Original Research Article

ANTIMICROBIAL RESISTANCE PATTERN OF SOME MICROORGANISMS ISOLATED FROM PACKAGED FOOD SAMPLES

ABSTRACT

Aims: The aim of this study was to determine the resistance pattern of microorganisms isolated from some selected packaged food samples. Six different packaged food samples that include noodles, spaghetti, tomato paste, sugar, corn flakes and whole corn mill were collected at major supermarkets in Ogbomoso for bacteria and fungi isolation and evaluation.
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 and identified by their microscopic, morphological and biochemical features. 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) to determine their sensitivity pattern. 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.

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

1. INTRODUCTION

Many packaged and quick-serviced food products have overwhelmed the retail outlet with spaghetti, noodles, flakes among the most populous food products. This seems to be as a result of 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 and consumption of fast food product or packaged food continue to rise. Nigeria is considered the largest consumer of packaged foods in Africa, with the

consumption of 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 consequently low water activity. Proper food packaging plays a vital role in preserving food quality throughout transportation, distribution, and storage (Kontominas, 2016).

Spoilage in packaged foods is due to the presence of bacteria and fungi (mycotoxins) or their metabolites that contaminate it during processing and handling. The ability of microorganisms to persist on the surfaces of food thus spread food borne 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). In food, cross-contamination by foodborne pathogens is a significant concern due to the increased health risks posed by the consumption of contaminated food. 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 get into 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 human. These microorganisms form biofilm and causes associated infections over time due to transfer of resistance gene through horizontal gene transfer (Bridier et al., 2015). Among the most relevant bacterial pathogens known to cause food-borne disease are Brucella spp., Campylobacter sp., Salmonella spp., Yersinia sp., Listeria sp., and Escherichia coli spp. (Scallan et al., 2011) and bacteria in the order Bacillus, Clostridium, Sporolactobacillus, Soporosarcin, and Desulfotomaculum are involved in most of the food-borne outbreaks registered in Europe in 2015.

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

The presence of biofilms offers protection to 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. 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 resistance to antibiotics (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 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, 30^{o}C) for 24 hours. The broth was then serially diluted and plated out on Nutrient agar and Potato Dextrose agar for bacteria and fungi isolation respectively. Bacteria culture plates were incubated at 37^{o}C for 24 hours and PDA was incubated at 25^{o}C 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 4^{o}C 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 the Bergey's Manual of Systematic Bacteriology (Bergey, 2000). The fungal isolates were identified based on their macroscopic and microscopic features as described by Gaddeyya et al. (2012).

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

Non biofilm formers = OD $\leq$ OD_{cut}
Weak biofilm formers = OD < $OD \leq 2 \times ODcut$
Moderate biofilm formers = 2× OD_{cut}< $OD \leq 4 \times OD_{cut}$
Strong biofilm formers = OD> 4 × 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) were swabbed unto the surface of sterile Muller Hinton agar plates, and antibiotic disc was placed on it after allowing the plates to rest for 10 minutes. Antibacterial disc used (product of Rapid Laboratories, UK) contain Augmentin (30µg), Erythromycin (5µg), Cloxicillin (5µg), Cefuroxime (30 µg), Gentamicin (10µg), Ceftazidime (30 µg), Ofloxacin (5µg) and Ceftriaxone (30 µg). The, plates containing antibiotic disc were then incubated at 37^{o}C overnight and 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 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 25^{o}C 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 antibiotic were performed using 16S rRNA sequencing, fungal isolates with > 50 % resistance were identified through sequencing of the ITS1 and ITS4 regions.

Statistical Analysis: The data obtained were analyzed as the 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. Their morphological, microscopic and biochemical identification are as shown in Table 1 and 2. The bacteria were 13 Gram positive and 4 Gram negative bacteria belonging to 6 genera namely, *Bacillus, Parapusillimonas, Bulkholderia, Pseudomonas, Azotobacter*and*Paenibacillus*(Table 1). Also, fungi isolated from these samples belong to 4 genera namely, *Fusarium, Aspergillus, Colleotrichum*and *Meyerozym*(Table 2). Considerable levels of bacteria and fungi contamination 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 and 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 with 70.58% occurrence while others have percentage occurrence 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. Akintobi et 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 that created favorable environments for spoilage microbes. The fungi's ability to produce spores and their ubiquitous nature, along with 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 found to be most prevalence among the bacterial genera with percentage occurrence of 70.58 % (Figure 1). The genus *Aspergillus* was the most prevalent fungi genera 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 due to the fact that some Gram positive bacteria, such as *Bacillus,* have spores forming ability which ensure their 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*

