Microbial Examination of Ready-to-eat Fruit Samples (Pineapple, Pawpaw and Watermelon) from Street Vendors in Ogbomoso Market, Oyo State, Nigeria.

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Abstract

This study examined ready-to-eat fruit samples which include pineapple, pawpaw and watermelon. The items were purchased in Ogbomoso market, Oyo state, Nigeria. The microbial analysis was carried out to include isolation of microbial load, biochemical characterization and antimicrobial sensitivity test using disc diffusion method. In the results, total bacterial count was 1.06 x 10^5 (cfu/g) for pineapple, 3.95 x 10^4 (cfu/g) for watermelon and 1.04 x10^4 (cfu/g) for pawpaw. The highest coliform present was 1.4 x10^5 (cfu/g) in water melon while the least 6.26 x10^4 (cfu/g) in pineapple. In characterization of the isolates, 14.3 % are positive to citrate utilization test, 42.9 % motile, 42.9 % were positive to spore staining, 100 % glucose fermenter, fructose; maltose; lactose and sucrose utilization were 71.4 %; 85.7 %, 71.4 %, 85.7 % respectively. Antimicrobial susceptibility test highest of 15 mm in chloramphenicol against S*taphylococcus aureus*, the least was 7.5 mm in gentamicin against *Bacillus cereus* and *Lactobacillus spp*. In gram negative bacteria, the highest susceptibility was 10 mm in gentamicin against *Erwinia spp* while the lowest susceptibility was seen in nitrofurantoin against *Clostridium spp* and *Acetobacter spp* in streptomycin. The percentage susceptibility was 37.5 %, 41.7 % for gram positive and gram negative respectively. This study has established the occurrence of antibiotic resistance in ready-to-eat fruit and calls for urgent improvement in processing and handling of such foods, public awareness and training of vendors is necessary.

Keywords: Microbes; fruits; street-vendors, processing and handling

Introduction

Ready-to-eat foods are majorly fruits and vegetables that are display already prepared for immediate consumption without further processing, by hawkers or vendors along the streets or by local markets. (Oranusi and Olorunfemi, 2011; Mengistu and Tolera, 2020). Fruits are an extraordinary dietary source of nutrients, micronutrients, vitamins and fiber for humans and are thus vital for health and well-being (Jabbar *et al*. 2014; Olatunji and Afolayan, 2018). Balanced diets, rich in fruits are especially valuable for their ability to prevent deficiency diseases, examples are vitamin C and vitamin A. Nutrients from fruits are reported to reduce the risk of several diseases (Kalia and Gupta, 2006; Yahia *et al.*, 2019). However, fruits are widely exposed to microbial contaminations through contact with soil, dust, water and through handling during harvest and postharvest processing. Fruits therefore harbor a diverse range of microorganisms including pathogens (Ray and Bhunia, 2007; Ofor *et al*., 2009; Taban and Halkman, 2011; Gudda *et al.*, 2020). Differences in microbial profiles of various fruits and vegetables result largely from unrelated factors such as resident micro-flora in the soil, nonresident micro-flora via animal manures, sewage or irrigation water, transportation and handling by individual retailers (Ray and Bhunia, 2007; Ofor *et al*., 2009; Mritunjay and Kumar, 2015; Zhang *et al.*, 2020). In developing countries such as Nigeria, continued use of untreated waste-water for irrigation farming and manure as fertilizers for the production of fruits and vegetables is a major contributing factor to contaminations (Olayemi, 1997; Amoah *et al*., 2009; Shrestha *et al.*, 2018).

