

A Survey on the Bacterial Load of Selected Fruits and Leafy Vegetables in Minna Metropolis of Niger State, Nigeria

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Abstract

A survey of bacterial load of selected fruits Tomato (*Solanum lycopersicum*); Garden egg (*Solanum melongena*) and Okra (*Abelmoscus esculentus*) and leafy vegetables Fluted pumpkins (*Telferia occidentalis*), Bitter leaf (*Vernonia amygdalina*) and Amaranth (*Amaranthus caudatus*), were collected from urban and rural farms and markets in Minna metropolis Niger State, Nigeria was examined. The result revealed the presence of pathogenic bacteria at all the locations. The rural farm and rural market shows a significance difference in the means bacterial count with Tomatoes having the highest bacterial load of 6.1×10^5 cfu/g, followed by Garden egg 4.5×10^5 cfu/ml and Okra 3.6×10^5 cfu/ml however, the leafy vegetable had Bitter leaf 7.5×10^5 cfu/ml and Amaranth had the lowest load of 3.4×10^5 cfu/ml. The urban farm and urban market shows the bacteria loads of Okra having the highest count of 6.0×10^5 cfu/ml, and 4.5×10^5 followed by tomatoe 3.0×10^5 cfu/ml and Garden egg 2.0×10^5 cfu/ml but the leafy bitter leaf had the highest bacteria load of 7.5×10^5 cfu/ml and Amaranth had the lowest of 3.4×10^5 cfu/ml. The comparison between the two location shows that the urban location had more bacteria load in the farm and market. The bacteria isolated and identified include species of *Streptococcus faecalis*, *Streptococcus epidimidis*, *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*. The presence of these bacterial poses a health hazard to the consumers of these fruits and vegetable when they are consume raw.

Key Words: Fluted pumpkins, bitter leave, pathogenic bacteria

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Introduction

Fruits and leafy vegetables are exceptional dietary source of nutrients, micronutrients, vitamins and fibre for human beings hence vital for health and fitness. Well balanced foods, rich in fruits and vegetables, are especially valuable for their ability to prevent deficiencies of vitamin C and vitamin A and also reduce the risk of several diseases (Kalia and Gupta, 2006). These fruits and leaves promote good health, they harbor a wide range of microbial contaminants, thus undermining their nutritional and health benefits, thereby increasing outbreaks of human infections associated with the consumption of fresh (or minimally processed) fruits and vegetables (Hedberg *et al.*; 1994; Altekruse and Swerdlow, 1996; Beuchat, 1996, Beuchat, 1998, Beuchat, 2002).

Bacteria, viruses and parasites on fruits and vegetables have been linked with illness (Sewell and Farber, 2001). Several cases of an outbreak of typhoid fever has been associated with eating contaminated vegetables grown in/or fertilized with contaminated soil or sewage (Beuchat, 1998). These outbreaks differ from a few persons to many thousands affected (SCF, 2002). Olsen *et al.*, (2000) reported that diseases associated with the consumption of fruits and vegetables doubled between 1973 – 1987 and 1988 – 1992. In Canada, 18 outbreaks were documented from 1981 to 2000, with approximately 2000 people affected and 18 deaths (Sewell and Farber, 2001). In developing countries such as Nigeria, continued use of untreated waste water and manure as fertilizers for the production of fruits and vegetables is a major contributing factor for the outbreak of diseases (Olayemi, 1997; Amoah *et al.*, 2009).

The differences in microbial profiles of various fruits and vegetables 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 (Ray and Bhunia, 2007; Ofor *et al.*, 2009).

The objective of the study is to determine the bacterial loads of each location under study. To

identify the bacteria present in the different samples and locations.

Materials and Methods

Collection of Samples

Three fruits and leafy vegetables each were used for this study include: Garden egg (*Solanum melongena*); Tomato (*Lycopersicon esculentum* and Okra (*Abelmoschus esculentus*), while the Leafy vegetables were Fluted pumpkins (*Telferia occidentalis*), Bitter leave (*Vernonia amygdalina*) and Amaranthus (*Amaranthus caudatus*). The samples were acquired from four different locations in Minna metropolis:

- i. Urban commercial garden at Chanchaga (Long $6^{\circ} 34'$ and Lat $9^{\circ} 31'$) and Gidan Kwano areas (Long. $6^{\circ} 26'$ Lat $9^{\circ} 31'$);
- ii. Urban market: sampled at Mobil market (Long. $6^{\circ} 33'$ and Lat. $9^{\circ} 36'$)
- iii. Rural farm at Garatu (Long. $6^{\circ} 26'$ and Lat. $9^{\circ} 29'$) and Maikunkele (Long. $6^{\circ} 28'$ and Lat. $9^{\circ} 39'$).
- iv. Rural market at Garatu (Long. $6^{\circ} 26'$ and Lat $9^{\circ} 28'$).

