

FOOD POISONING ASSOCIATED BACTERIA ON FRUITS IN THE OPEN MARKETS IN LAGOS NIGERIA

Avwioro TO¹, Ajayi MB², Onyije FM³, Onobrudu DA⁴

1. Department of Nursing, Obafemi Awolowo University Teaching Hospital, Ile-Ife, Nigeria
2. Department of Microbiology, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria
3. Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Bayelsa State, Nigeria
4. Department of Biochemistry, Faculty of Science, University of Port Harcourt, Rivers State, Nigeria

Corresponding author: Avwioro TO

Email: rukewe09@yahoo.com

ABSTRACT

Aim: To determine the presence of food poisoning bacteria on fruits in the open markets.

Method: A total of 1000 assorted fruits consisting 100 each of guava, carrots, oranges, mangoes, African cherry, pear, water melon, cashew, apple and garden eggs were randomly collected from open markets in Lagos, Nigeria. A sterile cotton wool tipped applicator pre-soaked in normal saline was used to rinse the fruits and subsequently cultured on deoxycholate agar, Selenite enrichment broth, mannitol salt agar, Robertson cooked meat medium, blood agar and Sorbitol McConkey agar. They were incubated aerobically and anaerobically. PCR, Gram, biochemical, enzymatic analyses as well as agglutination reactions with different sugars were used for the identifying the bacteria.

Results: Overall, 14.6% of the fruits had food poisoning bacteria. The most common food poisoning bacteria on fruits was found to be *Staph. aureus* (6%). This was followed by *E. coli* (3.8%). *Salmonella* (1.3%), *Shigella* (1.3), *Clostridium* spp (0.6) and *Campylobacter jejuni* (1.6%). The most infected fruit was water melon (2.5%), followed by mangoes (2.3%), African cherry (1.7%), oranges (1.4%), pear (1.4%), guava (1.3%), garden eggs (1.3%), cashew (1.1%), carrots (0.9%) and apple (0.7%).

Conclusion: Common fruits sold in the open markets may have been contaminated with food poisoning bacteria. They should be washed properly before consumption.

Key words: Food poisoning, Bacteria, Fruits

INTRODUCTION

Food borne illnesses may be due to intoxication or infection. Intoxication arises from ingestion of toxin produced by pathogens, while infection is as a result of ingestion of food and drinks containing living pathogenic organisms (Ajayi et al., 2013). Bacteria are the main causes of food borne illnesses. The symptoms of food borne illnesses include diarrhoea, vomiting, abdominal cramps, headache and nausea. The organisms mostly implicated are *Clostridium perfringens*, *E. coli*, *Salmonella* and *Staphylococci* (Addisand Sisay, 2015). Sources of contamination of food items include plant surfaces, animals, water, sewage, air and soil as well as food handlers and those processing foods (Adams and Moss 2008; Owolagba et al., 2009; Bean and Griffins 1990). *Salmonella* are

Gram negative rods. The primary habitats of *salmonella* species are the intestinal tracts of humans and animals. The organisms are excreted in faeces and are transmitted by insects and other living creatures to other places (Kalpelmecher 1993; Jay 2000; Radostits et al., 2007). There are several species of *Salmonella*. The ones that infect humans only include *S. Typhi*, *S. Paratyphi A*, *S. Paratyphi C*. They are the causative agents of typhoid and paratyphoid fevers. Others are *S. gallinarum* (poultry), *S. dublin* (cattle), *S. abortusequi* (equine), *S. abortus-ovis* (sheep) and *S. choleraesuis* (swine) (Quinn et al., 2001). *Salmonella* often enter the host by ingestion and move from the stomach into the small intestine (Bryan et al., 1971). The incubation period of *salmonella* is 12-36 hours, although in certain cases the symptoms resolve

