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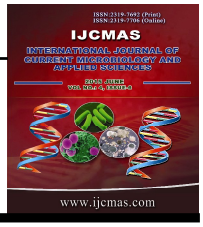


Original Research Article

Bacteria Contaminants on Surfaces of Some Edible Fruits Sold in Makurdi Metropolis, Benue State, Nigeria

C.U. Aguoru*, S.Maaji and J.O. Olasan

Department of Biological Sciences, Botany Unit, University of Agriculture, Makurdi, Nigeria

**Corresponding author*

Keywords

Fruit, Contaminants, Markets, Vendors, Diseases, Hygiene, Sanitary measures

A B S T R A C T

This study aimed at investigating bacteria contaminants on surfaces of some edible fruits sold in Makurdi metropolis in Benue state, Nigeria. Twenty (20) samples each of fresh fruit type namely: orange, garden egg, avocado pear, carrot, and mango were collected from each of the four market sampled (Wadata, High Level, Wurukum and North Bank markets). Fruits samples were serially diluted following standard bacteriological practices. Pure cultures of bacteria isolates were carried out for total viable counts followed by gram staining technique and biochemical characterization for identification purposes.Results showed that orange samples collected from Wurukum, Wadata and North-Bank markets recorded the highest volume of bacteria (9.0×10^7, 8.6×10^7 and 8.6×10^7cfu/g respectively). Mango, orange and garden egg fruits sold in High Level market also showed high level of contaminants ($8.4 \times 10^{7,}$ 7.6×10^7 and 6.4×107 respectively). Generally, avocado pears and carrots collected from different markets showed lower level of contaminants. Cultural and biochemical characterization of bacteria isolated from all the fruits revealed the presence of eight (8) species of bacteria, namely: *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* spp, *Pseudomonas aeruginosa*, *Streptococcus* spp, *Salmonella* spp, *Lactobacillus* spp and *Proteus* spp. In their percentage of occurrence, *S. aureus* was the highest (45%). *Klebsiella*, *P. aeruginosa*, *Streptococcus*, *Salmonella* and *Proteus* had 40% each. The detection of these opportunistic pathogens poses serious public health risks. This calls for urgent public sensitization and sanitary measures by appropriate regulatory bodies to oversee the activities of fruit vendors in the study area and other public places where fruits are sold in Nigeria.

Introduction

Edible fruits are those fruits that can be eaten directly from harvest. Most edible fruits have sweet taste, attractive aroma and quality nutritional properties. Fruits are important source of vitamins and to a lesser extent carbohydrate, fats and protein and as such they are indispensable food items needed for healthy living (Bevan, 2002; Bryan *et al.*, 2006; WHO, 2015). In Nigeria, fruits are commonly sold in most public

places such as the markets, road sides, streets and motor parks. In some cases, they are sold using mobile platforms like wheel barrows and trucks loaded with all kinds of fruits such as orange, banana, pawpaw, mango, pineapple, avocado pear, apple, garden egg and coconut. The fruits are sometimes loaded in trays and basin on vendors head. The choice of fruits sold by a particular marketer is influenced by the level of profitability and availability of the fruits as each fruit type is seasonal in nature.

The occasional presence of pathogenic bacteria, parasites and viruses capable of causing human infections has been documented in fruits (Adelana, 2004; Hassan *et al.*, 2006; Abdullahi and Abdulkareem, 2010). The safety of these fruits is thus called to question, as regulatory bodies in Nigeria do not enforce sanitary conditions in the handling and presentation of fruits. Also, without much formal education in hygiene and sanitation, the fruit vendors acquire their handling techniques traditionally, considering the above conditions in which these edible fruits are handled and sold, it is expected that the safety of these fruits is not guaranteed, thus not meeting the health standard. Since there is a rapid increase of the already large number of people involved in the consumption of edible fruits in the study area, there is every need to have first-hand information on the safety of these fruits by investigating the types and volume of potential pathogenic microbes present on the surfaces.

Materials and Methods

Study area

This research was carried out in Makurdi metropolis, the capital of Benue state, Nigeria. Makurdi is located on latitude 07°45' and longitude 08°30'. The town is dominated by guinea savanna types of vegetation. The vegetation cover is mostly made up of giant grasses, oil palm, and locust bean tree, among others. The mean annual rainfall is favourable for food production. It ranges between 150–180mm, while the mean annual temperature ranges between 27^0C–28^0C. The climate can be described as tropical. There are 2 seasons, the wet season and the dry season. The former starts from April to October while the latter start from October to April. The presence of River Benue in the study area also favours food production.

