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Full Length Research

Molecular characterisation and antibiotic susceptibility profiles of bacterial isolates from some selected fruits in Ibadan metropolis

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ABSTRACT: Fresh fruit and vegetables are essential components of a healthy and balanced diet; their consumption is encouraged by different organisations (WHO, FAO, USDA, EFSA) and nutrition experts to protect against a range of diseases. Fresh-cut fruits are eaten without any further sterilisation, which means that all microorganisms loaded on the produce will gain entry to the human body. It is the reason why pathogen contamination is of particular concern to consumers. Factually, foodborne illness caused by pathogens is not the only problem; antibiotic resistance carried by pathogenic/ nonpathogenic bacteria is also a severe problem. Hence, this study aims to microbiologically assess the safety of some commonly consumed fruits, namely watermelon, carrot, apple and tomato and to determine the antibiotic susceptibility of the isolated organisms. Isolation of pathogenic microorganisms was carried out using the standard isolation methods, after which the isolates were characterised molecularly. Antibiotic resistance of the isolates was tested against some readily used antibiotics. The result revealed the presence of pathogenic organisms, namely *Klebsiella variicola* (CP050958.1), *Enterobacter hormaechei* (CP109738.1), *Klebsiella pneumonia* (CP030320.1) and *Enterobacter bugandensis (*MN208217.1) and all test isolates were found resistant to the selected antibiotics. The occurrence and resistance of these microorganisms to antibiotics could pose serious health impacts, including potential long-term disability, and economic consequences such as medical costs, loss of productivity. Vulnerable groups, such as young children, the elderly, and those with weakened immune systems, will be at risk for severe outcomes.

Keywords: Antibiotics, foodborne, pathogens, molecular characterization.

INTRODUCTION

Fruits and vegetables can be defined as agricultural products having tissues that are subjected to normal living characteristics such as respiration, excretion, metabolism, etc. They are fleshy portions of plants with edible characteristics, which could be eaten whole, precut, or sliced. They could be served in retail outlets and in ceremonies. Fresh fruits and vegetables are widely available in various cities, towns, and villages in Nigeria (Odewale *et al.,* 2025). The consumption of fresh fruits and vegetables is increasing as consumers' bodies strive to eat healthy diets and benefit from the year-round availability of these products that, up until recently, were considered to be seasonal. White grapes, orange, mango, apple, red grapes, and dates are some of the fruits that are normally consumed raw in order to obtain their valuable nutrients in the best form. Fruits are good sources of nutrients for growth, repair and control of body processes as most of them contain sugar, vitamins, mineral elements and small quantities of protein and carbohydrates (Nur, 2020).

Consumption of fresh fruits and vegetables is encouraged worldwide by both government and privately-owned health agencies or groups. Fresh vegetables and fruits play a crucial role in human nutrition due to their high nutrient content, including water, dietary fibre, proteins, phytochemicals, vitamins, and minerals such as calcium, potassium, and magnesium. Their high cellulose and fibre contents also help in the regulation of the digestive system. International organisations, including the World Health Organisation (WHO), the Food and Agricultural Organisation (FAO), and the Centres for Disease Control and Prevention (CDC), have encouraged people to eat more fresh fruits and vegetables (Odewale *et al*., 2025).

The consumption of fresh fruits and vegetables in various countries is increasing, which stimulates their production, which has increased by more than 30% worldwide in recent years. However, due to anthropogenic activities, vegetables and fruits are often exposed to active bacterial contamination. The survival of microorganisms is facilitated by nutrients that are released from damaged tissues. Contamination of vegetables and fruits can occur during cultivation, during harvesting, transportation and in the consumer's kitchen. The soil can also be a source of pollution, which is often filled with manure containing various microorganisms. Contamination of vegetables and fruits can be facilitated by rainfall or irrigation in agriculture (Lykov *et al.,* 2021).