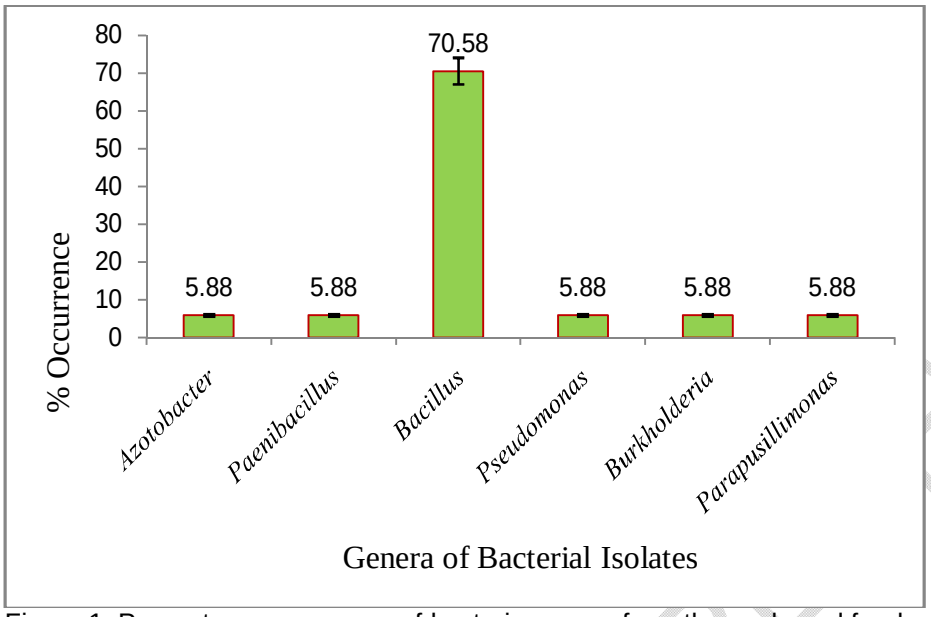

Figure 1: Percentage occurrence of bacteria genera from the packaged food samples

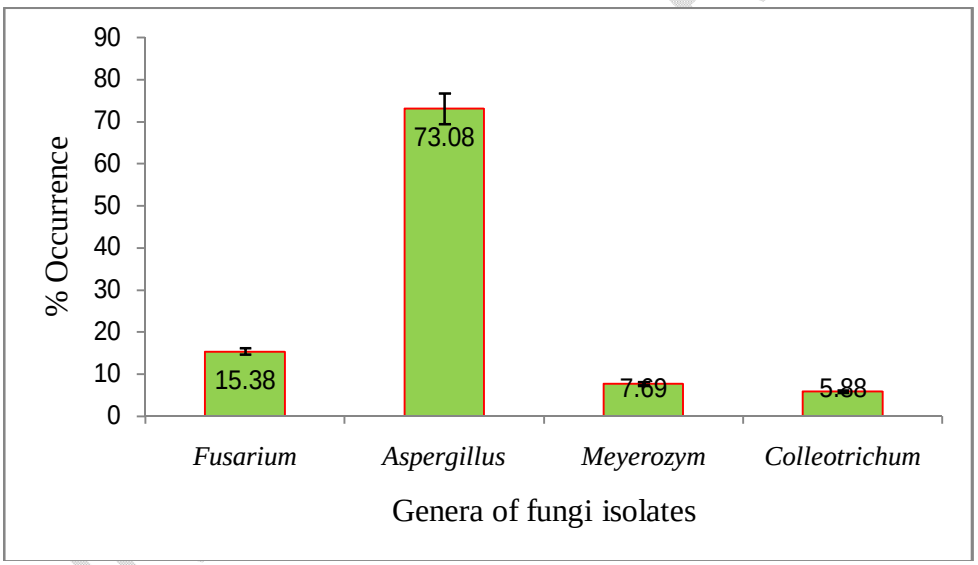

Figure 2: Percentage occurrence of fungi genera from the packaged food samples

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 the presence of multiple species at a given site, which can enhance interspecies communication and cross-feeding, thereby promoting biofilm biomass (Zupancic *et al.,* 2018).

The antimicrobial susceptibility test for all isolated bacteria and fungi were shown in Tables 4-6. From the result obtained, gentamicin and ofloxacin were active against the bacterial

isolates 100 %, erythromycin was active against the isolates at 92.31 % for all Gram positive bacteria while the least activities was 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 antibiotic used (Table 5). The spread of antibiotic resistance in bacteria is a significant public health concern in various environments. The consumption of foods without heat treatment, which may harbor 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).

