**THE POLYTECHNIC, IBADAN ACADEMIC BOOKS AND JOURNALS**

**REVIEWER’S ASSESSMENT FORM**

**INSTRUCTIONS TO REVIEWER**

1. PLEASE FREELY COMMENT ON THE MANUSCRIPT BY TRACKING YOUR SUGGESTED CHANGES ON THE MANUSCRIPT.
2. KINDLY STATE YOUR OPINIONS ON THE MANUSCRIPT BY FILLING THE FORM BELOW.

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| **Manuscript Number** | **STS2\_17** |
| **Manuscript Title** | **Antibiotic susceptibility patterns and molecular characterisation of bacterial isolates from certain fruits** |

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**(i) Is the Title appropriate? Yes No**

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| --- | --- |
| **Suggest Changes** |  |

(ii)

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| **Relevance and Contribution to Knowledge** |  |

**(iii) In-depth Treatment of the Topic (Comment on each of the sections)**

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| --- | --- |
| **Abstract** | Too shallow |
| **Introduction/Background** | Tautology and lots of sentences without proper references. |
| **Methodology** | The author was unable to describe appropriately |
| **Results/Findings** | Minimal result and description |
| **Inference/Discussion** | Very shallow |
| **References** | Too few. Not properly done |

(iv)

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| --- | --- |
| **Language** | The author should write using either American or British English, not a combination of both. |

(v)

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| --- | --- |
| **Presentation** |  |

(vi)

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| **General Comments** | The author’s use of English is poor.  The author did not take note of scientific names and how they should be written.  The use of punctuations was below average.  The use of references throughout the manuscript was very loose. I observed that numerous statements were mentioned flippantly and were not referenced.  Figure and Table heading were not descriptive enough.  I stopped making further comments in the manuscript, because I observed the entire manuscript had to be properly overhauled and resubmitted (if considered). |

**(vi) Conclusion: Tick any of the following:**

YES

**Accept Accept with major corrections**

**Accept with minor corrections Reject**

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| **Comments on Decision made** |  |

**Assessor’s name:**

**Signature: Date:**

**Antibiotic susceptibility patterns and molecular characterisation of bacterial isolates from certain fruits**

**Abstract**

A healthy and balanced diet must include fresh fruit and vegetables; nutrition experts and several organizations (WHO, FAO, USDA, EFSA, etc.) recommend consuming them to prevent a variety of ailments. Since fresh-cut fruits are consumed without being further sterilized, all of the germs present on the food will enter the body of the consumer. This is the reason that customers are especially concerned about pathogen contamination. In actuality, antimicrobial resistance by microbes and food borne illnesses caused by these microbes pose great threat to mankind. Hence, this research aims to isolate microrganisms from selected fruits and to ascertain their resistance or susceptibility to selected 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.

Key words: antibiotic resistance, pathogens, characterization, antibiotics

**Introduction**

Produce with tissues that experience regular living processes like respiration, excretion, metabolism, etc. is what is referred to as fruits and vegetables. These are plant parts that are meaty and edible; they can be consumed whole, chopped, or sliced. They could be offered at ceremonies and in retail establishments. Fresh fruits and vegetables are widely available in various cities, towns, and villages in Nigeria (Gbolabo *et al*.,2023. Fresh produce is becoming more and more popular as people try to maintain healthy diets and take advantage of the year-round availability of these once thought to be seasonal goods. Certain fruits, like dates, oranges, mangoes, apples, red grapes, and white grapes, are best ingested raw in order to absorb their essential elements. Fruits are rich in sugar, vitamins, minerals, and trace amounts of protein and carbs. As such, they are excellent providers of nutrients for bodily growth, repair, and regulation (ifra , 2020).

Around the world, health organizations that are privately owned as well as those run by governments promote the intake of fresh fruits and vegetables because they are abundant in water, dietary fiber, proteins, phytochemicals, vitamins, and minerals including calcium, potassium, and magnesium, fresh fruits and vegetables are essential to human nutrition. Their high cellulose and fiber contents aid in the digestive system's regulation as well (Gbolabo *et al*.,2023).

