Bacteriological Analysis of Ready-to-eat Foods from Morogoro Municipal Market

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SUMMARY

Street vendors supply large quantities of food at affordable prices in many places especially in developing countries. Street foods are common sources of bacteriological contamination causing food poisoning, diarrhea, cholera and typhoid fever. This study analyzed the bacteriological quality of ready-to-eat foods vended in Morogoro Municipal Market. A total of 70 samples from different street foods were randomly collected from different vendors and transported in cool boxes to the laboratory for bacteriological analysis. Standard microbiological methods were used for isolation, enumeration and identification of bacteria. Additional information regarding food preparation, storage and handling practices observed by vendors was noted to correlate with the extent of bacterial contamination. Majority (67.1%) of the ready to eat foods were contaminated with bacteria. Vegetable salads and Potato fries showed highest bacterial contamination rates (78.6%). *Escherichia coli* (49.2%) was the major isolate in all food types. Other bacteria isolated were *Bacillus cereus* (19.7%), *Staphylococcus aureus* (14.8%), *Klebsiella pneumoniae* (14.8%) and *Salmonella spp.* (1.6%). *E. coli* was resistant to some antimicrobials (carbenicilin, clindamycin and tetracycline). High levels of bacterial contamination were associated with poor hygiene of vendors, unsafe food handling practices and use of contaminated water in food preparation. Although the presence of the microorganisms is not necessarily a threat to human health, the fact that some microorganisms were resistant to some antibiotics is of concern. Provision of sanitation and hygiene education to vendors and regulations for implementation of good hygienic practices can improve quality of street foods.

Key words: *Street foods, bacterial contamination, antimicrobial resistance*, *E. coli*, *B. cereus*, *S. aureus*, *K. pneumoniae*

INTRODUCTION

Street food is any prepared ready-to-eat food or drink that is usually sold by a vendor on a street and in other public places, such as at market places, fairs or exhibitions. These foods and beverages are ready for consumption without the need for another process or preparation (Nonato *et al.,* 2016). A study reported by the Food and Agriculture Organization in 2007 estimated that 2.5 billion people worldwide eat street food every day (FAO, 2007). Many people prefer street foods as they are cheaper and easily accessible compared to most restaurants.

Street foods are sold in places like schools, around market, at the bus station or even near construction sites. Street food vending activities in Tanzania is predominantly done by women vendors commonly called "*Mama Lishe*" or "*Mama Ntilie*" in Swahili. Street vendors are mostly neither licensed nor trained in food sanitation (Nonga *et al.,* 2015). The risk of contamination for prepared street foods is high because most of them are prepared in poor environment with poor handling and poor storage facilities. Risk factors related to food contamination include lack of good social services such as portable water, refrigeration and waste disposal. Also most street vendors

have poor knowledge on food safety and hygiene and have insufficient resources for food inspection or food microbiological analysis (Nonato et al., 2016)

Food contamination is defined as foods that are spoiled or tainted because they either contain microorganisms, such as bacteria or parasites, or toxic substances that make them unfit for consumption (Hussain, 2016). Food can be contaminated at any stage during preparation and the contamination can cause effect to consumers such as food borne illness. Contaminants have several routes throughout the supply chain (from farm to fork) and can make the food unfit for consumption.

Bacteria contaminations are common food hazards which can lead to food borne illness (Nonato *et al.,* 2016). World Health Organization (WHO) has noted that foodborne diseases are a worldwide challenge since the numbers of people encountering foodborne diseases are increasing (Nonato et al., 2016).

Bacteria contamination in foods can occur due to different reasons and at different stages from preparation to consumption.

A fully cooked meal may be contaminated by coming into contact with other raw foods, drippings from raw foods that contain pathogen, from food handler fingernails or clothing or dirty utensils (Rheinländer et al., 2008). Other food contaminants are chemicals that can enter the food supply chain through various roots including pesticides used during farming processes, heavy metals in the environment, and other chemical agents (Rather *et al.,* 2017).

Bacteria are microscopic single-celled organisms that thrive in diverse environments. Although not all bacteria are harmful to humans, some can cause infections of the gastrointestinal tract (Oghene *et al.,* 2014). Most common food-borne diseases in Morogoro are diarrhoea, dysentery, typhoid and cholera but no clear data about the extent of these diseases have been contributed by eating contaminated street vended food.

Noting that street foods in Morogoro Municipal Market are prepared under different conditions with unknown hygienic levels, the study assessed the quality and determined the possible sources of bacterial contamination in the foods. This study will provide information on bacteriological quality of the street foods in similar places and help to raise public awareness and particularly to food vendor's on measures to avoid food contamination.

