Preliminary Investigation on the Prevalence and Antibiotics Susceptibility Pattern of Bacteria Associated with the Spoilage of Avocado Pear (*Persea americana*) Sold within Abakaliki, Ebonyi State, Nigeria

C. O. Okeh *, P. C. Igwe b, C. V. Uzoh a, O. S. Chukwu a, O. J. Owolabi a, B. Ugwu a, K. E. Aroh a and I. Yusuf a

*Department of Microbiology, Alex Ekwueme Federal University Ndufu Alike Ikwo, Ebonyi State, Nigeria.

b Department of Applied Microbiology, Ebonyi State University Abakaliki, Ebonyi State, Nigeria.

**Authors’ contributions**

This work was carried out in collaboration among all authors. Authors COO and PCI wrote the research concept and designed the study. Authors CVU and OSC collected materials and data. Authors OJO and BU managed the literature searches and article editing. Authors KEA and IY performed data analysis and interpretation. All authors read and approved the final manuscript.

**ABSTRACT**

The prevalence and antibiotic susceptibility pattern of bacteria associated with spoilage of avocado pear (*Persea americana*) were studied. A total of sixteen (16) avocado pear samples were collected from various markets in Abakaliki and these samples were bacteriologically examined. The five (5) bacterial isolates were characterized and identified on the basis of morphological, Gram staining and biochemical tests. The total aerobic plate count was $3.5 \times 10^5$ CFU/g. Out of sixteen samples of avocado pear examined, thirty two bacteria were identified which included *Staphylococcus aureus* 4 (12.5%), *Escherichia coli* 10 (31.2%), *Salmonella* species 7 (21.8%), *Shigella* species 5 (15.6%) and *Klebsiella* species 6 (18.7%). *Escherichia coli* 10 (31.2%) showed the highest percentage prevalence while *Staphylococcus aureus* 4 (12.5%) showed the least percentage.

*Corresponding author: E-mail:optchuks@yahoo.com;
prevalence. Bacteria isolated from the avocado pear samples were highly resistant to more than 55% of the antibiotics tested in this study. E. coli, Salmonella, Shigella and Klebsiella species isolated were also found to be 100% resistant to ceftazidime and oxacillin. The Salmonella and E. coli were found to be highly resistant to over 50% of the tested antibiotics. However, some of the Salmonella and E. coli isolates were also found to be susceptible to gentamicin, ciprofloxacin and ofloxacin. Shigella, Klebsiella and S. aureus were also found to be susceptible to some of the tested antibiotics including ofloxacin, ciprofloxacin and gentamicin. However, the high rates of resistance found in this study showed that some fruits were heavily contaminated with pathogens that may cause infections in humans when ingested.

Keywords: Prevalence; bacteriologically; avocado; antibiotics.

1. INTRODUCTION

Avocado pear (Persea americana), is a member of the family Lauraceae, which mainly contains shrubs and trees that yield resinous aromatic gum from their cut bark. It is among the well-known indigenous fruit trees in the tropical and subtropical rain forest zone of the Southern regions of West Africa (extending eastward from Sierra Leone to Nigeria and Western regions of Central Africa, which includes Cameroun, Equatorial Guinea, Gabon, Democratic Republic of Congo, Congo Brazzaville and Angola). The fruit is a pome and characterized by a central core surrounded by edible fleshy layers [1]. The trees are partially self-pollinating and are often propagated through grafting to maintain a predictable quality and quantity of the fruit [2]. The Avocado fruit has a pulpy mesocarp of 3 to 9 mm thickness, 7cm-20cm long, weighs 100g – 1000g and has a large central seed, 5cm – 6.4cm long. The skin texture is finely pebbled and dull green when ripe (Korsten, 1993). Spoilage is always a concern to anyone who purchases fresh fruits. An increase in local acceptability of fresh fruits indicates that a proper understanding of factors of spoilage or prolonging freshness of fruits is of increasing importance to consumers (Liao and Well, 2007). Spoilage of fresh avocado fruits usually occurs during storage and transport and while waiting to be processed unlike many other fruits [2]. Numerous species of microorganisms easily attack the fruit. The composition of the avocado fruit influences the likely type of spoilage [2]. The high spoilage rate of Avocado fruit coupled with its high nutritional contents pre-supposes that an array of microorganisms may be involved in its spoilage of Avocado fruits. However limited studies exist regarding the microflora associated with spoilage of the popular avocado fruits sold in Abakaliki. This study was undertaken to obtain relevant data on the bacteria that are responsible for the high perishability of the ripe Avocado pear fruits and to identify species that could pose a threat to food safety and health hazard to end consumers of spoilt fruits. The oils from the pulps and seeds are used in foods, pharmaceuticals and cosmetics manufacturing as well as numerous industrial uses. They are rich in monounsaturated fatty acids and are comparable to other currently used vegetable oils [3].

