and handling*. Annual Review of Food Science and Technology, 6, 157–176.DOI: 10.1146/annurev-food-022814-015616
6. Martinon, A., Cronin, U. P., & Wilkinson, M. G. (2012). *The survival and transfer of pathogens in food environments: A review*. Food Microbiology, 31(2), 99–114. DOI: 10.1016/j.fm.2012.02.004
7. Havelaar, A. H., Kirk, M. D., Torgerson, P. R., et al. (2015). *World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010*. PLOS Medicine, 12(12), e1001923.DOI: 10.1371/journal.pmed.1001923
8. Bridier, A., Sanchez-Vizuete, P., Guilbaud, M., et al. (2015). *Biofilm-associated resistance to antimicrobial agents in foodborne pathogens*. Food Microbiology, 45, 139–148.DOI: 10.1016/j.fm.2014.04.015
9. Scallan, E., Hoekstra, R. M., Angulo, F. J., et al. (2011). *Foodborne illness acquired in the United States: Major pathogens*. Emerging Infectious Diseases, 17(1), 7–15.DOI: 10.3201/eid1701.P11101
10. O'Neill, J. (2016). *Tackling drug-resistant infections globally: Final report and recommendations*. The Review on Antimicrobial Resistance.
11. Valeriano, C., De Oliveira, R. M., & Rios, E. M. (2012). *Biofilm formation in food processing environments: Implications for food safety*. Brazilian Journal of Microbiology, 43(2), 576–586.DOI: 10.1590/S1517-83822012000200020
12. Makovcova, J., Babak, V., & Slany, M. (2017). *Biofilm-forming ability of bacteria in industrial food processing environments*. Food Control, 76, 210–218.DOI: 10.1016/j.foodcont.2017.01.001
13. Bergey, D. H. (2000). *Bergey's manual of systematic bacteriology* (2nd ed.). Springer.DOI: 10.1007/978-0-387-29298-4

14. Gaddeyya, G., Nagamani, K., & Rao, P. (2012). *Microscopic and macroscopic methods for fungal identification: A practical guide*. Mycology Today, 4(1), 14–19.
15. Amao JA, Omojasola PF, Barooah M. (2019). Isolation and characterization of some exopolysaccharide-producing bacteria from cassava peel heaps. Sci Afr.;4:e00093. DOI: 10.1016/j.sciaf.2019.e00093
16. Singh, R. (2017). *Quantitative determination of biofilm-forming ability in microbial isolates: Methods and applications*. Advances in Microbiology, 7(6), 325–332.DOI: 10.4236/aim.2017.76026
17. CLSI (Clinical and Laboratory Standards Institute). (2018). *Performance standards for antimicrobial susceptibility testing* (28th ed.). CLSI Supplement M100.
18. Costa, D., Assunção, R., & Oliveira, J. (2020). *The role of preservatives in enhancing the microbiological safety of packaged foods*. Food Science and Technology, 58(2), 233–246.DOI: 10.1590/fst.05820
19. Akhigbemidu, W., Chukwuma, M., & Okeke, I. (2015). *Microbial evaluation of packaged noodles and food safety implications*. African Journal of Biotechnology, 14(30), 2398–2404.DOI: [10.5897/AJB2015.14687](https://doi.org/10.589
20. Asoso, E. F., Oke, A. S., &Fasehun, S. T. (2022). *Microbial analysis of packaged tomato paste in Nigeria*. Nigerian Food Journal, 40(2), 123–134.DOI: 10.4314/nfj.v40i2.4
21. Akintobi, O., Adekanmi, O., & Ogundipe, A. (2018). *Fungal contamination in tomato products: A case study in Nigeria*. Mycological Reports, 12(4), 201–208.DOI: 10.1080/21501203.2018.1451234
22. Amina, A., Yusuf, M., & Abdullahi, S. (2023). *Bacterial diversity and resistance profiles in packaged foods: A regional perspective*. Journal of Microbial Research, 45(1), 77–89.DOI: 10.1016/j.jmr.2023.01.005
23. Jacksch, L., Schwartz, C., &Nebe, J. (2021). *Biofilms in food processing environments and public health concerns*. Journal of Food Protection, 84(4), 627–636.DOI: 10.4315/JFP-20-456
24. Zupancic, T., Legat, A., & Mancini, D. (2018). *Interplay between bacterial communities in biofilm formation and resistance*. Frontiers in Microbiology, 9, 1521.DOI: 10.3389/fmicb.2018.01521
25. Fiedler, F., Miller, H., & James, R. (2019). *Antimicrobial resistance in foodborne bacteria: Implications for public health*. Trends in Microbiology, 27(3), 215–225.DOI: 10.1016/j.tim.2018.10.007
26. Agboola, J. O., Ayandele, A.A., &Amao, J. A. (2021). Isolation and Characterization of Bacteria with Multiple Drug Resistance from Pig Dung: doi.org/10.26538/tjnpr/v5i8.29. *Tropical Journal of Natural Product Research (TJNPR)*, *5*(8), 1506-1514. https://www.tjnpr.org/index.php/home/article/view/498
27. Jans C, Sarno E, Collineau L, Meile L, Stärk KDC and Stephan R (2018) Consumer Exposure to Antimicrobial Resistant Bacteria From Food at Swiss Retail Level. Front. Microbiol. 9:362. doi: 10.3389/fmicb.2018.00362
28. Fusaro, C., Miranda-Madera, V., Serrano-Silva, N., Bernal, J. E., Ríos-Montes, K., González-Jiménez, F. E., Ojeda-Juárez, D., &Sarria-Guzmán, Y. (2024). Antibiotic-Resistant Bacteria Isolated from Street Foods: A Systematic Review. *Antibiotics*, *13*(6), 481. https://doi.org/10.3390/antibiotics13060481
29. SafarpoorDehkordi F, Gandomi H, Basti AA, Misaghi A, Rahimi E. Phenotypic and genotypic characterization of antibiotic resistance of methicillin-resistant Staphylococcus aureus isolated from hospital food. Antimicrobial resistance & infection control. 2017 Dec;6:1-1
30. Pesavento G, Ducci B, Nieri D, Comodo N, Nostro AL. Prevalence and antibiotic susceptibility of Listeria spp. isolated from raw meat and retail foods. Food control. 2010 May 1;21(5):708-13.

31. Parvin MS, Talukder S, Ali MY, Chowdhury EH, Rahman MT, Islam MT. Antimicrobial resistance pattern of Escherichia coli isolated from frozen chicken meat in Bangladesh. Pathogens. 2020 May 28;9(6):420.
32. Bhutia MO, Thapa N, Tamang JP. Molecular characterization of bacteria, detection of enterotoxin genes, and screening of antibiotic susceptibility patterns in traditionally processed meat products of Sikkim, India. Frontiers in microbiology. 2021 Jan 11;11:599606.
33. Afshari A, Taheri S, Hashemi M, Norouzy A, Nematy M, Mohamadi S. Methicillin-and vancomycin-resistant Staphylococcus aureus and vancomycin-resistant enterococci isolated from hospital foods: Prevalence and antimicrobial resistance patterns. Current microbiology. 2022 Nov;79(11):326.
34. Abalkhail A. Frequency and Antimicrobial Resistance Patterns of Foodborne Pathogens in Ready-to-Eat Foods: An Evolving Public Health Challenge. Applied Sciences. 2023 Nov 30;13(23):12846.