Sample collection

Twenty (20) samples each of fresh fruit type namely: orange, garden egg, avocado pear, carrot, and mango were collected in sterile polythene bags from each of the market sampled (Wadata, High Level, Wurukum and North Bank markets) within Markurdi metropolis and transported to the Advanced Laboratory of the University of Agriculture, Markurdi Nigeria.

Laboratory work

All culture media for isolation of the organisms were prepared following standard microbiological practices (Aguoru and Katsa, 2009). Each of the fruit samples was rinsed in a beaker containing sterile distilled water followed by 10 folds serial dilution. 1ml of the rinsed water sample was aseptically transferred using a sterile pipette into 9ml of sterile distilled water in a test tube to give a 10^{-1} dilution. Serial dilutions were made up to 10^{-7} and 1ml each of the suspension was inoculated into a prepared molten nutrient agar plates and the inoculums were then incubated at 37^0C for 24hrs to allow colony formation of bacteria. Pure cultures were prepared from the

primary cultures of the bacteria and afterwards gram staining and biochemical characterization were done to identify the different bacteria present in the culture. Initial colonies on the different agar plates were determined by plate count method with the aid of a magnifying hand lens. Discrete colonies were then picked and streaked on fresh media where 0.1ml of the inoculums was carefully and evenly spread over the entire surfaces of MacConkey and CLED agar plates. The inoculated plates were incubated at 37^0C for 24 hours for isolations of bacteria. Total Viable Count (TVC) of bacteria colonies was carried out. Based on their microscopic, cultural and morphological characteristics, colonies were selected from each plate for gram staining and biochemical tests (catalase and coagulase).

Results and Discussion

In descending order of colony forming unit (cfu/g), orange samples collected from Wurukum, Wadata and Northbank markets recorded the highest volume of bacteria (9.0×10^7, 8.6×10^7 and 8.6×10^7respectively). Mango, orange and garden egg fruits sold in High Level market also showed high level of contaminants ($8.4 \times 10^{7,}$ $7.6 \times 10^7$and 6.4 × 107 respectively). Generally, avocado pears and carrots collected from different markets showed lower level of contaminants (table 1; figure 1). Cultural and biochemical characterization of bacteria isolated from all the fruits revealed the presence of eight (8) species of bacteria, namely: *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* spp, *Pseudomonas aeruginosa*, *Streptococcus* spp, *Salmonella* spp, *Lactobacillus* spp and *Proteus* spp (table 2). In their percentage of occurrence, *S. aureus* was the highest (45%). *Klebsiella*, *P. auroginosa*, *Streptococcus*, *Salmonella* and *Proteus* had 40% each. Lactobacillus was the least in occurrence (25%) (Table 3, Figure 2). *S. aureus*, *Klebsiella* and *Proteus* were observed in all samples of fruits while *P. aeruginosa* was found in all fruits except orange. *Streptococcus* was present in all fruits except in carrot while *Lactobacillus* was found only in avocado pear and mango fruits.

The presence of *Staphylococcus aureus* and its high occurrence (45%) in fruit samples is a serious health risk because of its ability to cause a wide variety of infection through toxin production. This bacterium has been implicated to cause boils, impetigo, food poisoning, cellulitis and toxic shock syndrome (MedicineNet, 2015). According to the United State Food and Drug Administration (USFDA, 2015), the presence of this bacterium or its enterotoxins in processed foods or on food processing equipment is generally an indication of poor sanitation. The agency emphasized that *S. aureus* can cause severe food poisoning as it has been identified as the causative agent in many food poisoning outbreaks in many parts of the world.

The presence of *Escherichia coli* in the fruit sample analysed is indicative of faecal contamination. *E. coli* is part of the normal flora of the human intestines. Some strains of *E. coli* have been linked to diarrhea gastro enteritis and urinary tract infections (Hassan *et al.,* 2006; Aguoru *et al.,* 2015). *Klebsiella spp*. is second only to *E. coli* as a urinary tract pathogen. It is a ubiquitous opportunistic pathogen which has been cultured from many sources such as soil, water, raw fruit and salads. This pathogen inhabits the upper respiratory tract and could cause diseases when there are opportunities to do so. *Salmonella spp* are non-lactose fermenters usually associated with contamination. Contamination with these organisms could arise from washing fruits with contaminated water or poor handling of fruits by vendors.

