Bacterial Evaluation of Ready-To-Eat Sliced Fruits Sold for Human Consumption in Ado-Ekiti, Nigeria

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ABSTRACT

Introduction: Safety after consumption of ready-to-eat sliced fruits often sold by road sides is of concern. The fruits are sliced by unschooled fruit sellers with little or no knowledge of hygienic protocols for achieving food safety. Consumption of such exposed food may not be without challenges in food safety. **Aim and Objectives:** The study aimed to evaluate the safety of ready-to-eat sliced fruit sold for human consumption. The objectives include finding out bacterial contamination of the fruits, bacterial load on the fruits and antibiotic sensitivity pattern of isolated bacteria. **Materials and methods:** Twenty samples each of sliced pawpaw, watermelon and pineapple were evaluated for bacterial contamination using standard microbiological procedures. Antimicrobial susceptibility pattern of isolated bacteria were tested. **Results:** Ninety bacteria isolates belonging to five bacterial genera were recorded. Of these isolates, *Staphylococcus aureus* accounted for 34.3 %, followed by *Escherichia coli* and *Klebsiella pneumoniae* [17.2 % each], *Salmonella typhi* [15.2%], *Pseudomonas aeruginosa* [9.1 %] and lastly other salmonellae [7.1 %]. The total aerobic counts range from 9.36 x 10^5 to 7.25 x 10^6/ml of fruit homogenate. Pawpaw recorded the heaviest contamination followed by watermelon and pineapple. Augmentin and cefuroxime recorded the highest mean percentage antibiotic resistance of 83.3 each, followed by ceftaxidime and cefixime [66.7 each], gentamicin [16.7]; ofloxacin and ciprofloxacin recorded no resistance against all the bacteria. **Conclusion:** The mean total aerobic microbial counts on sliced fruits were beyond acceptable limit for human consumption. Public enlightenment and training of fruit vendors is hereby advocated to reduce possible hazards due to consumption of these products in Nigeria and Ado-Ekiti in particular.

Keywords: Bacteria; Sliced fruits; Ready-to-eat; Contamination; Microbial load.

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INTRODUCTION

Sliced fruits [Ready-to-eat] are normally packaged ready for consumption in their raw state. There is an increasing rate of consumption of raw fresh products, like vegetables and fruits in many parts of the world and mainly because of changes in the human lifestyle and their tendency towards convenience and spending less time on preparing food (1,2,3). Furthermore, due to increased awareness of the benefits of fruits and vegetables especially in reducing the risk of some diseases, demand for fruits and vegetables has increased (4). In addition, consumption of fruits have increased significantly, because they are dietary sources of nutrients, micronutrients, vitamins and fiber for humans and therefore vital for wellbeing (5,6). Ready-to-eat [RTE] fruits are on high demand and they are considered as low-calorie meal, rich in fiber and provide a great variety of vitamins, minerals (7). RTE sliced fruits are readily available for low income earners who cannot afford the cost of the whole fruit. Outbreaks of human infections associated with the consumption fruits have increased due primarily to transmitting various pathogens to humans (8,9). This is a risk factor for the consumer's health and therefore a food safety challenge to the public (10). Salmonellosis has been associated with the consumption of cut watermelon in the United States of America (11). In Nigeria, particularly in Ekiti State, morbidity associated with ill health due to Salmonella has been reported to be on the increase and sometimes leading to death (12). One of the causes of Salmonellosis has been attributed to the consumption of contaminated foods (13). This is said to be responsible for a number of cases of illnesses and deaths in Nigeria (14).

Thus, safety is a challenge despite the benefits derived from eating raw fruits. Tissues of sliced fruits are exposed during processing thus allowing microorganisms to invade these richer sources of nutrients as compared to intact fruit surface (15). Besides this, the high water activity and low acidic tissue, as in many fruits, facilitates rapid microbial growth (16). These conditions provide a perfect "culture media" for a number of important human pathogens and spoilage microorganisms to contaminate sliced fruits, which results in a faster deterioration of sliced fruits compared to whole fruit.

Sliced fruits are packaged (ready-to-eat) in thin transparent cellophane bags and displayed beside the road by uneducated, untrained and unlicensed fruit vendors. Contamination of these sliced fruits may occur through pathogens already on the surface of the fruits from pre and post harvest stages (17,6). Further sources of microbial invasion of sliced fruits occur due to pathogens invading the interior surfaces of the fruit during washing, peeling, slicing, packaging, handling and marketing (18,19). Also, use of dirty utensils such as knives, trays as well as the open display of sliced fruits encourages sporadic visits by flies and other insects and dust that may contain soil microorganisms (20).

