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Research article

Antibiotic sensitivity of bacterial and fungal isolates from tomato (*Solanum lycopersicum* L.) fruit

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Abstract: Decayed ripened tomato fruit contaminated with spores and toxins with relatively heat resistant could poised food poisoning in humans and animals. This research investigated the effect of antibiotic sensitivity of fungi and bacteria isolated from tomato (*Solanum lycopersicum*) fruit in Osogbo markets, Nigeria. One hundred decayed fruit of tomato were procured from three main markets (Igbonna, Oja Oba and Sabo) within the metropolis. Fungi and bacteria were cultured on Sabourand dextrose, MacConkey and Tomato juice agar media. Eight species of bacteria (*Pseudomonas aeruginosa, Bacillus cereus, Bacillus subtilis, Proteus mirabilis, Salmonella typhi, Escherichia coli, Klebsiella aerogenes* and *Staphylococcus aureus*) and six fungi (*Rhizopus stolonifer, Fusarium* spp., *Mucor* spp., *Aspergillus niger, Saccharomyces cerevisiae* and *Penicillium* spp.) were isolated and characterized. Fungal isolates were highly virulent compared with bacteria in the decayed tomato fruit. Sabo market had the most prevalence fungi and bacteria isolates, while Igbonna and the Oja−Oba markets followed in that trend. *Mucor* spp. and *Bacillus subtilis* exhibited the highest fungal and bacterial counts of 42×10^4 cfu g^{-1} each in the Sabo market. Chloramphenicol was the most suitable antibiotic for controlling both micro flora. Except *B. subtilis*, varied degrees of antibiotic sensitivities and resistances were observed on all the bacteria. Technological improvement of harvesting, packaging, handling, storage and preservation could reduce tomato fruit losses and invariably enhance shelf life and quality.

Keywords: Decayed tomato - Bacteria - Fungal isolates - Antibiotics.

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a berry, annual, short−lived herbaceous plant of the Solanaceae family. It is usually sprawls on the ground, and could reach about 1−5 m height (Wogu & Ofuase 2014). It has a weak woody stem covered with glistering yellow to reddish glandular hairs, rarely vine over other plants. The leaves are between 10 and 25 cm long with 5–9 leaflets on the petioles, which are odd and pinnate. Each leaflet is about 8cm long with serrated margins. Flowers are yellow from 1–2 cm with fine and pointed lobes on its corolla (Ijato *et al.* 2011). The fruit is edible with a smooth epicarp, and varies in shape and size. Immature fruit is green and becomes yellow or bright red as it ripens (Chinedu & Enya 2014). Tomato plant is cultivated in the savannah agro-ecological zone of Nigeria during cropping season and dry season under furrow irrigation. The plant usually produces higher yield and better fruit qualities with minimal foliar diseases under irrigation compared to those cultivated during the cropping season.

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Tomato fruit ranks 7th as the most important staple crop worldwide, after wheat, maize, rice, soybeans, cassava and potatoes, with production estimate of approximately 160 million tonnes, cultivated on 4.8 million hectares in the year 2011 (FAOSTAT 2011, Ogunbanwo *et al.* 2014). The fruit is consumed as vegetable, dietary supplement, eaten raw as salad and for garnishing assorted cooked food or condiment, contributing to a healthy well−balanced diet. It is also valuable in the food industries (Fatima *et al.* 2015, Bello *et al.* 2016a). Ripened raw tomato fruit of 100 g constituents are carbohydrate 4 g, energy 75 kg (18 k), dietary fiber 1 g, sugar 2.6 g, fat 0.2 g, vitamin C (22%, 13 mg), protein 1g, and water 95 g (Ijato *et al.* 2011). Nutritionally, the fruit contains calcium, niacin, flavonoids, lycopene, beta-carotene, derivatives of hydroxycinnamic acid, high amount of water and vitamins, specifically A, C, and E which are very vital in metabolic activities of humans (Gerszberg *et al.* 2015). The deep red coloration of the fruit has been attributed to lycopene, a form of carotenoid pigment with a powerful antioxidant that protects humans against diabetes, cardiovascular diseases and anti-cancer as well as preventing blood clotting (Wu *et al.* 2011, Murray 2012, Raiola *et al.* 2014, Abdul−Hammed *et al.* 2015).