These were sampled separately and packed in pre-sanitized plastic containers and transported to the Microbiology laboratory of the Federal University of Technology, Minna. The samples were stored for analysis.

Media Preparation

The media were prepared according to the manufacturer's instruction, as reported by Eliner *et al.* (1992), Cheesbrough (2003) and Oyeleke and Manga (2008).

Total Bacterial Counts

The pour plate method was used. Ten ml of each sample was aseptically poured into sterile bottles and diluted serially in distilled water up to a 10-5 dilution. One ml of each the 10-4 and 10-5 dilutions were mixed with nutrient and MacConkey agar in Pertri dishes for determination of microbial content. The agar plates were allowed to solidify and incubated at 37°C for 24 h. The colonies that grew were counted and the values are expressed as colony forming units (cfu)/ml. Pure cultures of isolates were obtained by repeated subculturing

onto fresh media and the cultures were maintained on agar slants for further identification.

Characterization and identification of isolates

Bacterial isolates were characterized based on microscopic appearance, colonial morphology and biochemical tests. The isolates were identified by comparing their characteristics with those of known taxa, as described by (Eliner *et al.* (1992), Barrow and Feltham (1993), Cheesbrough (2003), Oyeleke and Manga (2008).

Data Analysis

Data generated was subjected to Analysis of Variance (ANOVA) using the Minitab Release 14 Computer Statistical software. Means were separated by LSD test (when significant differences occur between treatments).

Results and Discussion

Bacterial loads of fruits and leafy vegetables

The bacterial load on fruits and leafy vegetables detected between rural farms and markets in Minna metropolis are shown in Fig. 1. This result show significant difference among the means. The result for rural farm and market for the fruits showed that Tomatoes from rural market had the highest bacterial load of 6.1×10^5 cfu/ml, followed by Garden eggs with 45×10^4 cfu/ml (also in the market). Bitter leave had the highest bacterial load (6.0×10^5 cfu/ml) within the leafy vegetables at the market. This was followed by Teliferia from the farm (3.7×10^5 cfu/ml). However, Bitter leave from the rural farm had the lowest with 2.6×10^5 cfu/ml.

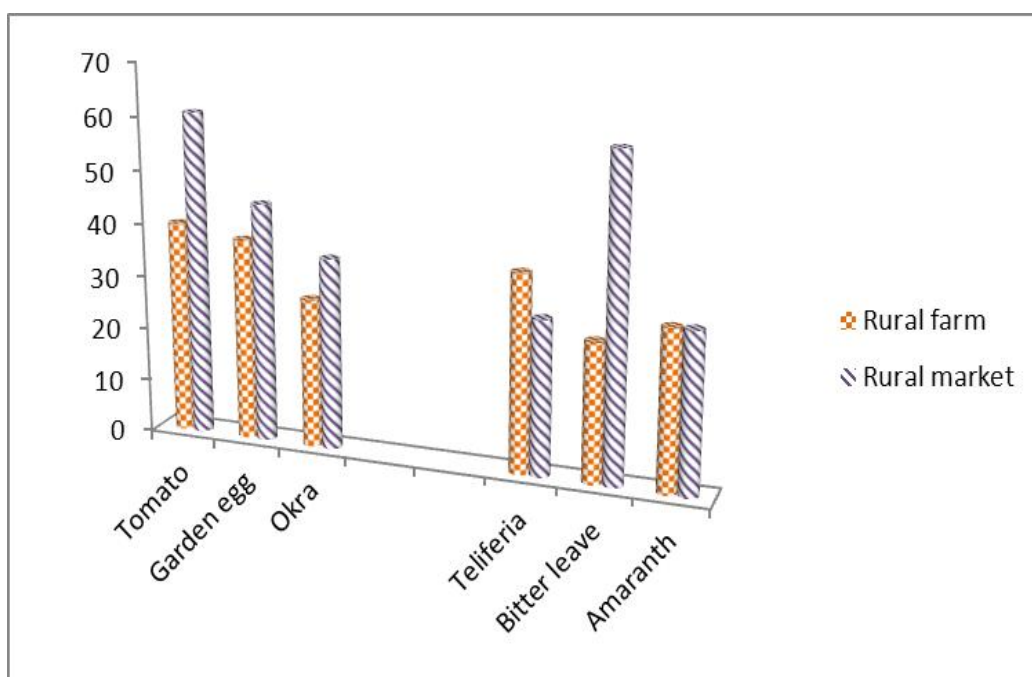


Fig. 1: The bacterial load of fruits and leafy vegetables from the rural farms and markets.