This study aimed at evaluating sliced pawpaw, watermelon and pineapple sold as ready-to-eat fruits in Ado-Ekiti to ascertain their safety. The total aerobic microbial count on the fruits and antibiotic sensitivity pattern of organisms isolated were evaluated

MATERIALS AND METHODS

Study area

The study was conducted in Ado-Ekiti, Ekiti State. Ado-Ekiti is a city in Southwest

Nigeria and lies on latitude 7°35 and 7°38 North of the equator and Longitude 5°10 and 5°15 East of the Greenwich Meridian (21). It is the capital city of Ekiti State. It has population of 424,340 (22). The city has two universities and a Federal Polytechnic. The inhabitants of the city are mainly traders, secondary and tertiary institution workers, workers of some firms and farmers. The food traders trade essentially on yam, beans, rice, fruits and vegetables.

Study site

The research was carried out in the Medical Microbiology Unit of the Department of Medical Laboratory Science, Afe Babalola University, Ado-Ekiti.

Ethical consideration

Ethical approval to conduct this research in Afe Babalola University was sought for and obtained from the Ethics and Research Committee of the College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti.

Sample size

A total of sixty [60] RTE sliced fruits were used in the study. This comprises of twenty pieces of each of the fruits.

Sample collection

Sliced watermelon, sliced pawpaw and sliced pineapple were bought from various local fruit sellers at ten different locations in Ado-Ekiti. The samples already in transparent nylon bags were collected into different sterile waterproof nylon bags and transported in ice pack to the laboratory for analysis within 30 minutes of collection.

Sample processing

The ready-to-eat sliced fruits were subjected to standard microbiological techniques such as colony forming count, culture, biochemical tests, and disc diffusion method of antibiotic sensitivity testing for the total aerobic plate count, isolation, identification and antibiotic susceptibility testing in the laboratory.

Colony forming unit (cfu) count

Pestle and mortar were washed and rinsed in sterile distilled water. One gram from each sample was crushed using the pestle and mortar and aseptically transferred into 9 ml of sterile distilled water in a sterile glass beaker and mixed well (23). Then, ten-fold serial dilution of the resultant homogenate were prepared by transferring 1 ml of the suspension into 9 ml of sterile distilled water to obtain dilutions of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. The total aerobic plate count for the samples were determined by plating 1 ml of the dilutions of the homogenates on sterile Nutrient agar using the pour plate technique and allowed to gel (24,25,23). The petri-dishes were incubated at 32°C for 24 hours for aerobic plate count. From the sample homogenate, MacConkey agar and Selenite F broth were inoculated and incubated for 24-48 hours at 37°C. After 24 hours, subcultures were made from the Selenite F broths unto Xylose-Lysine Dextrose Agar and incubated for 24 hours at 37°C for isolation of enteric pathogens.
Plates were brought out of the incubators and discrete colonies were counted using a colony counter device [Gallenkamp, England] and each count was expressed in colony forming unit per mililiter [cfu/ml] of the sample homogenate (25,23).

Identification of isolates

All isolates were characterized using standard microbiology and biochemical tests as described by Barrow and Feltam (26) and Cheesebrough (27). Bacterial isolates were identified according to Barrow and Feltam (28) and Garrity *et al.* (29). The following biochemical tests were carried out for the characterization and identification of the organisms: Gram staining, catalase, coagulase, citrate, utilization, urea decomposition, indole, motility, sugar fermentation and triple sugar iron tests.

Antibiotics sensitivity test.

Mueller Hinton agar plate was prepared aseptically and used for disc diffusion method of antibiotic sensitivity testing (27). A colony of the isolate was inoculated into peptone water and incubated overnight. This overnight culture broth was adjusted to match that of 0.5 McFarland standard by either diluting with distilled water or incubating further (30). With the aid of sterile cotton wool, the inoculum was evenly spread on Muller Hinton agar and antibiotic disc were placed aseptically at equidistance to each other on the plate and incubated for 24 hours at 37^{o}C. The antibiotics used were: gentamicin, [GEN 10 μg], augmentin [AUG 30 g], ofloxacin [OFL 5 μg], ceftazidime [CAZ 30 μg], cefuroxime [CRX 30 μg], ciprofloxacin [CFX 5 μg] and cefixime [CXM 5 μg] [Abtek Biological Ltd, Liverpool, L9 7AR, UK]. The zones of inhibition were measured and interpreted in accordance with the Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing (31).