Fruits are important parts of our daily diet to maintain a healthy life as they have nutritional value. But unfortunately, a number of foodborne diseases have been noticed due to the consumption of raw and contaminated fruits. Fresh-cut fruits are eaten without any further sterilisation, which means that all microorganisms loaded on the produce will enter the human body. It is the reason why pathogen contamination is of particular concern to consumers. Factually, foodborne illness caused by pathogens is not the only problem; antibiotic resistance carried by pathogenic/ nonpathogenic bacteria is also a severe problem (Yi *et al*., 2022).

Fruits provide the ideal environment for the survival and growth of many types of microorganisms, especially bacteria. The internal fruit tissues consist high concentration of various types of sugars, minerals, vitamins and amino acids. Spoilage refers to any change in the condition of food in which the food becomes undesirable or unacceptable for human consumption. Bacterial spoilage first causes softening of tissues as pectins are degraded, and the whole fruit may eventually degenerate into a slimy mass. Starch and sugars are metabolised next, and unpleasant odours and flavours develop along with lactic acid and ethanol. Some spoilage microbes are capable of colonising and creating lesions on healthy, undamaged plant tissue. Microbial fruit infection may occur during the growth season, harvesting, handling, transport and post-harvest storage and marketing conditions, or after purchasing by the consumer. Spoilage will cause fruits to become less palatable or even toxic to consumers. Previously, several studies have reported the occurrence of bacteria in spoiled fruits, including *Pseudomonas, Erwinia, Xanthomonas, Enterobacter, Flavobacterium, Chromobacter, Lactobacillus, Bacillus, and Clostridium*. Fruits are vital sources of nutrients for human beings. They give the body the necessary vitamins, fats, minerals, and oils in the right proportion for human growth and development. Fruits, however, have serious challenges to their existence (Hasan and Zulkahar, 2018).

Fruits and vegetables could be the sources of contamination in food preparation areas. Ready-to-eat fruits and vegetables may be sliced, peeled, shredded and washed/unwashed. The destruction of surface cells during processing can cause an exposure of the produce to the entry of microorganisms, which utilise the readily available nutrients compared to intact produce. In addition, the high-water activity of many fruits and the approximately neutral pH of vegetables encourage the rapid growth of microbes. Hence, this study aims to determine the microbiological safety of some consumed fruits in Ibadan, Oyo State, Nigeria, which will help enlighten consumers on the health impact associated with the consumption of some of these fruits.

MATERIALS AND METHODS

Collection of samples

A total of fifty samples comprising watermelon, carrot, apple and tomato were purchased from 10 stationary vendors in different markets in Ibadan metropolis. Samples were collected aseptically in a sterile bag and transported to the laboratory immediately for microbiological analysis.

Isolation of bacteria

All samples were chopped, and a 20 g sample was added to a sterile conical flask containing 180 mL of distilled water. A ten-fold serial dilution of diluent in folds of five and six was done for the enumeration of total viable bacteria (TVB). 0.1 mL of each sample was spread onto tryptic soy agar (TSA) and MacConkey agar, and plates were incubated at 37°C for 24 to 48 hours

Identification of bacterial isolates

Biochemical test (Gram staining)

Gram staining is a differential staining procedure that separates bacteria to either, Gram positive or Gram negative. It was done by using a sterile wire loop to drop a loop full of sterile distilled water on a clean, grease free glass slide. A colony of the bacteria was smeared on the slide and allowed to air dry. The smear was heat fixed by gently passing over flame thrice. The smear was flooded with crystal violet for 60 seconds and washed off immediately with water, it was then flooded with Lugols iodine for 60seconds and washed off with water. It was decolorized with acetone for few seconds and washed off. The smear was counterstained with neutral red for 60 seconds, then washed off with water. The slide was allowed to air dry. It was viewed under the X100 objectives with oil immersion. Gram positive cells picked up the color of the primary stain while Gram negative cells pick up the colour of the secondary stain (Smith & Hussey, 2016).