Table.1 Total viable counts of bacteria from fruits (in descending order)

Location	Fruit type	VC (CFU/g)
Wurukum	Orange	9.0×10^7
Wadata	Orange	8.6×10^7
North bank	Orange	8.6×10^7
High Level	Mango	8.4×10^7
High Level	Orange	7.6×10^7
High Level	Garden egg	6.4×10^7
North bank	Mango	4.6×10^7
Wadata	Garden egg	4.4×10^7
Wurukum	Mango	3.6×10^7
North bank	Carrot	2.9×10^7
Wadata	Carrot	2.9×10^7
North bank	Garden egg	2.4×10^7
Wadata	Avocado pear	2.4×10^7
High Level	Carrot	1.8×10^7
Wurukum	Carrot	1.6×10^7
Wadata	Mango	1.6×10^7
Wurukum	Garden egg	1.5×10^7
High Level	Avocado pear	1.4×10^7
Wurukum	Avocado pear	1.2×10^7
North bank	Avocado pear	1.0×10^7

Table.2 Cultural and biochemical characterization of bacteria isolates

Suspected Organism	Cultural Characterization on media			Gram Stain Reaction	MOT	CAT	COA
	NA	MA	CLED				
S.aureus	Golden-yellow, smooth, mucoid and raised	Pink colonies	Yellow colonies with pale periphery	+ve	-ve	+ve	+ve
E.coli	Flat and cream with uneven edge	Smooth pink colonies	Deep yellow colonies	-ve	+ve	+ve	N/A
Klebsiella	Very mucoid raised, milky and smooth	Mucoid pink colonies	Extremely mucoid, yellow to whitish colonies	-ve	+ve	+ve	N/A
Pseudomonas	Light-green colonies	Pale whitish colonies	green colonies	-ve	+ve	+ve	N/A
Streptococcus	Creamy-brown Rough flat and Shiny colonies	Light pink flat rough colonies	smooth and grayish yellow	+ve	-ve	-ve	-ve
Salmonella	Smooth, flat and creamy	Grayish red colonies	flat, blue, mucoid opaque and shiny colonies	-ve	+ve	+ve	N/A
Lactobacillus	Smooth, large Cracked, flat and creamy	deep pink dry dull, large and rough edges	Grey colonies with rougher surfaces	+ve	-ve	-ve	N/A
Proteus	Creamy with odour	pale mucoid and smooth colonies	translucent blue green colonies	-ve	+ve	N/A	N/A

Legend: N/A = Not applicable COA =Coagulase test, CAT = Catalase test, MOT = Motility test, MA = MacConkey Agar, NA = Nutrient Agar, CLED = Cystein Lactose Electrolyte Deficient.

Table.3 Percentage occurrence of bacterial species in all fruits

Isolated bacteria	(%) occurrence in all fruits
S. aureus	45
E. coli	30
Klebsiella	40
P. aeruginosa	35
Streptococcus	40
Salmonella	40
Lactobacillus	25
Proteus	40

