

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.

Abstract

The prevalence and antibiotic susceptibility pattern of bacteria associated with spoilage of avocado pear (*Persea americana*) was 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. This study showed the results of total aerobic plate count of 3.5×10^5 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%) respectively. *Escherichia coli* 10 (31.2%) showed highest percentage prevalence while *Staphylococcus aureus* 4 (12.5%) showed least percentage 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.

1.0 INTRODUCTION

Persea americana commonly called Avocado pear is a member of the family Lauraceae, which are mainly shrubs and trees that yield resinous aromatic gum from their cut bark (Liao and Well, 2007). 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, characterized by a central core surrounded by edible fleshy layers (Barry, 2001). The Avocado fruit has a pulpy mesocarp of 3 to 9 mm² 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). 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; identify species that could pose a threat to food safety and health hazard to end consumers of spoilt fruits.

MATERIALS AND METHODS

Study Area

This study was carried out in Abakaliki Local Government area of Ebonyi State. According to 1996 population and Housing census, Ebonyi State was made up of 1,064,156 males and 1,112,179 females. There was an estimated population of about 2.3 million people in Ebonyi State. The land mass of Ebonyi State is 5,935km². Approximately 30% of the population of Ebonyi State dwell in urban areas and are civil servants and students (Ezegwui *et al.*, 2013).



Figure 1: Map of Nigeria and Ebonyi State, showing Abakaliki the capital of Ebonyi State (Wikipedia.com, 2016).

Collection of Samples

A total of Sixteen (16) avocado pear samples (4 samples each) from four different markets 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 used in this study.

Bacteriological Analysis

Twenty five grams (25g) of each of the sample was weighed out 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 5th dilution was plated on Plate count agar and Nutrient agar, incubated at 37°C for 24 hours. The plates were removed and discrete colonies counted and multiplied by the dilution factor (Pellegrino *et al.*, 2002). Distinct colonies were subcultured on fresh nutrient agar plates to obtain pure cultures.

Characterization and Identification of Bacterial Isolates:

The bacterial isolates was identified on the basis of colonial morphology followed by microscopic examination after gram-staining. Biochemical tests including catalase, oxidase, motility, indole test, coagulase test and methyl red test was carried out to characterize the isolates as described by (Cheesbrough, 2006).

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 by dissolving 0.5g of hydrated barium chloride in 50ml of

distilled water . 0.6ml of barium chloride 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 (Onyeagba, 2004).

Standardization of test bacteria :

All the test bacteria isolated was 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 was carried out to obtain microbial population of 10^5 CFU/ml and compared with 0.5 McFarland turbidity standard (Cheesbrough, 2006).

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 (NCCLS, 2002). The following Oxoid antibiotics disk 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 (NCCLS, 2009).

RESULTS

The cultural, morphological and biochemical characteristics of the isolated bacteria, found in all avocado pear fruit samples purchased from four Markets Within Abakaliki.

Table 1: Morphological and Biochemical Characteristics of the Bacteria Isolated from avocado pear Purchased from four Markets Within Abakaliki.

S N	Biochemical							SHAPE	ARRANGEMENT	Morphological CHX	PROBABLE ORGANISM
	C A	C O	O X	IND	Motility	M R	GRA M RXN				
	+	-	-	-	+	-	-	Rod	Single	Pale white with black edges	<i>Salmonella</i> spp.
	-	-	-	-	-	-	-	Rod	Single	Pale white	<i>Shigella</i> spp.
	+	-	+	-	+	-	-	Rod	Single	Yellow-green	<i>Pseudomonas</i> spp.
	+	+	-	+	-	+	+	Cocci	Group	Yellow	<i>Staphylococcus aureus</i>
	-	-	-	-	+	-	-	Rod		Large, circular, gray smooth colonies	<i>Proteus</i> spp

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

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

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		AP 1	2.2×10^5
2		AP 2	3.8×10^5
3		AP 3	4.8×10^5
4		AP 4	3.8×10^5
	Presco		
5		AP 5	
6		AP 6	2.2×10^5
7		AP 7	5.0×10^5
8		AP 8	5.4×10^5
			2.2×10^5
	New Market		
9		AP 9	
10		AP 10	2.2×10^5
11		AP 11	3.8×10^5

12		AP 12	5.1×10^5 2.4×10^5
13	Kpirikpiri Market	AP 13	3.8×10^5
14		AP 14	5.8×10^5
15		AP 15	3.8×10^5
16		AP 16	3.9×10^5
		Mean (cfu/g)	3.5×10^5

This shows 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 5.8×10^5 cfu/g and lowest mean bacteria load of 2.2×10^4 were obtained (Table 2).

