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Bacterial Contamination of Ready to Eat Fruits Sold in and Around Ugbowo Campus of University of Benin (Uniben), Edo State, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Fresh fruits are major sources of nutrients and vitamins which help to promote good health. However, fresh fruits may also harbour a wide range of microbial contaminants of public health significance. To assess the bacterial contaminants of fruits sold in Ugbowo campus of University of Benin, 200 samples of replica of five fruits were purchased from four locations within the campus. Samples were analyzed to study the distribution of organisms based on the type of fruits and the locations in which the fruits were sampled. The results showed that 62.5% of the fruit samples yield bacterial growth, while 37.5% of the fruit samples yield no bacterial growth. The highest number of organisms was isolated from banana and pawpaw at the same rate (13% each), while oranges recorded the least number of isolates. Using non-parametric Wilcoxon statistics the results further showed that the number of samples with bacterial growth was significantly higher than that without bacterial growth (p=0.042). Four bacteria belonging to three genera were identified. The identified

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organisms were *Staphylococcus aureus*, Coagulase negative Staphylococci (CoNS), *Escherichia coli* and *Salmonella* Enteritidis. The highest number of organisms was isolated from sale points in junior staff quarters closely followed by sale points in anatomy gate. *S. aureus* has the highest incidence in all the locations followed by *E. coli*. However, the distribution of organisms isolated from all the locations showed no significant difference (p=0.996). *S. aureus* (46.4%) was the most frequently isolated organisms from all the fruit samples followed by *E. coli* (28.8%), while *Salmonella* Enteritidis was the least frequently isolated (11.2%).The results of the statistical analysis using Duncan multiple range test revealed that the distribution of *S. aureus* in all the fruit samples was significantly higher than that of CoNS (p=0.000), *E. coli* (p=0.003) and *Salmonella* Enteritidis (p=0.000). Also, the occurrence of *E. coli* was significantly higher than that of CoNS (p=0.008) and *Salmonella* Enteritidis. (p=0.003). The results further showed that the frequency of *Salmonella* Enteritidis. in all the fruit samples was not significantly different from those of CoNS (p=0.628). Thus, bacteriologically safe fruits are essential to maximize the health and nutritional benefits inherent in adequate consumption of these products. Therefore, proper decontamination through washing of fruits, hands and containing vessels is essential.

Keywords: Fruits; Bacterial contamination; Ugbowo campus; UNIBEN.

1. INTRODUCTION

Fruit is a part of flowering plant that derives from specific tissues of the flower, one or more ovaries, and in some cases accessory tissues. To a large extent, both humans and many animals have become dependent on fruits as a source of food [1]. The nutritional and health importance of fruits cannot be underestimated in that they contain substantial quantities of essential nutrients in a rational proportion. They are excellent sources of minerals, vitamins, enzymes and dietary fiber [2]. Other important nutrients supplied by fruits and vegetables include riboflavin (B_2), zinc, calcium, potassium, and phosphorus.

Despite the health benefits of fruits to healthy living, the contamination of these fruits had created another burden to consumers. These are as a result of their exposure to microbial contamination which could be from human handling, transport vehicles, insects, dust, and rinse water. Other sources of contamination are harvesting equipment, soil, faeces, irrigation water (including water used to apply fungicides and insecticides) manure, wild and domestic animals [3,4]. The surface of fruits harbours microorganisms depending upon the mechanical handling of the fruits. Microbes can adhere to surface, invade/penetrate fruits surface and multiply within the tissue. Fruits therefore harbour a diverse range of microorganisms including plant and human pathogens [5,6,7]. Differences in microbial profiles of various fruits result largely from unrelated factors such as resident microflora in the soil, application of non-resident microflora via animal manures, sewage or irrigation water, transportation and handling by individual retailers [8,9].

Experts say fruits are reservoirs of disease causing germs. In recent years there has been an increase in the number of reported cases of food borne illness linked to fresh fruits [4,6,10,11,12].

Therefore, this study was undertaken to determine the microbial contaminants of fruits sold within the Ugbowo campus of the University of Benin, Benin City, Edo State of Nigeria.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 200 samples comprising of five different types of fruit Apple (*Malus sylvestris*), Banana (*Musa paradisiacal*) Water Melon (*Citrullus lanatus*), Pawpaw (*Carica papaya*) and Oranges (*Citrus sinensis*) were collected from 4 different sources (location) within and around the Ugbowo campus of the University of Benin (UNIBEN). These four locations are designated as follows; Location A (Ekosodin gate), Location B (Main gate), Location C (Anatomy) and Location D (Junior Staff Quarters).

Location A: this location is outside UNIBEN campus, mainly populated by students. The main fruits buyers are students.
Location B: this location is the main entrance into UNIBEN. The fruits buyers are students, staff and their family and visitors of the university.

