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Article

Antimicrobial Resistance of Tomato-Spoiling Microorganisms from Ajegunle Market, Oyo Town, Oyo State, Nigeria

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Abstract

Pathogenic bacteria and fungi associated with tomato spoilage have the tendency of harbouring antimicrobial resistance genes that can pose significant health threats to consumers, hence the need to ascertain their antibiotic sensitivity. Five spoilt tomato samples were purchased from five different tomato sellers in Ajegunle market, Oyo Town, Oyo State, Nigeria. Isolation was carried out using the pour plate method. The antibiogram of selected antibiotics and antifungal drugs against the bacteria and fungi isolates was determined using the diffusion disk technique. Colony count for bacteria ranged from $1.0 \times 10^3 – 6.3 \times 10^3$ CFU/mL on Nutrient agar and $1.0 \times 10^3 – 9.6 \times 10^3$ CFU/mL on MacConkey agar while the fungal count ranged from $2.2 \times 10^5 – 5.1 \times 10^5$ CFU/mL on potato dextrose agar. The bacteria identified were *Staphylococcus aureus, Aeromonas veronii, Bacillus cereus, B. brevis, B. pumilus, B. licheniformis, B. subtilis, Corynebacterium xerosis, Corynebacterium kutscheri* and *Lactobacillus casei* with *B. licheniformis* being the most prevalent. Fungal isolates identified were: *Saccharomyces cerevisiae, Rhizopus* spp. and *Fusarium* spp. with *Saccharomyces cerevisiae* being the most prevalent. Most of the Gram-positive bacteria were resistant to Azithromycin (87.5%), Gentamycin (90.7%), Ofloxacin (93.7%) and Erythromycin (93.7%) while *Aeromonas veronii* was resistant to Gentamycin, Levofloxacin, Ofloxacin and Nitrofurantoin at 50% respectively, while *Fusarium* species showed resistance against Griseofulvin. The bacterial isolates' multi-antibiotic resistance (MAR) index ranged from 0.3 to 0.8. The resistance of the isolated microorganisms to commercial drugs could be risky to public health.

Keywords: Spoilt tomatoes, Ajegunle market, Bacteria, Fungi, Antibiotics sensitivity.

1. Introduction

Tomato (*Solanum lycopersicum* L.) which belongs to the *Solanaceae* family, is a pulpy edible popular berry and climacteric fruit that can be eaten raw or cooked [1, 2]. It has low sugar content, making them less sweet when compared to other fruits [3] and constitutes daily meal of every household [4]. It is eaten raw in salads, cooked as a vegetable, for making stew and soup, used as an ingredient in various dishes and sauces [5, 6]. Tomato juice, paste and powder are few of its domestic by-products while syrup, vitamin C, and puree are among its industrial by-products [4]. Tomato as one of the most nutritive fruits is rich in vitamins A, B, C, and E; carbohydrates, proteins, lipids, fibers, potassium and also contains a lot of lycopene, which has several health benefits such as preventing atherosclerosis, minimizing the risk of cancer and cardiovascular disease [6, 7, 8, 9, 10]. The rich nutritional content and medicinal properties of tomatoes and its products make them globally significant [11]. However, access to a ready market is a big challenge when dealing with highly perishable crops like tomatoes in most developing countries. Tomatoes have high water content, making them more prone to microbial deterioration especially by bacteria and fungi among others [8, 10]. Microbial attacks have mostly caused postharvest loss of tomatoes because of poor physiological and environmental factors [10]. Diseased and healthy tomato fruits reportedly showed similarity in their proximate composition, as there was only a difference in their moisture and crude protein contents [12]; however, consumption of spoilt or diseased tomatoes can cause food poisoning.

Economic hardship in Nigeria makes spoilt tomatoes appealing to many people for consumption because they are cheaper and come in larger quantities compared to fresh and whole tomatoes. However, the consumption of spoilt tomatoes could increase the risk of food poisoning and also cause grave health issues such as liver cancer [13]. Therefore, this research isolated and characterized different bacteria and fungi from spoilt tomatoes from Ajegunle market in Oyo and also investigated the antibiotic and antifungal sensitivity of the isolated microorganisms.

2. Materials and Methods

2.1 Sample collection, preparation of materials and media

Spoilt tomatoes were purchased from five different tomato sellers at Ajegunle market in Oyo. The samples were placed in sterile polythene bags and transported to the departmental laboratory for analyses. Nutrient agar (NA), MacConkey agar and potato dextrose agar (PDA) were prepared according to the manufacturer's instructions.

2.2 Isolation of bacteria and fungi from spoilt tomato samples

Five samples of spoilt tomatoes were transferred into a sterile mortar containing 10 mL of sterile distilled water and ground with a sterile pestle to dislodge the microorganisms present in the tomato samples. The aliquot obtained from the spoilt tomato solution sample was used in inoculation. One milliliter of dilution 10^{-3} and 10^{-5} of the sample was inoculated into a properly labelled sterile Petri dish using the pour plate technique. The NA plates were allowed to set and the plates were incubated in an inverted position for 24 hours at 37ºC. Discrete colonies that developed after incubation were counted and enumerated as colony-forming unit (CFU/mL) after multiplying with the dilution factor. Colonies from the primary plates were aseptically picked with a sterile inoculating needle and transferred into a nutrient agar plate, with a streaking technique such that discrete colonies appear at the ends of streaked lines after incubation. The sub-cultured plates were incubated at 37°C for 24 hours. Discrete colonies from the sub-cultured plates were aseptically transferred, streaked on slant and incubated for another 24 hours at 37°C. The number of distinct colonies present per plate was counted and the colony forming unit per milliliter (CFU/mL) was recorded [13].

The pour-plate method was used for the isolation of fungi on PDA. After the serial dilution, one milliliter of dilution 10^{-3} and 10^{-5} of the sample was inoculated into properly labelled sterile Petri dishes. The PDA plates were allowed to set and the plates were incubated in an inverted position at 28±2°C for five days. Colonies that developed after incubation were counted and enumerated as colony forming unit (CFU/mL) after multiplying with the dilution factor. Colonies from the primary plates were aseptically picked with a sterile inoculation needle, transferred into a freshly prepared sterile PDA (with 1mL streptomycin to inhibit bacteria growth) using the spread plate method and incubated for 3-5 days at 28±2°C [13].