Table 3: Percentage Prevalence of Bacteria Species Isolated from Cabbage Samples Purchased Within Abakaliki Metropolis.

Total Number of samples collected	Total Number of Bacteria Isolated	Type of Bacteria isolated	source				Number and % of Bacteria isolated
			Meat Market	Presco	Kpirikpiri	New Market	
16	32	<i>Staphylococcus aureus</i>	1	0	2	1	4(12.5 %)
		<i>Shigella</i> species	2	0	1	2	5(15.6 %)
		<i>Salmonella</i> species	2	1	2	2	7(21.8 %)
		<i>Escherichia coli</i>	3	2	2	3	10(31.2%)
		<i>Klebsiella</i> species	2	1	1	2	6 (18.7%)

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 most occurrence bacteria isolated were *Escherichia coli*, followed by *Salmonella* species, and *Staphylococcus aureus*

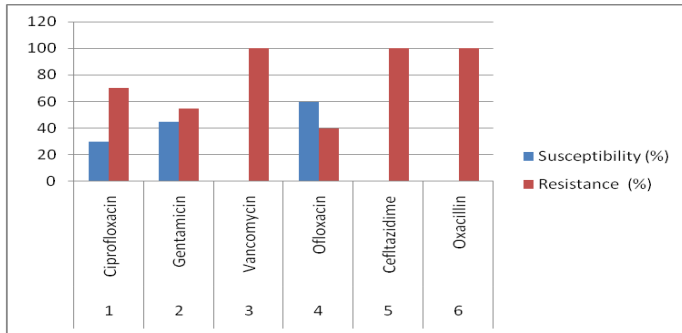
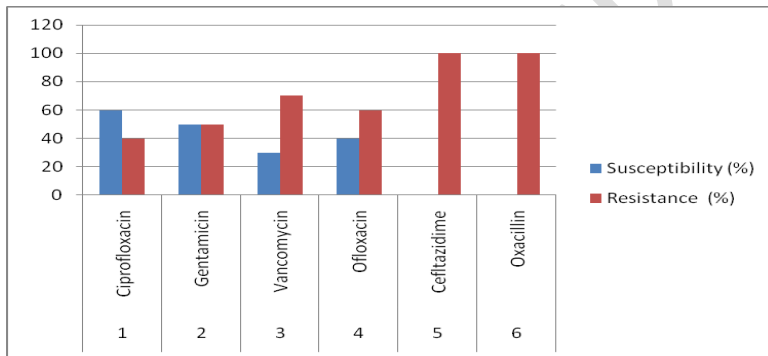


Figure 2 Antibiotic Percentage Resistance and Susceptibility of *Salmonella species* Isolated from Avocado pear Purchased from Selected Markets in Abakaliki.

Key: ceftazidime (CAZ), gentamicin (CN), ciprofloxacin (CIP), vancomycin (VA), oxacillin (OX) and ofloxacin (OFX).

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 ceftazidime. also *Salmonella species* isolation were susceptible to ciprofloxacin, ofloxacin and gentamicin with the percentage values of 30%, 45% and 60% respectively.



Figures 3 Antibiotic Percentage Resistance and Susceptibility of *Shigella species* Isolated from Avocado pear Purchased from Selected Markets in Abakaliki.

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

The result obtain from this study showed that *Shigella species* isolated from Avocado pear purchased from Abakaliki were 100% resistant to oxacillin and ceftazidime. also *Shigella species* isolation were susceptible to ciprofloxacin, gentamicin, vancomycin and ofloxacin with the percentage values of 60%, 50%, 30% and 40% respectively.

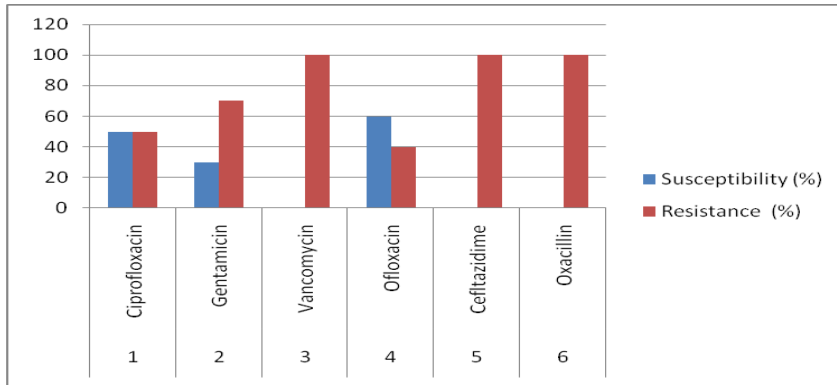


Figure 4 Antibiotic Percentage Resistance and Susceptibility of *Escherichia coli* Isolated from Avocado pear Purchased from Selected Markets in Abakaliki.

