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*) 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 x 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 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 gentanicin, ciprofloxacine 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

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 (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 and characterized by a central core surrounded by edible fleshy layers (Wogu and Ighile, 2014).The trees are partially self-pollinating and are often propagated through grafting to maintain a predictable quality and quantity of the fruit (Eze and Chimaeze, 2014). 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 (Eze and Chimaeze, 2014). Numerous species of microorganisms easily attack the fruit. The composition of the avocado fruit influences the likely type of spoilage (Eze and Chimaeze, 2014). 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 (Lopez, et al.,1996).

MATERIALS AND METHODS

Study Area

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

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 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.

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^{o}C for 24 hours. The discrete colonies on the plates were counted and multiplied by the dilution factor (Acharjee et al., 2013). Distinct colonies were subcultured on fresh nutrient agar plates to obtain pure cultures (Noor et al.,2013)

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, (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 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 (Onyeagba, 2004).

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 10^5CFU/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. 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), cefltazidime(CAZ,30 µg), gentamicin(CN, 10 µg), ciprofloxacine (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^{o}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

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

SN	Biochemical				Motility		GRAM RXN	SHAPE	ARRANGEMENT	Morphological CHX	PROBABLE ORGANISM
	CA	CO	OX	IND		MR					
	+	-	-	-	+	-	-	Rod	Single	Pale white with black edges	*Salmonella* spp.
	-	-	-	-	-	-	-	Rod	Single	Pale white	*Shigella* spp.
	+	-	+	-	+	-	-	Rod	Single	Yellow-green	*Pseudomonas* spp.
	+	+	-	+	-	+	+	Cocci	Group	Yellow	*Staphylococcus aureus*
	-	-	-	-	+	-	-	Rod		Large, circular, gray smooth colonies	*Proteus* 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
9	**New Market**	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 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 5.8×10^5 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	*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%)

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*

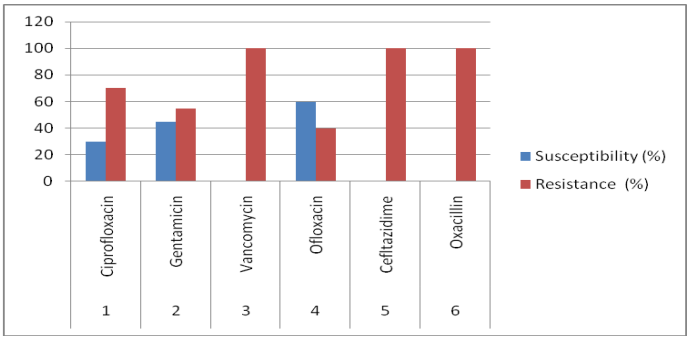
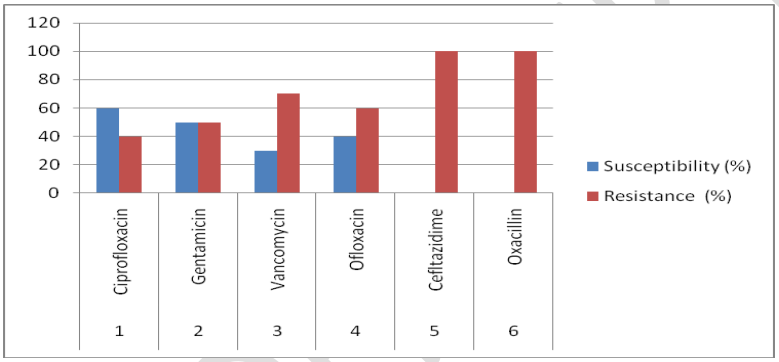


Figure 2 Antibiotic Percentage Resistance and Susceptibility of *Salmonella species* Isolated from Avocado pear Purchased from Selected Markets in Abakaliki.
Key: cefltazidime (CAZ), gentamicin (CN), ciprofloxacine (CIP), vancomycin (VA), oxacillin (OX) and ofloxacin (OFX).

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.



Figures 3 Antibiotic Percentage Resistance and Susceptibility of *Shigella 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 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%, 50%, 30% and 40% respectively.