2.3 Characterization of bacteria isolates from spoilt tomatoes

The morphology of isolates was determined by examining the macroscopic characteristics of the colonies like their form, consistency, margin etc. Gram staining [14] and endospore staining [15] were done.

2.4 Identification of bacteria isolates from spoilt tomatoes using biochemical tests

Catalase test [15], oxidase test [16], Voges Proskauer (VP) test [17], methyl red test [18], citrate utilization test [19], growth on 6.5% NaCl [20], growth at 55ºC [14], starch hydrolysis test [21], sugar fermentation test [22], triple sugar iron (TSI) test [14], coagulase test [14], indole production test [14], and motility test [14] were done.

2.5 Characterization and identification of fungal isolates from spoilt tomatoes

The macroscopic and microscopic examinations were carried out by observing the colony features on the plate which include colour, texture, pigment etc. [8].

2.6 Antibiotic susceptibility testing of bacterial isolates from spoilt tomatoes

McFarland standard (0.5) was prepared [23]. A sterile swab was dipped into the bacterial suspension (standardized inoculum) and it was pressed over the tube to reduce excesses and it was streaked over Mueller-Hinton agar. The antibiotic disk was placed on the surface of the agar; it was inverted and incubated at 37ºC for 24 hours, and the zones of inhibition were measured and interpreted according to Clinical and Laboratory Standard Institute (2015) [24].

2.7 Antifungal susceptibility testing of fungal isolates from spoilt tomatoes

Two hundred milligrams (0.2 g) of the commercially available antibiotic drugs (fluconazole, nystatin and griseofulvin) were separately dispensed into 10 mL of distilled water to make a stock solution of 200 mg/ml. PDA was used for the agar well diffusion and disc diffusion methods, and zones of inhibition were measured [25].

3. Results

The results of the microbial count from spoilt tomatoes from five different sellers in Ajegunle market, Oyo using nutrient agar is shown in Table 1. The spoilt tomatoes from the first seller (A) had 3.2 x 10^5 CFU/mL while those from the fifth seller (E) had 1.0 x 10^6 CFU/mL. Table 2 shows the microbial count using MacConkey agar; sample A had 2.0 x 10^6 CFU/mL while sample E had 3.8 x10^5 CFU/mL. Table 3 shows the microbial count of the fungi using PDA; sample A had 5.1 x 10^5 CFU/mL while sample E had 2.2 x 10^5 CFU/mL. Moreover, a total of 32 bacterial isolates and 16 fungal isolates were used for further studies. Table 4 shows the colonial and morphological features of the bacteria isolates. Table 5a shows the biochemical characterization of Gram-positive rod while Table 5b shows the biochemical characterization of Gram-negative rod and Gram-positive cocci isolated from the spoilt tomatoes. The ten bacteria identified were *Bacillus licheniformis, B. subtilis, B. brevis, B. pumilus, B. cereus, Corynebacterium xerosis, C. kutscheri, Lactobacillus casei, Staphylococcus aureus* and *Aeromonas veronii*.

Table 1: Total bacterial count of spoilt tomatoes from Ajegunle market, Oyo on Nutrient Agar

Samples	10^{-3} (CFU/mL)	10^{-5} (CFU/mL)
A	1.2 x 10^3	3.2 x 10^5
B	2.0 x 10^4	1.0 x10^5
C	6.2 x 10^3	5.2 x 10^3
D	1.2 x 10^3	1.0 x 10^4
E	5.0 x 10^4	1.0 x 10^6

CFU = colony forming unit; mL = milliliter.

Bacillus licheniformis had the highest occurrence of 41% while *L. casei, B. subtilis* and *S. aureus* had the lowest frequency of 3.1% (Figure 1). For the fungi isolates, *Saccharomyces cerevisiae* had the highest frequency (62.5%) while *Fusarium* species was the least (12.5%) (Figure 2). The activity of the antibiotics on the bacteria isolates from the spoilt tomatoes is shown in Table 6. *Bacillus licheniformis* had the highest multi-antibiotic resistance (MAR) index range 0.5 - 0.8, *C. xerosis* had 0.4 - 0.5 while *C. kutscheri* had 0.6

- 0.7. The MAR index was 0.7 for *B. cereus*, *B. pumilus*, *B. brevis*, *B. subtilis* and *S. aureus*; 0.6 for *L. casei* while 0.3 for *A. veronii*.

Table 2: Total bacterial count of spoilt tomatoes from Ajegunle market, Oyo on MacConkey Agar

Samples	10^{-3} (CFU/mL)	10^{-5} (CFU/mL)
A	1.4×10^2	2.0×10^6
B	1.2×10^2	1.2×10^4
C	1.0×10^3	7.4×10^5
D	9.6×10^3	3.2×10^5
E	9.6×10^3	3.8×10^5

CFU = colony forming unit; mL = milliliter.

Table 3: Total fungal count of spoilt tomatoes from Ajegunle market, Oyo on Potato Dextrose Agar

Samples	10^{-3} (CFU/mL)	10^{-5} (CFU/mL)
A	4.5×10^3	5.1×10^5
B	2.8×10^3	2.8×10^4
C	2.2×10^3	4.7×10^5
D	2.3×10^2	2.2×10^5
E	2.3×10^2	2.2×10^4

CFU = colony forming unit; mL = milliliter.

Table 4: Colonial and morphological features of bacteria isolated from spoilt tomatoes from Ajegunle market, Oyo