Catalase test

A drop of 3% hydrogen peroxide was made on a clean glass slide. With a wooden applicator, a colony of the test bacteria was brought in contact with the hydrogen peroxide and observed for bubble. The presence of bubble was a positive catalase test and no bubbles showed a negative catalase test (Reiner, 2016).

Coagulase test

Two drops of distilled water were made at the ends of a clean grease-free glass slide. A colony of the test organism was emulsified on each of the drops. To one of the suspensions a loop-full of plasma was added and mixed gently. The other suspension was left as the control. Observation of agglutination within ten (10) seconds of addition of plasma indicated a positive coagulase test. Agglutination indicates a positive test (UK Health Security Agency 2025).

Indole test

The isolate was inoculated in 5ml peptone water (tryptophan broth) and incubated at37°C for 24 hours. After 24 hours, 3 drops of Kovac's reagent was added to the inoculum, shaken and observed for reaction. A positive reaction is indicated by the development of a red colour in the reagent layer above the broth within one minute and a negative reaction is indicated by the reagent retaining its yellow colour (McWilliams, 2016

Citrate utilization

Citrate utilization test was done to differentiate among enteric organisms on the basis of their ability to ferment citrate as a sole source of carbon by the enzyme citrate permease. Simmons citrate agar slants of 2 ml in each vial was prepared by autoclaving at 15 psi 121°C. Using sterile technique, small amount of the experimental bacteria from 24-hours old pure culture was inoculated into the vials by means of a streak inoculation method with an inoculating needle and the vials were incubated for 48 hours at 37°C. Following incubation, citrate positive culture was recognized by the presence of growth on the surface of the slant of Simmons citrate agar and deep Prussian blue coloration of the medium (Aditi *et al*., 2017).

Sugar fermentation

Triple sugar iron test was done to differentiate among the different groups or genera of the Enterobacteriaceae based on the ability to reduce sulfur and ferment carbohydrates. Slants were prepared in the test tubes by autoclaving. Using sterile technique; small amount of the experimental bacteria from 24-hours old pure culture was inoculated into the tubes by means of a stab and streak inoculation method with an inoculating needle. The screw caps were not fully tightened and the tubes were incubated for 24 hours at 37°C. Fermentation is indicated by yellowing of the butt and the slant of Triple Sugar Iron (TSI) agar media. If gas was formed during the fermentation, it is shown in the butt either by the formation of bubbles or cracking of the agar.

Molecular identification

Extraction of genomic DNA from bacteria isolates

DNA was extracted at the African Bioscience Centre, Oyo State, Ibadan, using the Zymo Research extraction kit following the manufacturer's protocol. The quality and concentration of the extracted DNA were determined using a Nanodrop spectrophotometer, and the DNA was stored at -20°C for further downstream application.

Amplification of 16S rRNA of bacteria isolates

Extracted DNA was used as a template to amplify the 16S rRNA using the universal primers. PCR was performed in a final reaction volume of 25 μL in a PCR tube, containing 12.5 μL of master mix (mixture of dNTP, taq polymerase, MgCl and PCR buffer), 2 μL forward primer (AGAGTTTGATCCTGGCTCAG), 2 μL reverse primer (GGTTACCTTGTTACGACTT), 2 μL of extracted DNA and 6.5 μL of nuclease-free water. Thermal cycles for the amplification were set as: initial denaturation for 5 minutes at 94°C, 30 cycles of 40 seconds at 94°C, 30 seconds at 50°C, and 1 minute at 72°C and final extension for 5 minutes at 72°C. Thereafter, 5μl of each PCR product was analysed on 1 % (w/v) agarose gel supplemented with ethidium bromide. The amplicons were then visualised using a gel documentation system.

Nucleotide sequencing and alignment

16S rDNA sequencing of the isolated strain was carried out by dye terminator cycle sequencing (Quick Start kit).