High pH (4.9−6.5), water and nutrient contents enhance microbial growth such as bacteria and fungi, which degrade the nutrients through enzymes production (Trias *et al.* 2008, Matthew 2011, Ogunbanwo *et al.* 2014), and heighten spoilage susceptibility, thereby reducing the nutritional and market values. Contamination of tomato fruit by microbes is due to poor handling during the production chain, transportation, distribution, marketing and storage (Akinyele & Akinkunmi 2012). Environmental factors such as temperature, frost and rainfall constituted adverse effects on quality of the fruit and their storage shelf life (Akinyele & Akinkunmi 2012). Besides the damage to the fruit, microbial infections poise potential health hazards to animals and humans, as some of the organisms are pathogenic, producing toxins capable of causing diseases such as diarrhoea, gastroenteritis, respiratory infections and meningitis, if ingested (Barth *et al.* 2009).

The Centre for Disease Control and Prevention estimated 76 million cases of food borne diseases yearly, and the etiology is predominantly of microbial origin (Wokoma 2008). Ghosh (2009) reported that fungi were more virulent than bacteria in tomato fruit spoilage. The main tomato diseases in the forest and savanna ecologies of Nigeria are *Aspergillus niger*, *Pseudomonas solanacearum*, *Sclerotium rolfsii and Fusarium oxysporum* (Ogunbanwo *et al.* 2014). The need for an elaborate study of contaminating pathogens of the tomato fruit becomes essential, considering tomato fruit as a ready−to−eat food with minimal processing or eaten raw and can possess serious threats to food safety (Ofor *et al.* 2009). Similarly, the high price of fresh ripened tomato fruit sold in the Nigerian markets is a major concern. A relatively cheaper spoiled fruit consumed by the poor contain microbial infectious diseases. Based on these, a study was conducted to characterize and determine antibiotic sensitivity of fungi and bacteria isolated from tomato (*Solanum lycopersicum* L.) fruit in Osogbo markets, South Western Nigeria.

MATERIALS AND METHODS

Sample collection

One hundred decayed fruit of tomato were procured from the three main markets of Igbonna, Oja Oba and Sabo markets in Osogbo, Nigeria. The samples were collected with hand gloves into sterile polythene bags aseptically and immediately conveyed for analysis in the laboratory.

Isolation, cultural and morphological identification of fungi

A scalpel sterile blade was employed to cut off a segment of the tomato fruit samples of about 3−5 cm. The samples were stored in a clean chamber at room temperature for nine days. In the Petri dishes, the emerged fungi isolated aseptically were placed on the sterilized Potato Dextrose Agar and then incubated at 25°C for 6 days, as described by Amusa *et al.* (2002). The related pathogens were isolated using morphological and cultural characteristics by comparing with the culture that was collected from the seed health pathology laboratory, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, as identified by the International Mycological Institute, CABI Bioscience, Egham, UK (Amusa *et al.* 2002).

Isolation and morphological identification of bacteria

The fruit samples were cultured by swabbing the cut interior aseptically and they were plated on 2.7 mL Tomato juice and 4.5 mL MacConkey broth. The microbial growth detected as turbidity in the broth was thereafter sub-cultured on Cysteine Lactose Electrolyte Deficient agar (CLED) and incubated at 37°C for 24 hours (Amusa *et al.* 2002). Tentative classifications of isolates were conducted by gram staining, Oxidase and

motility tests as well as sub-cultures were carried out on CLED. These include deep yellow and opaque colonies of *S. aureus*, mucoid yellow to whitish blue colonies of *Klebsiella* spp., greenish blue or blue colonies of *P. aeruginosa*, greenish colour colonies of *Proteus* spp. and yellow coloured colonies of lactose fermenting *E. coli.* (Hi−Media manual 2003). Substantiation of bacterial pathogens diversity were carried out by sub-culturing on Xylose Lysine Deoxycholate agar (XLD agar; M1108, Himedia, Cetrimide Agar for *Pseudomonas* spp., Mannitol salt agar for *Staphylococcus aureus* and *Salmonella–Shigella*−agar (S−S agar M108, Himedia, Mumbai) for *Salmonella*, Mumbai). MacConkey agar was also sub-cultured for additional enteric pathogens and different biochemical tests. Furthermore, the swab samples were emulsified in normal saline (0.85% sodium chloride), covered and assessed for protozoa.

