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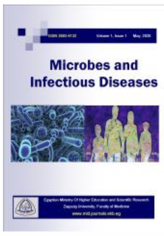

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Original article

Multidrug resistant bacteria associated with fresh fruits and vegetables sold in Lokoja markets, Kogi state, Nigeria

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ABSTRACT

Background: Fruits and vegetables are an extraordinary dietary source of nutrients, phytochemicals, vitamins, and fiber and are globally recommended as part of a healthy diet. Despite the nutritional benefits, their surfaces could become contaminated with pathogenic bacteria causing illness or death. This study aimed at identifying bacteria species associated with surfaces of fruits and vegetables sold in old and international markets in Lokoja. **Methodology:** Thirty fruits and vegetables were purchased from two major markets and processed according to standard techniques. Total aerobic plate count (TAPC) was estimated by culturing on selected media and isolates obtained were biochemically characterized. Resistance patterns of isolates were determined by the disk diffusion method using commercially available antibiotics. **Results:** The mean bacteria count from the old market ranged from 4.56 X 106 – 7.0 X 106, while that of international market ranged from 4.45 X 106 – 4.98 X 106. The prevalence of bacteria isolated from the fruits and vegetables were *Klebsiella pneumoniae* (28.2%), *Salmonella* species (sp.) (20.5%), *Escherichia coli* (15.4%), *Staphylococcus aureus* (10.2%), *Enterobacter* sp. (7.7%), *Pseudomonas aeruginosa* (5.1%), and *Bacillus* sp. (5.1%) and *Proteus vulgaris* (2.7%). All isolates were entirely resistant (100%) to ampicillin and oxacillin, gentamycin (94.9%), ciprofloxacin (69.2%), cefotaxime (51.3%), tetracycline (43.6%), while isolates were least frequently resistant to ceftazidime (41.0%). **Conclusion:** The presence of multidrug resistant isolates indicates that fresh fruits and vegetables sold in Lokoja are potential vehicles for transmitting food borne illnesses. Food safety measures should be taken by retailers and consumers to mitigate the spread of food borne illnesses.

Introduction

Fruits and vegetables could be described as agricultural products having tissues that are subjected to normal living characteristics such as respiration, excretion, metabolism, etc. [1].They are fleshy portions of plants with edible characteristics, which could be eaten wholly, precut, or sliced. They

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could be served in retail outlets and in ceremonies [2]. Fresh fruits and vegetables are widely available in various cities, towns, and villages in Nigeria [3]. Consumption of fresh fruits and vegetables is encouraged world-wide by both government and privately-owned health agencies or groups. Fresh vegetables and fruits play an important role in human nutrition due to their high nutrient content of water, dietary fiber, proteins, phytochemicals, vitamins and minerals such as calcium, potassium, and magnesium [4]. Their high cellulose and fiber contents also help in the regulation of the digestive system [5]. International organizations including the World Health Organization (WHO), the Food and Agricultural Organization (FAO), and Centers for Disease Control and Prevention (CDC) have encouraged people to eat more fresh fruits and vegetables [6].

Fresh fruits and vegetables provide a healthy and balanced diet and can prevent chronic diseases such as heart diseases, cancer, diabetics, cardiovascular diseases, chronic obstructive pulmonary diseases, osteoporosis, and obesity including several micronutrient deficiencies especially in developing countries [7]. Vegetables consumed raw are increasingly being recognized as important vehicles for the transmission of human pathogens [8]. As fresh vegetables are eaten raw or slightly cooked to preserve the taste and their nutrient contents, this serves as a potential source of various food-borne infections and disease outbreaks [9,10]. Harvesting/processing practices and the location of the edible part during growth affect their microbial status at the time of consumption [11]. Pathogens from the human and animal reservoir as well as other environmental pathogens can be found at the time of consumption. Insects, birds and rodents have also contributed to faecal contamination of fruits and vegetables and this has created several health hazards. However, epidemiological traceability is extremely difficult for fruits and vegetables as carriers of food borne pathogens [11].