Fresh produce is being consumed more often in many nations, which in turn is driving ~~up~~ global production, which has risen by over 30% in recent years. However, agricultural produce are prone to microbial contamination due to human activity. The nutrients that are liberated from injured tissues help bacteria survive. Fruits and vegetables can get contaminated in the kitchen, in transit, during harvesting, and during production. Another potential source of pollution is the soil, which is frequently contaminated by manure that contains a variety of microorganisms. Rainfall or irrigation in agriculture can facilitate the contamination of fruits and vegetables (Lykov *et al* 2021).

Because fruits may provide some nutritional benefits, they should be a regular component of our diet in order to stay healthy. Unfortunately, eating raw or infected fruits has been linked to a range of foodborne illnesses. Since fresh-cut fruits are consumed without being further sterilized, every microbe present on the produce will enter the body of the consumer. This is the reason that customers are especially concerned about pathogen contamination. In actuality, antibiotic resistance carried by pathogenic and nonpathogenic bacteria is a serious issue in addition to foodborne sickness brought on by pathogens (Lanhua *et al*., 2022).

A fruit's environment is perfect for the survival and growth of many different kinds of germs, particularly bacteria. High concentrations of different kinds of carbohydrates, minerals, vitamins, and amino acids can be found in the inside fruit tissues. Any alteration in the state of food that makes it unfit for human eating is referred to as spoilage. The deterioration of pectins brought on by bacterial rotting initially softens the tissues, and finally the entire fruit may turn into a slimy sludge. After starch and sugars are broken down, lactic acid, ethanol, and disagreeable flavors and odors emerge. Certain bacteria that cause spoiling have the ability to infect and cause lesions on plant tissue that is still healthy. During the growing season, harvesting, handling, transporting, and post-harvest storage, microbial fruit infections can happen ( Hasan and Zulkahar,2018)

Fruits and vegetables may be the cause of contamination in areas used for food preparation. Fruits and vegetables that are ready to eat can be chopped, peeled, shredded, and cleaned or left unwashed. When surface cells are destroyed during processing, it can allow microorganisms to enter the food and take advantage of the easily accessible nutrients more so than when the product is intact. Furthermore, the almost neutral pH of vegetables and the high water activity of many fruits promote the rapid growth of microorganisms.

**Materials and methods**

**Collection of samples**

Ten samples of each fruit—watermelon, water, carrot, apple, and tomato were purchased from ten stationary vendors in several Ibadan city markets. Samples were gathered aseptically in a sterile bag and sent right away to the lab for microbiological examination.

**Isolation of Bacteria**

Isolation of bacteria from the samples was done using the method described by Mohammed*et al*., 2022

**Identification of Bacterial Isolates**

Bacteria identification was done using biochemical and molecular methods.

**Molecular identification**

Molecular identification of the isolates was done using the method ofSelvam Arjunan *et al*., 2014

**Antibiotic susceptibility test for bacterial isolates**: 0.5 Mcfarland turbidity stand was use to compare three (3) culture of each bacteria plate. Bacteria isolate was into each sterile muller Hinton agar and were allowed to stand at room temperature (27%) to 30mins to allow inoculated organism to pre diffuse in the prepared media. The disc contain antibiotics septrin, chloramphenicol, ciprofloxacin, sparfloxacin, amoxillin, gentamycin Augmentin, pefloxacin, ofloxacin and Streptomycin, erythromycin, Ciprofloxacin, were carefully place aseptically on Mueller Hinton agar plates. All plates were placed in an incubator and allowed to stand for 24 hours at 37OC. tone on inhibition was measured in millimeters to meet the guidelines set by the clinical standard laboratory institution (CLSJ, 2017).

**Results**

A total of sixty bacterial isolates were isolated and four of the isolates were selected, characterized molecularly and used for antibiotic sensitivity test.

