



## Microbiological contamination of ready-to-eat sliced pineapple sold in commercial parts of Ibadan, Oyo State Nigeria

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### Abstract

This study evaluated the microbiological contamination of ready-to-eat sliced pineapple sold in Apete, Sango, and University of Ibadan markets. The anaerobic plate counts for all samples exceeded international standards. A total of eighteen bacterial species were identified, with *Lactobacillus* species being the most prevalent, accounting for 35% of the isolates. Six fungal species were also isolated, with *Penicillium* species being the most common, occurring at a frequency of 50%. Antibiotic sensitivity tests revealed that Ofloxacin, Gentamycin, and Ciprofloxacin demonstrated the greatest inhibitory properties against the isolates. The high levels of microbial contamination and the presence of potential pathogens pose significant risks to public health, underscoring the importance of enhanced hygiene practices and increased consumer awareness. These findings imply that Ofloxacin, Gentamycin, and Ciprofloxacin could be effective treatments for food borne infections resulting from the consumption of contaminated ready-to-eat sliced pineapple.

**Keywords:** Sliced fruits, biofilm, microbial contamination, pathogen

### Introduction

Fresh fruits that are ready to consume are often purchased from street vendors, hawkers, or local markets and can be consumed without any additional preparation, such as cutting, peeling, or rinsing, as they have already been processed by the vendors. These fruits are prone to microbial contamination due to contact with soil, dust, water, and handling during harvest or post-harvest processing. They can harbor a diverse range of microorganisms, including both plant and human pathogens (Nguyen-the and Carlin, 1994; Carmo *et al.*, 2004) [4, 10]. The level of microbial contamination various fruits can vary significantly based on several unrelated factors, including the presence of resident microflora due to animal manures, sewage, or irrigation water, transportation, and individual retailer handling (Ray and Bhunia, 2007; Ofor *et al.*, 2009) [11].

In developing nations like Nigeria, the persistent usage of untreated wastewater and manure as fertilizer for fruit production significantly contributes to contamination (Amoah *et al.*, 2009) [1]. Despite the nutritional and health benefits associated with consuming fresh or minimally processed fruits, recent years have seen an increase in outbreaks of human infections due to contaminated produce. For instance, several cases of typhoid fever have been linked to consuming contaminated vegetables grown in soil polluted with harmful microorganisms (Benchart, 2002). Enteric pathogens, such as *Escherichia coli* and *Salmonella*, are a major concern during foodborne outbreaks (Buck *et al.*, 2003) [3]. Pathogens can invade the internal parts of fruits during various stages of production, including washing, peeling, slicing, trimming, packaging, handling, and marketing (Kalia and Gupta, 2006) [7]. Moreover, sliced fruit street vending without proper storage conditions exacerbates the problem, as it exposes the sliced fruit to flies and other disease-causing agents. Unlicensed vendors with poor education levels and inadequate training in food

hygiene often process and sell sliced or peeled fruits (Muinde and Kuria, 2005) [9].

This study focused on pineapple, a popular herbaceous plant and fruit consumed fresh or in processed form in Ibadan, Nigeria. Scientifically known as *Ananas Comosus*, it belongs to the Bromeliaceae family and contains beneficial nutrients for human health. Pineapple is composed of moisture, sugars (sucrose, glucose, and fructose), and minerals like calcium, potassium, magnesium, and manganese. This tropical delight is also rich in enzymes, bromelain, and the antioxidant vitamin C, which play crucial roles in the body's healing process (Joy and Minu, 2013) [6].

Given the increasing demand for ready-to-eat fruits and the associated health risks posed by bacterial pathogens, this study was designed to evaluate the microbiological contamination, biochemical analysis, and antibiotic susceptibility testing of isolates from ready-to-eat sliced pineapple in Apete, Sango, and the University of Ibadan (UI) markets.

### Materials and Methods

Samples were collected from different marketplaces (sources) in Ibadan, Oyo State namely, Apete, Sango and UI. The locations were selected because they are commercial hubs well known in the city. A total number of six samples of sliced pineapple (*Ananascomosus*) were purchased randomly, collected in sterile plastic and transported to the laboratory for processing.