Location C: this location serves as entrance into UNIBEN through University of Benin teaching hospital (UBTH) the fruits buyers here are mainly Medical students and UBTH students and staff
Location D: this location is mainly occupied by UNIBEN junior staff, the fruits buyers are junior staff occupants, students in the hall of residence and other staff of UNIBEN.

The oranges were peeled while the water melon and pawpaw were sliced and ready to eat. All the samples were collected in sterile universal containers and plastic bags and transported to the laboratory for processing.

2.2 Isolation of Organisms

Twenty-five grams (25 g) of each sample was weighed and washed in 100 ml of sterile distilled water. Using pour plate, 1 ml of each of the rinse water was inoculated into molten cooled Nutrient agar and replicated in McConkey agar and Mannitol salt agar for differential purpose. The plates were allowed to solidify, inverted and incubated at 37ºC for 24 h for colony formation. All pure isolates from the media were sub-culture into nutrient agar slant, labelled appropriately and refrigerated for further purposes [12].

2.3 Identifications of Bacterial Isolates

Stock cultures of bacterial isolates with different morphological characteristics stored on Nutrient agar slants were identified based on standard method [13]. The identification of the bacterial isolates were carried out using the following biochemical test, preliminary Gram staining, Catalase and coagulase test, Motility, use of Kligler iron agar (for sugar fermentation, gas production and hydrogen sulphide production), Voges-proskauer test, Methyl-red test.

2.4 Statistical Analyses

Non parametric Wilcoxon test and Duncan multiple range test (DMRT) were used to test for significance difference. All statistical analyses were carried out using the SPSS 17.0 window based program. Significance difference and non-significance difference was defined when $p \leq 0.05$ and $p > 0.05$respectively.

3. RESULTS

The results of this study revealed that most of the fruit samples were highly contaminated as shown in Table 1. The results showed that 62.5% of the fruit samples yield bacterial growth, while 37.5% of the fruit samples yield no bacterial growth. Using non-parametric Wilcoxon statistics the results further showed that the number of samples with bacterial growth is significantly higher than that without bacterial growth ($p=0.042$).

The frequencies of all the isolates in the replica of the sampled fruits were presented in Table 2. The results showed that *S. aureus* has the highest occurrence in all the fruits samples, followed by *E. coli,* while *Salmonella* Enteritidis has the least incidence rate. The highest number of organisms was isolated from banana and pawpaw at the same rate (13% each), while oranges (18.4%) recorded the least number of isolated organisms. The results of the statistical analysis using Duncan multiple range test revealed that the distribution of *S. aureus* in all the fruit samples was significantly higher than that of CoNS ($p=0.000$), *E. coli* ($p=0.003$) and *Salmonella* Enteritidis ($p=0.000$). Also, the occurrence of *E. coli* was significantly higher than that of CoNS ($p=0.008$) and *Salmonella* Enteritidis. ($p=0.003$). The results further showed that the frequency of *Salmonella* Enteritidis. in all the fruit samples was not significantly different from that of CoNS ($p=0.628$).

Table 3 shows the distribution of organisms isolated based on the locations in which the fruit samples were collected. Based on cultural, morphological and biochemical characteristics of the organisms isolated, a total of four bacteria were identified and number of the different bacteria isolated from each of the samples varied. The isolated organisms include *S. aureus*, Coagulase negative Staphylococci (CoNS), *E. coli* and *S.* Enteritidis. The highest number of organisms were isolated from location D closely followed by location C. *S. aureus* has the highest incidence in all the locations followed by *E. coli*. However, the distribution of organisms isolated from all the locations showed no significant difference ($p=0.996$).

4. DISCUSSION

This study revealed that 125 (62.5%) of the fruits sampled were grossly contaminated. The microorganisms present in fruits are a direct reflection of the sanitary quality of the cultivation water, harvesting, transportation, storage, and processing of the produce [14,8]. Also, the high microbial contamination observed in the fruits of

Table 1. Growth of microorganisms isolated from different fruits

S/no	Fruits	No. with growth (%)	No. without growth (%)	Total (%)
1	Apple	25 (12.5)	15 (7.5)	40 (20)
2	Banana	26 (13)	14 (7)	40 (20)
3	Orange	23 (11.5)	17 (8.5)	40 (20)
4	Pawpaw	26 (13)	14 (7)	40 (20)
5	Water melon	25 (12.5)	15 (7.5)	40 (20)
	Total	125(62.5)	75 (37.5)	200 (100)

Table 2. Frequency distributions of microorganisms isolated from different fruits

