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Bacteriological Assessment of Ready-to-Eat Pawpaw (*Carica papaya*) Sold in Selected Locations in Benin City

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ABSTRACT: This study was carried out to assess the microbiological quality of ready-to-eat papaya fruit from selected locations in Benin City. Standard bacteriological techniques using selective/differential media were employed for the isolation, purification and putative identification of selected bacterial isolates present in vended papaya fruits. The susceptibility and resistance of these bacterial isolates to common antibiotics was accessed. The public health implication of the identified isolates were also evaluated using their multiple antibiotic resistance (MAR) index. Pawpaw samples obtained from three locations had colonies which were too numerous to count while samples obtained from eight other locations had counts which ranged from 2.33×10^4 - 1.89×10^5 cfu/g. Total staphylococcal count ranged from 8.2×10^2 - 8.21×10^4 cfu/g. Coliform count ranged between 4.8×10^3 - 1.68×10^4 cfu/g. *Salmonella* count obtained from the pawpaw samples were in the range of 2.03×10^2 - 1.86×10^4 cfu/g while *Pseudomonas* counts were within the range of 4.2×10^3 - 6.7×10^3 cfu/g. The putative bacterial isolates from vended pawpaw fruit were *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica, Klebsiella oxytoca* and *Enterococcus faecalis*. The isolates were found to be resistant to some of the antibiotics such as Ampicillin, Amoxicillin, Eythromycin and Cefixime. They however, were susceptible to Gentamicin and Ciprofloxacin and were least susceptible to Amoxicillin, Ampicillin and Cefixime. Results obtained are of public health importance as the isolates were found to have an MAR index greater than 0.2.

Keywords: Public health, MAR index, Pawpaw, Papaya, Coliform, Vended fruits

Introduction

Nigeria is the third largest producer of papaya in the world with 703,800 metric tons (FAO, 2010). Consumption of sliced fruits has been on the increase since they are easily accessible, convenient and most especially cheaper compared to purchasing the whole fruit (Nwachukwu *et al.,* 2008). It has been reported by Barro *et al.* (2006) and WHO (2002) that vended fruits or street foods are commonly associated with gastrointestinal diseases due to poor hygiene (improper handling and serving practices). However, sliced fruits are commonly processed and sold by unlicensed vendors with no or poor level of education as well as no training in food hygienic practices (Barro *et al.*, 2007). There are several concerns over the health of consumers as a result of the unhygienic conditions of the vended fruits (Tambekar *et al.,* 2009). According to Daniel *et al.* (2014), unsanitary processing, poor preservation methods, use of contaminated trays for the display of fruits and cross-contamination of sliced fruits further increases the risk of contamination. The aforementioned fact was also as noted by Bryan *et al.* (1992), who opined that open display of vended fruits encourages sporadic visits by flies, cockroaches, rodents and dust. Poorly processed street vended produce have been identified as an important cause of deaths in developing countries (Mensah *et al.,* 2002). Bacteria causing gastroenteritis can contaminate the sliced produce, thus exposing consumers to greater risk of infection (Jolaoso *et al.,* 2010). The increase in the consumption of vended fruits in most developing countries is attributed to lack of formal jobs for the working age groups. Sales of sliced fruits can contribute significant income for households and at the same time providing a source of inexpensive nutritious meal (Mosupye and Von Holy, 2000). Outbreak of infections due to the consumption of vended fruits had been reported in literatures (Ries *et al.,* 1992; Swerdlow *et al.,* 1992; Weber *et al.,* 1994; Bean and Griffin, 1996; Koo *et al.,* 1996). The increase in consumption of sliced fruits has been linked with a parallel increase in food borne illness (Estrada-Garcia *et al.*, 2004).

Fruits are known to carry a natural non-pathogenic microflora, and have an epidermal layer of cells which provides a barrier for penetration of microorganisms. Cutting and slicing can eliminate the protections and microbes can invade the internal tissue (Barro *et al.*, 2007). Bacteria such as *Salmonella* sp., *Shigella* sp., *Campylobacter* sp., and *Escherichia coli* can contaminate sliced fruits through contact with sewage and contaminated water (Gayler *et al.*, 1995). These microorganisms, under the influence of environmental factors (temperature, pH, oxygen, moisture content) pose serious threats to papaya fruit production. Besides the losses in income to the papaya fruit marketers, the rotten fruits could also cause health hazard to consumers. Krogh (1992) had earlier reported that most microbes infecting plant tissues often produced secondary metabolites in their hosts, which are known to be hazardous to animals including man. Some of these metabolites include the ergot alkaloids on cereals by *Clavicaep* spp., fumonisin on maize by *Fusarium spp*, aflatoxins and ochratoxins on several plants products by *Aspergillus* spp. (Prasad, 1992).