S/N	Isolate code	Margin	Colour	Elevation	Texture	Shape
1	$A3^{1NA}$	Filiform	Cream	Flat	Moist	Filamentous
2	$A3^{2NA}$	Undulate	Cream	Cream	Moist	Irregular
3	$A3^{4NA}$	Entire	White	Raised	Slimy	Circular
4	$A3^{5NA}$	Undulate	White	Flat	Dry	Irregular
5	$A4^{1Mac}$	Entire	White	Raised	Slimy	Circular
6	$A5^{1NA}$	Filiform	White	Raised	Dry	Filamentous
7	$A5^{2Mac}$	Filiform	White	Raised	Dry	Filamentous
8	$A5^{3NA}$	Entire	Cream	Raised	Moist	Circular
9	$B3^{Mac}$	Entire	Translucent	Raised	Shiny	Circular
10	$B3^{1NA}$	Undulate	Cream	Flat	Dry	Circular
11	$B3^{2NA}$	Filiform	Filiform	Flat	Dry	Filamentous
12	$B3^{3NA}$	Undulate	Translucent	Raised	Dry	Irregular
13	$B5^{1NA}$	Filiform	Translucent	Flat	Moist	Filamentous
14	$B5^{1\ Mac}$	Entire	Translucent	Raised	Shiny	Circular
15	$B5^{2NA}$	Filiform	Translucent	Flat	Dry	Filamentous
16	$B5^{2\ Mac}$	Filiform	Translucent	Flat	Dry	Filamentous
17	$B5^{4NA}$	Filiform	Translucent	Raised	Dry	Filamentous
18	$C3^{Mac}$	Entire	Cream	Raised	Moist	Circular
19	$C3^{1Mac}$	Entire	Cream	Raised	Moist	Circular
20	$C3^{1NA}$	Entire	Cream	Raised	Dry	Circular
21	$C3^{2NA}$	Entire	Translucent	Raised	Dry	Circular
22	$C5^{1Mac}$	Undulate	Cream	Raised	Moist	Irregular
23	$C5^{2Mac}$	Entire	Pink	Raised	Moist	Circular
24	$C5^{1NA}$	Undulate	Cream	Raised	Moist	Irregular
25	$C5^{2NA}$	Entire	Cream	Flat	Shiny	Circular
26	$D3^{Mac}$	Entire	Pink	Raised	Moist	Circular
27	$D3^{1Mac}$	Entire	Cream	Umbonate	Shiny	Irregular
28	$E3^{1Mac}$	Entire	Cream	Raised	Moist	Circular
29	$E3^{2Mac}$	Lobate	Translucent	Pulvinate	Dry	Irregular
30	$E5^{1Mac}$	Entire	Opaque	Opaque	Shiny	Circular
31	$E5^{2Mac}$	Filiform	Translucent	Flat	Moist	Rhizoid

A, B, C, D, E = tomato samples from the first, second, third, fourth and fifth sellers, respectively; 1 – 5 = sample representatives; Na = Nutrient agar; Mac = MacConkey agar.

Table 5a: Biochemical characterization of gram-positive rod bacteria from spoilt tomatoes from Ajegunle market, Oyo

S/N	Isolate code	Gram reaction	Cellular morphology	Catalase	Endospore	Starch hydrolysis	Citrate	6.5% NaCl	Growth at 55ºC	MR	VP	Glucose	Mannitol	Arabinose	Cell diameter (µm)	Probable organism
1	$A3^{1NA}$	+	Rod	+	-	-	-	+	+	+	+	A/NG	A/G	A/G	<1	*Corynebacterium xerosis*
2	$A3^{2NA}$	+	Rod	+	+	+	+	+	+	+	+	A/G	A/G	A/NG	>1	*Bacillus cereus*
3	$A3^{4NA}$	+	Rod	+	+	-	-	+	+	+	+	A/G	A/G	A/NG	<1	*Bacillus pumilus*
4	$A3^{5NA}$	+	Rod	+	+	+	+	+	+	+	+	A/NG	A/G	A/NG	>1	*Bacillus licheniformis*
5	$A4^{1Mac}$	+	Rod	+	+	+	+	+	-	+	-	A/NG	A/G	A/NG	>1	*Bacillus brevis*
6	$A5^{1NA}$	+	Rod	+	+	+	+	+	+	-	+	A/G	A/G	A/NG	>1	*Bacillus cereus*
7	$A5^{2Mac}$	+	Rod	-	-	+	+		+	+	+	A/NG	A/G	A/NG	1	*Lactobacillus casei*
8	$A5^{3NA}$	+	Rod	+	+	+	+	+	+	+	+	A/NG	A/NG	A/G	>1	*Bacillus licheniformis*
9	$B3^{Mac}$	+	Rod	+	+	-	+	-	+	-	-	A/NG	-	A/G	>1	*Bacillus brevis*
10	$B3^{1NA}$	+	Rod	+	-	-	+	+	+	+	+	A/G	A/G	A/G	<1	*Corynebacterium xerosis*
11	$B3^{2NA}$	+	Rod	+	+	+	+	+	+	-	-	A/G	A/NG	A/NG	>1	*Bacillus brevis*
12	$B3^{3NA}$	+	Rod	+	+	+	+	+	+	+	+	A/G	A/G	A/G	>1	*Bacillus licheniformis*
13	$B5^{1NA}$	+	Rod	+	+	-	-	+	+	-	+	-	A/G	A/NG	<1	*Bacillus pumilus*
14	$B5^{2NA}$	+	Rod	+	-	+	+	+	+	+	+	A/G	A/NG	A/G	1	*Corynebacterium kutscheri*
15	$B5^{2Mac}$	+	Rod	-	-	+	+	+	-	+	+	A/NG	A/G	A/NG	1	*Corynebacterium kutscheri*
16	$B5^{4NA}$	+	Rod	+	+	-	+	+	+	+	+	A/NG	A/NG	A/NG	>1	*Bacillus cereus*
17	$C3^{1Mac}$	+	Rod	+	+	+	+	+	+	+	+	+	A/NG	A/NG	>1	*Bacillus licheniformis*
18	$C3^{1NA}$	+	Rod	+	+	+	+	+	+	+	+	+	A/NG	A/NG	>1	*Bacillus licheniformis*
19	$C3^{2NA}$	+	Rod	+	+	+	+	+	+	+	+	A/NG	A/G	A/NG	>1	*Bacillus licheniformis*
20	$C5^{1Mac}$	+	Rod	+	+	+	+	+	+	+	+	A/NG	A/G	A/NG	>1	*Bacillus licheniformis*
21	$C5^{2Mac}$	+	Rod	+	+	+	+	+	-	+	+	A/NG	A/NG	A/NG	<1	*Bacillus subtilis*
22	$C5^{1NA}$	+	Rod	+	-	+	+	+	+	+	+	A/NG	A/NG	A/NG	1	*Corynebacterium kutscheri*
23	$C5^{2NA}$	+	Rod	+	+	+	+	+	+	+	+	A/G	A/G	A/G	>1	*Bacillus licheniformis*
24	$D3^{Mac}$	+	Rod	+	+	+	+	+	+	+	+	A/NG	A/G	A/NG	>1	*Bacillus licheniformis*
25	$D3^{1Mac}$	+	Rod	+	+	+	+	-	+	+	+	A/NG	A/G	A/NG	>1	*Bacillus licheniformis*
26	$E3^{1Mac}$	+	Rod	+	+	-	+	+	+	+	+	A/G	A/G	A/G	>1	*Bacillus licheniformis*
27	$E3^{2Mac}$	+	Rod	+	+	+	+	+	+	+	+	+	A/G	A/NG	<1	*Bacillus licheniformis*
28	$E5^{1Mac}$	+	Rod	+	+	+	+	+	+	+	+	A/G	A/G	A/NG	<1	*Bacillus licheniformis*
29	$E5^{2Mac}$	+	Rod	+	+	+	+	+	+	+	+	A/G	A/G	-	1	*Corynebacterium kutscheri*