MN208217.1 *Enterobacter bugandensis* strain 118-b blue

AB548579.1 *Escherichia coli* strain: JCM 24006

JN102565.1 *Staphylococcus aureus* strain FUA2080

CP109738.1 *Enterobacter hormaechei* strain 2017-45-33

CP050958.1 *Klebsiella variicola* strain FDAARGOS 628

CP030320.1 *Klebsiella pneumoniae* strain 23

86

100

100

0.16

0.42

0.03

0.02

0.80

0.71

0.03

0.11

0.08

0.51

Figure 1: Phylogenetic tree of the Isolates

**Table 1.1: 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

**Discussion**

Fruits and vegetables have their own microbiota, which includes bacteria that cause spoiling and yeast. They may also contain parasites and dangerous bacteria like Salmonella, Shigella, Yersinia enterocolitica, Clostridium botulinum, Bacillus cereaus, Escherichia coli, and Listeria monocytogenes. Kumar and Mritunjay (2015). Non-pathogenic microbes have been shown to accelerate the rate of spoiling, lowering the produce's quality and market value. During the harvesting process, feces, human handling, harvesting equipment, transport containers, domestic and wild animals, air, transport vehicles, ice, or water can all introduce harmful bacteria into fruits and vegetables (Beuchat, 1995). Because they contain microorganisms, fruits and vegetables aid in the dissemination of bacteria throughout the meal preparation process (Altieri and Nicholls, 2017). Food-borne illnesses are causing an increasing amount of public health problems, societal unrest, unwarranted financial burdens, and avoidable deaths.

The study's findings indicate that ready-to-eat food samples—fruits—that were purchased from vendors in various Ibadan markets contained dangerous pathogens. Klebsiella varricola, Enterobacter hormaechi, Klebsiella pneumoinia, and Enerobacte bugandensis were the isolated organisms. Fresh food may have been cross-contaminated by the vendor or the surroundings because the business is typically kept in an unsanitary state.

The results of comparable investigations conducted in Nigeria are comparable to the high bacterial counts found in sliced fruits. Oladele and Aladesanmi (2020) conducted a study that revealed the presence of bacteria isolates, including Providencia rettgeri, Escherichia coli, Bacillus cereus, Proteus mirabilis, and Kersytersia gyiorum, in sliced water melon acquired from Akure.

According to Eni et al. (2010), the microbes found in the samples are as a result of the hygienic conditions of the water used for growing, harvesting, transporting, storing, and processing the produce. As a matter of fact, the microbial loads in fruits rise six- to seven-fold when they are chopped in different ways. This is due to the fact that fruit prep tools like cutters and slicers can be strong sources of contamination because they Furthermore, bacteria including Klebsiella, P. mirabilis, and E. coli were recovered by Mohammed et al. (2021) from bitter leaves and pumpkin. Carrots purchased in Zaria, Nigeria, were found to have Proteus, Citrobacter, Klebsiella, and Salmonella species, according to Blessing and Ojodomo (2023).

The emergence of antibiotic resistance in pathogenic bacteria, which is ultimately creating issues with the treatment process, is currently one of the biggest worries facing the entire world (Uddin et al., 2011).

Fruit germs that are resistant to antibiotics are becoming more prevalent worldwide. The amount of fresh fruit being consumed is rising concurrently. Drug-resistant pathogen ingestion will unavoidably rise when fresh fruit is consumed. The emergence of antibiotic resistance in pathogenic bacteria, which is ultimately creating issues with the treatment process, is currently one of the biggest worries facing the entire world (Jabin et al., 2022). The findings of the test for antibiotic susceptibility indicated that each of the four isolates that were chosen had an antibiotic resistance. There has occasionally been a rise in the antimicrobial resistance of bacteria that have been identified from food and other sources to widely used antibiotics. Kindu et al. (2019) discovered that every harmful bacterium was resistant to erythromycin in a related study.

**Conclusion**

. The findings of this research showed that the local fruit samples had a high microbial load and were resistant to several medications. Therefore, ready-to-eat fruits and vegetables that are bought from various Ibadan markets are unsafe for ingestion by humans and put their health at risk due to the microbiological quality. High microbial loads on fruits and vegetables are mostly caused by a combination of causes, including environmental factors, non-hygienic packaging techniques, contamination from farms or producing areas, and inappropriate food handling during processing. This study demonstrates the urgent need for a regulatory body to inspect food vendors in order to encourage an increase in the standard of quality and health of its consumers.