The glassware used were soaked and washed with detergent and disinfectant, and thoroughly rinsed in water. The McCartney bottles with their content were autoclaved at 121°C for 15 minutes. The culture media such as Nutrient Agar, Eosine Methelene Blue Agar, *Salmonella Shigella* Agar, and Saboraud Dextrose Agar were all prepared according to manufacturer's specification in conical flask,

covered with cotton wool and aluminum foil, and sterilized before used.

### 1. Microbiological analysis/isolation of bacterial

Microbiological analysis was carried out using serial dilution method with the aid of weighing balance. 1.0g from each sample was obtained using sterile knife and wearing hand gloves and transferred into 9ml of sterile distilled water in McCartney bottle. The bottle was shaken thoroughly; serial dilution of the solution was done from  $10^1$  to  $10^{-10}$ . Sterilization was done by autoclaving at  $121^\circ\text{C}$  for 15 minutes.  $10^{-3}$ ,  $10^{-5}$ , and  $10^{-6}$  dilution factor was plated into sterile petri dishes labeled  $10^{-3}$ ,  $10^{-5}$ , and  $10^{-6}$  respectively using pour plate techniques and was incubated anaerobically i.e. (oxygen free environment) for 24 hours (Cheesbrough 2005) [5].

### 2. Maintenance of Pure Culture

Following the incubation period, the plates were inspected for growth, and individual colonies were chosen from those that exhibited distinct characteristics on the medium. These organisms were then placed on the inclined surfaces of their respective media and stored in the refrigerator for subsequent examination.

The colonies that formed on the plates were classified based on their morphological features, such as elevation, size, surface texture, color, opacity, and edge. A variety of biochemical tests were conducted on the isolated bacteria to facilitate their identification, which included the following

#### 2.1 Gram staining techniques

A thin smear of the bacterial of 24 hours old culture isolate was made on clean glass slide and heat fixed by flaming. Few drops of crystal violet are added to the smear for one minute. The crystal violet was washed with lugol's iodine and stained with fresh tap water and then decolorized by adding few drops of 95% alcohol until there is no more violet liquid observed (Cheesbrough 2005) [5].

#### 2.2 Catalase test

To test for the presence of catalase enzyme, 24 hours old culture was flooded with 3% hydrogen peroxide solution. Evolution of gas bubbled indicated the presence of catalase enzyme.

#### 2.3 Oxidase test

This test was carried out using Whatman number 1 filter paper by adding few drops of oxidase reagent (1% aqueous tetramethyl 1-p-phenylene diamine dihydrochloride) on the Whatman paper. The paper was smeared with different isolates using a sterile wire loop. The fermentation of a very deep purple coloration within 10 seconds indicates a positive reaction while absence of deep coloration indicated a negative reaction.

#### 2.4 Indole test

Isolate were grown in a sterile peptone broth for 24 hours. After incubation of the pure bacteria isolates, five (5) drops of Kovac's reagent (isoamyl alcohol, paradimethyl 1 aminobenzaldehyde, concentrated hydrochloric acid) was added to the broth culture. A positive reaction appears as a presence of red color in the surface alcohol layer of the broth. A negative reaction appears yellow color in the surface alcohol layer of the broth (Cheesbrough 2005) [5].

### 2.5 Coagulase test

A drop of physiological saline was placed on each end of a sterile slide, with a straight sterile wire loop, the isolated colony was picked and put on each drop on the slide then emulsify. A drop of human plasma was put on one of the suspensions and mix gently, no plasma was put on the second suspension to differentiate any granular from true coagulase clumping. A clumping of organism within 10 seconds indicate coagulase positive.