Table 5b: Biochemical characterization of gram-negative rod and gram-positive cocci bacteria from spoilt tomatoes from Ajegunle market, Oyo

S/N	Isolate code	Gram reaction	Cellular morphology	Oxidase	Catalase	Indole	Motility	Coagulase	MR	VP	Citrate	Glucose	Mannitol	Lactose	Sucrose	Probable organism
1	A5[1Mac]	-	Rod	+	NR	+	+	NR	-	+	+	+	NR	NR	NR	*Aeromonas veronii*
2	B5[1Mac]	-	Rod	+	NR	+	+	NR	+	+	+	+	NR	NR	NR	*Aeromonas veronii*
3	C3[Mac]	+	Cocci	NR	+	NR	NR	+	+	+	+	+	+	NR	NR	*Staphylococcus aureus*

A, B, C, = tomato samples from the first, second, third, fourth and fifth sellers, respectively; 1, 5 = sample representatives; Mac = MacConkey agar; A/NG = Acid (Yellow) and no gas; A/G = Acid (Yellow) and gas; NR = Not required.

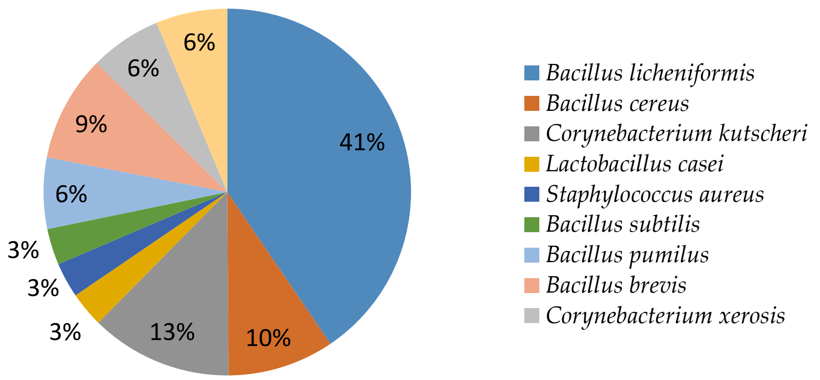

Figure 1: Occurrence of bacteria isolated from spoilt tomatoes from Ajegunle market, Oyo

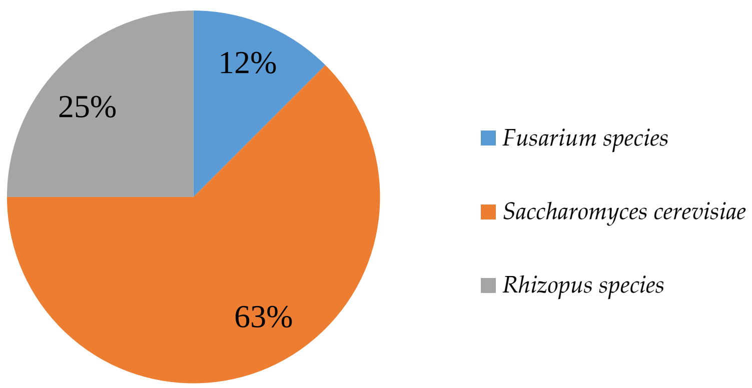

Figure 2: Occurrence of fungi isolated from spoilt tomatoes from Ajegunle market, Oyo

Table 6: Antibiotic activity on the isolated bacteria from the spoilt tomatoes from Ajegunle market, Oyo

S/N	Bacterial isolates	Resistance pattern	MAR index
1	*Corynebacterium xerosis*	CIP, IMP, CXM, AUG, CTX	0.4
2	*Bacillus cereus*	CIP, IMP, CXM, AUG, CTX, CRO, LBC, ZEM	0.7
3	*Bacillus pumilus*	CIP, IMP, CXM, AUG, CTX, CRO, LBC, ZEM	0.7
4	*Bacillus licheniformis*	CIP, CXM, AUG, CTX, CRO, ZEM	0.5
5	*Bacillus brevis*	CIP, IMP, CXM, AUG, CTX, CRO, LBC, ZEM	0.7
6	*Aeromonas veronii*	NA, CXM, CRO, CTX, ZEM, NF, AUG	0.6
7	*Lactobacillus casei*	CIP, IMP, CXM, AUG, CTX, CRO, ZEM	0.6
8	*Bacillus licheniformis*	CIP, IMP, CXM, AUG, CTX, CRO, LBC, ZEM	0.7
10	*Bacillus brevis*	CIP, IMP, CXM, AUG, CTX, CRO, LBC, ZEM	0.7
11	*Corynebacterium xerosis*	CIP, IMP, CXM, AUG, CTX, ZEM	0.5
12	*Bacillus brevis*	CIP, IMP, CXM, AUG, CTX, CRO, LBC, ZEM	0.7
13	*Bacillus licheniformis*	CIP, IMP, CXM, AUG, CTX, CRO, LBC, ZEM	0.7
14	*Bacillus pumilus*	CIP, IMP, CXM, AUG, CTX, CRO, LBC, ZEM	0.7
15	*Corynebacterium kutscheri*	CIP, IMP, CXM, AUG, CTX, CRO, ZEM	0.6
16	*Aeromonas veronii*	CTX, ZEM, ACX, AUG	0.3
17	*Corynebacterium kutscheri*	CIP, IMP, CXM, AUG, CTX, CRO, ZEM	0.6
18	*Staphylococcus aureus*	CIP, IMP, CXM, AUG, CTX, CRO, LBC, ZEM	0.7
19	*Bacillus subtilis*	CIP, IMP, CXM, AUG, CTX, CRO, LBC, ZEM	0.7
20	*Corynebacterium kutscheri*	CIP, IMP, CXM, AUG, CTX, CRO, GN, ZEM	0.7
21	*Bacillus licheniformis*	CIP, IMP, CXM, AUG, CTX, AZN, CRO, LBC, ZEM	0.8
22	*Bacillus licheniformis*	CIP, IMP, CXM, AUG, CTX, CRO, LBC, ZEM	0.7
23	*Bacillus licheniformis*	CIP, IMP, CXM, AUG, CTX, AZN, CRO, LBC, ZEM	0.8
24	*Bacillus licheniformis*	IMP, CXM, AUG, CTX, CRO, ZEM	0.5
25	*Bacillus licheniformis*	CIP, IMP, CXM, AUG, LBC, ZEM	0.5
26	*Corynebacterium kutscheri*	CIP, IMP, CXM, AUG, CTX, CRO, ZEM	0.6