Statistical analysis

Data generated were subjected to analysis using statistical package for social science [SPSS] version 20. The frequencies of each organism were recorded in percentages [%], mean and standard deviations.

RESULTS

A total of 99 isolates belonging to five bacteria genera were recorded from the 60 samples of sliced fruits. *Staphylococcus aureus* recorded the highest occurrence of 34.3 % followed by *Escherichia coli* and *Klebsiella pneumoniae* [17.2 % each], *Salmonella typhi* [15.2 %], *Pseudomonas aeruginosa* [9.1 %] and other salmonellae [7.1 %].

The microbial loads on the sliced fruits range from 9.36 x 10^5 to 7.25 x 10^6 /ml of fruit homogenate [Table 1]. The most contaminated of the three sliced fruits was pawpaw followed by watermelon and pineapple [Table 1].

Table 1: Mean aerobic plate count of bacteria on the sliced ready-to-eat fruits.

S/N	Sliced fruits	Mean total aerobic plate count [cfu/ml] of fruit homogenate.
1	Watermelon	1.04×10^6
2	Pawpaw	7.25×10^6
3	Pineapple	9.36×10^5

Table .2: The rate of occurrence of different isolated bacteria on the ready-to-eat sliced fruits.

Organisms	Occurrence (%) of organisms on RTE sliced fruits			
	Watermelon n=20	Pawpaw n=20	Pineapple n=20	Total n=60
Salmonella typhi	5 (25.0)	6 (30.0)	4 (20.0)	15 (25.0)
Other salmonellae	2 (10.0)	3 (15.0)	2 (10.0)	7 (11.7)
Staphylococcus aureus	12 (60.0)	14 (70.0)	8 (40.0)	34 (56.7)
Escherichia coli	8 (40.0)	5 (25.0)	4 (20.0)	17 (28.3)
Klebsiella pneumoniae	6 (30.0)	7 (35.0)	4 (20.0)	17 (28.3)
Pseudomonas aeruginosa	3 (15.0)	4 (20.0)	2 (10.0)	9 (15.0)
Total	36	39	24	99

In table 2, of the 60 sliced fruits, *Staphylococcus aureus* was the most frequent, occurring 34 times (56.7 %) followed by *E. coli* and *K. pneumoniae,* 28.3 % [17 times each], *S. typhi,* 25.0 % [15 times], *Pseudomonas aeruginosa,* 15.0 % [9 times] and the least was other salmonellae, 11.7 % [7 times]. Out of 20 samples of watermelon, *Staphylococcus aureus* recorded the highest frequency of 60 % [12 times], followed by *E. coli,* 40 % [8 times], *K. pneumoniae,* 30 % [6 times], *S. typhi,* 25.0 % [5 times], *P. aeruginosa,* 15.0 % [3 times] and other salmonellae, 10.0 % [2 times]. Of 20 samples of pawpaw, the highest bacterial frequency was *S. aureus,* 70.0 % [14 times], followed by *K. pneumoniae,* 35.0 % [7 times], *S. typhi,* 30.0 % [6 times], *E. coli,* 25.0 % [5 times], *P. aeruginosa,* 20.0 % [4 times] and other salmonellae, 15.0 % [3 times]. *Staphylococcus aureus* equally recorded the highest frequency of occurrence, 40.0 % [8 times] in sliced pineapple, followed by *E. coli, K. pneumoniae,* and *S. typhi,* 20.0 % [4 times each], P. aeruginosa and other salmonellae, 10.0 % [2 times each].

Of the three sliced RTE fruits, pawpaw was mostly contaminated with *S. aureus,* followed by watermelon and then pineapple [Table 2]. Watermelon was mostly contaminated with *E. coli* followed by pawpaw and then pineapple. *K. pneumoniae* mostly contaminated pawpaw followed by watermelon and then pineapple. *K. pneumoniae* mostly contaminated pawpaw, followed by watermelon and then pineapple. Finally, of the three fruits, *P. aeruginosa* occurred more in pawpaw, followed by watermelon and then by pineapple [Table 2]. Augmentin and cefuroxime recorded the highest mean percentage antibiotic resistance of 83.3 each, followed by ceftaxidime and cefixime [66.7 each], gentamicin [16.7]; ofloxacin and ciprofloxacin recorded no resistance against all the bacteria. In table 3, *Salmonella typhi* was resistance to augmentin, ceftaxidine, cefuroxime and cefixime but sensitive to gentamicin, ofloxacin and ciprofloxacin. Other salmonellae are generally resistance to gentamicin, augmentin, cefuroxime and cefixime. *S. aureus* was resistance to augmentin, ceftaxidime, cefuroxime and cefixime but sensitive to gentamicin, ofloxacin, and ciprofloxacin [Table 3].