S/no	Fruits	Microorganisms (%)				
		S. aureus	**CoNS**	***E. coli***	***S.* Enteritidis**	**Total**
1	Apple	8(6.4)	4(3.2)	8(6.4)	5(4)	25(20)
2	Banana	13(10.4)	5(4)	7(5.6)	1(0.8)	26(20.8)
3	Orange	10(8)	3(2.4)	9(7.2)	1(0.8)	23(18.4)
4	Pawpaw	14(11.2)	2(1.6)	7(5.6)	3(2.4)	26(20.8)
5	Water melon	13(10.4)	3(2.4)	5(4)	4(3.2)	25(20)
	Total	58(46.4)	17(13.6)	36(28.8)	14(11.2)	125(100)

Table 3. Number of microorganisms isolated from fruits based on location of sampling

S/No	Location	Microorganisms (%)				
		S. aureus	**CoNS**	***E. coli***	***S.* Enteritidis**	**Total**
1	A	12(9.6)	3(2.4)	10(8)	5(4)	30(24)
2	B	12(9.6)	5(5)	9(7.2)	4(3.2)	30(24)
3	C	19(15.2)	2(1.6)	9(7.2)	2(1.6)	32(25.6)
4	D	15(12)	7(5.6)	8(6.4)	3(2.4)	33(26.4)
	Total	58(46.4)	17(13.6)	36(28.8)	14(11.2)	125(100)

Key: A (Ekosodin gate), B (Main gate), C (Anatomy) and D (Junior Staff Quarters).

this study may be a reflection of storage conditions and how long these produce were kept before they were obtained for sampling. Bacteria on storage materials may transfer to produce and cross contamination between produce is probable particularly where produce are pre-washed with the same wash water by the vendor or processor. More importantly, bacteria on the produce may multiply over time depending on the storage conditions especially those that are psychrophilic [15,16]. Raw fruits are known potential for a wide range of microorganisms, including human pathogens [17]. The survival or growth of these organisms on intact fruit surfaces will be dependent on the extrinsic factors of available nutrient, temperature, presence of scales and fibres, gaseous atmosphere, mechanical handling and moisture. Fruits on display for sale are often visited by many hands of the customers and by the vendors. These individuals pick and drop as many fruits as are available, to enable them make a choice. Poor handling by unhygienic hands is a factor contributing to the high microbial load. The dusty environments of the motor parks, busy roads and campuses/institutions, coupled with water of questionable quality which often is used to sprinkle the fruits to keep fresh are contributing factors that could aid the survival and possible multiplication of contaminants on fruit surfaces [18].

The findings in this study that *Staphylococcus aureus* was the most frequently isolated organisms in all the fruits was in accordance to the report of Eni et al. [12]. *Staphylococcus aureus* isolated from most of the fruits may have entered the fruits during packaging or handling since the organisms are normal flora of the human skin and nasal cavity [19]. The presence of *Staphylococcus aureus* in fruits is of public health significance because it is usually responsible for staphylococcal food poisoning [20], severe soft tissue infections, and toxic shock syndrome (TSS) [21,22].

Coagulase-negative Staphylococci (CoNS) previously dismissed as contaminants are now emerging as important potential pathogens [23]. In the last two decades, CoNS have also

emerged as significant pathogens, especially in immunocompromised patients, premature newborns, urinary tract infections, arthritis, and infections of prosthetic joints [24].

Most *E. coli* are not pathogenic and are part of the normal human and animal gut flora. Thus, isolation of *E. coli* in this study may reflect poor sanitary condition, faecal contamination of water use for processing or washing of fruits [25] and improper handling of fruits from processors and consumers. Enteropathogenic *E. coli* are usually associated with diarrhoea due to the presence of virulent factors including toxin production, adhesins and invasiveness

Salmonella spp. is an important cause of gastrointestinal illness in humans. *Salmonella* Enteritidis and *Salmonella* Typhimurium are the most frequently reported non-typhoidal serotypes in many countries and outbreaks have been associated with a diverse range of food vehicles. However, a wide range of serotypes have been associated with outbreaks involving fresh products. Salmonellosis is characterised by diarrhoea, fever, abdominal cramps and vomiting usually lasting 4-7 days [26]. Although most *Salmonella* infections are self-limiting, in a small proportion of cases these may lead to bacteraemia. The case-fatality rate in industrialised countries is less than 1% [27].

The presence of *S. aureus*, a pathogenic organism of public health concern, in most of the samples and the presence of other pathogenic and opportunistic bacteria like *E. coli*, *Salmonella* Enteritidis and CoNS in some of the fruits, further highlights the need to safeguard the health of the consumers by proper washing and decontamination of these produce which are consumed without heat treatment.

5. CONCLUSION

Fruit processors should be educated on the adverse effect of using untreated or polluted water for processing as these could serve as sources of contamination. Processors/vendors should also observe strict hygienic measures to ensure that they do not serve as source of chance inoculation of microorganisms and contamination. There is also the need to make a law compelling vendors, in Nigeria, to transport/sell sliced ready to eat fruits and vegetables in cool temperature controlled carts similar to those used for the transportation/sales of yogurts and ice creams [12].

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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