CIP = Ciprofloxacin; IMP = Imipenem; AUG = Amoxicilin Clavulanate; CTX = Cefuroxine; AZN = Azithromycin; CRO = Ceftriaxone Sulbactam; ERY = Erythromycin; GN = Gentamycin; LBC = Levofloxacin; ZEM = Cefixime; OFX = Ofloxacin; GN = Gentamycin; NA = Nalidixic acid; CXM = Cefuroxine; CTX = Cefotaxime; ACX = Ampicllox; IMP = Imipenem; NF = Nitrofurantoin; MAR = Multi Antibiotic Resistance.

Figures 3 and 4 respectively show the resistance pattern of Gram-positive and Gram-negative bacteria isolated from the spoilt tomatoes. Most of the Gram-positive isolates were resistant to Azithromycin (87.5%), Gentamycin (90.7%), Ofloxacin (93.7%) and Erythromycin (93.7%). However, seven of the antibiotics showed resistance against the microbes; Levofloxacin (68.7%), Ceftriaxone Sulbactam (90.6%), Ciprofloxacin (96.8%), Imipenem (96.8%), Cefuroxine (96.8%), Cefixime (96.8%) and Amoxicilin Clavulanate (100%). In Gram-negative bacteria (*A. veronii*), Gentamycin, Levofloxacin, Ofloxacin and Nitrofurantoin showed 50% resistance against the microbes while Nalidixic acid, Cefuroxine, Ceftriaxone Sulbactam, Cefotaxime, Cefixime, Ampicllox and Amoxicilin Clavulanate were 100% resistant to *A. veronii*.

The colonial and morphological features of fungi isolates are shown in Table 7. The antibiotic susceptibility of *Fusarium* species to fluconazole, nystatin and griseofulvin is shown in Table 8. In the agar well diffusion method, the zone of inhibition was 11 mm for Fluconazole but there was no zone of inhibition in Griseofulvin; however, in the disc diffusion method, Griseofulvin shows 6 mm. The same zone of inhibition (8 mm) was recorded for Nystatin in the two methods.

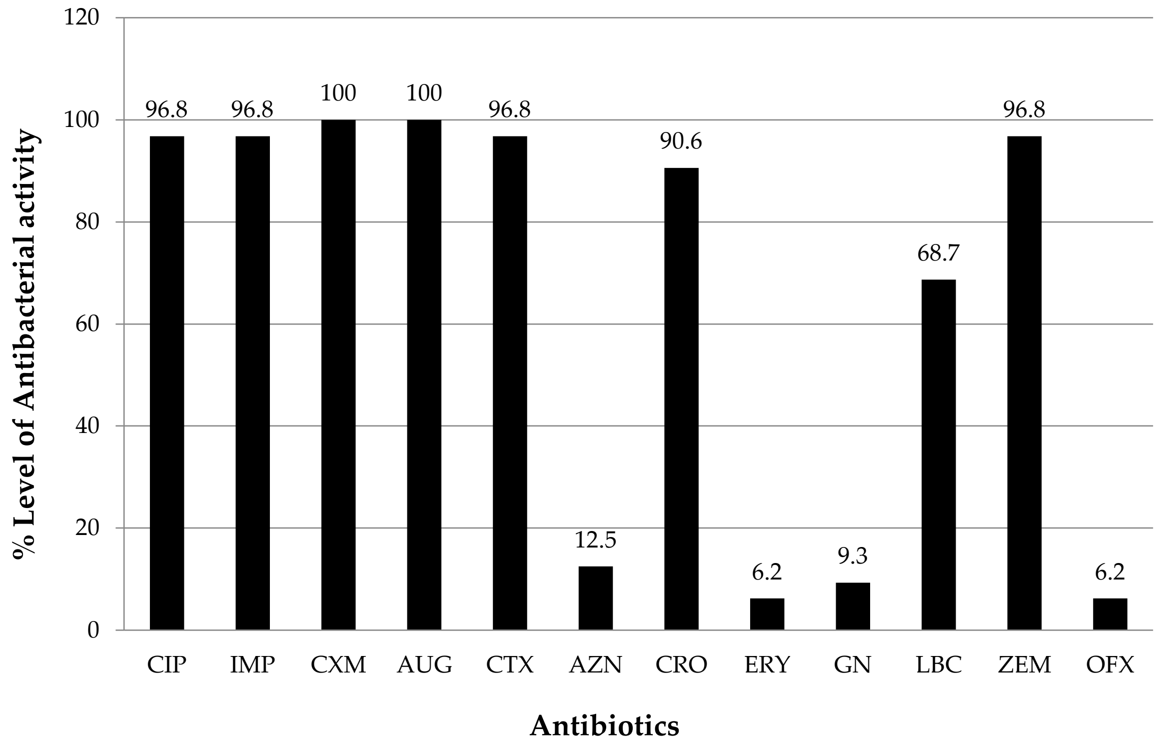

Figure 3: Resistance pattern of gram-positive bacteria isolated from spoilt tomatoes from Ajegunle market, Oyo. *CIP = Ciprofloxacin; IMP = Imipenem; CXM = Cefuroxine; AUG = Amoxicilin Clavulanate; CTX = Cefotaxime; AZN = Azithromycin; CRO = Ceftriaxone Sulbactam; ERY = Erythromycin; GN = Gentamycin; LBC = Levofloxacin; ZEM = Cefixime; OFX = Ofloxacin.*