Table 3: Sensitivity pattern of *Salmonella typhi*, other salmonellae, and *Staphylococcus aureus* against tested antibiotics.

Antibiotics	Mean zone of Inhibition (mm) of bacteria ± standard deviation					
	Salmonella. typhi	**Sensitivity**	**Other Salmonellae**	**Sensitivity**	***S. aureus***	**Sensitivity**
GEN (10)	17.0±0.5	S	17.1±0.8	S	17.0±0.4	S
AUG (30)	9.3±0.4	R	0.0±0.0	R	18.3±0.7	S
OFL (5)	16.6±0.8	S	18.8±0.6	S	19.7±0.6	S
CAZ (30)	6.0±0.2	R	18.0±0.4	I	4.0±0.5	R
CRX (30)	3.1±0.2	R	2.5±0.1	R	3.8±0.1	R
CPR (5)	21.0±0.6	S	19.3±1.0	S	18.1±0.8	S
CXM (5)	18.8±0.4	S	1.6±0.03	R	4.0±0.3	R

KEY: GEN = gentamicin, AUG = amoxicillin/clavulanic acid, OFL = ofloxacin, CAZ = ceftazidime, CRX = cefuroxime, CPR = ciprofloxacin, CXM = cefixime, R = resistance, S = sensitive, I = intermediate sensitivity.

Table 4: Sensitivity pattern of *Escherichia coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* against tested antibiotics.

Antibiotics	Mean zone of Inhibition (mm) of bacteria ± standard deviation					
	E. coli	Sensitivity	*K. pneumoniae*	Sensitivity	*P. aeruginosa*	Sensitivity
GEN (10)	17.0±0.3	S	13.2±0.6	I	10.0±0.3	R
AUG (30)	3.70±0.2	R	3.5±0.2	R	0.0±0.0	R
OFL (5)	19.5±0.7	S	20.0±0.5	S	17.8±0.6	S
CAZ (30)	19.5±0.4	R	18.2±0.8	S	4.1±0.5	R
CRX (30)	4.5±0.4	R	17.8±0.1	S	0.0±0.0	R
CPR (5)	23.7±0.07	S	23.4±0.6	S	22.7±035	S
CXM (5)	8.1±0.6	R	18.0±0.4	S	0.0±0.0	R

KEY: GEN = gentamicin, AUG = amoxicillin/clavulanic acid, OFL = ofloxacin, CAZ = ceftazidime, CRX = cefuroxime, CPR = ciprofloxacin, CXM = cefixime, R = resistance, S = sensitive, I = intermediate sensitivity

In table 4, *E. coli* was generally resistance to augmentin, ceftaxidime, cefuroxime and cefixime but sensitive to gentamicin, ofloxacin and ciprofloxacin. *K. pneumoniae* was resistance to augmentin, ceftaxidime and cefuroxime but sensitive to ofloxacin, ciprofloxacin and showed intermediate sensitivity to gentamicin and cefixime. *P. aeruginosa* was resistance to all the antibiotics except ofloxacin and ciprofloxacin [Table 4].

DISCUSSION

The organisms isolated in the present study were also among the organisms isolated on street vended pineapple, pawpaw and watermelon in Ota, Ogun State, Nigeria; the frequency of occurrence also was most common in *Staphylococcus aureus*, followed by *E. coli*, *Salmonella species*, *Klebsiella pneumoniae*, and then

Pseudomonas aeruginosa (32). Adesetan *et al*. (25) also recorded similar organisms on street vended fruits in Ijebu area of Ogun state, Nigeria. The types of bacteria present on sliced fruits in the present study also affirmed the previous study by Odebisi-Omokanye *et al.* (33). Thus, similar organisms contaminate RTE sliced fruits in different places in Nigeria.

