Full Length Research Paper

Antimicrobial sensitivity of bacterial isolates from street vended fruits in Ijebu area of Ogun state, Nigeria

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ABSTRACT

Street vended fruits are fruits which are sold by street vendors and require no further processing like peeling, slicing and washing before consumption. A total of seventy-five (75) samples of sliced fruits: pineapples (20), pawpaw (25), watermelons (15) and coconut (15) were purchased from different vendors in Ijebu area of Ogun State and pour plate method was used to analyze the samples to determine their microbiological safety. Identification of gram negative, oxidase negative bacteria was done using API 20E kit and the conventional biochemical testing for gram positive bacteria. Antibiotic sensitivity test was carried out by disc diffusion method. The microbial load for pawpaw was in the range of 2.6-8.0 × 10^5 cfu/ml, Pineapple 1.2 – 2.3 ×10^5cfu/ml, Coconut 0.5 – 5.6 × 10^5 cfu/ml and watermelon 3.0 – 9.3 × 10^5 cfu/ml Bacteria isolated include *Staphylococcus aureus, Micrococcus* sp, *Bacillus subtilis, Lactobacillus* spp, *Streptococcus* spp, *E. coli, Bacillus cereus, Klebsiella pneumoniae, Serratia plymuthica, Serratia ficaria, Proteus mirabilis* and *Enterococcus faecalis.* Antibiotic sensitivity test carried out on the isolates revealed that many of the bacteria showed an intermediate to sensitive reaction to most of the antibiotics tested. All the gram positive bacteria were sensitive to Ciprofloxacin, Streptomycin, Cloxacillin and most were resistant to cefuroxime. All the gram negative isolates were sensitive to chloramphenicol. *K. pneumoniae* was resistant to pefloxacin and co-trimoxazole. *S. plymuthica* was sensitive to all the antibiotics. Transmission of resistant bacteria is a potential concern with unhygienic handling of sliced fruits. Better handling process and health education is crucial to preventing spread of resistant bacteria and food poisoning through these otherwise commonly consumed fruits.

Keywords: Pineapple, pawpaw, watermelon, coconut, bacteria, antibiotics.

INTRODUCTION

Street vended fruits are fruits prepared by vendors for immediate consumption. According to Oranusi and Olorunfemi (2011), ready-to-eat fruits are fruits that can be bought directly from street vendors or hawkers or at local markets and eaten immediately i.e. without necessarily having to cut, peel or rinse them before consumption as they have already been prepared by the vendors.

In common language usage, "fruit" normally means the fleshy seed-associated structures of a plant that are sweet or sour and edible in the raw state, such as apples, oranges, grapes, strawberries, bananas, and lemons (Schlegel, 2003). Fruits may be eaten, pre-cut or sliced

into pieces. Pre-cut fruits refer to fruits that have been cut open, sliced into pieces but remain in the fresh state and are stored or displayed for sale or for serving in retail outlets (fresh fruit packs in supermarkets, cut fruits in buffet) assorted fruits offered by restaurants and vendors (Kaplan and Campbell, 1982; Lund, 1992; De Roever, 1998).

Fruits are an extraordinary dietary source of nutrients, micronutrients, vitamins and fiber for humans and are thus vital for health and well-being. Well balanced diets, rich in fruits are especially valuable for their ability to prevent vitamin C and vitamin A deficiencies and are also reported to reduce the risk of

several diseases (Kalia and Gupta, 2006).

Over the years, there has been a significant increase in the consumption of sliced/ready to eat fruit because they are easily accessible, convenient, nutritious and, most especially, cheaper than the whole fruits (Nwachukwu et al., 2008). Regular consumption of fruit is associated with reduced risks of cancer, cardiovascular disease (especially coronary heart disease), stroke, Alzheimer disease, cataracts, and some of the functional declines associated with aging (Liu, 2003).

Raw foods, especially ready-to-eat salad vegetables, sprouts and cut fruits have been implicated in outbreaks of food borne diseases in both developed and developing countries (WHO, 1998).