These microorganisms, under the influence of environmental factors, pose serious threats to public health of those who consume pawpaw fruit. The study was carried out to evaluate the bacteriological quality of ready-to-eat papaya fruits sold in selected locations in Benin City.

Materials and methods

Study Design: Thirty-one (31) vended papaya samples were purchased from different vendors at ten different locations and analyzed in the month of June 2017. The vendors were orally quizzed about the time of preparation of the vended samples, their level of education, awareness of hygienic practices. A control sample containing whole fresh papaya fruit was aseptically prepared in the laboratory and analysed. A four-fold serial dilution was used to enumerate the total heterotrophic bacterial, coliform, staphylococcal and Pseudomonas count present in the vended papaya fruit samples. The four-fold dilution was employed in order to detect more accurately population of bacteria which may be present in the apparently sterile ready to eat fruit samples. Standard susceptibility testing using Kirby Bauer method (Bauer *et al.,* 1996) was employed to determine the level of resistance of the putative bacterial isolates.

Sample Processing and Quantitative Detection of Bacteria: Samples were processed immediately after collection in the Department of Microbiology, University of Benin. A sterile blade was used to cut 25 g from each of the ready-to-eat papaya sample and aseptically transferred into a 150 mL conical flask and then sealed with foil paper to prevent contamination. The sample in the conical flask was vigorously shaken. 75 mL of normal saline (which was prepared by dissolving 8.5g of NaCl in 1 L of distilled water and then autoclaving at 121 ºC for 15 min) was added to enhance the homogenization of the sample and to dissolve the sample. The resultant mixture was regarded as the stock suspension with a total volume of 100 mL. Subsequently, 2.5 mL of suspension was taken from the stock suspension and added to a test tube containing 7.5ml of normal saline and shaken properly to ensure homogeneity. Another 2.5 mL was pipetted out of the first dilution test tube and added to a second test tube containing 7.5 mL of normal salins. This was done continuously until the fifth serial dilution. From the appropriate dilution, 200 µL each was inoculated on the different media for analysis.

Identification of Bacteria: Relevant staining and biochemical tests were carried out on isolates recovered from selective cum differential agar used in the study. Catalase, coagulase, oxidase, citrate, indole, urease and sugar fermentation test were used to confirm the putative identity of the bacterial isolates after positive growth on differentia agar.

Antibiotics Susceptibility Test: The antimicrobial agents were chosen on the basis of their importance in treating human or animal infections caused by Gram positive and Gram negative bacteria as well as a broad spectrum antibiotic of choice for multi-resistant pathogen. The already putatively identified bacterial isolates were made to undergo antibiotics susceptibility testing using the standard Kirby-Bauer disc diffusion technique (Bauer, 1966). A loopful of each test bacterial corresponding to 10^8 cells/mL were evenly streaked on Mueller-Hinton agar and the streaked plate was impregnated with different antibiotic discs manufactured by Oxoid Limited which include: Cephazolin (30 µg), Ampicillin (10 µg), Cefixime (5 µg), Ciprofloxacin (5 µg), Ceftriaxone (30 µg), Erythromycin

(15 µg), Vancomycin (30 µg), Gentamicin (10 µg), Cefuroxime (30 µg) and Amoxicillin (25 µg). The plates were incubated at 37 ºC for 24 h after which the zones of inhibition were measured and interpreted as Resistant (R), Intermediate resistant (I) or Sensitive (S) in conformity with the recommended standards established by the Clinical Laboratory Standards Institute (2017).

Multiple Antibiotic Resistances (MAR) Index: The MAR index is a good tool for health risk assessment which identifies if the isolates are from a region of high or low antibiotic usage. A MAR index of 0.2 and above indicates a 'high-risk' source of contamination (Davis and Brown 2016). The multiple antibiotic resistance MAR index was determined for each isolate using the methods delineated by Chitanand *et al.* (2010) by dividing the percentage of antibiotic resistance of the total percentage of antibiotics used in the study. However, the total percentage resistance of certain multi-resistant organisms' was calculated and used as the numerator.