Biochemical Tests

Biochemical tests such as catalase, sugar, indole and coagulase tests were carried out as described by Holt *et al.* (1994). Enzymatic assays for catalase and coagulase as well as tests for constituent sugar and indole were also carried out, as described by Holt *et al.* (1994).

Assessment of colony form units (CFU ml^{-1})

Bacterial strains were earlier preserved on nutrient agar at 4°C (Noor *et al.* 2013). After two hours of growth in 5 ml of the nutrient broth as pre−culture, the OD of the culture broth was measured at 600 nm (OD_{600}) and thereafter adjusted to 0.1. Thirty (30) µL was introduced into three different sets of 30 ml of the nutrient broth and incubated at 37°C. These were then shaken at 0, 100 and 200 rotations per minute (rpm) (Noor et al., 2013). At 12−hour interval, the growths were monitored by measuring OD_{600}. The colony form units (CFU)/ml were estimated by counting colonies on nutrient agar at 24 hours (Noor *et al.* 2013).

Antibiotic sensitivity test

The standardized disc diffusion method was adopted by applying the zone size interpretation chart to evaluate bacteria sensitivity on the selected antibiotics. The sensitivity tests were carried out following M2−A6 disc diffusion method using nutrient agar, as suggested by the National Committee for Clinical Laboratory Standards (NCCLS 1997). Identified bacteria were cultured overnight with nutrient agar, then suspended in a sterile physiological saline (0.9%w/v NaCl) to achieve an equivalent of 0.5 McFarland turbidity standards. A sterile, non−toxic cotton swab was plunged into the standardized innocula and used to spread the entire surface of Mueller Hinton agar plates (NCCLS 2002). Antibiotic discs (PS003G−VE, polytes Laboratory, Enugu, Nigeria) were position aseptically on the surface of the agar plates using sterilized forceps, and thereafter incubated at 37^{o}C for 24 hours, while zones of inhibition were measured and classified as either sensitive or resistant, as described by CLSI (2012). The antibiotics screened were Ampicilin (Sam−Ace Ltd., Akoda−Ede, Nigeria, 30µg mL^{-1}), Ampiclox (Me−cure Industries Ltd., Lagos, Nigeria, 30µg mL^{-1}), Ciprofloxacin (Tuyil Pharmaceutical Industries Ltd., Ilorin, Nigeria, 10µg mL^{-1}), Amoxicillin (Michelle Laboratories Ltd., Enugu, Nigeria, 30µg mL^{-1}), Streptomycin (North China Pharmaceutical Co., Ltd., Shijiangzhuang, China, 10µg mL^{-1}), Septrin (Tuyil Pharmaceutical Industries Ltd., Nigeria, 30µg mL^{-1}), Augumentin (Tuyil Pharmaceutical Industries Ltd., Ilorin, Nigeria, 10µg mL^{-1}), Chloramphenicol (Maxheal Pharmaceuticals, India, 30µg mL^{-1}), and Gentamycin (Furen Pharmaceutical Group Co., Ltd., Henan, China, 10µg mL^{-1}).

RESULTS AND DISCUSSION

Fungal culture

Morphological and cultural features of fungal isolates revealed *Fusarium* spp., *Rhizopus stolonifer*, *Aspergillus niger*, *Saccharomyces cerevisiae*, *Mucor* spp., *and Penicillium* spp. in the decayed tomato fruit samples (Table 1). This corroborates with the findings of several researchers (Ghosh 2009) who reported the presence of *Fusarium* spp., *Penicillium* spp. and *Aspergillus niger* as the prominent isolates in tomato fruit spoilage. These results also agree with the reports of an independent researcher, Akinmusire (2011) who reported that *Rhizopus stolonifer*, *Mucor* spp. and *Fusarium oxysporum*as as major spoilage organisms of ripe tomato fruit from selected markets in Maiduguri, North Eastern Nigeria. On the other hand, Oyemaechi *et al.* (2014) isolated *Aspergillus phoenicis* in Onitsha, Nigeria.