### 2.6 Sugar fermentation

This test was performed to determine the ability of different isolates to ferment sugar. The sugar used include: glucose, fructose, galactose, lactose, sucrose, and maltose. The basal medium used was peptone water and the indicator used was phenol red. 1 g of each sugar and 1 g of peptone water were dissolved in 100 ml of distilled water separately. 10 ml from the broth sugars were aspirated into appropriate test-tubes. A Durham tube were introduced into each test tube in an inverted position. The tubes were corked and sterilized for 15 minutes at  $121^\circ\text{C}$  using an autoclave. After sterilization, the tubes were allowed to cool and 24 hours old culture was introduced. The tubes were incubated for 24 hours at  $37^\circ\text{C}$ . The tubes were then examined for color changes and production of gas.

### 3. Antibiotic sensitivity test

This test was conducted to evaluate the susceptibility of each isolate to commonly available antibiotics. The disc diffusion method was employed for this assessment. Mueller Hilton agar was prepared according to the manufacturer's specifications and sterilized in an autoclave at  $121^\circ\text{C}$  for 15 minutes. The isolated organisms were introduced into the agar using a swab stick. Each antibiotic disc was aseptically placed on the surface of the plate with sterile forceps to ensure complete contact with the agar surface. The plates were incubated for 24 hours at  $37^\circ\text{C}$ . The clear zone of incubation (measured in millimeters (mm) around the antibiotic disc on the plate) indicated the susceptibility of the isolate to each antibiotic. This information was used to categorize the isolate into sensitivity and resistance isolates.

## Results and Discussion

The total anaerobic plate count for sliced pineapple sold in Sango, Apete and UI market, Ibadan as shown in Table 1 ranges from  $4.5 \times 10^{-5}$  cfu/g to  $1.1 \times 10^{-3}$ . International Microbiological standard recommends unit of bacterial contamination for food in the range of less than 10 cfu/ml of food for total bacteria count. It was observed that almost all the fruits examined had bacterial counts above the acceptable limit and it is therefore microbiologically unacceptable.

Eighteen bacterial organisms were identified from the sliced ready-to-eat pineapple, and eight of these were characterized and named, specifically: Lactobacillus species, Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Proteus, Pseudomonas, salmonella, and Shigella species. This finding is consistent with studies conducted by other researchers (Oranusi and Olorunfemi, 2011) [12]. E. coli counts in fruits are commonly used and accepted as

indicators of fecal contamination (Kalia and Gupta, 2006) [7]. *Staphylococcus aureus* could have originated from the handlers, as it is a common flora found on human skin. The morphological and biochemical properties of the bacteria are detailed in Table 2.

**Table 1a:** The total viable count of bacteria on Nutrient agar

Lab code	No. of organism	Cfug/ml
SP <sub>1</sub> (10 <sup>-3</sup> )	80	8.0 x 10 <sup>-4</sup>
SP <sub>1</sub> (10 <sup>-4</sup> )	45	4.5 x 10 <sup>-5</sup>
SP <sub>1</sub> (10 <sup>-2</sup> )	75	7.5 x 10 <sup>-3</sup>
AP <sub>2</sub> (10 <sup>-3</sup> )	20	2.0 x 10 <sup>-4</sup>
AP <sub>2</sub> (10 <sup>-2</sup> )	22	2.2 x 10 <sup>-3</sup>
SP <sub>1</sub> (10 <sup>-4</sup> )	33	3.3 x 10 <sup>-5</sup>
UP <sub>2</sub> (10 <sup>-2</sup> )	47	4.7 x 10 <sup>-3</sup>
UP <sub>2</sub> (10 <sup>-3</sup> )	32	3.2 x 10 <sup>-4</sup>

**Table 1b:** The total viable count of bacteria on Eosine methylene blue agar

Lab code	No. of organism	Cfug/ml
AP <sub>2</sub> (10 <sup>-2</sup> )	25	2.5 x 10 <sup>-3</sup>
SP <sub>2</sub> (10 <sup>-2</sup> )	11	1.0 x 10 <sup>-3</sup>
SP <sub>1</sub> (10 <sup>-2</sup> )	100	1.00 x 10 <sup>-4</sup>
SP <sub>1</sub> (10 <sup>-4</sup> )	23	2.3 x 10 <sup>-5</sup>
UP <sub>2</sub> (10 <sup>-3</sup> )	20	2.0 x 10 <sup>-4</sup>
SP <sub>1</sub> (10 <sup>-3</sup> )	38	3.8 x 10 <sup>-4</sup>