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).

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

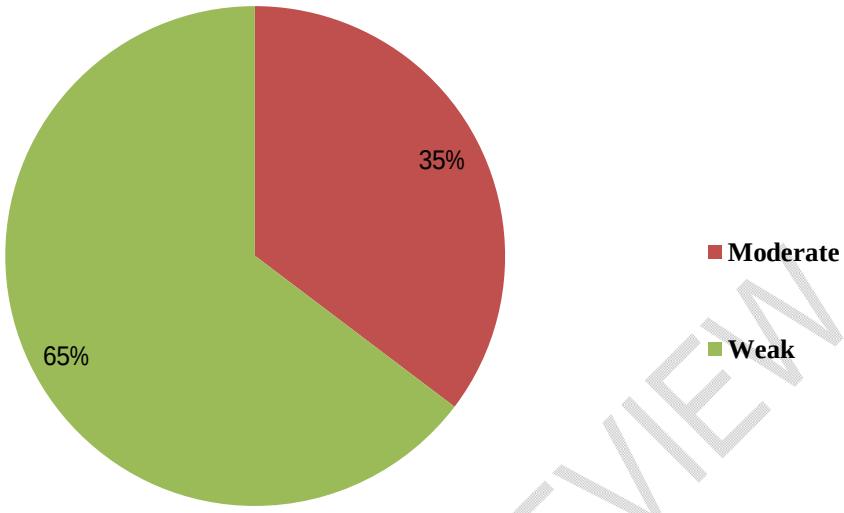

Figure 3: Percentage occurrence of biofilm producers among isolated bacteria

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 the Figure 4b, the tree was divided into 4 clades, with isolate InfC3f in the same clade with *Aspergillus oryzae* (EU680477.1) and GolN2f was 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*

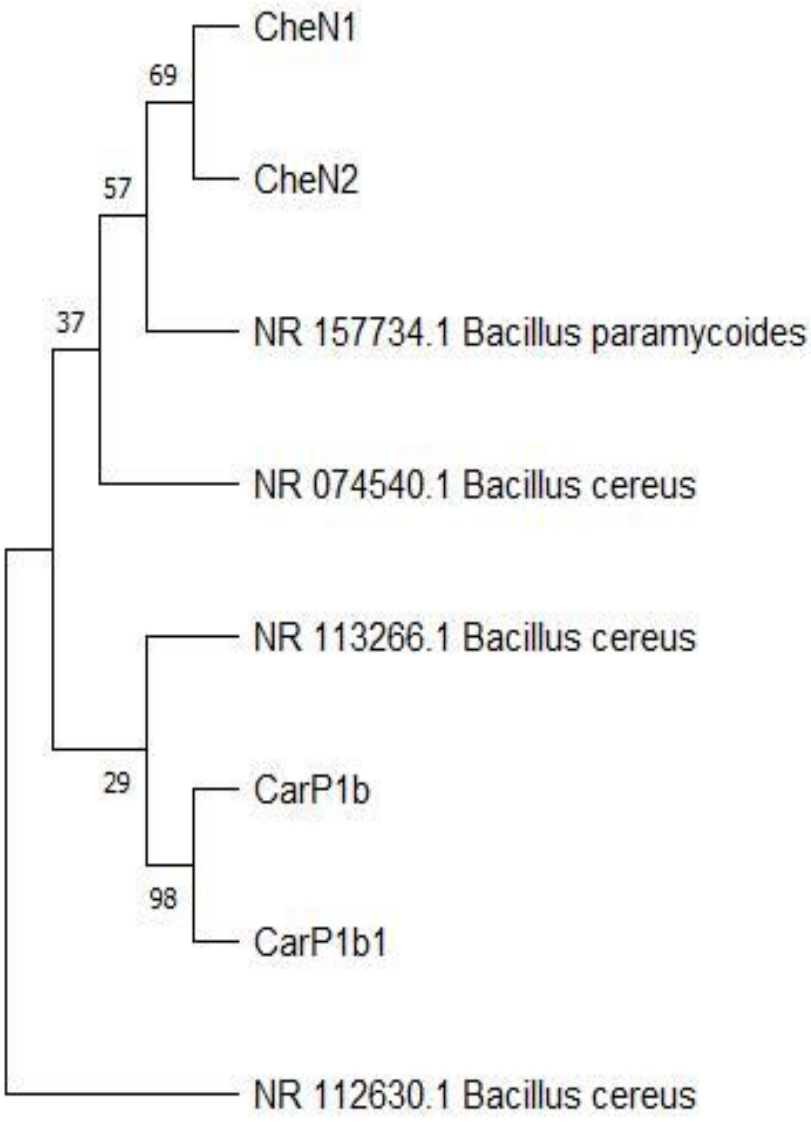

Figure 4a: Phylogenetic relatedness of selected isolated bacteria from packaged food samples