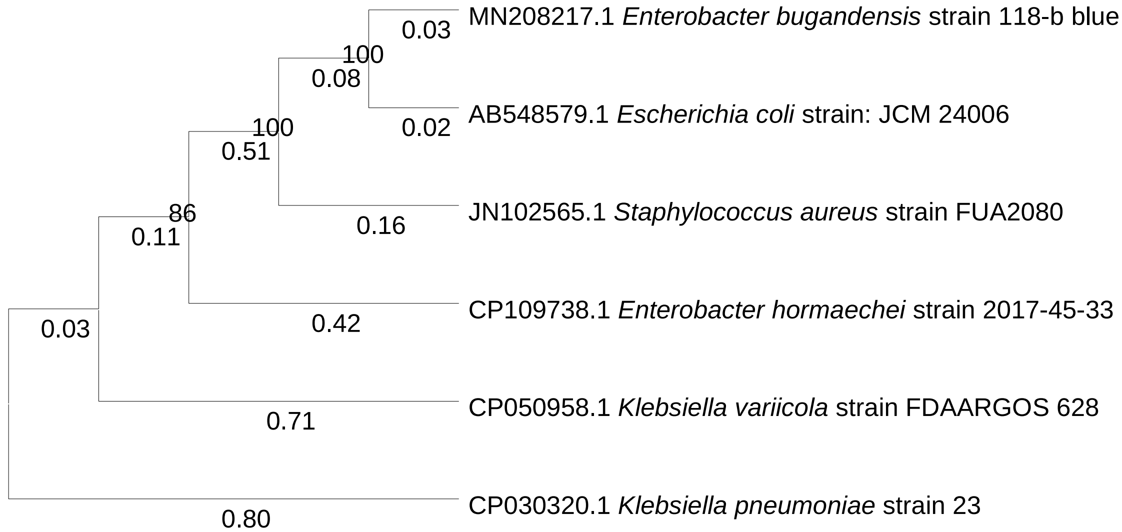

Figure 1. Phylogenetic tree was constructed based on the nucleotide sequences of 16–23S rRNA region of and those reported from other countries using Neighbor-joining method.

The gene sequences of each isolate obtained in this study were compared with the known nucleotide database at the National Centre for Biotechnology Information (NCBI) by using their worldwide website, and the BLAST (Basic Local Alignment Search Tool) algorithm.

Antimicrobial susceptibility testing

The antibiotic susceptibility testing was done as per Clinical and Laboratory Standards institute (CLSI) guidelines using Kirby–Bauer's method. Inoculum was prepared for each bacterial isolate by matching the turbidity to 0.5 McFarland standard and spreading on Muel-ler-Hinton agar (MHA) plate. Paper disc which contains antibiotics (septrin, chloramphenicol, ciprofloxacin, sparfloxacin, amoxillin, gentamycin Augmentin, pefloxacin, ofloxacin and Streptomycin, erythromycin, Ciprofloxacin) were kept on the top of the MHA plate and incubate at 37°C for 24 hours. According to CLSI M100Guideline 2022, the size of the zones of inhibition was classified as sensitive, moderate, or resistant to the antibiotics tested.

RESULTS AND DISCUSSION

A total of sixty bacterial isolates were isolated, and four of the isolates were selected, characterised molecularly and used for an antibiotic sensitivity test (Figure 1). The result from this study reveals the presence of harmful bacteria in ready-to-eat food (fruit) samples purchased from vendors in different markets in Ibadan. The isolated organisms were *Klebsiella varricola, Enterobacter hormaechi, Klebsiella pneumoinia* and *Enerobacte bugandensis*. Cross contamination of fresh produce could have been from the vendor or the environment since the operating premises are usually kept unclean.