Over the last few years, there has been a significant increase in the consumption of sliced/ ready-to-eat fruits such as Pineapple (*Ananas* comosus); Watermelon (*Citrullus* lanatus) and Pawpaw (*Carica papaya*) in Nigeria. These ready-to-eat fruits are easily accessible, convenient, and most importantly cheaper than whole fruits and most times presented in assorted form (Gupta, and Rana, 2003, Orji *et al.*, 2016). Due to the high price of whole fruits relative to the income of the majority of people in Nigeria, the demand for ready-to-eat processed fruits is flourishing as a business. Economic factors are therefore the major reasons that make people succumb to consuming the already cut or sliced fruits (Nwachukwu *et al*., 2008).

Despite the nutritional and health benefits of this food source, outbreaks of human infections associated with the consumption of fresh or minimally processed fruits and vegetables have increased in recent years (Altekruse and Swerdlow, 1996; Beuchat, 1996, Beuchat, 2002; Oluwatoyin *et al.,* 2015; Oje *et al.*, 2018). Enteric pathogens such as *Escherichia coli* and *Salmonella* are among the greatest concerns during food-related outbreaks (Threfall *et al.*, 2000; Buck *et al*., 2003; Araújo *et al.*, 2017). Several cases of typhoid fever outbreak have been associated with eating contaminated vegetables grown in or fertilized with contaminated soil or sewage (Beuchat, 1998; FDA, 1998; Thompson and Powell, 2000; Oje *et al.*, 2018). These increases in fruits and vegetables-borne infections may have resulted from increased consumption of contaminated fruits and vegetables outside the home as most people spend long hours outside the home. In Nigeria for instance, street vending of handy ready-to-eat sliced fruit and vegetables has recently become very common and the market is thriving (Orji *et al*., 2016; Oku *et al.*, 2020).

Safe fruits and vegetables are essential to good health, to maximize the health benefits promised by adequate consumption of ready-to-eat fruits, proper washing of fruits and vegetables with clean safe water is essential for decontamination. Water treated with varying concentrations of organic acids, such as acetic, citric and sorbic acids, has been shown to reduce microbial loads on fruits and vegetables (Karapinar and Gonul 1992; Beuchat, 1998; WHO, 2006; Swallah *et al.*, 2020). There are different sources of microbial invasion of sliced produce; pathogens may invade the interior surfaces of the produce during washing, peeling, slicing, trimming, packaging, handling and marketing (Khali and Mazhar, 1994; Barro *et al*., 2007; Oku *et al.*, 2020). The use of dirty utensils, as well as the open display of street food encourages sporadic visits by flies, cockroaches, other insects, and dust (Bryan *et al*., 1992; Nelson, 2006; Orji *et al.*, 2016; Balali *et al.*, 2020). Holding of sliced fruits that requires no further processing before consumption at ambient temperatures during retail maintains the produce at optimum temperatures for proliferation/invasion by pathogenic mesophilic organisms (Muinde and Kuria, 2005; Oje *et al.*, 2018). Bacteria like *Salmonella spp.*, *Shigella spp.*, *Campylobacter spp*. and *Escherichia coli* can contaminate sliced fruits through contact with sewage and contaminated water (Fredlund *et al*., 1987; Blostein, 1991; Beuchat, 1995; Gayler *et al*., 1995; Barro *et al.*, 2006; Oje *et al.*, 2018; Zhang *et al.*, 2020). Therefore, the objective of this study was to examine microbial loads of sliced, ready-to-eat pawpaw, pineapple and watermelon samples from street vendors in Ogbomoso Market, Nigeria.

Materials and Methods

Materials

Ready-to-eat fruit samples namely pawpaw, pineapple and watermelon were used. They were purchased in Sabo market in Ogbomoso, Oyo state, Nigeria. These samples were brought to the microbiology laboratory in the new laboratory complex of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso for microbiological analysis.