In the decayed tomato samples assessed, fungi isolates were more prevalent than bacteria. However, there were wide variations in microbial population, with *Mucor* spp. being predominant (28 isolates), followed by *A. niger* (11 isolates) and *Penicillium* spp. (8 isolates) (Fig. 1). These results corroborate previous studies of

Ogunbanwo *et al.* (2014) and Fatima *et al.* (2015) who reported that fungi were more prevalent compared to bacteria in Nigeria. Amongst the three major markets investigated however, Sabo market had the highest fungi and bacteria isolation rates, while Igbonna and the Oja−Oba markets followed in that order (Table 2). *Mucor* spp. had the highest average fungal value of 42×10^4 in Sabo market compared to other two markets. Wogu & Ofuase (2014) and Oyemaechi *et al.* (2014) opined that the storage conditions, poor handling, transportation, distribution and marketing practices could increase the level of microbial contamination of tomato fruit. These products are very rich in carbohydrates content and poor in the level of proteins with a pH value ranging from slightly acidic to neutral, which enhance enabling niche to several pathogenic bacteria and fungi (Trias *et al.* 2008, Ogunbanwo *et al.* 2014). The microbial spoilage of tomato fruit has also been obsereved to contain Aflatoxin which possibly being a source of potential health hazards to humans, as it produces mycotoxins which induce mycotoxicosis disease (Oyemaechi *et al.* 2014).

Table 1. Cultural and morphological characters of fungal isolates in tomato fruit samples from three major markets in Osogbo, Nigeria.

Fungal isolates	Morphological characteristics	Cultural characteristics
Rhizopus stolonifer	Hyphae were non−septate and there were enlarged condiophores at the tip, producing round vesicle−like chains.	Profuse proliferation of filamentous condiophores.
Fusarium spp.	Condiophores produced conidia in clusters or single forms. Septate hyphae with canoe−shaped macroconidia.	At first, the conidia were cottony and white, and thereafter changed to pink.
Saccharomyces cerevisiae	Hyphae were absent, but *Saccharomyces* spp. produced ascospores, particularly in the V−8 medium of acetate ascospore agar. Multilateral budding was usually rudimentary Pseudohyphae.	Very fast growing colonies with moist, flat, smooth, dull, tannish cream or glistening in color.
Penicillium spp.	From a specialized conidiogeny, chains of single−celled conidia (Ameroconidia) were produced in basipetal succession called a phialide.	Prolific production of colonies with filamentous, cottony, velvety, flat or woolly in texture.
Mucor spp.	Hyphae were non−septate and branched. Long sporangiophores with non−septate terminal spore sporangia.	Profuse proliferation of sporangiophores that covered agar surface with white fluff that shortly turned grey. The reverse side was white.
Aspergillus niger	Branched and septatehyphae condiophore with secondary branches. Enlarged condiophore at the tip, producing round vesicle−like chains.	Greenish, filamentous condiophore, with rapid growing of black velvety spores.