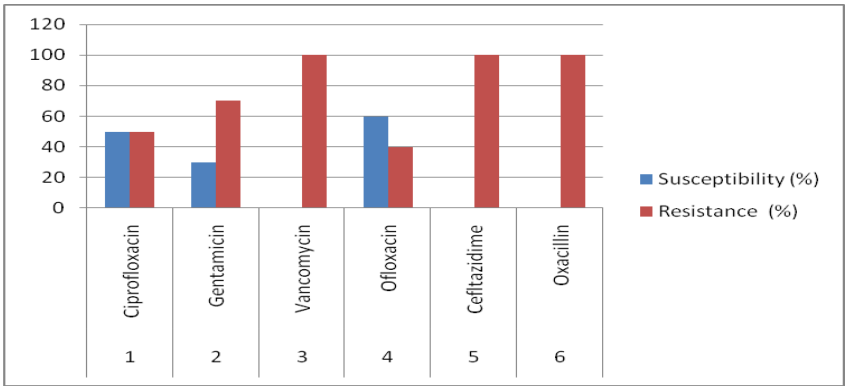


Figure 4 Antibiotic Percentage Resistance and Susceptibility of *Escherichia coli* Isolated from Avocado pear Purchased from Selected Markets in Abakaliki.
Key: cefltazidime (CAZ), gentamicin (CN), ciprofloxacine (CIP), vancomycin (VA), oxacillin (OX) and ofloxacin (OFX)

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%).

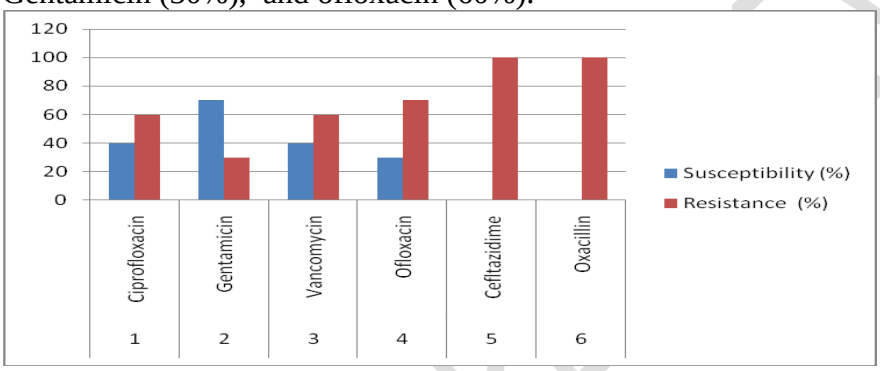


Figure 5 Antibiotic Percentage Resistance and Susceptibility of *Staphylococcus Aureus* isolated from Avocado pear purchased from Selected Markets in Abakaliki

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

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

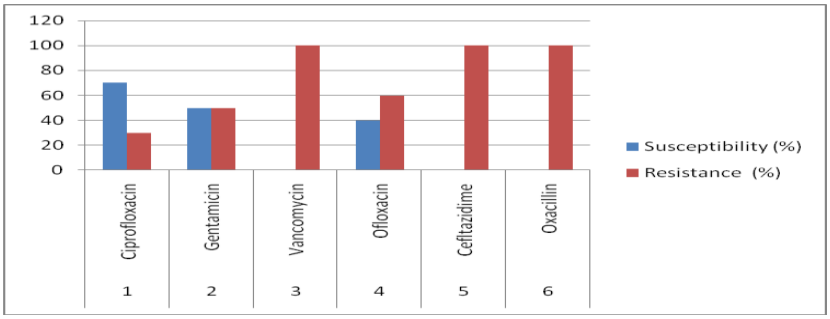


Figure 6 Antibiotic Percentage Resistance and Susceptibility of *Klebsiella* species isolated from Avocado pear purchased from Selected Markets in Abakaliki.
Key: cefltazidime (CAZ), gentamicin (CN), ciprofloxacine (CIP), vancomycin (VA), oxacillin (OX) and ofloxacin (OFX).

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

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, (1998) 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* 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 the highest percentage prevalence while *Staphylococcus aureus* 4 (12.5%) showed the least percentage prevalence. These results corroborate the work of Ikenebomeh and Chikwendu, (1997)who identified similar microbes. This was similar to the works of Ahmed et al.,2013; Rahman and Noor, 2012 who isolated same organisms. This was similar to the works of Akpoka et al.,2020 who reported same organisms in addition to *Erwinia* and *Phytophthora* isolated from Avocardo Pear in Benin City. The findings of this study was in harmony with the works of Afroz et al.,2015 who isolated same 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 (2014) 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 (Dreher and Davenport, 2013). Pre-harvest and post-harvest factors, consisting of the farm soil-type, storage conditions, and handling practices, maybe the likely source of these microbes (Leff and Fierer, 2013). 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 gentanicin, ciprofloxacine 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. 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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