Occasional reports of multistate outbreaks of salmonellosis in the United States associated with contaminated fresh fruits and vegetables have coincided with increased consumption of fresh produce in recent years due to changing consumer preferences, greater selections, wider distribution, and year-round availability (Hedberg et al., 1994). In Nigeria for instance, street vending of handy ready-to-eat sliced fruit and vegetables has recently become very common and the market is thriving (Eni et al., 2010). Street foods are perceived to be a major public health risk due to lack of basic infrastructure and services, difficulty in controlling the large numbers of street food vending operations because of their diversity, mobility and temporary nature(Ghosh et al., 2007; de Sousa, 2008) and are untrained in food hygiene (Barro et al., 2006). Sliced fruits commonly consumed in Nigeria include paw-paw, pineapple, watermelon, salad vegetables, cucumbers, carrots and pears. Their increased consumption, coupled with the associated risk of disease to which consumers may be exposed, is a matter of great concern. This increase in the consumption of sliced fruit has been linked with a parallel increase in food-borne illness (Estrada-Garcia et al., 2004, Mensah et al., 1999).

Microbiological studies from many developing countries, carried out on street vended food articles have revealed a high bacteria count. Salmonella species, S. aureus and members of the family Enterobacteriaceae were common pathogens found in such food items (Bryan et al., 1997; Mosupye and von Holy, 1999). Enteric pathogens such as E. coli and Salmonella are among the greatest concerns during food related outbreaks (Buck et al., 2003).

Contamination or cross-contamination of street foods, especially sliced fruits and vegetables are increased by unsanitary processing and preservation methods. The use of dirty utensils, as well as the open display of street food produce encourages sporadic visits by flies, cockroaches, rodents and dusts (Bryan et al., 1992). Food contamination with antibiotic resistant bacteria can be a major threat to public health, as the antibiotic resistance determinants can be transferred to other pathogenic bacteria potentially compromising the treatment of severe bacterial infections.

The prevalence of antimicrobial resistance among food pathogens has increased during recent decades (Davis et al., 1999; Garau et al., 1999; Threfall et al., 2000 and Chui et al., 2002). Despite the extensive studies, the antibiotic sensitivity of theses bacteria has not been well studied especially in a developing country. The aim of this research is therefore threefold: isolate bacteria from sliced fruits sold by street vendors in south-west Nigeria, characterize the bacteria isolates by using both Analytical Profile Index (API 20E) and conventional method for gram negative and gram positive bacteria respectively and perform antimicrobial sensitivity test on the bacteria.

MATERIALS AND METHOD

Collection of Samples

A total of seventy-five (75) samples of sliced fruits: pineapples (20), pawpaw (25), watermelons (15) and coconut (15) were purchased from different fruit vendors in Ijebu Area of Ogun State in South-West, Nigeria. Each sample was placed separately in sterile polythene bags and transported to the laboratory for processing within one hour of collection.

Isolation of Bacteria

Ten grams (10g) of each fruit was weighed using a sterile knife and pounded in a sterile ceramic mortar and pestle, and then dissolved in a conical flask containing 90ml of sterile distilled water. The resultant homogenate was diluted $10^{-2},$ 10^{-3}, 10^{-4} and 10^{-5}. From the appropriate dilution, 1ml of the inoculum was placed inside separate sterile Petri-dish. Plate Count Agar (PCA), Nutrient agar and MacConkey agar that have been allowed to cool were carefully poured in the plates. Extreme care was taken not to spill the media or allow it to get on the lid of the Petri dishes. The media with the inocula in the plates were allowed to solidify and incubated invertedly at 37^{0}C for 24-48 hours. Discrete colonies on the different media were isolated and purified by repeated sub culturing on fresh agar to obtain pure cultures of the different isolates and stored at 4^{0}C for further tests.

Enumeration of Bacteria

Colonies were counted at the expiration of incubation period. Counts were expressed as colony forming unit per ml of sample homogenates.

Characterization of Bacteria Isolates

All bacteria isolates were gram stained. Gram positive bacteria were characterized biochemically using the conventional method while Gram negatives were characterized using API 20E kit. The biochemical tests used to further characterize bacteria are: catalase, methyl-red, oxidase, citrate utilization, coagulase and indole tests. Oxidase test was also carried out on the Gram negative isolates to know if they are oxidase positive or negative. Only oxidase negative Gram negative bacteria isolates were used for the Analytical Profile Index 20E.