In Nigeria, ready to-eat fruit and vegetables are becoming popular due to their high patronage [12]. Surprisingly, some of these fruits and vegetables are not washed before being consumed. Also, most vendors are not educated on personal and public hygiene because such products are exposed to contaminated air, unclean environments and packaging materials [13]. While there is an increase in global consumption of fresh fruits and vegetables, this is greatly threatened by an upsurge of microbial contamination [14]. It has been reported that consumption of raw vegetables without proper washing is an important route in the transmission of diseases [15].

This has aroused researchers' interest in investigating the microbial safety profile of ready-to-eat fruits and vegetables. Several studies have been carried out on the microbial safety of fruits and vegetables. Thus, this study assessed the level of bacterial contamination of fruits and vegetables sold in Lokoja, Nigeria.

Materials and methods

Study area

The study was carried out in old market, and international market. Lokoja, Kogi state, Nigeria. Lokoja, the capital of Kogi state is located on latitude 7º45'27.56″-7º51'04.34″N and longitude 6º41'56.64″-6º45'36.58″E and lies at an altitude of 45 to 125m above sea level. Kogi state is one of the six states located in the North Central zone of Nigeria.

Collection of samples

A total of 30 undamaged samples of fresh fruits and vegetables comprising of ten samples each for tomatoes, cucumber, and carrots were purchased at random from traders at old and international markets, all situated in Lokoja metropolis from May – July2021.Onpurchase, the samples were immediately packaged separately in sterile nylon bag and labeled with a unique number of collection and transported to the Biological Sciences Laboratory, Federal University Lokoja and analyzed immediately.

Isolation and biochemical characterization

For the isolation of bacterial strain from different samples, 100g of each whole sample (tomatoes, cucumber and carrots) bought from each location was weighed and diluted in 900 ml of buffered peptone water (BPW) in a sterile tube and centrifuged at 10,000 rpm for 2 minutes before making dilutions. A 1ml aliquot of the homogenate mixture was used for serial dilutions. MacConkey agar, nutrient agar, mannitol salt agar and eosin methylene blue agar were inoculated with 1ml of sample taken using the pour plate method. It was left to solidify and plates were incubated for 24 hours at 37^0C. Distinct colonies were observed and further subcultured by streaking on freshly prepared media to obtain pure culture. The pure strains were subjected to Gram's reaction and biochemical tests such as, catalase, oxidase, methyl red, indole, citrate, lactose fermentation test, hydrogen sulfide production test; Voges-Proskauer test, nitrate, urease, motility test and Starch hydrolysis.

Total aerobic plate count of bacteria

One hundred grams of the sample was washed in sterile 900 ml buffered peptone water from which 1ml was transferred to the first test tube containing 9mls of the diluent. This was repeated for the other three sets of tubes to dilute to 10^{-5} . From the last test tube, 1ml was pipetted and dropped in pre-sterilized plates in duplicate. A molten agar was poured into the petri dishes and gently rocked to spread and cooled. The plates were incubated at 37^0 C for 24hrs after which colonies were counted and multiplied by the dilution factor.

Antibiotic susceptibility testing

Antimicrobial susceptibilities were determined by the Kirby- Bauer disc diffusion method in accordance to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2018). The following antibiotic disks(Oxoid Ltd, Basingstoke, Hampshire, UK) were used: ceftazidime (CTZ, 30μg), cefotaxime (CTX, 30μg), ciprofloxacin (CIP, 5μg), tetracycline (TET, 30μg), ampicillin (AMP, 10 μg), oxacillin (OX, 5 μg) and gentamicin (CN, 30 μg). All plates were incubated at 37^0C for 24 hours. The diameters of zones of inhibition was measured to the nearest millimeter using a ruler. Control strain *Escherichia coli* ATCC 25922 was used in the testing to validate the results of the antibiotic discs. Approved [16] susceptibility zone diameter interpretative standard was used.