Methods: Isolation of microorganisms present in the ready-to-eat-fruits

Potato Dextrose Agar (PDA), Nutrient agar (NA) and MaCconkey agar (MAC) were employed in isolation and microbial counts in this study to isolate both fungi and bacteria. All media used were prepared according to the manufacturer's specification. Isolation and identification of microorganisms were done by standard microbiological methods. Antibiotic Susceptibility Test (AST) of bacterial isolates was done with disc diffusion method for the sensitivity test (Beathy *et al*., 2004). For Anti-microbial Sensitivity Test (AST) determination CDS (Calibrated Dichotomous Sensitivity) method was used for standard interpretation of the zones of inhibition. Annular Radius ≥ 6mm = Susceptibility; ≤ 6mm = Resistant (Bell *et al*., 1975; Bell *et al*,. 2016).

Results and Discussion

In this present research work, the following organisms were isolated; *Staphylococcus aureus; Lactobacillus spp.; Bacillus cereus; Acetobacter spp.; Erwinia spp; Bacillus subtilis Bacillus spp* prevalence of these organisms has been documented by many authors (Oranusi and Olorunfemi, 2011). From ready- to- eat fruit samples analyzed, there were general bacteria, the coliform bacteria but fungi growth was negligible after four days of incubation and this indicated that the samples were free of mold growth. From the watermelon isolate; the organisms identified include *Staphylococcus aureus; Lactobacillus spp* and *Bacillus cereus.* In pineapple the following were isolated: *Lactobacillus spp.; Bacillus cereus; Acetobacter spp.* while in pawpaw *Erwinia spp; Bacillus subtilis; Bacillus spp.* were identified, several authors have indicated prevalence of similar pathogens in fruits (Oluwatoyin *et al.*, 2015; Orji *et al.*, 2016).

From the ready-to-eat fruit sample analysis as presented in Table 1; Pineapple had the highest total bacterial count of 1.06×10^5 cfu/g and the least bacterial count 1.04×10^4 cfu/g was from pawpaw. Watermelon has the highest total coliform count of 1.4×10^5 cfu/g followed by pawpaw with 1.0×10^5 cfu/g while pineapple has the lowest count of 6.26×10^4 cfu/g. The result was in a range of similar work (Orji *et al.*, 2016; Oje *et al.*, 2018). The mold growth was negligible hence effort was focused on the organisms that are of economical importance. Hence further work was on enumeration of bacterial contaminants for probable organisms.

Table 1: Microbial count of fruit samples

Sample	Total bacterial count (cfu/g)	Coliform count (cfu/g)	Fungal count (cfu/g)

Watermelon	3.95×10^4	1.4×10^5	0.001×10^3
Pawpaw	1.04×10^4	1.0×10^5	-
Pineapple	1.06×10^5	6.26×10^4	0.001×10^3

Biochemical characterization of bacteria isolates was carried out to identify the organism present as shown in Table 2. The result showed most species are rod shaped, can hydrolyze starch, majority of the organisms catalase positive, positive to capsule staining and ferment several carbohydrate type; glucose; fructose; maltose lactose, some produce gas while others produce gas and acid as shown in the table. Though the leading species of coliform bacteria like *E. coli* was not present, the attributes that implicate many of the isolates as coliform bacteria are evident such as ability to utilize a variety of substrate, ability to produce gas and acid from sugar. The characteristics listed above implicated some of the organisms are non-fecal coliforms that grow at ranges of low temperature and are an important cause of some food spoilage because of their ability to utilize different substrate; carbohydrates and other organic compounds as food for energy. Three out of the organisms are motile, three are spore staining positive (Frazier and Westhoff 2005; Achi and Madubuike 2007).