in 2-3 days without any complication (Bean and Griffins, 1990) *Staphylococcus aureus* are Gram positive cocci which usually occur clusters, although some occur in singles or short chains. The strains that produce enterotoxin are the ones that cause food poisoning. *S. aureus* produces six types of enterotoxins (A, B, C1, C2, D and E). Most food poisoning is caused by enterotoxins A and D. These enterotoxins stimulate the Central Nervous Systems vomiting center and inhibit water and sodium absorption in the small intestine (Anderson and Pritchard (2008). The incubation period of *S. aureus* is 2-4 hours. The onset is sudden and is characterized by vomiting and diarrhea without fever (Adams and Moss, 2008). *Clostridium botulinum* are Gram positive anaerobic spore bearing bacilli found in soil, sediments of lakes, ponds and decaying vegetation. Most infections are associated with fish and sea food products. There are seven strains of the organism numbered A, B, C, D, E, F, G classified on the basis of serology and other neurotoxin. Botulism in animals is predominantly due to types C and D (Hall et al., 1985). *C. botulinum* produces a highly potent neurotoxin that causes neuroparalysis (Jay, 2000). The incubation period of *C. botulinum* is 12-36 hours. The symptoms include vomiting, constipation, thirst, dryness of mouth, blurred-vision, and difficulty in speaking, breathing and swallowing. Death can occur within 7 days as a result of respiratory paralysis. *Clostridium perfringens* is a Gram-positive anaerobic spore bearing bacillus that is abundant in the environment, vegetation, sewage, soils, water and animal faeces. Enterotoxins produced by *Perfringens* are numbered A to G. Food poisoning is most commonly caused by organisms that produce type A enterotoxin. The other types are relatively safe and do not usually cause food poisoning (Labbe and Nolan, 1981). The incubation period of *C. perfringens* is 8-24 hours. The symptoms are acute abdominal pain and diarrhoea. *Escherichia coli* are Gram negative motile rods which ferment glucose and a wide range of sugars (Quinn et al., (2001). *E. coli* O157:H7 is one of the several serotypes of verotoxin producing *E. coli*. It produces cytotoxic shiga like toxins (SLT). The symptoms include diarrhea with abdominal cramps, bloody diarrhea without fever. *Shigella* are Gram-negative non motile, non spore forming rods, facultative anaerobes transmitted from infected persons, contaminated foods, water and by fecal-oral spread to persons. With the help of Shiga toxin and adhesins which

promote binding to epithelial cell surfaces, the bacteria penetrate the epithelium of the intestines and breakdown the lining, resulting in haemorrhage (CDC). The symptoms include abdominal pain, cramps, fever, diarrhea, vomiting, and bloody stools (Scallan et al., 2011; Mead et al., 1999). *Campylobacteriosis* is caused by the motile bacteria of the genus *Campylobacter*. There are several species of *Campylobacter*, but the most prevalent species that causes human illness is *C. jejuni* (Jay, 2000; Radostits et al., 2007) where it causes gastroenteritis. It can be transmitted from poultry where it is found to be abundant and asymptomatic (Quinn et al., 2001). The symptoms are fever, nausea, vomiting, abdominal cramping, diarrhea, enteritis and malaise. The illness which may last for 7-10 days begins within 2-5 days after ingestion of the organisms (Walderhaug, 2007). The organisms can also cause meningitis, urinary tract infections, short-term reactive arthritis and Guillain Barré syndrome (CDC, 2011). *Listeria monocytogenes* are the causative agents for listeriosis. They are facultative anaerobic bacteria one of the most virulent food-borne pathogens, with between 20 to 30% of infections resulting in death (Jay, 2000). The symptoms which may last 7-10 days include muscle aches, fever and vomiting and less commonly diarrhoea. It may also lead to meningitis (Adams and Moss, 2008; Bean and Griffins, 1990). Good sanitation is necessary for the prevention of the disease. (CDC, 2011). Bacterial food poisoning is wide spread and it is fast becoming a major public health challenge in certain parts of the developing countries. Lagos, Nigeria is a highly populated area with several people and diverse living manners. Hygiene is a major problem in this part of Nigeria and the government of Lagos State is concerned about it. It was for this reason that this work was done.

METHODS

Collection and treatment of samples

A total of 1000 assorted fruits consisting 100 each of guava, carrots, oranges, mangoes, African cherry, pear, water melon, cashew, apple and garden eggs were randomly collected from Lagos Nigeria. A sterile cotton wool tipped applicator pre-soaked in normal saline was used to rinse the fruits and subsequently cultured in culture media.