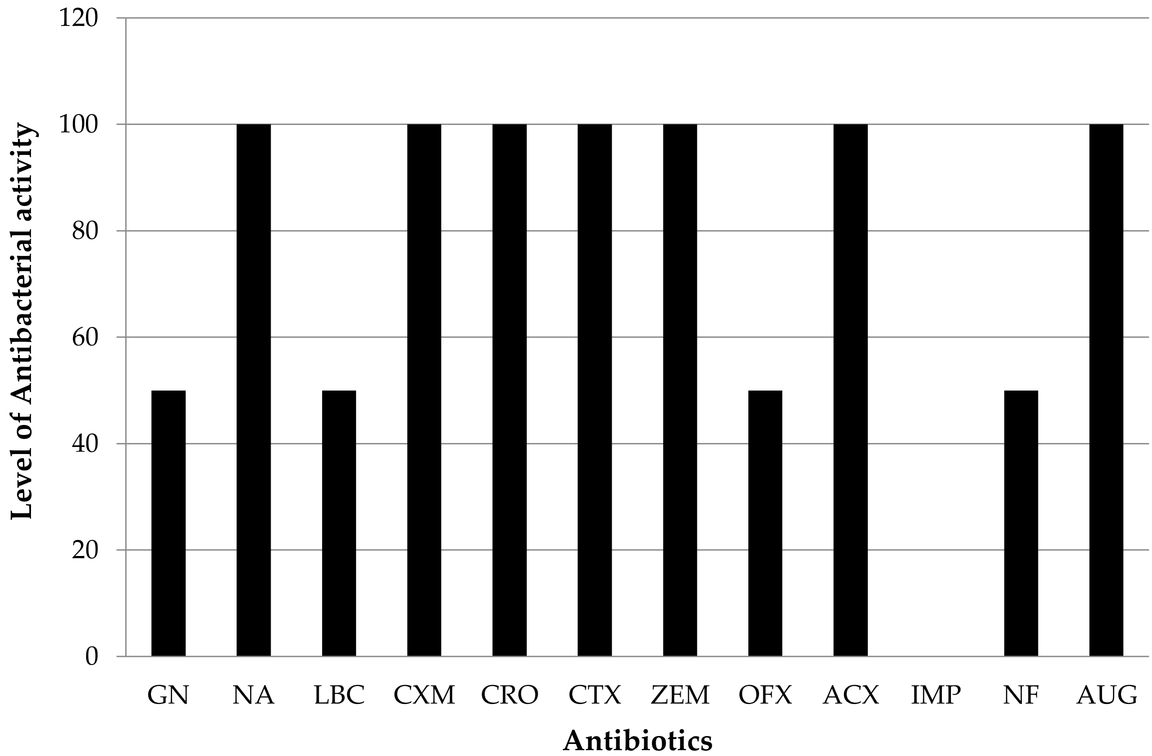

Figure 4: Resistance pattern of gram-negative bacteria isolated from spoilt tomatoes from Ajegunle market, Oyo. *GN = Gentamycin; NA = Nalidixic acid; LBC = Levofloxacin; CXM = Cefuroxine; CRO = Ceftriaxone Sulbactam; CTX = Cefotaxime; ZEM = Cefixime; OFX = Ofloxacin; ACX = Ampicllox; IMP = Imipenem; NF = Nitrofurantoin; AUG = Amoxicilin Clavulanate*

Table 7: Colonial and morphological features of fungal isolates from spoilt tomatoes from Ajegunle market, Oyo

S/N	Isolate code	Colour	Opacity	Texture	Growth rate	Microscopic features	Probable organism
1	$A3^1$	Cream	Translucent	Dry, smooth colonies, irregular in shape	Slow growth	Budding yeast cells, oval/ cocci in shape	*Saccharomyces cerevisiae*
2	$A3^2$	Cream	Translucent	Dry, smooth colonies, irregular in shape	Rapid growth around the plate	Budding yeast cells, cocci in shape, two to three ascus	*Saccharomyces cerevisiae*
3	$A3^3$	Cream	Opaque	Dry colonies, irregular	Heavy growth on the surface of the plate	Spherical cells packed in clusters	*Saccharomyces cerevisiae*
4	A5	White	Opaque	Wooly/ Cottony	Rapid growth within three days	Colourless sickle-shaped conidia, with distinct foot cells divided by several cross walls, branching septate hyphae	*Fusarium* species
5	$B3^1$	Cream	Translucent	Dry colonies	Rapid growth around the plate	Oval shape, budding yeast cells	*Saccharomyces cerevisiae*
6	$B3^2$	Cream	Translucent	Dry colonies	Rapid growth around the plate	Oval shape, budding yeast cells	*Saccharomyces cerevisiae*
7	$B3^3$	Cream	Opaque	Copious cottony and aerial mycelia growth with dark globules	Colonies were fast growing and sporulating	Dark sporangium containing spores, a large collumella and a root-like rhizoids	*Rhizopus* species
8	C3	Cream	Opaque	Copious cottony and aerial mycelia growth with dark globules	Colonies were fast growing and sporulating	Dark sporangium containing spores, a large collumella and a root-like rhizoids	*Rhizopus* species
9	$C5^1$	Cream	Translucent	Dry, smooth colonies, irregular in shape	Slow growth	Budding yeast cells, oval/ cocci in shape, three to four ascus	*Saccharomyces cerevisiae*
10	D3	Cream	Opaque	Copious cottony and aerial mycelia growth with dark globules	Colonies were fast growing and sporulating	Dark sporangium containing spores, a large collumella and a root-like rhizoids	*Rhizopus* species
11	$D3^4$	Cream	Opaque	Copious cottony and aerial mycelia growth with dark globules	Colonies were fast growing and sporulating	Dark sporangium containing spores, a large collumella and a root-like rhizoids	*Rhizopus* species
12	$D5^1$	Cream & white	Opaque	Dry and round colonies	Slow growth	Oval shape, budding yeast cells	*Saccharomyces cerevisiae*
13	$D5^2$	Cream	Opaque	Dry and irregular colonies	Slow growth	Oval shape, budding yeast cells	*Saccharomyces cerevisiae*
14	E3	Cream	Opaque	Copious cottony spread across the surface of the plate	Colonies were fast growing and sporulating	Budding yeast cells, oval/ cocci in shape	*Saccharomyces cerevisiae*
15	$E3^1$	Cream	Opaque	Wooly/Cottony	Rapid growth within three days	Colourless sickle-shaped conidia, with distinct foot cells divided by several cross walls, branching septate hyphae	*Fusarium* species
16	$E5^3$	Cream, round, dry, cottony, translucent	Opaque	Dry, Cottony and round colonies	Rapid growth	Budding yeast cells, oval/ cocci in shape, three to four ascus	*Saccharomyces cerevisiae*

A, B, C, D, E = tomato samples from the first, second, third, fourth and fifth sellers, respectively; 1 – 5 = sample representatives.