Statistical Analysis: The obtained data were exposed to version 21.0 of SPSS statistical package. Descriptive statistics were employed to both determine the level of contamination as well as the susceptibility profile of obtained isolates.

Results

The results for total heterotrophic, staphylococcal, coliforms, *salmonella,* and *Pseudomonas* count is presented in Table 1. Some pawpaw samples had colonies too numerous to count while the other samples had counts which ranged from 2.33 x 10^4 - 1.89 x 10^5 cfu/g. Total staphylococcal count ranged from 8.2 x 10^2 - 8.21 x 10^4 cfu/g. Coliform count ranged between 4.8 x 10^3 - 1.68 x 10^4 cfu/g. *Salmonella,* obtained from the fruit samples ranged between 2.03x10^2 to 1.86x10^4 while similar scenario was observed for *Pseudomonas aeruginosa* with values within the range of 4.2x10^3 - 6.7x10^3 cfu/g (Table 1).

Table 1: Total heterotrophic, Staphylococcal, coliforms, *Salmonella,* Enterococcal and *Pseudomonas* count from vended papaya fruits sold in selected locations in Benin City.

Locations of Vended Fruits	**Total Bacterial Count (X10^4)** cfu/g				
	Heterotrophic	**Staphylococcal**	**Coliform**	***Salmonella***	***Pseudomonas***
A	TNC	8.21	0.57	0	0.53
B	6.67	0.042	0.59	0	0
C	4.67	0	0	0	0
D	2.09	0	0	0	0
E	TNC	1.678	0	1.89	0.42
F	TNC	2.51	1.68	1.68	0.67
G	6.67	0	0	0	0
H	13.67	3.0	0	0	0
I	18.99	2.03	0	0	0.44
J	2.33	0.082	0.48	0	0
K	0.0052	0.0041	0	0	0

Legend: A= Hawker, B=Anatomy gate, C= Unrinsed, D=Rinsed, E=Hall 4. F= Lab, G=New Benin, H= Ekosodin, I= BDPA, J = Control, TNC = Too numerous to count

Amount of microorganism in CFU/g of sample is given by the formula

$$\frac{cfu}{g} = \frac{no.\ of\ colonies\ X\ dilution}{voulume\ of\ inoculum}$$

Figure 1 reveals the percentage occurrence of bacterial isolates from vended papaya fruits obtained from different locations in Benin City. *Pseudomonas aeruginosa, Enterococcus, Klebsiella* and *E. coli* were found to have 36.36% while *Staphylococcus* had 70.72%.

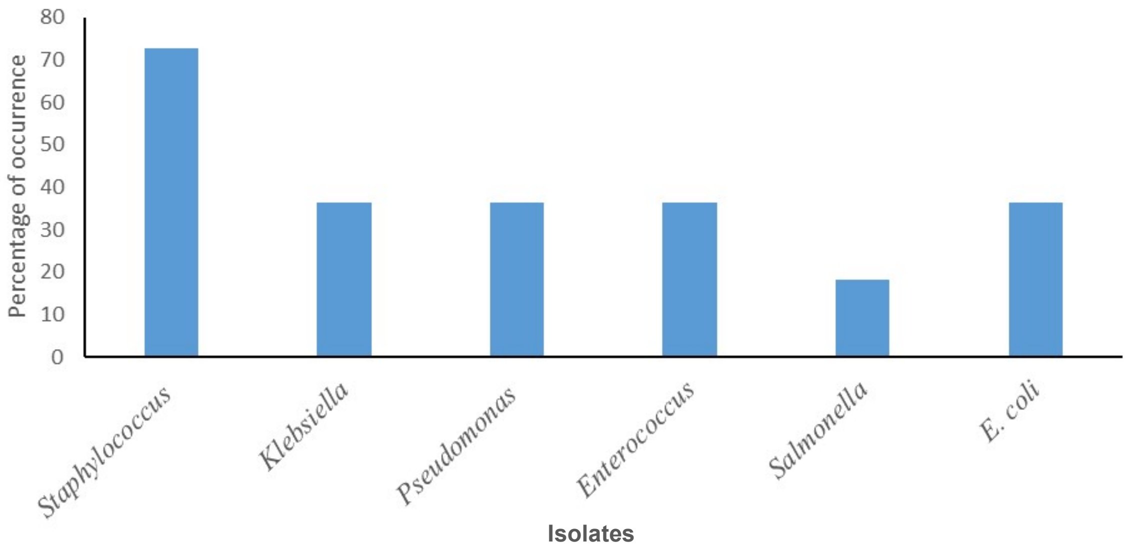

Fig. 1. Percentage occurrence of bacterial isolates from vended papaya fruits

The antibiotic sensitivity and resistance pattern of isolated bacterial species from papaya fruit is shown in Table 2. The isolated bacterial species were found to be resistant to most of the antibiotics commonly used. However, all isolates were susceptible to Gentamicin (100%) and Ciprofloxacin (100%) while they were least susceptible to Amoxicillin, Ampicillin and Cefixime.