MATERIALS AND METHODS

Study design and study area

This was a cross sectional study conducted at Morogoro Municipal Market (Mawenzi) located in Morogoro between March and May, 2019. Morogoro Town is located 196 km west of Dar-es-salaam and 260 km east of Dodoma, the country's capital city. Annual temperature ranges from 16°C-33°C and average annual rainfall is between 821-1505 mm. According to the National Population Census (2012), Morogoro Municipality has a population of 315,866. Mawenzi Market serves a large population of the people working and/ or residing within and close to Morogoro Municipality.

Sampling procedure and sample size

The sampling was random where five different ready-to-eat food types were sampled. The selected food types were boiled rice, beef soup, stiff porridge, raw vegetable salads, and fried potato chips. These were the commonest foods consumed around. Eight samples were collected for each type of food and from different vendors. A total of 70 samples were collected in sterile containers and transported within 2 hrs of collection on ice in cool boxes to the Microbiology Laboratory of the College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture.

Microbial analysis

Samples (100gm) were grinded by using Laboratory blender under sterile conditions and well mixed. Serial dilution was prepared by taking 1g of sample dissolved in 9ml of 0.5% normal saline and then by using pour plate technique 0.1ml was taken from each serial diluted samples and poured in Blood agar, Nutrient agar and Mac Conkey media (Hi Media Laboratories) and incubated at 37°C for 24 hrs as described by Lepp (2010).

The concentration number of bacteria at different factor of dilution was recorded where the low dilution factor from 10^{-7}-10^{-10} gave the minimal number of bacteria count. Sub culturing was done by using streak plate method to obtain discrete colonies by streaking into four quadrants of the petri dish and incubated at 37°C for 24 after the incubation, the different cultured petri dish were examined for microbial growth.

Identification of isolated organisms was carried out using colony morphology where by colonies were identified based on their shape, texture, smell and colour. Gram's staining was performed to differentiate between Gram Positive and Gram Negative bacteria supplemented by Methyl Red, Indole Test, Voges Proskauer, Citrate Test and Triple Sugar Ion Test (Hi Media Laboratories) biochemical tests as described by Lepp (2010). Microbial enumeration was done by pour plate colony counting.

Antimicrobial Susceptibility test

RESULTS

Microbial contamination

The results recorded in Table 1 show that majority (67.1%) of the ready to eat foods were contaminated with bacteria. Vegetable salads and potato fries showed high bacterial

After isolation and identification of bacteria from samples the pure colony was obtained then bacterial suspension was prepared to match the turbidity of the 0.5 McFarland turbidity standards. Disk diffusion method was used to determine the efficiency of antibiotics to the different bacteria identified.

The antibiotics used included Cefoxitin (FOX), Chloramphenicol (C), Ciprofloxacin (CIP), Clindamycin (DA), Erythromycin (E), Gentamycin (GN), Penicillin G (P) and Tetracycline (TET) as these are among the commonly prescribed antibiotics in Morogoro. An antimicrobial agent of a specified concentration (0.5McFarland) was added into the Muller-Hinton agar medium and followed by diffusion of antibiotics disks into the agar and incubated at 37°C for 24hrs. An inhibition zone was recorded which was directly proportional to bacterial susceptibility to the antimicrobial present in the disk.

Data analysis

Quantitative data was collected after performing bacteriological assessment.

Number of contaminated samples were counted and recorded; data was analyzed by using descriptive statistics whereby frequency and mean calculated for continuous variables and the findings were presented in tables

contamination rates (78.6%). Least contaminated food in this study was beef soup with 50% contamination rate.

Table 1. Microbial Contamination in different types of food

Food Type	Total Contaminated Samples	Percentage Contamination (n=14)
Raw Vegetable Salad (n=14)	11	78.6
Rice (n=14)	9	64.3
Beef soup (n=14)	7	50.0
Stiff Porridge (n=14)	9	64.3
Fried Potato Chips (n=14)	11	78.6
Total Contamination (n=70)	47	67.1

Different bacteria were isolated where *Escherichia coli* (49.2%) was the major isolate in all food types. Other bacteria isolated were *Bacillus cereus* (19.7%), *Staphylococcus aureus* (14.8%), *Klebsiella pneumoniae* (14.8%) and *Salmonella spp.* (1.6%). A total of 14 out of 70 food samples had mixed bacterial contamination (Table 2).