The microbial load on pawpaw in the present study was higher than 8.0 x 10^5 reported by Adesetan *et al*. (25) and 2.1 x 10^6 reported by Oranusi and Olorunfemi (32). The microbial load on sliced watermelon in the present study was higher than the 9.3 x 10^5 reported by Adesetan et al. (25) but lower than the 8.2 x 10^8 reported by Oranusi and Olorunfemi (32). The microbial load on sliced pineapple in the present study was higher than 2.3 x 10^5 reported by Adesetan *et al* (25) and 2.0 x 10^6 reported by Oranusi and Olorunfemi (32). The microbial load on the sliced ready-to-eat fruits is a function of the hygienic nature of fruit sellers and the sanctity of materials employed in the preparation and packaging of the products. Ready-to-eat foods with plate count between 0-10^3 are acceptable, between 10^4 and $\leq 10^5$ is tolerable and 10^6 and above is unacceptable according to the recommendations of International Commission on Microbiological Specifications for Foods (34). Rating the findings of the present study therefore, the sliced pawpaw, watermelon are unacceptable for human consumption while sliced pineapple may be tolerable (but is at the upper limit of the tolerable level).

Staphylococcus aureus and *Pseudomonas aeruginosa* are environmental organisms and can be found on soils, surfaces of articles and on human's body (35). Its recurrent isolation on the sliced fruits could be a pointer to an unhygienic human handling of the fruits (36). Use of contaminated knives and tables may contribute to the contamination of the fruits. *Staphylococcus aureus* is a normal flora of the hands and skins of humans (37) and this organism may have been introduced by body contact of sellers of the fruits. Some of the organisms are causative agent of food poisoning.

K. pneumoniae, *Salmonella species*, *E. coli* are enteric organisms and their occurrence on RTE sliced fruits could be an indication of indirect fecal contamination from the hands of the fruit sellers (38) or directly from the surface of the fruits into the tissues of the fruits possibly due to reuse of contaminated water. The common occurrence of *E. coli* in faeces and its survival characteristics in water led to its adoption as an indicator of faecal contamination (39). There are recent reports of increased cases of typhoid fever in Ado-Ekiti (40,41). These researchers' reports appear to be supported by the findings of high level of contamination of RTE sliced fruits in the present study by *Salmonella typhi*, the common causative agent of typhoid fever. The prevalence of salmonella and *E. coli* food poisoning appear also supported by the occurrence of the causative agents in the present study.

Proper disinfection or decontamination procedures are required in ensuring the safety of sliced fruits. Washing and sanitization steps before cutting will lower the microbial contamination on the fruits. Contaminations due to wash water may result in raised microbial load on the surface of whole fruit and hence in the final slice fruits. Infiltration of wash water into the intercellular spaces will certainly pave way to pathogens entering into the fruit tissue (19). Such infiltration of pathogens from wash water to fruit is an important safety concern and needs to be avoided always. Sliced fruit samples used in the present study showed no visible signs of spoilage. Therefore, the appearance of the products

may not be a good yardstick for determining the microbial quality of the sliced fruits. To a large extent, the vendors' hygiene and preparation procedures will determine suitability of sliced fruits for human consumption.

The antibacterial resistance recorded in the present study agreed with the report of Okeke *et al*. (42) most especially against enteric pathogens. One hundred percent antibiotic susceptibility recorded against ofloxacin and ciprofloxacin agree with previous published report (43). Infections due to consumption of sliced fruits in Ado-Ekiti may pose some treatment challenges due to antibiotic resistance.

In conclusion, a total of 99 bacterial isolates belonging to five bacteria genera were recorded from the 60 samples of sliced fruits. *Staphylococcus aureus* recorded the highest occurrence of 34.3 % followed by *Escherichia coli* and *Klebsiella pneumoniae* [17.2 % each], *Salmonella typhi* [15.2 %], *Pseudomonas aeruginosa* [9.1 %] and other salmonellae [7.1 %]. The microbial loads on the sliced pawpaw, watermelon and pineapple sold in Ado-Ekiti were generally above acceptable limit for human consumption. The organisms generally showed gross degree of resistance to antibiotics. A comprehensive health education program should be given to the general population on the health risks associated with the consumption of ready-to-eat sliced fruits. Vendors of such fruits should be given training on proper handling of sliced fruits to be sold to the general public. The limitation to the present study was that the farmers, vendors and their environments were not investigated as possible sources of the contaminating bacteria. The fruit farmers were not known and the fruit vendors were not willing to be subjected to investigations together with their work environments at the time of sample collection for security reasons. Future studies in this direction might trace the sources of contaminating bacteria to the farmers, the vendors or their work environment including materials employed in processing their products.

Conflict of interest

The authors declare no conflict of interest in this work.

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