Table 2. Antibiotic susceptibility profile of putative bacterial isolates from vended papaya fruits

	Antibiotic Susceptibility of Isolates (%)									
Isolates	CIP	AML	AMP	CFM	CXM	E	CRO	CN	VA	KZ
Klebsiella (4)	100	50	25	50	50	75	60	100	50	100
Staphylococcus (6)	100	83.3	83.3	33.3	83.3	50	66.7	100	50	100
Enterococcus (4)	100	80	80	25	80	50	25	100	60	80
Salmonella (2)	100	0	0	50	100	0	-	100	0	0
Pseudomonas (4)	100	0	0	25	25	50	75	100	50	50
E. coli (4)	100	0	0	50	50	0	100	100	50	50
Total (%)	100	35.6	31.4	38.9	64.7	37.5	54.5	100.0	43.3	63.3

The antibiotic resistance patterns exhibited by bacterial species isolated from vended papaya fruit is shown in Table 3. The isolates showed multi-resistance, *Klebsiella* (48.5%) and *Staphylococcus aureus* (35.7%) showing resistance each to three antibiotics such as to AML, AMP, CFM, CXM, E, VA, and CRO respectively.

Table 3. Antibiotic resistance pattern exhibited by the bacterial isolates obtained from vended papaya fruits

Isolates	**Antibiotics Resistance Patterns**	**Isolates Percentage (%)**
Klebsiella	AML, AMP, CFM, CXM, E, VA, CRO	48.5
Staphylococcus	AML, AMP, CFM, CXM, E, VA, CRO	35.7
Enterococcus	AML, AMP, CFM, CXM, E, VA, KZ, CRO	45.7
Salmonella	AML, AMP, CFM, CXM, E, VA, KZ, CRO	55.5
Pseudomonas	AML, AMP, CFM, CXM, E, VA, KZ, CRO	91.6
E. coli	AML, AMP, CFM, CXM, E, VA, KZ, CRO	65.6

The MAR index of the putative bacterial isolates is shown in Figure 1. The results revealed that all isolated bacteria were obtained from sources of high contamination. The isolates were found to be of public health concern as they all had an MAR index greater than 0.2 with *Salmonella, Pseudomonas* and *E. coli* having MAR index of 0.610, 0.525, and 0.500 respectively. Several researchers have hinted that with MAR index greater than 0.2, one need to worry about the public health safety of the hosts of these multi resistant bacteria.

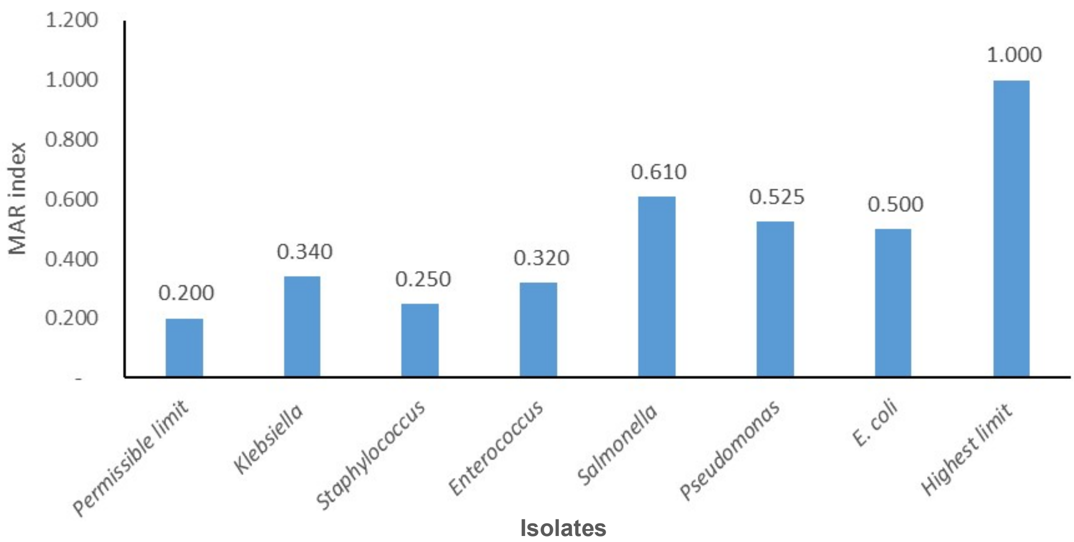

Figure 2. Multiple antibiotic resistance index of putative bacterial isolates from vended pawpaw fruits