**Table 1c:** The total viable count of bacteria on Salmonella Shigella agar

Lab code	No. of organism	Cfug/ml
SP <sub>1</sub> (10 <sup>-2</sup> )	18	1.8 x 10 <sup>-3</sup>
UP <sub>2</sub> (10 <sup>-4</sup> )	40	4.0 x 10 <sup>-5</sup>
SP <sub>1</sub> (10 <sup>-4</sup> )	20	2.0 x 10 <sup>-5</sup>
SP <sub>2</sub> (10 <sup>-4</sup> )	15	1.5 x 10 <sup>-5</sup>

**Table 2:** The Morphological and Biochemical analysis of bacteria isolate

LAB Code	Morphology	Gram reaction	Catalase	Oxidase	Indole	Coagulase	Lactose	Glucose	Sucrose	Fructose	Maltose	Galactose	Probable Organism
SP <sub>1</sub> 10 <sup>-3</sup>	R	-	+	-	-	-	+	+	+	+	±	±	<i>Escherichia coli</i>
SP <sub>2</sub> 10 <sup>-4</sup>	R	-	+	-	-	-	+	+	±	±	+	+	<i>Escherichia coli</i>
SP <sub>1</sub> 10 <sup>-2</sup>	R	-	+	-	-	-	-	+	-	±	-	-	<i>Lactobacillus spp.</i>
AP <sub>2</sub> 10 <sup>-3</sup>	C	+	+	+	-	+	±	+	-	-	-	+	<i>Staphylococcus aureus</i>
AP <sub>2</sub> 10 <sup>-2</sup>	R	-	+	-	-	-	-	-	+	-	+	+	<i>Lactobacillus spp.</i>
SP <sub>1</sub> 10 <sup>-4</sup>	C	+	+	-	-	+	-	+	+	+	-	-	<i>Staphylococcus aureus</i>
UP <sub>2</sub> 10 <sup>-2</sup>	R	+	+	-	-	-	±	±	±	±	±	±	<i>Bacillus subtilis</i>
UP <sub>2</sub> 10 <sup>-3</sup>	C	+	+	+	-	-	+	+	-	+	+	-	<i>Staphylococcus aureus</i>
AP <sub>2</sub> 10 <sup>-2</sup>	R	-	-	-	-	-	+	+	+	+	+	+	<i>Lactobacillus spp.</i>
SP <sub>2</sub> 10 <sup>-2</sup>	R	+	+	+	-	-	+	+	+	+	+	+	<i>Bacillus subtilis</i>
SP <sub>1</sub> 10 <sup>-4</sup>	R	-	-	-	-	-	+	-	+	-	-	-	<i>Proteus</i>
SP <sub>1</sub> 10 <sup>-2</sup>	R	-	+	-	-	-	+	+	+	+	+	+	<i>Lactobacillus spp.</i>
UP <sub>2</sub> 10 <sup>-3</sup>	R	-	+	+	-	-	±	±	±	±	±	±	<i>Pseudomonas aeruginosa</i>
SP <sub>1</sub> 10 <sup>-3</sup>	C	+	-	-	-	-	-	+	-	+	-	-	<i>Lactobacillus sp.</i>
UP <sub>2</sub> 10 <sup>-4</sup>	R	-	+	+	-	-	+	-	-	-	+	-	<i>Shigella spp.</i>
SP <sub>1</sub> 10 <sup>-2</sup>	R	-	+	-	-	-	+	+	-	+	+	+	<i>Salmonella spp.</i>
SP <sub>1</sub> 10 <sup>-4</sup>	R	-	+	-	-	-	+	+	+	+	+	+	<i>Lactobacillus spp.</i>
SP <sub>2</sub> 10 <sup>-4</sup>	R	-	+	-	-	-	+	+	+	+	+	+	<i>Lactobacillus spp.</i>