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

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

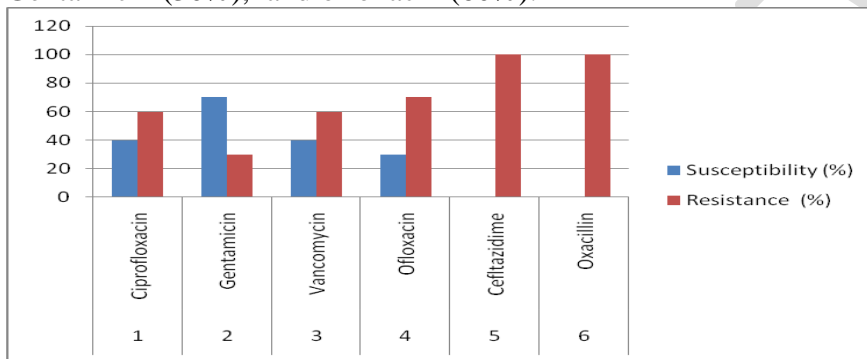


Figure 5 Antibiotic Percentage Resistance and Susceptibility of *Staphylococcus Aureus* isolated from Avocado pear purchased from Selected Markets in Abakaliki

Key: ceftazidime (CAZ), gentamicin (CN), ciprofloxacin (CIP), vancomycin (VA), oxacillin (OX) and ofloxacin (OFX).

The result obtain from this study showed that *Staphylococcus Aureus* isolated from Avocado pear purchased from Abakaliki were 100% resistant to oxacillin and ceftazidime. also *Shigella* species isolation were susceptible to ciprofloxacin, gentamicin, ofloxacin vancomycin and ofloxacin with the percentage values of 60%, 70%, 40% and 30% respectively.

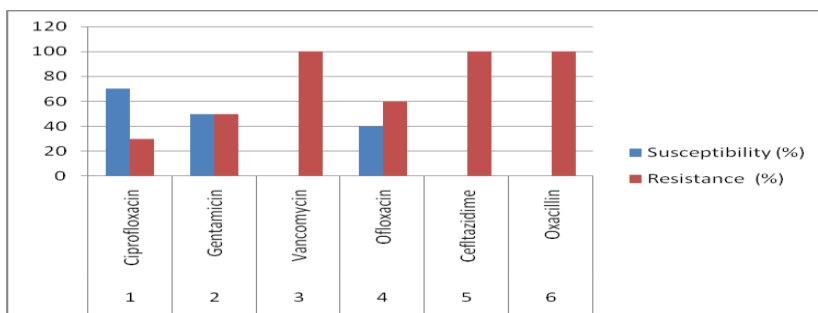


Figure 6 Antibiotic Percentage Resistance and Susceptibility of *Klebsiella* species isolated from Avocado pear purchased from Selected Markets in Abakaliki.

Key: ceftazidime (CAZ), gentamicin (CN), ciprofloxacin (CIP), vancomycin (VA), oxacillin (OX) and ofloxacin (OFX).

The result obtain from this study showed that *Klebsiella* species isolated from Avocado pear purchased from Abakaliki were 100% resistant to oxacillin, vancomycin and ceftazidime. also *Shigella* species isolation were susceptible to ciprofloxacin, gentamicin and ofloxacin with the percentage values of 70%, 50% and 40% respectively.

Discussions

In this study which was 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 these samples were bacteriologically examined for the isolation of common bacteria associated with avocado pear samples. The five (5) bacterial isolates were characterized and identified. This study showed the results of total aerobic plate count of avocado pear samples purchased from four different markets within Abakaliki with an average mean bacterial load of 3.5×10^5 CFU/g. This study was in line with previous studies from Frazier and Weshoff, (1998) who recorded highest total heterophilic counts of 3.8×10^5 cfu/g for foods in India. 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%) respectively. *Escherichia coli* 10(31.2%) showed highest percentage prevalence while *Staphylococcus aureus* 4 (12.5%) showed least percentage prevalence. These results corroborates the work of Ikenebomeh and Chikwendu, (1997) who identified similar microbes in his study. These organisms were equally reported as the causative agents for bacterial soft rots. 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. In this study, the *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. This result is in agreement with the work of Khatib *et al.*, (2014) in Lebanon. Khatib *et al.*, (2014) 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. There were many reports that enteric and non-enteric bacteria are fast-becoming resistant to some commonly used and available antibiotics (Salvador *et al.*, 2016). 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.

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.

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