Culture

The specimens were cultured on deoxycholate agar, Selenite enrichment broth, mannitol salt agar, Robertson cooked meat medium, blood agar and Sorbitol McConkey agar. They were incubated aerobically and anaerobically at 37°C for 12 to 48 hours. The organisms were subcultured to obtain pure colonies. Gram, biochemical, enzymatic and agglutination reactions as well as their reactions with different sugars were used for the identification of the organisms by standard methods.

Genomic DNA Preparation

The isolates of the following: *S. aureus*-enterotoxins A and D, *Clostridium botulinum* C and D, *E. coli* O157:H7, *Campylobacter jejuni*, *Salmonella* and *Shigella* were grown in Tryptone soy broth at 37°C for 24 h. Thereafter, 1ml of the liquid culture was transferred to micro tube of 1.5 ml volume. Bacterial cells were harvested by centrifugation at 10,000 rpm for 2 min. The supernatant was discarded and the pellets washed twice with sterile distilled water and resuspended in 1.5 ml sterile distilled water. The bacteria suspension was boiled for 10 min to lyse the cells and release the DNA followed by a cold shock treatment in ice for 10 min, centrifuged at 12,000 rpm for 5 min and the clear supernatant transferred to a new micro

tube. The supernatant containing bacterial template DNA was used directly in specific PCR for the detection of PCR fingerprinting.

PCR Amplifications and Cycling Conditions

PCR amplifications were performed on a thermocycler (Eppendorf Mastercycler Pro, Hamburg, Germany.). The specific primer pairs were used. The reaction volume was 25ul and consisted of 10x PCR buffer, 25mM MgCl₂, 10 mM dNTPs mixture, 5U/ul of Taq DNA polymerase (Fermentals, USA), 10 pmol of each primer set. And 5ng of extracted bacteria DNA. Amplifications were performed as follows: an initial denaturation at for 4 min followed by 35 cycles defined by denaturation at 94°C for 1 min, primer annealing at 60°C for 1 min, the extension at 72°C for 1 min followed by a final elongation at 72°C for 5 min.

Agarose Gel Electrophoresis

The PCR amplification products were fractionated by electrophoresis through 1.2% agarose in 1 x TAE buffer (0.04 M tris- acetate, 0.001 M EDTA (pH 8.0) visualized by staining the gel with ethidium bromide (10ug/ml). The gel pictures were taken using a Gel Documentation System (Clinix, Model 1500, China). A 100 bp DNA marker (Promega, USA) was used as a molecular weight marker.

RESULTS

Table 1. Percentage of certain food poisoning bacteria found on fruits

	Salmonella	Shigella	Staph aureus	Clostridium botulinum	E. coli	Campylobacter jejenu	Total
Guava	1	1	5	0	4	2	13
Carrots	0	0	5	0	4	0	9
Oranges	1	1	6	0	4	2	14
African cherry	2	2	6	1	4	2	17
Pear	1	1	4	0	5	1	14
Water melon	3	3	8	3	5	3	25
Cashew	0	0	6	0	4	1	11
Apple	0	0	5	0	2	0	7
Mangoes	3	3	9	1	4	3	23
Garden eggs	2	2	6	1	2	2	13
Total	13	13	60	6	38	16	146

Overall, 14.6% of the fruits had food poisoning bacteria. The most common food poisoning bacteria on fruits was found to be *Staph. aureus* (6%). This was followed by *E. coli* (3.8%). *Salmonella* (1.3%), *Shigella* (1.3), *Clostridium* spp (0.6) and *Campylobacter jejenu* (1.6%). The most infected fruit was water melon (2.5%), followed by mangoes (2.3%), African cherry (1.7%), oranges (1.4%), pear (1.4%), guava (1.3%), garden eggs (1.3%), cashew (1.1%), carrots (0.9%) and apple (0.7%).