High bacteria counts observed in sliced fruits are similar to the results obtained in similar studies in Nigeria. In a similar study, Oladele and Aladesanmi (2020) reported the contamination of sliced water melon with *Providencia rettgeri, Escherichia coli, Bacillus cereus*, *Proteus mirabilis* and *Kersytersia gyiorum* as identified bacteria isolates in the sliced fruits obtained in Akure. Similarly, Eni *et al*. (2010) reported that the bacterial loads present in fruits are a direct reflection of the sanitary quality of cultivation water, harvesting, transportation, storage, and processing of produce. In fact, fruits exposed to various types of cutting result in a six- to seven-fold increase in microbial loads. This is because cutters and slicers used in the preparation of fruits can be potent sources of contamination, since they usually provide inaccessible sites, which harbour bacteria. Also, Muhammad *et al*. (2021) isolated bacteria, namely *Klebsiella*, *P. mirabilis* and *E. coli* from pumpkin and bitter leaves. Ojodomo and Ashar (2023) reported the occurrence of *Proteus* species, *Citrobacter* species, *Klebsiella* species, and *Salmonella* species from carrots obtained in Zaria, Nigeria.

Food can be contaminated with antibiotic-resistant bacteria. It is one of the foremost threats to public health (Tabassum and Uddin, 2016). Nowadays, one of the major global concerns is the development of antimicrobial resistance by pathogenic bacteria, which is ultimately causing a problem in the treatment procedure (Uddin *et al*., 2011).

Table 1 showed that the bacterial isolates were all resistant to the selected antibiotics. Antibiotic resistance is far reaching threat to global health. Antibiotic-resistant bacteria are rising all over the world. At the same time,

Table 1. Antimicrobial susceptibility test**.**

Isolate	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E
Klebsiella variicola	MR	R	R	R	R	MR	MR	R	R	R
Enterobacter hormaechei	MR	R	R	MR	MR	MR	MR	MR	MR	R
Klebsiella pneumoniae	R	R	R	R	R	R	R	R	R	MR
Enterobacter bugandensis	MR	R	R	R	R	MR	MR	MR	MR	R

Keys: PEF: Pefloxacin, APX: Ampiclox, Z: Zinnacef , SXT: Septrin; AM: amoxicillin, R: Rocephine SP, sparfloxacin; CPX, ciprofloxacin; CN: gentamycin; S, streptomycin, E: Erythromycin The zones of inhibition (mm) were measured following incubation. According to the approach of Yasmin et al., (2020), the antibiotic susceptibility testing was divided into three categories, less than 7mm resistant, 7-10mm moderately resistant and 10mm susceptible.

consumption of fresh-cut fruit is increasing. Inevitably, ingestion of drug-resistant pathogens as those found in fresh-cut fruit, will increase. Nowadays, one of the major global concerns is the development of antimicrobial resistance by pathogenic bacteria, which is ultimately causing a problem in the treatment procedure (Jabin *et al*., 2022). The result of the antibiotic susceptibility test showed that all four selected isolates are resistant to the antibiotics. The antimicrobial resistance of bacteria isolated from food and other sources against commonly used antibiotics has increased from time to time. In a similar work by Geta *et al*. (2019), all of the pathogenic bacteria were found to be resistant to erythromycin. The use of antibiotics in agricultural practices might be largely responsible for the development of resistant foodborne pathogens. For instance, antibiotics are routinely used in livestock, poultry and fisheries, with their wastes serving as manure for crops. More so, wastes and effluents from farms could find their way into water bodies, which could be used for irrigation purposes; consequently, spreading resistance to antibiotics in raw vegetables Odewale *et al*. 2023). The result of this study suggests growing multidrug resistance in bacterial isolates from fresh fruits and vegetables, and could be a potential source of the spread of multidrug-resistant bacteria.

Conclusion

The current study shows the microbiological attributes of fresh fruits and vegetables collected from vendors in Ibadan. The results revealed the presence of a high microbiological load in the local fruit samples and their resistance to selected antibiotics. Hence, ready-to-eat fruits and vegetables purchased from different markets in Ibadan are not safe for human consumption, and consumers are at health risk in terms of microbial quality. Contamination from farms or production areas, improper food handling while processing, non-hygienic practices while packaging and environmental conditions are major factors responsible for high microbial load on fruits and vegetables. This study shows that there is an urgent need for a regulatory agency to vet food vendors in order to promote improvement in quality standards and food safety of ready-to-eat foods.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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