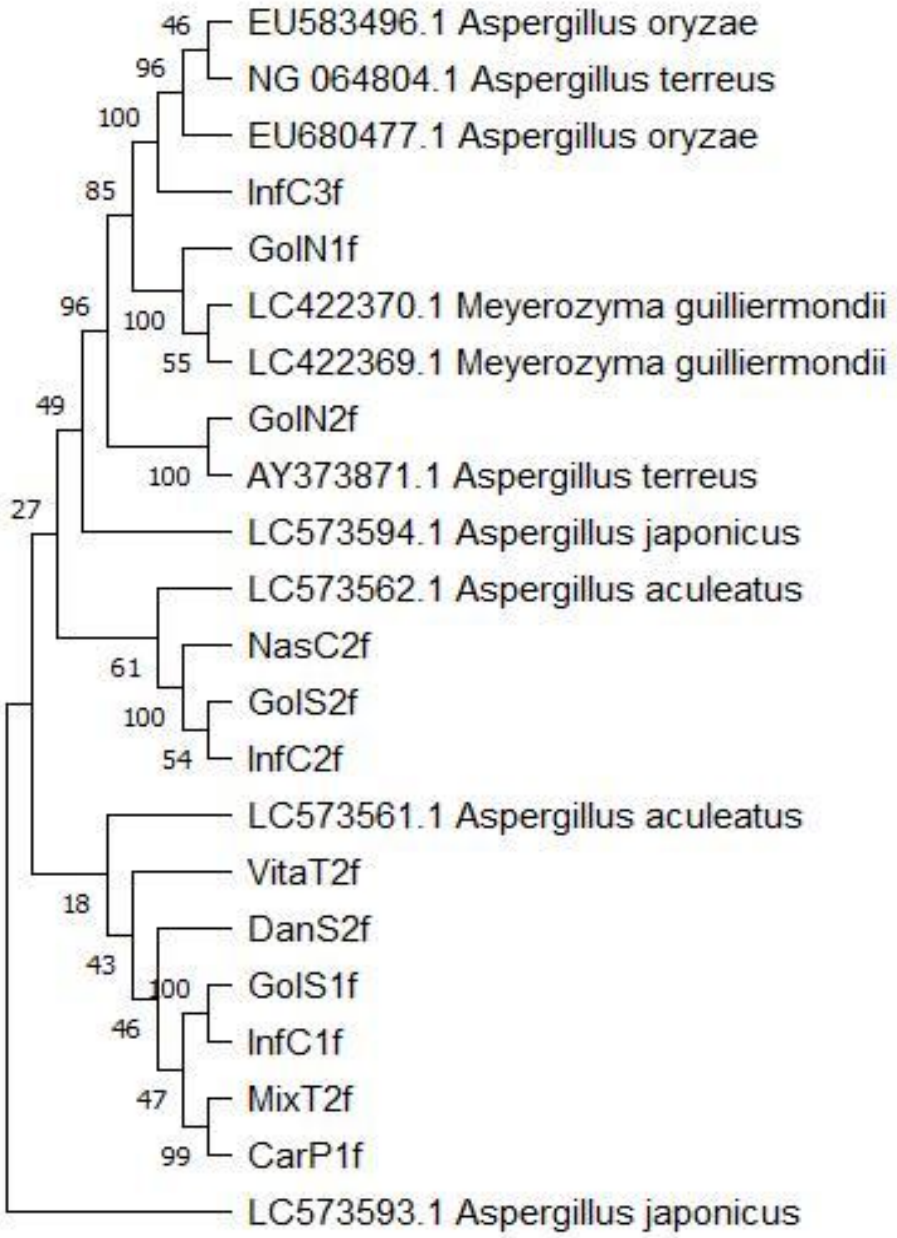

Figure 4b: Phylogenetic related of selected isolated fungi from packaged food samples

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. The predicted three dimensional protein structures for all isolated bacteria and their significant biochemical function were determined. Packaged

foods are important source that harbor different pathogenic microorganisms which may have implications on the health of the public.

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