Antibiotic Susceptibility Testing

The antibiotic sensitivity testing was carried out using the disc diffusion method as described by Bauer *et al.* (1966) on Mueller-Hinton agar and were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (2002). The antibiotics used for Gram positive bacteria were Ampiclox, Cefuroxime, Amoxicillin, Rocephin, Ciprofloxacin, Streptomycin, Co-trimoxazole, Erythromycin, Pefloxacin and Cloxacillin while the antibiotics used for Gram negative bacteria were Ciprofloxacin, Amoxicillin, Amoxicillin-clavulanate, Cloxacilin, Pefloxacin, Ofloxacin, Streptomycin, Co-trimoxazole, Chloramphenicol, and Sparfloxacin.

RESULTS

The microbial loads of the fruits samples are presented on Table 1. The result showed that watermelon has the highest microbial load of 9.3×10^5 cfu/ml followed by pawpaw with load of 8.0×10^5 cfu/ml, coconut 5.6×10^5 cfu/ml and pineapple with the least microbial load of 2.3×10^5 cfu/ml.

Biochemical Characterization for Gram positive bacteria using conventional methods revealed the presence of six gram positive bacteria namely: *Staphylococcus aureus*, *Micrococcus* sp., *Bacillus subtilis, Lactobacillus* sp., *Streptococcus* sp. and *Bacillus cereus* while the gram negative bacteria using Analytical Profile Index (API 20E) also reveals the presence of six species of bacteria namely: *Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis, Serratia plymuthica, Serratia ficaria* and *Enterococcus faecalis*.

Table 2 below shows the occurrence of bacteria isolates from street vended fruits samples. The result showed that *S. aureus* has the highest incidence of 13.4% followed by *Micrococcus* and *Lactobacillus* with an incidence of 12.0%. The bacteria with the least incidence were *E. faecalis, S. plymuthica, S. ficaria* and *B. cereus* with an incidence of 5.3% each.

Table 3 below shows the result of antibiotic test performed on Gram positive bacteria. All the gram positive isolate were +sensitive to cloxacillin. *Micrococcus, B. subtilis, B. cereus* and *Lactobacillus* were resistant to cefuroxime. *S. aureus* and *Micrococcus* sp. were intermediate to sensitive to all the antibiotics except amoxicillin. *B. subtilis* showed resistance to cefuroxime and amoxicillin; *Streptococcus* sp was resistant to amoxicillin while *B. cereus* was resistant to cefuroxime and erythromycin

Table 4 below shows the result of the antimicrobial test performed on gram negative bacteria. *S. plymuthica* was sensitive to all the antibiotics while *S. ficaria, E. faecalis* and *E. coli* were intermediate to sensitive to all the antibiotics tested. *K. pneumoniae* was resistant to pefloxacin and co-trimoxazole; *Proteus mirabilis* showed resistance to co-trimoxazole only.

DISCUSSION AND CONCLUSION

The microbial loads for the sliced fruits are shown in Table 1. The microbial load for pawpaw was in the range of 2.6-8.0×10^5 cfu/ml, Pineapple $1.2 - 2.3 \times 10^5$ cfu/ml, Coconut $0.5 - 5.6 \times 10^5$ cfu/ml and watermelon $3.0 - 9.3 \times 10^5$ cfu/ml. These results conform to those obtained by Nwachukwu *et al.* (2008), Farzana *et al.* (2011), Oranusi and Oluwafemi (2011) who recorded microbial load in fruits in the range of $10^4 - 10^9$ cfu/ml.

A total of 11 different bacteria species were isolated from the seventy (75) sliced fruits samples analysed and they are *Staphylococcus aureus, Micrococcus, B. subtilis, Lactobacillus, Streptococcus, B. cereus, Klebsiella pneumoniae, Serratia plymuthica, S. ficaris, Enterococcus faecalis, Proteus mirabilis* and *E. coli.* The isolation of these organisms is supported by the work of Eni *et al.* (2010); Jolaoso *et al.* (2010) who isolated *S. aureus, Klebsiella* sp, *Salmonella, Escherichia coli* from fruits. Daniyan and Ajibo (2011) also isolated *S. aureus, S. epidermidis, Bacillus* sp., *E. coli* and *Enterobacter aerogenes* from sliced fruits sold in Minna metropolis. This is further supported by the work of Oranusi and Olurunfemi (2011) who isolated *Bacillus, S. aureus, E. coli, Enterobacter, Salmonella, Klebsiella, P. aeruginosa, Proteus, Micrococcus* and *Lactobacillus* sp. from vended ready to eat fruits sold in Ota, Ogun State. Tambeker *et al.* (2009) also isolated *E. coli, P. aeruginosa, Salmonella, Proteus, S. aureus, Klebsiella* and *Enterobacter* from street vended fruits juices in Amravati city, India. *E. coli, K. aerogenes, P. mirabilis, S. aureus, Lactobacillus* were also recovered from sliced water melon (Nwachukwu *et al.*, 2008).