Table 2: Biochemical characterization of bacteria isolates for identification

Identity on sub.	*Lact. spp*	*Staph. a.*	*E. spp*	*B. sub.*	*B. cerius*	*C. spp*	*A. spp*	*%*
Citrate Utilization	-	+	-	-	-	-	-	14.3
Motility	-	-	+	+	+	-	-	42.9
S. S	-	-	·	+	+	+	-	42.9
Glucose	A	A	A	A	AG	AG	A	100
Fructose	AG	-	A	A	AG	A	-	71.4
Maltose	A	A	A	A	AG	AG	-	85.7
Lactose	A	AG	-	AG	-	A	A	71.4
Sucrose	A	A	A	A	A	A	-	85.7

Key -*; negative, +; positive, A; acid, AG; acid and gas, Lact. Spp. -Lactobacillus spp, Staph.. a-Staphylococcus aureus, E. spp-Erwina spp, B. sub.-Bacillus subtilis, B. cerius -Bacillus cerius, C. spp-Clostridium spp, A. spp- Acetobacter spp.,*

The probable organisms from the fruits sample are listed in Table 3, this include *Staphylococcus aureus*, *Lactobacillus spp.*, *Bacillus subtilis*, *Bacillus cereus*. *Lactobacillus strains* are rods, usually long and slender, form chains in most strains. The characteristics that make Lactobacillus important in food are the ability to ferment sugar to produce lactic acid, the attribute that may cause undesirable off flavor in fruits but may be desirable in cheese production and other dairy activities (Pahumunto *et al*., 2020). *Staphylococcus* strain is facultative; coagulase positive strains are pathogenic and may produce enterotoxin which causes food poisoning. *Bacillus* species are probably contaminants from soil, *Acetobacter* are known to produce acetic acid especially from ethyalcohol and cause sour taste in spoilt fruits especially when it is used for beverages. *Erwinia* species are common plant pathogens that cause different diseases in plants (Frazier and Westhoff 2005; Ya'ar Bar *et al*., 2021).

Table 4 presents gram positive bacteria with varying zones of inhibition, the highest zone of inhibition was seen in staphylococcus aureus on chloramphenicol with 15 mm zone. The list was 7.5 in *Bacillus cereus on gentamicin;* Lactobacillus spp on gentamicin; *Bacillus* on erythromycin respectively. All the gram positive bacteria identified in this study: *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus and lactobacillus spp* were susceptible to chloramphenicol and gentamicin antibiotics. Any antibiotic annular radius greater than 6 mm has antibiotic inhibition property according to Antibiotic Susceptibility test (AST) by the Calibrated Dichotomous Sensitivity Method (CDSM) (Bell *et al.*, 2016). All gram positive bacteria were resistant to ciprofloxacin, augmentin, amoxicillin, tetracycline, there were no zone of inhibition.

Table 3: Probable organism isolated from ready-to- eat fruits

Probable organism	Water melon	Pineapple	Pawpaw
Staphylococcus aureus	+	-	+
Lactobacillus spp.	+	+	+
Bacillus cereus	+	+	-
Acetobacter spp	-	+	-
Bacillus subtilis	-	-	+
Erwinia spp	-	-	+
Bacillus spp	-	-	+

All the gram positive bacteria with exception of *Lactobacillus spp* were susceptible to erythromycin. Table 5 showed all gram positive bacteria were resistant to ciprofloxacin, augmentin, amoxicillin and tetracycline. Only *Bacillus subtilis* was susceptible to cotrominidazo, the rest organisms were not susceptible.

Table 4: Gram positive bacteria sensitivity test

Antibiotics	Zone of inhibition (mm)			
	B. subtilis spp	*B. cereus*	*Staphy. aureus*	*Lactobacillus spp.*
Chloramphenicol	9.0	9.0	15	9.5
Gentamicin	9.5	7.5	9.0	7.5
Ciprofloxacin	-	-	-	-
Augmentin	-	-	-	-
Amoxicillin	-	-	-	-
Tetracycline	-	-	-	-
Erythromycin	8	7.5	9.5	-
Cotrominidazo	10	-	-	-