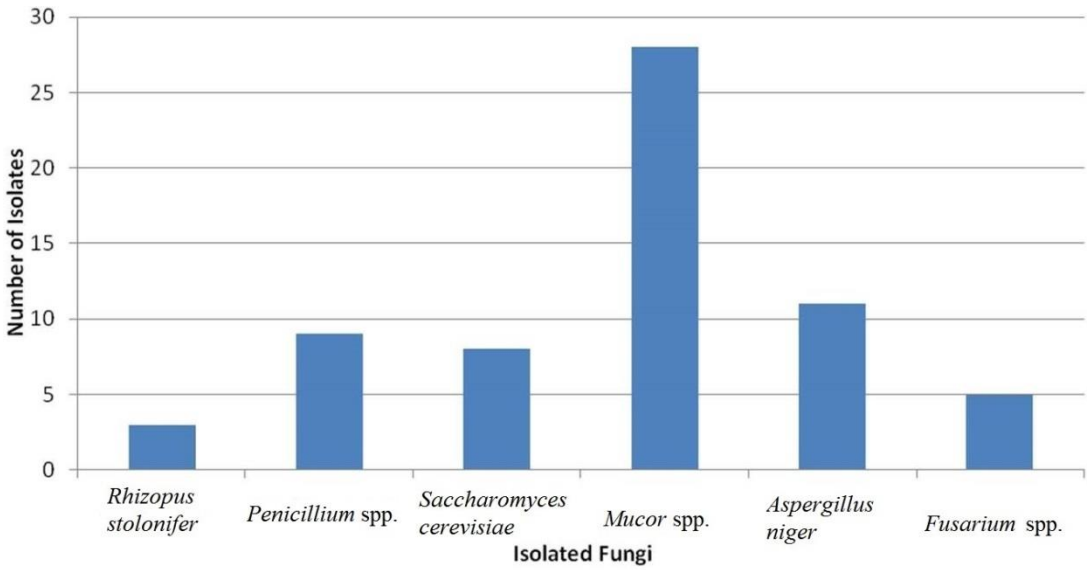

Figure 1. Distribution of fungal isolates in tomato fruit samples from three major markets in Osogbo, Nigeria.

Table 2. Mean fungal count of tomato fruits from three major markets in Osogbo, Nigeria.

Isolated fungi	Sabo	Igbonna	Oja Oba
Rhizopus stolonifer	16×10^4	12×10^4	11×10^4
Penicillium spp.	41×10^4	31×10^4	30×10^4
Saccharomyces cerevisiae	15×10^4	10×10^4	9×10^4
Mucor spp.	42×10^4	40×10^4	38×10^4
Aspergillus niger	35×10^4	32×10^4	30×10^4
Fusarium spp.	38×10^4	30×10^4	23×10^4

Based on the incidence and characterization of bacterial isolates in tomato fruit samples (Table 3), eight probable bacteria identified were *S. aureus*, *S. typhi*, *P. mirabilis*, *P. aeruginosa*, *K. aerogenes*, *E. coli*, *B. subtilis* and *B. cereus*. The occurrence and distribution of the bacterial isolates from decayed tomato fruit samples in the three major markets of Osogbo metropolis (Fig. 2) revealed that out of 61 isolates observed, *B. subtilis* was prominent with the highest value of 30 isolates, followed by *P. aeruginosa* with 8 isolates, while *P. mirabilis and K. aerogenes* had one isolate each.

Table 3. Characterization of bacterial isolates in tomato fruit samples from three major markets in Osogbo, Nigeria.

Features	Isolate descriptions							
Cultural								
Shape	Cocci	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Margin	Smooth	Smooth	Entire	Entire	Entire	Entire	Smooth	Smooth
Color	Yellow	Creamy	White	White	White	Pink	White	White
Morphological								
Motility test	–	–	+	+	+	–	+	+
Gram reaction	+	–	–	–	–	–	+	+
Shape	Cocci	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Cell arrangement	Cluster	Single	Single	Single	Single	Single	Single	Single
Sugar fermentation test								
Lactose	–	–	–	–	+	+	–	–
Glucose	A	A	A	A	AG	AG	A	A
Biochemical test								
Indole	–	–	–	–	–	–	–	–
Oxidase	–	–	–	–	+	–	–	–
Catalase	+	–	+	+	+	+	+	+
Coagulase	–	–	+	–	–	–	–	–
Feasible bacteria	*Staphylococcus aureus*	*Salmonella typhi*	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Klebsiella aerogenes*	*Escherichia coli*	*Bacillus cereus*	*Bacillus subtilis*

Note: – = Negative, + = Positive, AG = Acid and gas production, A = Acid production only.