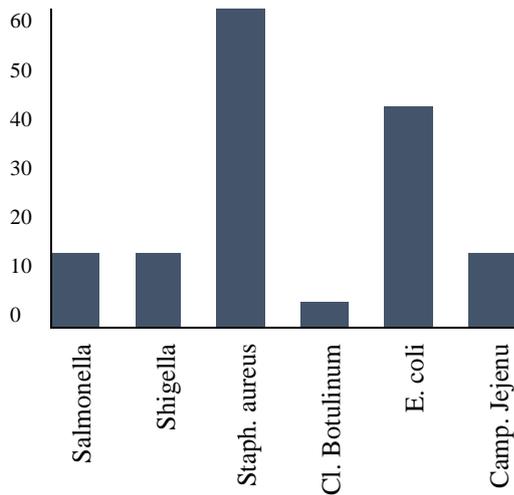


Fig. 1 Comparison of food poisoning bacteria on fruits

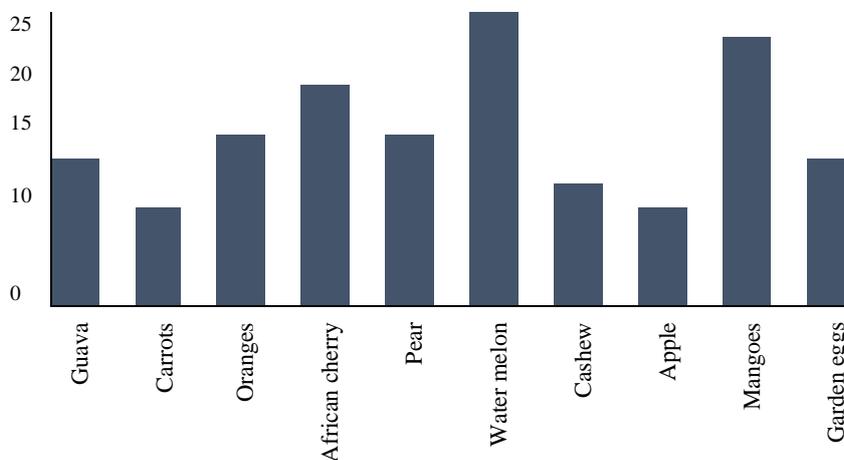


Fig. 2 Rate of infection of fruits with food poisoning bacteria

DISCUSSION

Bacteria are the main microorganisms that cause food borne illnesses (Oduola et al., 2010; Addis and Sisay, 2015) and have been a major challenge globally. In this study, Staph. aureus and E. coli were the most dominant bacteria on the raw fruits. This is in line with the report of Mathur et al., (2014) that infectious bacteria such as Staph. aureus were the most found bacteria on the surfaces of fruits and vegetables. These microbes are associated with symptoms which vary from one organism to another but may include diarrhea and abdominal discomfort. Some species of the organisms are very harmful while others are less harmful without symptoms (Quinn et al., 2001). For instance *S. aureus* produces enterotoxins A, B, C1, C2, D and E but most food poisoning is caused by enterotoxins A and D. Similarly, there are seven strains of *Clostridium botulinum* numbered A, B, C, D, E, F and G but most infections are due

to types C and D (Hall et al., 1985). Carriers and infected persons excrete these organisms in faeces from where they are transmitted to humans and other living creatures (Radostits et al., 2007). Their incubation periods also vary from 12 hours to 48 hours. Although several organisms can cause food poisoning, the target organisms in this research were Salmonella, Shigella, Staph aureus, E. coli and Clostridium. These organisms are often implicated in bacterial food poisoning in this part of Nigeria. *Escherichia coli* O157:H7 produces cytotoxic shiga like toxins which leads to diarrhea, abdominal cramps bloody diarrhea without fever in patients (Robinson et al., 2000). *Shigella* are transmitted from infected persons, contaminated foods, water and by fecal-oral spread to persons. The symptoms are diarrhea, abdominal pain, cramps, fever, vomiting, and bloody stools (Mead et al., 1999). The most prevalent specie of *Campylobacter* that causes human illness is

C. jejuni (Jay, 2000; Radostits et al., 2007). It is abundant in poultry and widely transmitted from there (Quinn et al., 2001). The symptoms include fever, nausea, vomiting, abdominal cramping, diarrhea, malaise and enteritis. (Walderhaug, 2007). *Listeria* is one of the most virulent food-borne pathogens with several infections resulting in death (Jay, 2000). The symptoms are muscle aches, fever and vomiting and sometimes diarrhea and meningitis (Adams and Moss, 2008; Bean and Griffins, 1990). Good sanitation is necessary for the prevention of the diseases. (Fowotade et al., 2017; CDC, 2011). Bacteria food poisoning is widespread and has become a public health challenge. Therefore, proper washing of fruits with clean and fresh water will minimize the rate of infection by these bacteria.

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