Table 8: Antifungal susceptibility of *Fusarium* species isolated from spoilt tomatoes from Ajegunle market, Oyo

Method	Nystatin	Fluconazole	Griseofulvin
	Zones of inhibition (mm)		
Disc diffusion	8	6	6
Agar well diffusion	8	11	-

Cork borer value (8 mm) is deducted; - means no zone.

4. Discussion

The type and number of microorganisms on any food substance depends on the nutritional composition supporting their growth. Pathogenic attacks cause tomatoes to have a relatively short shelf life [26], and a reduction in their market values and nutritional qualities [6]. In this research, the Gram-positive and Gram-negative bacteria isolated from spoilt tomatoes from Ajegunle market in Oyo were *Corynebacterium xerosis, C. kutscheri, Bacillus cereus, B. subtilis, B. pumilus, B. brevis*, *B. licheniformis, Lactobacillus casei, Staphylococcus aureus* and *Aeromonas veronii*. Similar to this work, Wogu and Ofuase [27] isolated *B. subtilis* and *S. aureus* from spoilt tomatoes while Chinedu and Enya [28] reported *B. subtilis* and *B. cereus* from spoilt tomatoes. Contrarily, Wogu and Ofuase [27] reported *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Proteus mirabilis* from spoilt tomatoes in Benin City; also, Ekundayo *et al*. [13] reported *Proteus* spp., *Salmonella* spp., *Klebsiella* spp., *Shigella* spp. and *Escherichia coli* in spoilt tomatoes from three markets in Ondo Town, but these microbes were not found in the spoilt tomato samples from Ajegunle market in Oyo.

Moreover, *B. licheniformis*, *C. kutscheri* and *B. cereus* were the predominant bacteria in this study; however, Obeng *et al*. [29] reported *Klebsiella* sp., *Enterobacter* sp. and *Citrobacter* sp. as the predominant bacteria isolated from spoilt tomatoes. Also, *Escherichia coli* and *Salmonella* sp. from spoilt tomatoes were reported by Samaila [6], but were absent in this research. *Bacillus subtilis* and aforementioned bacteria were isolated from spoilt tomatoes in this study but Ogofure and Ologbosere [12] reported *B. subtilis*, *Serratia marcescens*, *Leclercia adecarboxylata*, *Pectobacterium carotovorum* and *Salmonella enterica* from molecular characterization of bacterial isolates from tomatoes. The presence of all these bacteria in spoilt tomatoes reported from different places could be from poor hygienic practices of the farmers and sellers.

For fungal isolates, *Saccharomyces cerevisiae*, *Fusarium* sp. and *Rhizopus* sp. were isolated in this study. However, isolation of *S. cerevisiae*, *Fusarium* sp., *Aspergillus* sp. and *Penicillium* sp. from spoilt tomato was reported by Wogu and Ofuase [27]; *F. oxysporum*, *A. niger*, *R. stolonifer* and *Alternaria alternata* were reported from spoilt tomatoes by Okolo *et al*. [10]; *R. stolonifer*, *A. flavus* and *Candida tropicalis* were among the fungi reported from spoilt tomatoes by Danaski *et al*. [26]; *Penicillium* sp., *A. niger* and *A. flavus* from spoilt tomatoes were reported by Samaila [6] while *Rhizopus* sp., *Alternaria* sp., *Penicillium* sp. and *Aspergillus* sp. were reported by Ekundayo *et al*. [13]. Fungi are usually found in the soil; their spores are air-borne and thus can infect exposed tomato fruit. Microbial contamination of tomatoes decreases their market demand and reduces their shelf life, leading to loss and wastage of products [8].

The Gram-positive isolates were resistant to four of the antibiotics (Azithromycin, Gentamycin, Ofloxacin and Erythromycin) but susceptible to seven antibiotics (Levofloxacin, Ceftriaxone Sulbactam, Ciprofloxacin, Imipenem, Cefuroxine, Cefixime and Amoxicilin Clavulanate). Likewise, *Aeromonas veronii* (Gram-negative isolate) was resistant to four antibiotics (Gentamycin, Levofloxacin, Ofloxacin and Nitrofurantoin) but susceptible to seven antibiotics (Nalidixic acid, Cefuroxine, Ceftriaxone Sulbactam, Cefotaxime, Cefixime, Ampicllox and Amoxicillin Clavulanate). All the eight Gram-positive bacteria and *Bacillus subtilis* were resistant to Gentamicin by 90.7% (that is, 9.3% susceptibility), while Ogofure and Ologbosere [12] reported 100% susceptibility of *B. subtilis* to Gentamicin. The resistance may be associated with the genetic state of the bacterial species.

High level of poverty forces low income earners to look for alternate means of survival, thus buying spoilt tomatoes. However, the presence of bacteria with antibiotic resistance associated with the spoilt tomatoes sampled in this study highlights the potential risk of buying spoilt tomatoes. Therefore, the consumption of spoilt tomatoes should be avoided while fresh ones should be thoroughly washed with clean water before consumption.

5. Conclusion

Ten bacterial species and three fungal species were isolated and identified from spoilt tomato samples from Ajegunle market, Oyo; which could be risky to consumers' health as shown by their antimicrobial resistance to some of the selected antibiotics and antifungal drugs. Moreover, genetic studies of the isolated antimicrobial-resistant microorganisms from the spoilt tomatoes might be informative for antimicrobial drug development.