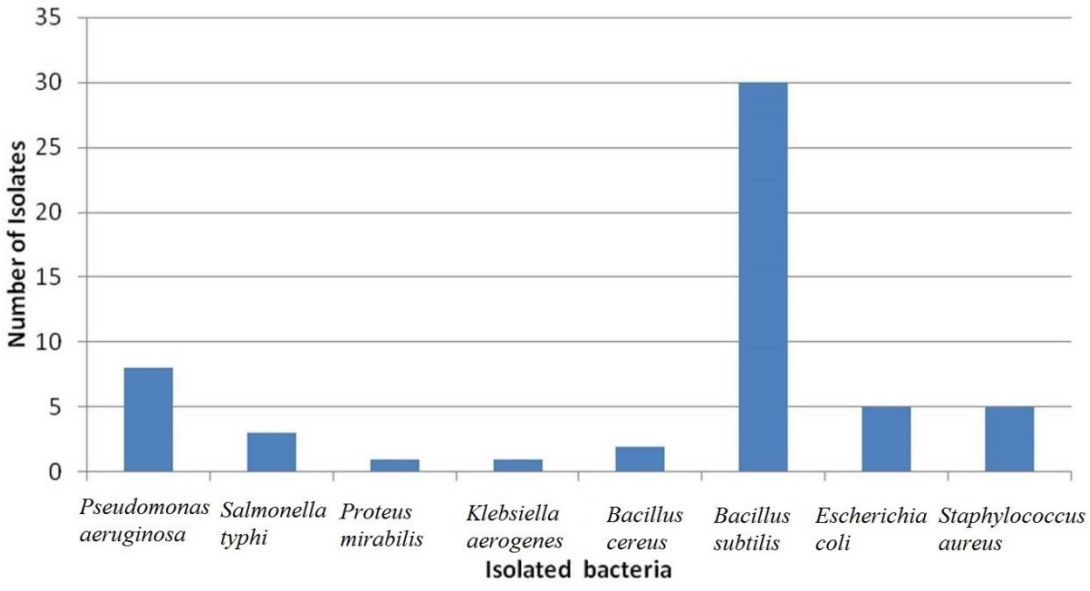

Figure 2. Distribution of bacterial isolates in tomato fruit samples from three major markets in Osogbo, Nigeria.

Regarding the three markets, Sabo market exhibited the highest bacterial and fungal counts, while by Igbonna and Oja Oba followed in that order (Table 4). *B. subtilis* was prominent with the value of 42×10^4 cfu g^{-1} in Sabo market, while the lowest count of 1×10^4 cfu g^{-1} was obtained for *B. cereus.* Observations made under this context signified that high rate of *B. subtilis* probably due to opportunistic contaminations through poor handling processes of tomato fruit. The occurrence of *S. aureus* that is usually associated with faecal matter also affirmed poor hygiene in the markets. This is consistent with the studies of Oyemaechi *et al.* (2014), who suggested that the presence of *S. aureus* in the decayed tomato fruit possibly due to contamination with organic manure and/ or faecal matter. It is noteworthy that the observations in this study negate some other researchers' reports outside Nigeria. For instance, Garg *et al.* (2013) isolated lactic acid bacteria, *Vibrio furnissii, Serratia marcescens* and *Aeromonas hydrophila* in India. The reasons adduced to these are that varied geographical and seasonal weather as well as inconsistencies in the agronomic practices (cultivation, harvesting, handling and packaging) involved in tomato production could result to these differences in the microbial isolates.

Table 4. Mean bacterial count of tomato fruit from three major markets in Osogbo, Nigeria.

Isolated bacteria	Markets/ CFU/g		
	Sabo	Igbonna	Oja Oba
Pseudomonas aeruginosa	21×10^4	11×10^4	5×10^4
Salmonella typhi	10×10^4	8×10^4	2×10^4
Proteus mirabilis	3×10^4	Nil	Nil
Klebsiella aerogenes	25×10^4	3×10^4	Nil
Bacillus cereus	1×10^4	Nil	Nil
Bacillus subtilis	42×10^4	34×10^4	15×10^4
Escherichia coli	13×10^4	7×10^4	3×10^4
Staphylococcus aureus	19×10^4	11×10^4	9×10^4

With the exception of *B. subtilis* isolates, the other seven bacteria had heterogeneous degrees of sensitivity and resistance to antibiotics, similar to the observation of Ghosh (2009) in Nigeria (Table 5). Wogu & Ofuase (2014) reported that existence of bacteria with multiple antibiotic sensitivities and resistances in the tomato spoilage indicated high risks and potential hazards on consumption. Bruises and damages inflicted on fruit

Table 5. Patterns of antibiotic sensitivity of bacterial isolates in tomato fruit samples from three major markets in Osogbo, Nigeria.