Statistical analysis

The diameters of the zones of inhibition (mm) of the organism to the antibiotics tested were interpreted as signifying susceptibility, intermediate or resistant according to the approved [16]susceptibility zone diameter interpretative standard. Descriptive statistics was carried out using the Microsoft Excel Software.

Results

Microbial load of the fruits and vegetable samples

Both markets recorded high bacteria load ($\geq 6\times10^6$) on all the samples. However, the bacteria load varied with the market type and sample type **(Table 1).** Carrots had the highest average TAPC of (9.34×10^6); followed by cucumber (8.81×10^6) while tomatoes have the least count (8.62×10^6). The TAPC of the fruits and vegetable indicate that cucumber purchased from old market showed the highest TAPC (11.26×10^6cfu/g) followed by tomatoes (10.14×10^6) and carrot (9.44×10^6) while cucumber from international market showed the least TAPC (6.36×10^6cfu/g).

The prevalence of bacteria isolates present in the fruits and vegetables samples

A total of 39 bacteria isolates were recovered from the samples. The bacteria isolates included *Klebsiella pneumoniae* which recorded the highest percentage of occurrence (28.2%), followed by *Salmonella*sp. (20.5%), *Escherichia coli* (15.4%), *Staphylococcus aureus* (10.2%), *Enterobacter*sp *Enterobacter aerogenes* (7.7%), *Enterobacter* sp.(5.1%), *Pseudomonas aeruginosa* (5.1%), and *Bacillus* sp.(5.1%) and *Proteus vulgaris* (2.7%). **Table 2** shows the distribution of bacterial isolates in the different samples.

Antibiotics resistance pattern of bacteria isolates

Figure 1 shows that the highest antibiotic resistance pattern was displayed against ampicillin and oxacillin each (100%), followed by gentamycin (94.9%), ciprofloxacin (69.2%), cefotaxime (51.3%), tetracycline (43.6%), while the lowest antibiotic resistance was displayed against ceftazidime (41.0%). The resistance pattern of bacterial isolates to the various antibiotics is shown in **table (3).**

Table 1. The total aerobic bacteria count in the fruits and vegetables samples.

Sample type	Sample market	Total aerobic plate count	Average total aerobic plate count
Carrot	old market international market	9.44×10^6 9.24×10^6	9.34×10^6
Cucumber	old market international market	11.26×10^6 6.36×10^6	8.81×10^6
Tomatoes	old market international market	10.14×10^6 7.09×10^6	8.62×10^6