**Reference**s

Ajiboye, A.E. and Emmanuel Assessment of Bacterial Contamination in Ready-to-Eat Fruits and Vegetables Sold at Oja-Oba Market, Ilorin, Nigeria Afr. J. Biomed. Res. Vol. 24 (May, 2021); 203- 209 Research Article

Amirul Islam, Reaz Mohammad Mazumdar, Fakruddin, Saiful Islam, Meher Nigad Nipa, Asif Iqbal and Habibur R. (2010). Multiple antibiotic resistant bacteria on fruits from different markets of chittagong city in Bangladesh. Bangladesh research publications Volume: 4, Issue: 4, Page: 332-340

Eni, A.O ., Oluwawemitan, I.A. and Oranusi, U.S. (2010). Microbial quality of fruits and vegetables sold in Sango Ota, Nigeria. African Journal of Food Science 4(5) 291-296

Gbolabo Odewale , Motunrayo Yemisi Jibola-Shittu , Nosakhare Stephenie Omosule , Titilope Blessing Esan (2023) Multidrug resistant bacteria associated with fresh fruits and vegetables sold in Lokoja markets, Kogi state, Nigeria Microbes and Infectious Diseases 2023; Article-In-Press, DOI: 10.21608 mid.2023.198302.1486

Infra tun Nur (2020): Isolation of pathogenic microorganisms from fresh fruits and screening the efficacy of different disinfectant solution against the pathogens. Journal of food safety and hygiene 6(3):133-144

Jabin, T., Hossain, M.M., Nasrin, S.,Tabassum, R., Rahman, M.A. and 1,Uddin, M.A (2022). Microbiological assessment and detection of drug resistant bacterial isolates in some vended fresh fruit juice samples in Dhaka city, Bangladesh Journal of Food Research 6 (4) (2022) 413 – 419 Food Research 6 (4) : 413 – 419

Kindu Geta, , Ameha Kebede, Meseret Chemedissa 2019 Antibiotic Susceptibility Test of Bacteria Isolated From Fruit Juices Sold in Cafes and Restaurants of Debre-Markos Town, North Western Ethiopia Kindu Geta1, \*, Ameh WNOFNS 24 (2019) 366-372

Lanhua Yi a,c,, Sirui Chen , Guang Li , Junhe Ren , Ruotao Zhou a , Kaifang Zeng (2022). prevalence of antibiotic resistance pathogens in online fresh-cut fruit from Chongqing, China and controlling Enterococcus faecalis by bacteriocin - Food Science and Technology 165 (2022) 113678

Lykov, N., Kakharova,M.A., Kureber, V.S. and A E Yurova 2021 Research of antibiotic resistance of microorganisms isolated from fruits and vegetables IOP Conf. Series: Earth and Environmental Science 839 (2021) 0

Muhammad, A.S., Ani, A.and Nasiru, A.M. (2021). Isolation and Identification of Bacteria Responsible for the Spoilage of Fluted Pumpkin (Telfaria occidentalis) and Bitter Leaf (Vernonia amygdalina) in Sokoto Metropolis EPRA International Journal of Multidisciplinary Research (IJMR)

Oladele O.O and Aladesanmi O.C (2020). microbial safety and molecular characterization of microorganisms associated with sliced water melon fruits in selected markets in Akure, Nigeria World News of Natural Sciences 32 (2020) 10-20

Salam Abbas, Radhakrishnan Senthilkumar and Selvam Arjunan (2014): Isolation and molecular characterization of microorganisms producing novelantibiotics from soil sample. European. Journal of Experimental. Biology. 4(5):149-155

Mohammed, N; Abagai, Rt; Mohammed, Y. (2022): Isolation and Characterization of Bacteria Species Related to Food Contact Surfaces in Selected Cafeterias in a Tertiary Institution Campus in ABU, Zaria, Nigeria Journal of Applied Science and Environmental Management.

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