Gram negative test indicated that the highest zone of inhibition of 10 mm was seen in gentamicin against *Erminia spp*, the lowest zone of inhibition was found to be 7.5mm *clostridium spp*. against nitrofurantoin. The zones of inhibition is less than 6mm in tetracycline against the tested organisms hence no susceptibility (Bell *et al.*, 2016). Gram negative bacterial sensitivity test indicated that no

zone of inhibition was noted in ampicillin, controminidazo and colchicine against all tested gram negative isolates. *Erwinia spp*, *Acetobacter spp* were susceptible to nalidixic.
The variation in susceptibility and resistance of the isolates to different antibiotics could be attributed to source of isolates and drug resistance transfer due to indiscriminate use and uncontrolled disposal of waste (Shewmake and Dillon, 1998; Inyang, 2009; Okonko *et al*., 2009, 2010; Orji *et al.*, 2016; Ersoy *et al.*, 2017).

Table 5: Gram negative bacteria sensitivity test

Antibiotics	Zone of inhibition in mm		
	Erwinia spp.	*Clostridium spp.*	*Acetobacter spp.*
Nalidixic	8	6	8
Gentamicin	10	8	9.5
Ampicillin	-	-	-
Cotrominidao	-	-	-
Colchicine	-	-	-
Tetracycline	< 6	< 6	< 6
Streptomycin	< 6	< 6	7.5
Nitrofuranton	8	7.5	8

There are high percentage multiple drug resistance in this study and the summary table shown in Table 6 clearly indicated 62.5 % drug resistance by gram positive bacteria, 58.3 % in gram negative isolates. Emergence of multiple resistances to antibiotics by organisms has been documented by many authors (Cheesebrough, 2006; Chikere *et al*.,2008; Okonko *et al*., 2009, 2010; Medved'over, *et al*., 2017). As it was observed in this work, in which many of the isolates were resistant to most of the antibiotics used many authors have also identified prevalence microbes such as *Staphylococcus aureus*, *Lactobasillus spp*, *Bacillus subtilis* among other (Achi and Madubuike 2007; Oranusi and Olorunfemi 2011; Gajdacs *et al*., 2017). The cause may be the wrong use of antibiotics and indiscriminate disposal of drugs (WHO 2018). According to Suchitra and Lakshmidevi (2009), intensive medical therapies and frequent use of antimicrobial drugs are capable of creating resistant microbial flora. This also points to the fact that the prevalence of such multidrug resistant organisms should be checkmated by careful and strict use of antibiotics for recommended purposes only since their economic implication cannot be over emphasized (Okonko *et al*., 2010). Health implication of these ready-to-eat-fruit with multi-drug resistance in most of the bacteria isolated is a signal that urgent need for public awareness and training of food vendors is necessary.

Table 6: Antimicrobial susceptibility test summary

Number of test isolate	Number of antibiotic used	Susceptibility %	Resistant %
4 gram +	8	37.5	62.5
3 gram-	8	41.7	58.3

Conclusions

The microbial isolates found were attributed to the level of hygiene practices, handling or health of workers. The presence of some of the isolate like *Saphylococcus* strain may cause food poisoning because the organism is capable of producing enterotoxin (Medved'ova *et al.* 2017). Moisture laden fruits favored the growth of many of this contaminant and may lead to spoilage. Antimicrobial sensitivity test carried out is a means of determining the best treatment needed in case of health challenges traced to food contamination of the sampled. However, the antibiotic resistance level observed in this study calls for concern, and the cause is multidimensional; misuse of antibiotics, overuse and genetic change may be involved (WHO, 2018; Oyedele *et al.*, 2020).

Authors' Contributions

The contributions of authors to the manuscript should be specified in this section; according to the type of contribution (choosing only the appropriate ones), the authors are mentioned by initials: Conceptualization: AO and EM; Data curation: AO and AOO; Formal analysis: CA and AO; Investigation: EM and AO; Methodology: AO and GM; Project administration: AO and AOO; Resources: AO and EM; Software: AOO and EM; Supervision: GM and EB; Validation AO, GM and EM; Visualization: GM and EM; Writing - original draft: AO and EM; Writing - review and editing: AO and CA. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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