References

1. FAOSTAT (2019). Available at: http://www.fao.org/faostat/en/#home. Accessed April 15, 2019.
2. Li, S., Chen, K. and Grierson, D. (2019). A critical evaluation of the role of ethylene and MADS transcription factors in the network controlling fleshy fruit ripening. *New Phytol*. 221, 1724-1741.
3. André, S., Vallaeys, T. and Planchon, S. (2017). Spore-forming bacteria responsible for food spoilage. *Research in Microbiology* 168 (4): 379 – 387.
4. Olanrewaju, T. O., Durojaiye, L. O., Wobo, I. G. and Abudu, S. (2023). Performance evaluation of an automatic tomato paste-making machine, *FUOYE Journal of Engineering and Technology (FUOYEJET)* 8 (2): 131 – 135.
5. Onuorah, S. and Orji, M. U. (2015). Fungi associated with the spoilage of post-harvest Tomato fruits sold in major markets in Akwa, Nigeria. *Universal Journal of Microbiology Research* 3 (2): 11 – 16.
6. Samaila, S. (2023). Identification and isolation of microorganisms responsible for spoilage of tomatoes (*Lycopersicon esculentum*) fruit and phytochemical analysis of the fruit in Maiduguri. *International Journal of Pure and Applied Science Research* 13: (1) 50 – 58.
7. Hazewindus, M., Haenen, G. R., Weseler, A. R. and Bast, A. (2014). Protection against chemotaxis in the anti-inflammatory effect of bioactives from tomato ketchup. *PLoS ONE*. 9 (12): e114387.
8. Obunkwu, G. N., Dike, K. S. and Nwkwasi, G. E. (2018). Isolation and identification of microbial deteriogens of fresh tomatoes stored at ambient temperature. *Microbial Research Journal International* 26 (1): 1 – 8.
9. Ali, M. D., Sina, A. A. I., Khandker, S. S., Neesa, L., Tanvir, E. M., Kabir, A., Khalil, M. I. and Gan, S. H. (2020). Nutritional composition and bioactive compounds in tomatoes and their impact on human health and disease: A Review. *Foods* 10 (1): 45.
10. Okolo, J. C., Igborgbor, J. C., Eze, E. M., Ogu, G. I. and Jonah, G. U. (2022). The shelf life of tomato fruits (*Solanum lycopersicum* L.) treated with extracts of two medicinal plants: *Azadirachta indica* and *Vernonia amygdalina*. *International Journal of Environment* 11: 2.
11. Terna, T. P., Anyam, R. W., Ekefan, E. J., Bem, A. A. and Okoro, J. K. (2019). Isolation and identification of fungi associated with soils and diseased tomato plants on farmers' fields in Benue state Nigeria. *FUDMA Journal of Sciences* 3 (2): 275 – 287.
12. Ogofure, A. G. and Ologbosere, A. O. (2023). Microbiological and proximate properties of healthy and diseased/spoilt (broken) tomatoes (*Lycopersicum esculentum* L.) sold in open markets in Benin City: public health implications. *J. Mater. Environ. Sci*., 14 (4): 395 – 409.
13. Ekundayo, E. A., Olapade, C. A., Ekundayo, F. O., Anuoluwa, I. A. and Adewoyin, H. O. (2024). Microbial assessment of spoilt tomatoes (*Solanum lycopersicum* Linn) being sold in some markets in Ondo City, Nigeria. *ABUAD International Journal of Natural and Applied Sciences* 4 (1): 9-17. https://doi.org/10.53982/aijnas.2024.0401.02-j.
14. Mbajiuka, S. C. and Emmanuel, E. (2014). Isolation of microorganisms associated with deterioration of Tomato and pawpaw. *International Journal of Current Microbiology and Applied Sciences* 3 (5): 501-512.
15. Tille, P. M., author. Bailey and Scott's Diagnostic Microbiology. St. Louis, Missouri: Elsevier, 2014.
16. Sandle, T. (2016). Microbial identification. *Pharmaceutical Microbiology*, pp.103 – 113. https://www.sciencedirect.com/science/article/pii/B9780081000229000098.
17. Reiner, K. (2010). Catalase test protocol. *American Society for Microbiology* 2016, 1 – 6.
18. Jesumirhewe, C., Ogunlowo, P. O., Olley, M., Springer, B., Allerberger, F. and Werner, R. (2016). Accuracy of conventional identification methods used for *Enterobacteriaceae* isolates in three Nigerian hospitals. Department of Pharmaceutical Microbiology, Igbinedion. Peer j; e2511.
19. McDevitt, S. (2009). Methyl Red and Voges-Proskauer Test Protocols. *American Society for Microbiology* 2016, 1 – 9.
20. Egbebi, O. A. and Muhammad, A. A. (2016). Microbiological analysis of ready-to-eat suya meat sold in Owo, Ondo State, *International Journal of Innovative Biochemistry and Microbiology Research* 4 (2): 11 – 15.
21. Rahman, S. S., Siddique, R. and Tabassum, N. (2017). Isolation and identification of halotolerant soil bacteria from coastal Patenga area. *BMC Res Notes*. 10: 531
22. Lal, A. and Cheeptham, N. (2012). Starch Agar Protocol. *American Society for Microbiology* 2016, 1 – 11.
23. Reiner, K. (2012). Carbohydrate Fermentation Protocol. *American Society for Microbiology* 2016, 1 – 10.
24. Hudzicki, J. (2009). Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. *American society for Microbiology* 2016, 1 – 23.
25. CLSI, *Performance Standards for Antimicrobial Disk Diffusion Tests. Approved Standard*, CLSI documents MO2, Clinical and Laboratory Standards Institutes, Wayne, PA, USA, 12th edition, 2015.

26. Oghenejobo, M., Uvieghara, K. E. and Omughele, E. (2013). Antimicrobial activities and phytochemical screening of the leaf extract of *Mitracarpus scaber*. *Sch. Acad. J. Biosci*. 1 (6): 227-230.
27. Danaski, A. I., Shugaba, A., Milala, M. A., Ndirmbula, J. B. and Gidado, A. (2022). Isolation, identification and pathogenicity study of the microbes causing tomato post-harvest spoilage in Maiduguri Metropolis, Maiduguri, Nigeria. *Nigerian Journal of Biochemistry and Molecular Biology* 37 (4): 303 – 313.
28. Wogu, M. D. and Ofuase, O. (2014). Microorganisms responsible for the spoilage of tomato fruits, *Lycopersicum esculentum*, sold in markets in Benin City, Southern Nigeria. *Scholar's Academic Journal of Bioscience*, 2: 459-466.
29. Chinedu, S. M., Enya, E. (2014). Isolation of Microorganisms associated with deterioration of tomato (*Lycopersicon esculentum*) and pawpaw (*Carica papaya*) fruits. *Int. J. Curr. Microbiol. App. Sci*.; 3 (5):501-512.
30. Obeng, F. A., Gyasi, P. B., Olu-Taiwo, M. and Ayeh-kumi, F. P. (2018). Microbial assessment of tomatoes (*Lycopersicon esculentum*) sold at some central markets in Ghana. *BioMed Research International* 2018: 7.

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