Table 2. Occurrence of different bacteria in the contaminated samples

Food Type	*Klebsiella*	*Salmonella*	*E. coli*	*Staph.*	*Bacillus*	*Mixed contam.*
Raw Vegetable Salad (n=14)	3	1	8	2	1	4
Rice (n=14)	3	0	6	0	3	3
Beef soup (n=14)	0	0	4	3	2	2
Stiff Porridge (n=14)	0	0	5	2	3	1
Fried Potato Chips (n=14)	3	0	7	2	3	4
Total Contamination (n=70)	9	1	30	9	12	14
% Isolated bacteria (n=61)	14.8	1.6	49.2	14.8	19.7	

E. coli had the highest bacterial counts (1.84×10^7 cfu/ml) in potato chips followed by *S. aureus* (2.92×10^6 cfu/ml) in stiff porridge. Lowest level of bacterial count was recorded in salad for *S. aureus* at 6.6×10^3 cfu/ml (Table 3).

Table 3. Highest and lowest bacterial counts (cfu/ml) in different foods

Food type (Bacteria counted)	High no of cfu/ml	Low no of cfu/ml
Salad (*S. aureus*)	1.2×10^6	6.6×10^3
Potato Chips (*E. coli*)	1.84×10^7	6.0×10^4
Beef soup (*K. pneumonia*)	1.96×10^6	8.4×10^3
Stiff porridge (*S. aureus*)	2.92×10^6	1.25×10^5
Rice (*B. cereus*)	1.16×10^5	9.6×10^3

Antimicrobial susceptibility

All isolated bacteria were susceptible to chloramphenicol and resistant to cefoxitin and penicillin G. In addition, *E. coli* was susceptible to ciprofloxacin and erythromycin; but resistant to carbenicilin, clindamycin and tetracycline. On the other hand, *S. aureus* was susceptible to clindamycin, ciprofloxacin, tetracycline, gentamycin, and erythromycin (Table 4).

Clindamycin was only effective against *S. aureus* but did not inhibit growth of *E. coli*, *K. pneumonia* or *B. cereus*

Table 4. Antimicrobial susceptibility test results showing the zones of growth inhibition

Antibiotic	*E. coli*	*K. pneumonia*	*S. aureus*	*B. cereus*
Carbenicilin (CAR)	0	0	NT	NT
Cefoxitin (FOX)	0	0	0	0
Chloramphenicol (C)	31	18	22	22
Ciprofloxacin (CIP)	32	NT	24	NT
Clindamycin (DA)	0	0	30	0
Erythromycin (E)	13	NT	24	10
Gentamycin (GN)	NT	14	24	24
Penicillin G (P)	0	0	NT	0
Tetracycline (TET)	0	7	26	11

Food vending environment

The market area including the food vending sites were observed to have poor environment with poor waste disposal facilities. The market has no reliable source of portable water and the vendors are forced to fetch water from neighboring buildings for both cooking and washing the utensils.

Some of the vendors had improper food handling practices and poor food storage facilities. Some vendors prepared the food at home and bring it to the market in big thermos flasks or plastic buckets.

Some vendors however prepare the food right there at the market and the foods were sold and eaten hot and fresh.

DISCUSSION

The current study was conducted to assess the bacteriological quality of street foods in Morogoro Municipal market. The study has showed that majority of street food (67.1%) are contaminated with bacteria such as *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella*, *Staphylococcus aureus* and *Bacillus* species.

Previously, Nonga *et al.* (2015) noted that the street food prepared in Morogoro are of poor quality after assessing their physiochemical and microbiological qualities. Bacteria contamination in foods can occur due to different reasons and at different stages from preparation to consumption. A fully cooked meal may be contaminated by coming into contact with other raw foods, drippings from raw foods that contain pathogen, from food handler fingernails or clothing, or dirty utensils (Rheinländer *et al.,* 2008). *E. coli* was the major isolate (49.2%) in all the foods sampled in this study.

E.coli and *Salmonella* were also reported in other studies (Gitahi, 2012; Nonga *et al.,* 2015). In another study conducted in street foods of Cape Coast Ghana all sampled foods tested positive for *E. coli* (Annan-Prah *et al.,* 2011). About 64.3% of rice and stiff porridge samples were contaminated.

Cooked rice samples were mostly found contaminated with *E.coli* which is an indicator of fecal contamination in water (Nonga *et al.*, 2015).

Poor hygienic practices during handling and preparations of food has been highly associated with contamination in cooked foods (Barro *et al.*, 2006).

Raw vegetable salads and potato chips in this study had high percentage of contamination (78.6%). Among the 11 contaminated salad samples, one was contaminated with salmonella species. Salmonella is a zoonotic agent found worldwide.