Fig.1 Regions of low and high fruit contaminants in the study area

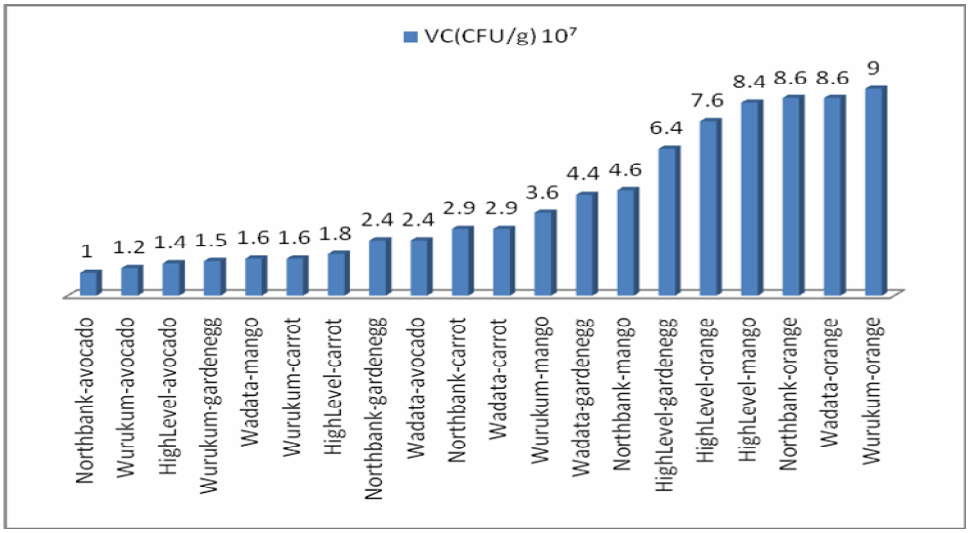
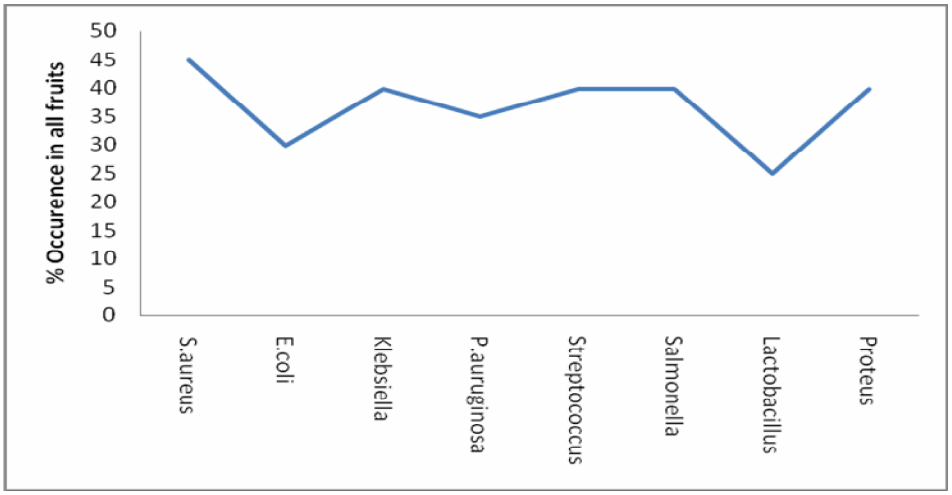


Fig.2 Percentage occurrence of contaminants in all fruits collected



According to the WHO (2002), the effect of bacteriological hazards such as *salmonella* on food safety is now a major public health concern worldwide. The most common disease associated with salmonella is gastro enteritis, commonly referred to as *salmonellosis*. The most serious forms of *salmonellosis* are typhoid and paratyphoid fever caused by *salmonella typh*i and *Salmonella paratyphi* serotypes respectively (Aguoru and Katsa, 2009).

The isolation of *Pseudomonas aeruginosa* in some fruit samples is also of public health concern. The most serious infections include malignant external otitis, endophthalmitis, endocarditis, meningitis, pneumonia, and septicemia (Bodey, 1983). According to Bodey (1983), it causes between 10% and 20% of infections in most hospitals. *Streptococcus* is known to be pathogens of the respiratory tract while some species of *Proteus* cause diseases only in host with impaired resistance (Uzeh *et al.,* 2009).

Using a bench mark of 4.4×10^7microbial load for instance, oranges collected from the four markets are unsafe for direct consumption. Mango and garden egg sold in High Level markets are also heavily contaminated whereas those sold in Wadata and Wurukum markets recorded low microbial loads. The safest fruits in this study are the avocado pears collected from NorthBank, Wurukum and High Level markets. In conclusion, high bacterial load and the presence of opportunistic pathogens imply that appropriate microbiological standards are lacking in the packaging and handling of fruits in the markets. Contamination could also be due to exposure of the fruits as well as the unhygienic water used in washing the fruits by the vendors. Thus, they are unsafe for direct human consumption unless appropriate measures are taken to reduce microbial contamination of fruits. This calls for urgent public education and sanitary measures by appropriate regulatory bodies to oversee the activities of fruit vendors in the study area and other public places where fruits are sold in Nigeria.

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