**KEY:** R - Rod C - Cocci + Positive – Negative

Table 3 indicates the fungal count of sliced pineapple sold at Sango, Apete, and UI market in Ibadan, with a range of 1.3 x 10<sup>-5</sup>cfu/g to 2.0 x 10<sup>-3</sup>. Six fungi organisms were characterized as *Aspergillus niger*, *Saccharomyces cerevisiae*, and *Penicillium* species. The presence of these fungi may be due to soil contaminated materials used during the processing of the fruits into cut produce. The ingestion of aflatoxins, which are produced by *Aspergillus* species and are carcinogenic, has been linked to a major risk factor for the development of liver cancer (Mensah *et al.*, 2002) [8]. The presence of *Saccharomyces cerevisiae* may be attributed to the nutritional composition and available water in the fruits.

The percentage occurrence of bacteria and fungi isolated in sliced ready-to-eat pineapple is presented in Table 4, showing that *Lactobacillus* species had the highest occurrence of bacteria isolates (35%), while *Penicillium* species had the highest occurrence of fungal isolates (50%). The antimicrobial sensitivity test was performed on the microorganisms

isolated from the samples, revealing variability in the susceptibility of the isolates to various antibiotics. Ofloxacin, Gentamycin, and Ciprofloxacin exhibited the highest inhibitory properties against all isolates, while Nitrofurantoin showed the lowest inhibitory properties, as shown in Table 5. All isolates demonstrated high resistance to Ampicillin, Ceftazidime, Cefuroxime, and Augmentin. The results of this study indicate that Gentamycin, Ciprofloxacin, and Ofloxacin are suitable options for treating infections.

**Table 3:** The total viable count of fungi on Saboured Dextrose Agar

Lab code	No. of organism	Cfug/ml
AP <sub>1</sub> (10 <sup>-3</sup> )	10	1.0 x 10 <sup>-4</sup>
SP <sub>2</sub> (10 <sup>-2</sup> )	20	2.0 x 10 <sup>-3</sup>
UP <sub>2</sub> (10 <sup>-4</sup> )	13	1.3 x 10 <sup>-5</sup>
AP <sub>1</sub> (10 <sup>-3</sup> )	20	2.0 x 10 <sup>-4</sup>
UP <sub>1</sub> (10 <sup>-3</sup> )	15	1.5 x 10 <sup>-4</sup>
SP <sub>1</sub> (10 <sup>-3</sup> )	22	2.2 x 10 <sup>-4</sup>

**Table 3b:** Morphological characteristics of fungi isolated in sliced pineapple.

S/n	Lab. Code	Shape	Opacity	Elevation	Form	Probable Organism
1.	AP <sub>2</sub> (10 <sup>-3</sup> )	Circular	Opaque	Convex	Smooth	<i>Aspergillus niger</i>
2.	SP <sub>2</sub> (10 <sup>-2</sup> )	Circular	Transparent	Convex	Rough	<i>Penicillium sp.</i>
3.	UP <sub>2</sub> (10 <sup>-4</sup> )	Circular	Transparent	Flat	Rough	<i>Penicillium sp.</i>
4.	AP <sub>1</sub> (10 <sup>-3</sup> )	Circular	Transparent	Convex	Smooth	<i>Penicillium sp.</i>
5.	UP <sub>1</sub> (10 <sup>-3</sup> )	Circular	Transparent	Convex	Smooth	<i>S. cerevisiae</i>
6.	SP <sub>1</sub> (10 <sup>-3</sup> )	Circular	Opaque	Convex	Smooth	<i>S. cerevisiae</i>