The differences in bacterial load between Urban locations (Farms and Markets)

There were significant difference among the mean bacterial load between fruit samples collected from both the urban farm and market (Fig.2). Okra had the highest bacterial load of 6.0 and 4.5×10^5 cfu/ml between the farm and market samples respectively. This was followed by the load on Tomato fruits with 3.0×10^5 cfu/ml at urban farm.

Garden eggs from the market were the least with bacterial load of 2.0×10^5 cfu/ml).

Among the leafy vegetables, bitter leave from the farm had the highest bacterial load of 7.5×10^5 cfu/ml. Amaranth from the market was lowest with a bacterial load value of 3.4×10^5 cfu/ml.

The comparisons of the bacterial load between sampled areas

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There was no significant difference recorded between the sampled locations - mean bacterial loads of either the fruit or leafy vegetables between rural and urban locations (see Fig. 3). After a close observation of this sampled areas however, farms in the urban area had a higher bacterial load of 5.6×10^5 cfu/ml compared to 4.4×10^5 cfu/ml at market of rural locations.

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State; reveals the presence of bacteria on fruits and leafy vegetables in Minna. From two locations i.e. rural and urban areas. There was no differences in the type of bacteria isolated and identified however some were more prominent on samples from urban (especially farms) than in rural locations. The bacterial isolated and identified includes: *Streptococcus faecalis*, *Streptococcus epidimidis*, *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* (Table 1).

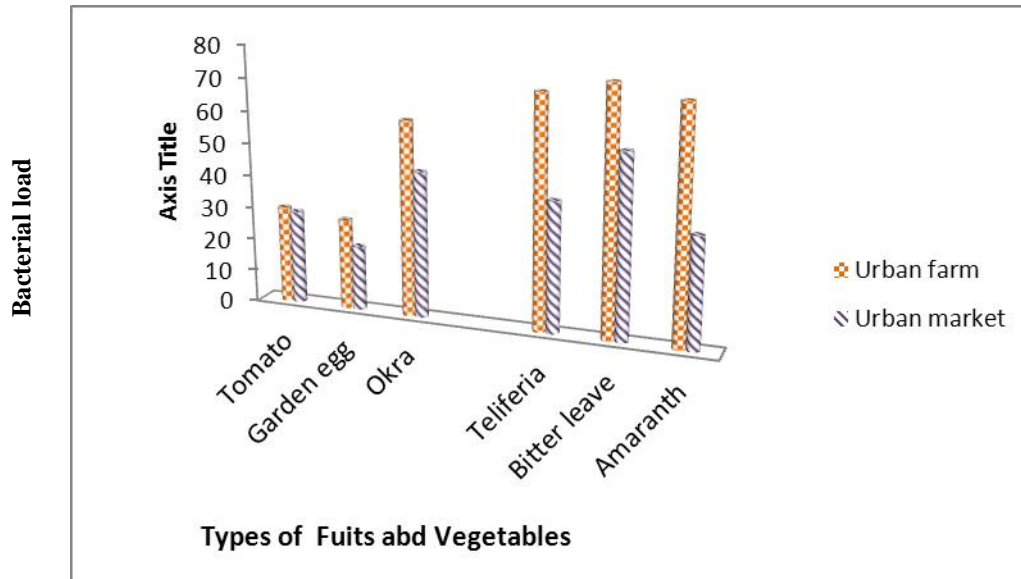


Fig. 2: Differences in the bacterial load of fruits and leafy vegetables from Urban locations.