The incidence of the different bacteria isolated is presented on table 2. The table showed that *S. aureus*(13.4%), *Micrococcus*(12.0%) and *Lactobacillus*(12.0%) are the bacteria frequently isolated from the sliced fruits. The bacteria least isolated were *E. faecalis, S. plymuthica, S. ficaria* and *B. cereus* with an incidence of 5.3% each. Jolaoso *et al.* (2010) and

Table 1. Range of Microbial load of street vended fruit samples

Fruit samples	Microbial load (10^5cfu/ml)
Pawpaw	2.6 – 8.0
Pineapple	1.2 – 2.8
Coconut	0.6 – 5.6
Watermelon	3.0 – 9.3

Table 2. Incidence of bacteria isolates in fruit samples

Sample name	Total no of samples	S. aureus	Streptococcus	Micrococcus	B. subtilis	B. cereus	Lactobacillus	P. mirabilis	E. coli	K. pneumoniae	S. plymuthica	S. ficaris	E. faecalis
Pawpaw	25	2 2.7%	3 4.0%	2 2.7%	2 2.7%	1 1.3%	2 2.7%	3 4.0%	2 2.7%	3 4.0%	2 2.7%	3 4.0%	2 2.7%
Pineapple	20	3 4.0%	1 1.3%	- -	1 1.3%	2 2.7%	3 4.0%	2 2.7%	2 2.7%	3 4.0%	1 1.3%	- -	2 2.7%
Coconut	15	2 2.7%	2 2.7%	4 5.3%	2 2.7%	- -	1 1.3%	- -	1 1.3%	- -	1 1.3%	1 1.3%	- -
Watermelon	15	3 4.0%	2 2.7%	3 4.0%	1 1.3%	1 1.3%	3 4.0%	1 1.3%	-	-	-	-	-
Total	75	10 13.4%	8 10.7%	9 12.0%	6 8.0%	4 5.3%	9 12.0%	6 8.0%	5 6.7%	6 8.0%	4 5.3%	4 5.3%	4 5.3%

Table 3. Antimicrobial Sensitivity Test for Gram positive Bacteria Isolates

ISOLATES	APX	CRX	AMX	CEF	CPX	STR	COT	E	PEF	CXC
S. aureus	I	S	R	S	I	S	I	I	I	S
Micrococcus sp	I	R	I	I	S	S	S	S	S	S
B. subtilis	S	R	R	I	S	S	S	I	S	S
Lactobacillus sp	S	R	S	I	S	R	S	I	I	S
Streptococcus sp	I	S	R	I	S	S	I	I	S	S
B. cereus	S	R	S	S	S	S	S	R	S	S

KEY
R = Resistance, I = Intermediate, S = Sensitive.
APX - Amplicox, CRX – Cefuroxime, AMX – Amoxicillin, CEF - Ceftriaxone, CPX-Ciprofloxacin,
STR-Streptomycin, COT-Cotrimoxazole, E - Erythromycin, PEF-Pefloxacin, CXC-Cloxacillin

Table 4. Antimicrobial Sensitivity Test for Gram negative Bacteria Isolates

ISOLATES	CPX	AMX	AUG	CXC	PEF	OFX	STR	COT	CHL	SP
K. pneumoniae	S	I	S	I	R	S	I	R	I	S
S. plymutica	S	S	S	S	S	S	S	S	S	S
S. ficaria	I	S	S	S	I	I	I	S	S	I
E. faecalis	I	S	S	S	I	I	I	S	S	I
P. mirabilis	S	I	I	I	I	S	I	R	S	S
E. coli	S	S	S	S	I	I	S	S	S	S