**Table 3c:** The biochemical characterization of fungi isolates

S/n	Lab. Code	Glucose	Fructose	Sucrose	Galactose	Maltose	Lactose	Probable organism
1.	AP <sub>2</sub> (10 <sup>-3</sup> )	+	+	+	+	-	+	<i>Aspergillus niger</i>
2.	SP <sub>2</sub> (10 <sup>-2</sup> )	+	+	+	+	+	+	<i>Penicillium sp.</i>
3.	UP <sub>2</sub> (10 <sup>-4</sup> )	+	+	+	+	+	-	<i>Penicillium sp.</i>
4.	AP <sub>1</sub> (10 <sup>-3</sup> )	+	+	+	+	+	-	<i>Penicillium sp.</i>
5.	UP <sub>1</sub> (10 <sup>-3</sup> )	+	+	+	+	+	+	<i>S. cerevisiae</i>
6.	SP <sub>1</sub> (10 <sup>-3</sup> )	+	+	+	+	+	+	<i>S. cerevisiae</i>

**Table 4a:** The total percentage occurrence of bacteria in pineapple

Organism	No. of organism	Percentage (%) of organism
<i>Escherichia coli</i>	2	12
<i>Lactobacillus species</i>	6	35
<i>Staphylococcus species</i>	3	18
<i>Bacillus subtilis</i>	2	12
<i>Proteus</i>	1	5
<i>Pseudomonas species</i>	1	5
<i>Shigella species</i>	1	5
<i>Salmonella species</i>	1	5

**Table 4b:** Total percentage occurrence of fungi isolated in sliced pineapple

Organism	No. of organism	Percentage (%) of organism
<i>Aspergillus niger</i>	1	16
<i>Saccharomyces cerevisiae</i>	2	33
<i>Penicillium species</i>	3	50

**Table 5:** Antimicrobial activities of bacteria isolated from sliced Pineapple

Isolate	Gen.	Cpr.	Amp.	Nit.	Ofl.	Caz.	Crx.	Aug.
<i>Escherichia coli</i>	+	++	-	+	++	-	-	-
<i>Lactobacillus sp.</i>	++	++	-	++	+	-	-	-
<i>Staphylococcus sp.</i>	+++	+++	-	+++	+++	-	-	-
<i>Bacillus subtilis</i>	+	++	-	++	++	-	-	-
<i>Proteus</i>	-	++	-	-	++	-	-	-
<i>Pseudomonas Aeruginosa</i>	+	++	-	+	++	-	-	-
<i>Shigella species</i>	+++	++	-	-	++	-	-	-
<i>Salmonella species</i>	++	+	-	++	++	-	-	-

**Key:** + Weak inhibition (diameter <8mm), ++ Moderate inhibition (diameter >8-12mm)

+++ Strong inhibition (diameter >12-above), - No inhibition

Caz Cefazidime 30ug

Crx Cefuroxime 30ug

Gen Gentamycin 10ug

Cpr Ciprofloxacin 30ug

Ofl Ofloxacin 30ug

Aug Augmentin 30ug

Amp Ampicillin 10ug

Nit Nitrofurantoin 30ug

### Conclusion

Our investigation focused on the microbiological contamination of ready-to-eat sliced pineapple available in Apete, Sango, and University of Ibadan Markets. The

findings from our study revealed that the ready-to-eat sliced pineapple in these markets exhibited higher microbial loads than the Microbiological Standard for fruits. Among the bacteria and fungi detected were *Escherichia coli* (12%), *Staphylococcus aureus* (18%), *Bacillus subtilis* (12%), *Proteus* (5%), *Pseudomonas* (5%), *Salmonella* (5%), and *Shigella species* (5%). *Lactobacillus species* (35%), *Aspergillus niger* (16%), *Saccharomyces cerevisiae* (33%), and *Penicillium species* (50%) were the microorganisms with the highest percentage of occurrence. These results pose significant public health risks, which necessitate urgent public sensitization. Our study found that Ofloxacin, Gentamycin, and Ciprofloxacin exhibited the strongest inhibitory properties against all the isolates, making them suitable choices for treating foodborne infections resulting from consuming contaminated ready-to-eat sliced pineapple.

## Author statements

### Authors and contributors

Adejuwon worked on microbiological analysis/Isolation of bacterial/fungi, Sugar fermentation and antibiotic Sensitivity test. Oluwatobi worked on Gram staining techniques, catalase test, oxidase test, indole test, coagulase test.

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