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out in Abakaliki Local Government area of Ebonyi State.
2.2 Collection of Samples

A total of Sixteen (16) avocado pear samples from four different markets (4 samples each) in Abakaliki metropolis were purchased. The avocado pear samples collected were fresh, undamaged, firm, healthy and ripe. The samples were dispensed into clean bags and then brought to Applied Microbiology Laboratory of Ebonyi State University, Abakaliki for microbiological analysis. The samples were left at a dust and insect free section at room temperature for 5-6 days to undergo natural process of spoilage before using.

2.3 Bacteriological Analysis

Twenty five grams (25g) each sample was weighed and placed in a sterile electric blender with 100ml of 0.1% peptone water for homogenization. The resulting wash was serially diluted to $10^{-5}$ in peptone water and 0.5ml of $5^{th}$ dilution was placed on Plate count agar and Nutrient agar, and incubated at 37°C for 24 hours. The discrete colonies on the plates were counted and multiplied by the dilution factor [4]. Distinct colonies were subcultured on fresh nutrient agar plates to obtain pure cultures [5].

2.4 Characterization and Identification of Bacterial Isolates

The bacterial isolates were identified on the basis of colonial morphology, microscopic examination, Gram staining and other biochemical tests as described by Cheesbrough, [6].

2.5 Preparation of 0.5 McFarland Equivalent Standard

A 1% v/v solution of sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99ml of water and 1% v/v solution of barium chloride was prepared by dissolving 0.5g of hydrated barium chloride in 50ml of distilled water. Barium chloride (0.6ml) of was added to 99.4 ml of the sulphuric solution and mixed. A small volume of the turbid solution was transferred to a screw capped bottle of the same type as used in preparing the test inoculum [7].

2.6 Standardization of Test Bacteria

All the test bacteria isolated were standardized before use by inoculating 5ml normal saline in sterile test tubes with loopful of a 24hrs culture of the test bacteria from a nutrient agar slant. The dilutions using loopful of the test bacteria and sterile water were carried out to obtain microbial population of $10^5$CFU/ml and compared with 0.5 McFarland turbidity standard [6].

2.7 Antibiotics Susceptibility Testing

The susceptibility and resistant testing of isolates was determined by the Kirby-Bauer susceptibility test method as recommended by the National Committee for Clinical Laboratory Standards. The following oxoid antibiotics disks were used: Amoxicillin/clavulanic acid (AMC, 20 µg), Ampicillin (AMP, 10 µg), ceftriaxone (CRO, 30 µg), erythromycin (E, 15 µg), oxacillin (OX, 1 µg) vancomycin (VA, 30 µg), ceftazidime (CAZ,30 µg), gentamicin(CN, 10 µg), ciprofloxacin (CIP, 5 µg) and ofloxacin ( OFX, 5µg). Muller Hinton agar was prepared and 0.5 Mcfarland standard of the test organisms were inoculated on the surface of the agar plates using sterile swab sticks. Test antibiotics listed above were aseptically placed on the inoculated agar plates and incubated at 37°C for 18-24 hrs. Inhibition zone diameters were measured and recorded as susceptible or resistant based on National Committee for Clinical Laboratory Standards [8].

3. RESULTS

The probable bacteria species which were isolated include: *Escherichia coli*, *Pseudomonas* species, *Shigella* species, *Staphylococcus aureus*, *Klebsiella* species and *Salmonella* species.

This showed the results of total aerobic plate count of avocado pear samples purchased from four different markets within Abakaliki metropolis. It revealed the highest mean bacterial load of $5.8 \times 10^5$ cfu/g and lowest mean bacteria load of $2.2 \times 10^4$ were obtained (Table 2).

The percentage frequency of Bacteria species isolated from avocado pear samples. It revealed that out of sixty samples of avocado pear analyzed, thirty-two bacteria were identified which include *Escherichia coli* 10(31.2%), *Pseudomonas* species 2 (6.4%), *Shigella* species 5(15.6%), *Staphylococcus aureus* 4(12.5%), *Klebsiella* species 6(18.7%) while *Salmonella* species were 7(21.8%) respectively. The bacteria of most occurrence were *Escherichia coli*, *Salmonella* species and *Staphylococcus aureus*
Table 1. Morphological and biochemical characteristics of the bacteria isolated from avocado pear purchased from four markets within abakaliki

| SN | Biochemical IND | MR Gram RXN | SHAPE | Arrangement | Morphological CHX | Probable organism |
|----|-----------------|-------------|--------|-------------|-------------------|-------------------|
|    | CA | CO | OX | Motility |                |                   |                   |
| 1  | +  | -  | -  | +         | -             | Rod               | Pale white with black edges | Salmonella spp. |
| 2  | -  | -  | -  | -         | -             | Rod               | Pale white          | Shigella spp.   |
| 3  | +  | -  | +  | +         | -             | Rod               | Yellow              | Pseudomonas spp. |
| 4  | +  | -  | -  | +         | +             | Cocci             | Yellow              | *Staphylococcus aureus* |
| 5  | -  | -  | -  | +         | -             | Rod               | Large, circular, gray smooth colonies | Proteus spp |