Discussion

The supposed increase in the consumption of sliced fruits has been linked with a parallel corresponding increase in food borne illnesses as reported in the studies carried out by Mensah *et al.* (1999) and Estrada-Garcia *et al.* (2004). The result obtained in this study is in agreement with previous report that vended fruit can serve as a vehicle of transmission of foodborne pathogens to consumers. Fruit produce is known to carry a natural nonpathogenic microflora and has an epidermal layer of cells which provides a barrier for penetration of microorganisms. Cutting and slicing usually eliminate the natural protective barriers of the fruit thus paving way for microbial cells to invade the internal tissues of the fruit (Barro *et al.*, 2007). The result obtained in this study shows that certain bacterial contaminants such as *Staphylococcus aureus*, *Salmonella, Pseudomonas* and *Escherichia coli* are a ready contaminants of vended papaya fruits and the presence of these bacteria in the fruit samples is a public health problem especially for the elderly, the children or immunocompromised in the community. Orji *et al.* (2016) studied the microbial contamination of ready-to-eat vended fruits in Abakpa main market, Abakaliki, Ebonyi state. They reported five bacterial isolates; *Salmonella*, *Pseudomonas*, *Escherichia coli, Shigella* and *Staphylococcus aureus* which was also in tandem with the findings in this study. Daniel *et al.* (2014) also had similar findings with the result obtained in this study where *Salmonella*, *Escherichia coli,* and *Staphylococcus aureus* were recovered from the vended fruits. *Enterobacter* and *Streptococcus* species which were not found in this study probably because they were not screened for. Similar reports were also evident in the studies of Odebisi-Omokanye *et al.* (2015) and Oranusi and Olorunfemi (2011). In both studies, they isolated *Salmonella*, *Escherichia coli,* and *Staphylococcus aureus* in addition to several other bacterial species of public health importance which were not screened for in this study.

Bryan *et al*. (1992) reported that unsanitary processing and preservative methods could increase the possibility of contamination as open display of street food or fruits encourage the sporadic visits by flies, cockroaches, rodents and dust which are potent carriers of microbial contaminants. These findings correlated with the result obtained in this study where the control sample prepared under the aseptic conditions in the laboratory had few or no bacterial population compared to other samples with counts ranging between 2.03 x 10^4 - over 1 x 10^6 cfu/g (depicted as too numerous to count (TNC)). The laboratory control samples were also devoid of several other contaminants of public health importance such as *Salmonella, Pseudomonas* and coliforms. Bacteria such as *Salmonella*, *Pseudomonas*, and *Escherichia coli* usually contaminate sliced fruits through contact with sewage and contaminated water (Gayler *et al.*, 1995). *Staphylococcus aureus* which is a normal flora of the skin, is usually introduced through unclean hands of the vendor or materials used during preparation or processing of vended fruits. *Salmonella* and *Pseudomonas* could have been introduced from water during washing or by soil and flies as reported by Gayler *et al*. (1995). The presence of *Salmonella* could also be possibly due to fecal contamination of water and hands of latent carriers as a consequence of poor personal hygiene as reported by Jolaoso *et al.* (2010). *E. coli* had also been associated with fecal contamination and its presence poses a serious threat to public health. Its presence in the vended fruit is also indicative of secondary contamination. All the isolated bacterial pathogens have shown by their antimicrobial susceptibility patterns that they are potential pathogens of public health importance. The multiple antibiotic resistance (MAR) index is a good tool for health risk assessment which identifies if the isolates are from a region of high or low antibiotic usage. An MAR index of 0.2 and above is indicative of 'high-risk' source of contamination as reported by Davis and Brown (2016) and Chitanand *et al.* (2010). The MAR index of isolated bacterial pathogen ranged between 0.25 - 0.60 (Figure 2).

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