KEY: R- Resistance, I- Intermediate,S- Sensitive.
CPX-Ciprofloxacin, AMX-Amoxicillin, AUG-Augmentin, CXC-Cloxacillin, PEF-Pefloxacin, OFX-Ofloxacin, STR-Streptomycin, COT- Cotrimoxazole, CHL-Chloramphenicol, SP-Sparfloxacin

Nwachukwu *et al.* (2008) recorded low level of *Lactobacillus* from sliced water melon. Some of the bacteria isolated in this sliced fruits may be contaminants from soil, the environment, during transportation or handling. Most of the organism isolated in this study might have been introduced into these fruits from faecally polluted water used for washing utensils (e.g. Knives, trays, and pans), wrapping material and the exposure of these products to low temperature (Daniyan and Ajibo, 2011).

The low frequency of *E. coli* recorded may be because some of the fruits were not washed in water after they were sliced but were packaged directly into the polythene bags. According to CDC (2011) the main mode of transmission of *E. coli* is through contaminated food or water. *E. coli* are found mostly in polluted water.

The presence of *S. aureus* may be explained by the fact that human beings that are processors or vendors carry this organism on/in several parts of their bodies (Nester *et al.,* 2001).

B. subtilis was isolated from all the samples. Oranusi and Olorunfemi (2011) recorded 100% occurrence for *Bacillus* in the street vended fruits. *Bacillus* are spore formers and are known environmental contaminants, they have been indicted as food borne pathogens.

The result of the antimicrobial sensitivity testis presented on Tables 3 and 4. Many of the bacteria show an intermediate to sensitive reaction to most of the antibiotics tested. All the gram positive bacteria were sensitive to Ciprofloxacin, Streptomycin and Cloxacillin. They showed an Intermediate reaction to Erythromycin except for *Micrococcus* which was sensitive to it. Only S. *aureus* and *B. cereus* were sensitive to ceftriaxone while *S. aureus* and *Streptococcus* were sensitive to cefuroxime. These findings is supported by the works of Daniyan and Ajibo (2011) who reported that *S. aureus* was resistant to pefloxacin but susceptible to Streptomycin, Ciprofloxacin, ceftriaxone and cefuroxime. Srinu *et al.* (2012) also reported that *S. aureus* was sensitive to Streptomycin, cotrimoxazole and Ciprofloxacin. Agwa *et al.* (2012) found that *B. cereus*

was susceptible to erythromycin, ciprofloxacin and streptomycin but resistant to ampiclox although our findings showed that *B. cereus* was sensitive to ampiclox but resistant to erythromycin.

Among the gram negative bacteria, *K. pneumoniae* was resistant to pefloxacin and cotrimoxazole ,*S. plymuthica* was sensitive to all the antibiotics while *S. ficaria* and *Enterococcus faecalis* and *E. coli* shows an Intermediate to sensitive reaction to the antibiotics. *P. mirabilis* was resistant to cotrimoxazole. According to Stock and Wiedemann (2001), *Klebsiella* spp. were naturally sensitive or intermediate to several penicillins, all tested cephalosporins, aminoglycosides, quinolones, tetracyclines, trimethoprim, cotrimoxazole, chloramphenicol and nitrofurantoin. *K. pneumoniae* in this research was resistant to cotrimoxazole. Marwa *et al.* (2012) reported that most *E. coli* isolates from food were sensitive to Amoxicillin/clavulanic acid, cefuroxime, ciprofloxacin, ofloxacin , sparfloxacin and cotrimoxazole. Srinu *et al.* (2012) also reported that *E. coli* was sensitive to Streptomycin, cotrimoxazole and ciprofloxacin. Osterbald *et al.* (1999) reported that *Escherichia* spp and *Klebsiella* sp shows high sensitivity to Amoxycillin/clavulanic acid and that 12% of *E. coli* isolates from vegetable were resistance to chloramphenicol.

There is need for the vendors to practice good hygiene to reduce contamination of street vended fruits with foodborne pathogens. Consumers should wash fruits with clean water before consumption. Vendors and consumers should be educated on the implication of foodborne pathogens in food.

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