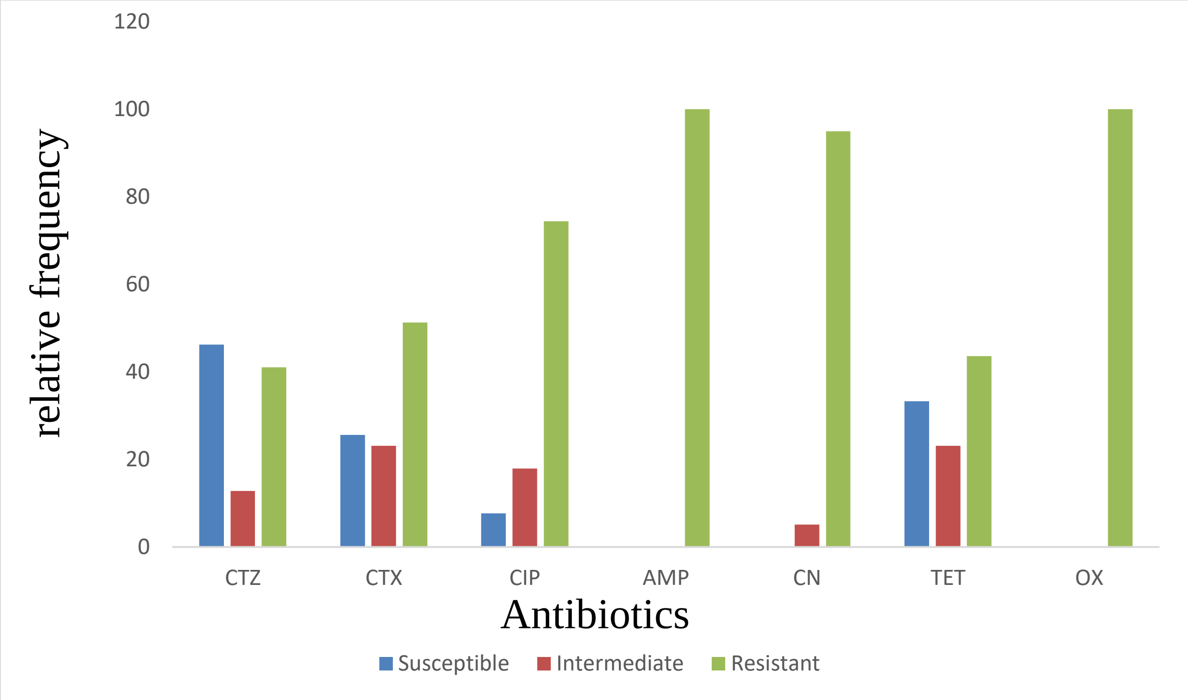
Table 2. Occurrence of bacterial species in the fruits and vegetables from Lokoja markets.

Bacterial isolates	Frequency	Percentage (%)
Klebsiella pneumoniae	11	28.2
***Salmonella* sp.**	8	20.5
Escherichia coli	6	15.4
Staphylococcus aureus	4	10.2
Enterobacter aerogenes	3	7.7
***Enterobacter* sp.**	2	5.1
Pseudomonas aeruginosa	2	5.1
***Bacillus* sp.**	2	5.1
Proteus vulgaris	1	2.7
Total	39	100

Table 3. Antibiotics resistance patterns of bacteria isolates from fruits and vegetables samples.

BACTERIA ISOLATE	CTZ	CTX	CIP	AMP	CN	TET	OX
***K. pneumoniae* (11)**	4(36.4)	6(54.6)	8(72.7)	11(100)	10(90.9)	5(45.5)	11(100)
***Salmonella* sp. (8)**	3(37.5)	3(37.5)	7(87.5)	8(100)	8(100)	2(25)	8(100)
***E. coli* (6)**	3(50)	4(66.7)	2(33.2)	6(100)	5(83.3)	4(66.7)	6(100)
***Staph. aureus* (4)**	2(50)	3(75)	4(100)	4(100)	4(100)	3(75)	4(100)
***Ent. aerogenes* (3)**	1(33.3)	1(33.3)	2(66.7)	3(100)	3(100)	1(33.3)	3(100)
***Enterobacter* sp. (2)**	1(50)	1(50)	1(50)	2(100)	2(100)	1(50)	2(100)
***P. aeruginosa* (2)**	2(100)	0.00	2(100)	2(100)	2(100)	1(50)	2(100)
***Bacillus* sp. (2)**	0.00	1(50)	1(50)	2(100)	2(100)	0.00	2(100)
***Proteus vulgaris* (1)**	0.00	1(100)	0.00	1(100)	1(100)	0.00	1(100)
Total (39)	16(41.0)	20(51.3)	27(69.2)	39(100)	37(94.9)	17(43.6)	39(100)

Figure 1. Antibiotics susceptibility pattern of bacterial isolates.