Key: CA = Catalase test, OX = Oxidase test, IND = Indole test, -= Negative, += Positive, MR=methyl red

Table 2. Bacterial load of avocado pear samples purchased in abakaliki metropolis

| S/N | Sample Source Meat Market | Sample code | Total aerobic plate count (cfu/g) |
|-----|---------------------------|-------------|----------------------------------|
| 1   | Presco                    | AP 1        | 2.2 x 10^5                       |
| 2   |                           | AP 2        | 3.8 x 10^5                       |
| 3   |                           | AP 3        | 4.8 x 10^5                       |
| 4   |                           | AP 4        | 3.8 x 10^5                       |
| 5   |                           | AP 5        |                                  |
| 6   | New Market                | AP 6        | 2.2 x 10^5                       |
| 7   |                           | AP 7        | 5.0 x 10^5                       |
| 8   |                           | AP 8        | 5.4 x 10^5                       |
| 9   |                           | AP 9        | 2.2 x 10^5                       |
| 10  |                           | AP 10       | 2.2 x 10^5                       |
| 11  |                           | AP 11       | 3.8 x 10^5                       |
| 12  |                           | AP 12       | 5.1 x 10^5                       |
| 13  | Kpirikpiri Market         | AP 13       | 2.4 x 10^5                       |
| 14  |                           | AP 14       | 3.8 x 10^5                       |
| 15  |                           | AP 15       | 3.8 x 10^5                       |
| 16  |                           | AP 16       | 3.9 x 10^5                       |
|     | Mean (cfu/g)              |             | 3.5 x 10^5                       |
| Total Number of samples collected | Total Number of Bacteria isolated | Type of Bacteria isolated | Source Meat Market | Presco | Kpirikpiri | New Market | Number and % of Bacteria isolated |
|----------------------------------|----------------------------------|---------------------------|--------------------|--------|------------|------------|----------------------------------|
| 16                              | 32                               | *Staphylococcus aureus*   | 1                  | 0      | 2          | 1          | 4(12.5 %)                        |
|                                 |                                  | *Shigella species*        | 2                  | 0      | 1          | 2          | 5(15.6 %)                        |
|                                 |                                  | *Salmonella species*      | 2                  | 1      | 2          | 2          | 7(21.8 %)                        |
|                                 |                                  | *Escherichia coli*        | 3                  | 2      | 2          | 3          | 10(31.2%)                        |
|                                 |                                  | *Klebsiella species*      | 2                  | 1      | 1          | 2          | 6 (18.7%)                         |
3.1 Antibiotic Percentage Resistance and Susceptibility of *Salmonella* species Isolated from Avocado pear Purchased from Selected Markets in Abakaliki

The result obtain from this study showed that *Salmonella* species isolated from Avocado pear purchased from Abakaliki were 100% resistant to oxacillin, vancomycin and cefltazidime. also *Salmonella* species isolation were susceptible to ciprofloxacine, ofloxacin and gentamicin with the percentage values of 30%, 45% and 60% respectively.

It showed that *Escherichia coli* from those market were 100% resistance to oxacillin, vancomycin and cefltazidime it also showed susceptibility to ciprofloxacine (50%), Gentamicin (30%), and ofloxacin (60%).

The result obtain from this study showed that *Shigella* species isolated from Avocado pear purchased from Abakaliki were 100% resistant to oxacillin and cefltazidime. also *Shigella* species isolation were susceptible to ciprofloxacine, gentamicin, vancomycin and ofloxacin with the percentage values of 60%, 70%, 40% and 30% respectively.
Fig. 4. Antibiotic percentage resistance and susceptibility of *Escherichia coli* isolated from avocado pear purchased from selected markets in abakaliki

Key: cefltazidime (CAZ), gentamicin (CN), ciprofloxacine (CIP), vancomycin (VA), oxacillin (OX) and ofloxacin (OFX)

Fig. 5. Antibiotic percentage resistance and susceptibility of *Staphylococcus Aureus* isolated from avocado pear purchased from selected markets in abakaliki