Isolated bacteria	Antibiotics									Total	
	CH	CP	AM	GE	ST	SE	AU	AX	AN		
	Markets										
	A B C	A B C	A B C	A B C	A B C	A B C	A B C	A B C	A B C	S No (%)	R No (%)
Pseudomonas aeruginosa	S R R	R R R	S S S	S R R	S S R	S R R	S S S	S S S	S S S	17(47)	19 (53)
Salmonella typhi	S R R	R R R	S S S	R R R	S R R	S R R	S S S	S S S	S S S	15 (42)	21 (58)
Proteus mirabilis	S S R	S S R	S S S	S S R	S S S	S S S	S S S	S S S	S S S	24 (67)	12 (33)
Klebsiella aerogenes	S R R	S S S	S S S	S R R	S R R	S R R	S S S	S S S	S S S	19 (53)	17(47)
Bacillus cereus	S S R	S S R	S S S	R R S	S S R	S S R	S S S	S S S	S S S	21 (56)	15 (56)
Bacillus subtilis	S S S	S S S	S S S	S S S	S S S	S S S	S S S	S S S	S S S	36 (100)	0 (0)
Escherichia coli	S R R	S S S	S S S	S S S	S S R	S S S	S S S	S S S	S S S	33 (92)	3 (8)
Staphylococcus aureus	R R R	S S S	S S S	S S S	S S R	R R R	S S S	S S S	S S S	29 (81)	7 (9)

Note: Antibiotics: CH= Chloramphenicol, CP= Ciprofloxacin, AM= Amoxicillin, GE= Gentamycin, ST= Streptomycin, SE= Septrin, AU= Augumentin, AX= Ampiclox, AN= Ampicilin; Test results: R= Resistant, S= Sensitive; Markets: A= Sabo, B= Oja Oba, C= Igbonna.

during harvest and handlings could enhance proliferation of microbes as a vehicle of infections of such damaged tissue, thereby causing fruit decay. This confirmed the assertion of Matthew (2011) that spoilage microbes often gain entry into the fruit through wounds. Ghosh (2009) also suggested that the prevalence of microbial contamination could be aggravated by poor sanitation including cross−contamination with other products in

transit. Tomato traders always display ripened tomato fruit in the open, and the heat of sun rays increase the rate of rotting. Previous researchers suggested that high temperatures may encourage deterioration of tomato fruit (Matthew 2011), and speed up the physiologic processes, leading to accumulation and sub−oxidation of metabolic by−products (Fatima *et al.* 2015). A range of temperatures between 7.2^{o}C and 10^{o}C were recommended for the storage of ripened tomato fruit, while a range of 12.8−21.1 ^{o}C is appropriate for matured green fruit. Matthew (2011) also observed that supply of tomatoes all year round may not be attainable through production alone, but an integrated approach of preservation and storage of excess at harvest could improve the shelf life. The quality of vegetables and fruit can only be maintained after harvest; thus it is absolutely imperative to harvest promptly especially at the peak quality period (Bello *et al.* 2016b). This is because overripe or immature fruit may have short shelf life in storage compared with those picked at appropriate maturity levels (Eni *et al.* 2010).

CONCLUSION

Fungi isolates were more prevalent than bacteria in the decayed tomato samples. Sabo market had the most prevalence of both fungi and bacteria isolates, while Igbonna and the Oja−Oba markets followed in that order. *Mucor* spp. exhibited the highest average fungal value in the Sabo market. Chloramphenicol was the most suitable antibiotic for controlling both microflora. Except *B. subtilis*, varied degrees of antibiotic sensitivities and resistances were observed on all the bacteria. Technological improvement of harvesting, packaging, handling, storage and preservation could reduce tomato fruit losses and invariably enhance shelf life and quality.

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