Discussion

The increasing consciousness of people to nutrition has resulted in the increased consumption of fresh unprocessed vegetables and fruits. These foods carry indigenous micro flora besides pathogenic microorganisms. A number of diseases outbreaks due to consumption of these produce have been reported. As indicated in this study, the TAPC from the produce tested namely carrots, cucumbers, and tomatoes were high, and this was recorded for both sampled markets, especially at old market. This suggests an unsatisfactory bacteriological quality that could pose a health challenge when consumed without further decontamination. The high bacterial contamination obtained from our study is similar to the bacterial count detected in a study conducted by [17] on some edible fruits sold in Makurdi, In addition, studies conducted by [12,18] recorded bacterial counts exceeding 2.5×10^6 CFU/mL in fruits and vegetables. Higher contamination of fruits and vegetables were recorded in the old market, which could be as a result of its nearness to river Niger; serving as irrigation source for the vegetables and also as wash water by vendors. Also, the high microbial contamination observed in the fruits and vegetables in this study may be a reflection of storage conditions and how long these fruits and vegetables were kept before they were obtained for sampling. The bacterial load recorded in this study may be part of the natural flora of the fruits and vegetables from air, soil, irrigation water, and the environment during transportation, rinsing water or during storage [19].

The presence of *Staphylococcus auerus*, *Salmonella* sp. and *Klebsiella pneumoniae* in the fruits and vegetables, 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. *Klebsiella pneumoniae* was the most frequently isolated bacteria, and its high presence in fruits and vegetables is of serious public health concern, as consumption of vegetables contaminated with *Klebsiella* sp. has been identified to cause acute pneumonia especially in immunocompromised individuals [20]. Furthermore, the presence of *Escherichia coli* in fruits and vegetables is worrisome, as it is an indicator of faecal contamination and shows poor hygienic practices among vendors. *Pseudomonas* sp. and *Bacillus* sp. could be part of the natural flora and are among the most common vegetable spoilage bacteria [21], though some *Bacillus* sp. could cause food borne illness. Moreover, the result of this study is in line with the report of [22] from air contaminated vended foods sold in Lokoja, Kogi state.

All bacterial isolates were entirely resistant to ampicillin and oxacillin. The use of antibiotics in agricultural practices might be largely responsible for the development of resistant food borne pathogens [23]. For instance, antibiotics are routinely used in livestock, poultry and fisheries with their wastes serving as manure for crops. More so, wastes and effluents from farms could find their way into water bodies which could be used for irrigation purposes; consequently spreading resistance to antibiotics in raw vegetables [24]. **Golly et al.** [25] also reported a similar resistance of *Escherichia coli* and *Salmonella* species from fresh vegetables to ampicillin. Furthermore, all bacterial isolates displayed resistance to three or more classes of antibiotics. This suggests growing multidrug resistance in bacterial isolates from fresh vegetables and could be a potential source of spread of multidrug resistant bacteria. Similarly, multidrug resistant bacteria have been reported in fresh vegetables [10,26]. In contrast with the findings of [23], this study found bacterial isolates to be most susceptible to ceftaxidime, among the test-antibiotics which suggests the effectiveness of third-generation cephalosporins against bacterial isolates.

Conclusion

This study has established that fruits and vegetables sold in old and international markets in Lokoja harbor a high number of pathogenic bacteria and the bacterial load was beyond the permissible limits. The causes of such high microbial contamination could be poor or unfitting sanitary conditions in and around the markets and poor agronomic practices during cultivation, harvesting, transportation, and prolonged storage. Cross contamination between produce may also occur if they are washed with the same water. Most of these vendors get their water from unclean sources such as river Niger that is very close to the old market. However, regulation bodies should educate retailers on good agricultural practices during cultivation, transportation, washing and storage to improve the microbial quality of fresh produce.

Authors' contribution

Conception and design of the study: G Odewale, MY Jibola-Shittu

Acquisition of data:G Odewale, MY Jibola-Shittu, NS Omosule

Analysis and interpretation of data: G Odewale, MY Jibola-Shittu, NS Omosule

Drafting the article and revising critically for intellectual content: G Odewale, MY Jibola-Shittu

Final approval of version to be submitted: All authors have approved the submission of the final article.

Disclosure of potential conflicts of interest

The authors report that there were no conflicts of interest.

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