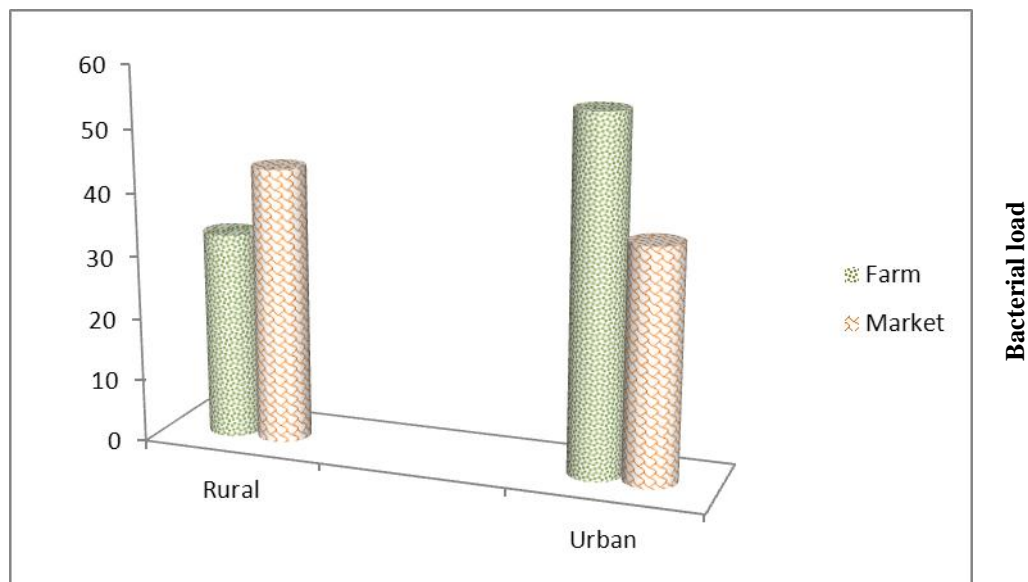


Fig. 3. Mean bacterial load between the sampled areas.

Table 1: Bacteria Isolated and Identified from samples based on Locations

Sampled area	Sampled Bacterial organisms				
	<i>Streptococcus feacalis</i>	<i>S.epidamidis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>
Rural farm	Teliferia	-	All samples	Tomato	All samples
Rural market	-	-	All samples	-	All samples
Urban farm	-	-	All samples	Tomato	All samples
Urban market	-	Bitter leave and okra	All samples	Tomato	All samples

The urban farm recorded a greater bacterial loads this could be as a resulted of waste water used to water the plant e.g. sewage water for watering the field, manure used for fertilization and the hygienic condition of the area where the vegetables were produced. These support the claim of Beuchat and Ryu, (1997) and Beuchat (1998) that the presence of many pathogens in the soil was thought to be from historical application or environmental presence of fasces or untreated sewage. Also Benjamen (2005) said, pathogens existing in the soil or water can be the source of both pre- and post-harvest contamination. These higher bacterial loads in urban area might also not be unconnected with the life style (comprising of throwing rubbish anyhow) thereby quickly increasing the concentration of pollution in the cities. Out of all these three vegetables Bitter leave and *Teliferia occidentalis* consistently had the highest bacterial load almost throughout the sampled areas in Minna metropolis. *Staphylococcus aureus* appeared to be the most common species of *Staphylococci* to cause Staph infections. *Staphylococcus aureus* can cause a range of illnesses from minor skin infections, such as pimples to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS) (Bowersox, 1999). *Streptococcus feacalis* can cause endocarditis and bacteraemia, urinary tract infections (UTI), meningitis, and other infections in humans (Murray, 1990). A recently recognized strain, *E. coli* 0157:H7, produces high levels of toxins that can cause kidney damage, as well as septicaemia, or blood poisoning. Symptoms can include diarrhoea, chills, headaches, and high fever, and in some cases the infection can lead to death, even with medical intervention

Minhas and Samra, (2004) reported that leafy vegetables when eaten raw, can transmit contamination from farms to consumers. Thus,

these higher bacteria load on leafy vegetables calls for caution, because many people use Bitter leave and *Teliferia occidentalis* for medicine where they only squeeze out the juice and drink it raw, as well as its use for salads. Other isolated bacterial include *Bacillus substillis* which may contaminate food but rarely causes food poisoning (Ryan and Ray, 2004) and *Staph epidermidis* which is not usually pathogenic, but patients with a compromised immune system are often at risk for developing an infection (Salyers and Whitt, 2002). Highest bacterial load for fruit and vegetables also occurred in Tomato and Okra intermittently for both the rural and urban sample location of Minna metropolis. The organisms *E. coli* was isolated from Okra.

Mukherjee *et al.*, (2004), reported that while all samples were virtually free of pathogens, *E. coli* was 19 times more prevalent on produce acquired from the organic farms . They estimated that this was due to the common use of manure aged less than a year. The use of cattle dung's manure enhanced this practice. Thus, for any farm where manure is to be used, the demand for well-rottened manure should not be used only for preventing ammonia build up and disease infestation on crops but for postharvest hygienic status of the produce. This finding is in no way far from what was observed in the current study for Garatu sampled location, where cattle are reared in abundance. This could have been the reason for the presence of *E. coli* in the Tomato sampled from this area. Another reason for tomato may be attributed to the fact that as most consumers of this fruit prefer it being sold ripe there was the potential for the fruit to pike up microorganisms as it is being ripened. The riper a fruit is, the closer it is to bacterial build up.

In the light of the foregoing; the following recommendations are made to help in safeguarding the health of end users of fruits and leafy vegetable in Minna metropolis:

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Farmers should ensure that vegetables are grown hygienically. The use of sewage or waste water with potential risks of transmitting infectious pathogens should be minimized or avoided. Fruits and leafy vegetable sellers and consumers should endeavor to thoroughly wash all product before consumption. This could be achieved through proper washing in clean or chlorinated water.

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