Key: cefltazidime (CAZ), gentamicin (CN), ciprofloxacine (CIP), vancomycin (VA), oxacillin (OX) and ofloxacin (OFX)

Fig. 6. Antibiotic percentage resistance and susceptibility of *Klebsiella* species isolated from avocado pear purchased from selected markets in abakaliki

Key: cefltazidime (CAZ), gentamicin (CN), ciprofloxacine (CIP), vancomycin (VA), oxacillin (OX) and ofloxacin (OFX)
The result obtained from this study showed that Klebsiella species isolated from Avocado pear purchased in Abakaliki were 100% resistant to oxacillin, vancomycin and cefltazidime. Also, Shigella species isolation was susceptible to ciprofloxacin, gentamicin and ofloxacin with the percentage values of 70%, 50% and 40% respectively.

4. DISCUSSIONS

This study aimed at determining the prevalence and antibiotic susceptibility pattern of bacteria associated with the spoilage of avocado pear (Persea americana) sold in Abakaliki, Ebonyi State. Sixteen (16) avocado pear samples were collected from various markets in Abakaliki and were bacteriologically examined for the isolation of common bacteria associated with avocado pear samples. The five (5) bacterial isolates were characterized and identified. The average mean bacterial load of 3.5x10^5 CFU/g was recorded as the total aerobic plate count from different markets in Abakaliki. This study was in line with previous studies from Frazier and Weshoff, [9] who recorded high total heterophilic counts of 3.8 x 10^5CFU/g for foods in India. Out of sixteen samples of avocado pear examined, thirty-two bacterial isolates were identified, which included Staphylococcus aureus (12.5%), Escherichia coli (31.2%), Salmonella species (21.8%), Shigella species (15.6%) and Klebsiella species (18.7%) respectively. Escherichia coli (31.2%) showed the highest percentage prevalence while Staphylococcus aureus (12.5%) showed the least percentage prevalence. These results corroborate the work of Ikenebomeh and Chikwendu, [10] who identified similar microbes. This was similar to the works of Ahmed et al., [11]; Rahman and Noor, [12] who isolated similar organisms. This was similar to the works of Akpoka et al., [13] who reported similar organisms in addition to Erwinia and Phytophthora isolated from Avocado Pear in Benin City. The findings of this study were in harmony with the works of Afroz et al., [14] who isolated similar organisms as well as Vibrio sp which was not isolated in this study. These organisms were equally reported as the causative agents for bacterial soft rots. Eze and Chimaeze [2] also reported the presence of some of these spoilage organisms in avocado pear. This indiscriminate exposure coupled with the high nutritional content of the pear often resulted in an increased likelihood of contamination of the avocado pear that was propagated by flies, airborne dust, unhygienic human contacts, and damages to the fruit’s outer surface [15,16]. Pre-harvest and post-harvest factors, consisting of the farm soil-type, storage conditions, and handling practices, maybe the likely source of these microbes [17]. Bacteria isolated from the avocado pear samples bacteriologically analyzed in this study were highly resistant to more than 55% of the antibiotics tested. In this study, the E. coli, Salmonella, Shigella and Klebsiella species isolated were also found to be 100% resistant to cefltazidime and oxacillin. The Salmonella and E. coli were found to be highly resistant to over 50% of the tested antibiotics. However, some of the Salmonella and E. coli isolates were also found to be susceptible to gentamicin, ciprofloxacin and ofloxacin. Shigella, Klebsiella and S. aureus were also found to be susceptible to some of the tested antibiotics including ofloxacin, ciprofloxacin and gentamicin. This result is in agreement with the work of Khatib et al., [18] in Lebanon. Khatib et al., [18] showed that the Gram-negative bacteria isolated from several fresh farm produce from various groceries in Lebanon were multidrug resistant in nature and were resistant to more than 50% of the antibiotics tested. However, the high rates of resistance found in this study showed that some fruits are heavily contaminated with potent bacterial pathogens that cause infections in humans.

5. CONCLUSION

To reduce the rate of contamination which in turn leads to the spoilage of Avocado pear fruits and also lead to the ingestion of contaminated fruits by consumers, it is important that the sellers should be properly educated and sensitized on the need to improve their own personal hygiene which is one of the factors that affect post harvest of Avocado pear fruits, thereby introducing contaminants which leads to spoilage. This research has been able to isolate and identify bacteria associated with the spoilage of avocado pear fruits and also presented some steps to be taken in order to reduce the presence of microorganisms capable of causing harm to the consumer. It is suggested that proper handling would ensure a better quality of Avocado pear fruits being sold in our local markets.

CONSENT

It is not applicable.
ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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