Many foods particularly of animal origin and those subjected to sewage pollution are likely to pose risk to humans if contaminated with Salmonella. For instance, Salmonella was the most frequent cause of bacterial foodborne illness in the US and France, in the 20th century (Vaillant *et al.*, 2005).

The bacteria on raw vegetable salads may have originated from contaminated water used in washing the vegetables. Some studies in developing world have noted the unavailability of potable water for various activities at food vending site. The shortage of clean potable water forces some vendors to re-use the water, especially for cleaning utensils and used dishes (Rane, 2011).

The contamination could also be attributed to poor salad preparation practices, including poor hygienic conditions of the premises. The presence of *K. pneumonia* in salad indicates sources of contamination from animal waste manure, contaminated irrigation water and post-harvest washing using contaminated water (Puspanadan *et al.*, 2012).

The current study has revealed the presence of *Staphylococcus aureus* and Bacillus species contaminating beef soup. *S. aureus* may cause gastroenteritis as a result of consumption of contaminated food

containing staphylococcus enterotoxins (Le Loir *et al.*, 2003). *S. aureus* counts in salads and stiff porridge was 1.2×10^6 and 2.96×10^6 respectively. Guidelines for microbiological quality of ready-to-eat foods have set limits where the food with colony counts beyond 10^4 is unacceptable and hazardous (Gilbert *et al.*, 2000).

The presence of *S. aureus* in food should be a matter of public concern since it may cause staphylococcal food poisoning, a form of gastroenteritis with rapid onset of symptoms (Le Loir *et al.*, 2003). *B. cereus* was found in about 19.5% of the sampled foods in this study. In another study in Zaria, Nigeria, out of 160 food samples tested, 42 (26.3%) were contaminated with *B. cereus* (Umoh and Odoba, 1999).

Some foods showed high colony counts (1.16×10^5 to 1.84×10^7 cfu/g) compared to what is set for microbiological quality of ready-to-eat street foods (10^5 cfu/g) (Gilbert *et al.*, 2000). The levels of contamination on the sampled street foods were high partly due to poor handling, preparation and storage of the foods.

In some cases however, the chances of contamination are always there especially in street foods prepared at environments where food hygienic practices are not properly adhered to (Barro *et al.*, 2006).

The food vending environment in the current study lacked important and adequate facilities to provide food hygiene and safety. Some of the necessary facilities for street food vending include water supply and waste disposal site, most of vendors searching water from neighbor places, but the reliability and safety of this water is not known.

Also unhygienic practices of selling-places were some of the major problems, for example foods were sold on open areas without being well covered hence exposed to dirt and dust. Apart from poor quality of raw materials, it has been reported that preparation of food long before its

consumption, storage at ambient temperature, inadequate cooling and reheating and undercooking are some of the reasons for contamination of ready-to-eat foods (Rane, 2011).

The consumption of street food cannot be stopped on hygienic grounds (Tambekar *et al.*, 2009). These practices could be improved to ensure more safety. Proper ways to avoid contaminations of street foods start with following hygiene principles such as vendors wearing clean garments, remove unnecessary accessories such as jewelry as they can be source of contaminant, finger nails short trimmed, hands washed before and after handling the foods, regular cleaning of all food contact surfaces (Rheinländer *et al.*, 2008).

Although the presence of the microorganisms is an indicator of poor hygiene and not necessarily a threat to human health, the fact that some microorganisms were resistant to some antibiotics brings an even greater risk. Pathogenicity of bacteria is known to be due either to the amount of bacteria ingested, the susceptibility of the person or the resistance of strains to the antibiotics (Moussé *et al.*, 2016).

In this study, for instance, *E. coli* that was the major isolate in the sampled foods, was susceptible to ciprofloxacin and erythromycin; but resistant to carbenicilin, clindamycin and tetracycline.

This study has isolated different bacteria from five types of street foods (salad, rice, stiff porridge, beef soup and fried potato chips).

The contamination levels are varied according to the foods types, and possibly by vendors. Sufficient hygiene and sanitation measures should be provided, including provision of potable water and waste removal facilities around the market. Successful food hygiene education and knowledge about food hygiene practices will help in the prevention of foodborne diseases around Morogoro Region.

The contamination rates are high and possibly pose a risk to health of the individuals and could contribute to risks of antimicrobial resistance.

It is recommended to develop microbial contamination prevention guidelines for street foods to reduce microbial contaminated street food in Tanzania.

With possible development of antimicrobial resistance if the use of antimicrobials is unchecked, it recommended that prescribing of antimicrobial treatment to the patient should be done after identifying the respective pathogenic microorganisms and susceptibility to antimicrobials.

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