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Assessment of Aerobic Plate Counts, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* in Meat Sold by Street Vendors in the Eastern Cape Province, South Africa

B.E. Mazizi[1,*], V. Muchenje[1], M. Makepe[2], G. Mutero[2]

[1]Department of Livestock and Pasture Science, University of Fort Hare, Alice, South Africa
[2]Veterinary Laboratory, Department of Rural Development and Agrarian Reform, Grahamstown, South Africa
*Corresponding author: 201101708@ufh.ac.za

Abstract The study was carried out to determine the aerobic plate counts (APC), *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* contamination levels in cooked (n=24) and raw (n=24) beef, pork and mutton samples, surface contact plates (n=48) and water samples (n= 40) from street vendors. A total of 8 street vendors who were willing to participate in the study were randomly selected. After biochemical tests, no significant differences were found in the microbial counts of meat sold by street vendors in Alice and King Williams town. Furthermore, no significant differences were found in the mean scores of raw beef, mutton and pork where APC (4.8, 3.7 and 2.8 Log CFU/g), *Staphylococcus aureus* (3.3, 3.7 and 2.8 Log CFU/g) and *E. coli* (1.0, 0.6 and 0.3 Log CFU/g) respectively. *Salmonella* tested negative in all the samples tested in the study. The results in the study were associated with cross-contamination during processing and storage. However, the levels of contamination in cooked meat were lower when compared to the standards set by Commission Regulation for determining the microbiological quality of ready-to-eat foods. Overall, poor hygiene of the street vendor, utensils, and holding area were major sources of contamination. It was therefore concluded that there were no differences in the microbial counts of meat sold in the informal markets of Nkonkobe and Buffalo City Municipalities in the Eastern Cape Province, South Africa. Improved sanitation facilities, hygiene tools, and training will promote the production of safer food by the street vendors.

Keywords: *meat, street vendors, hygiene, Staphylococcus aureus, Escherichia coli and Salmonella*

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1. Introduction

Foodborne illnesses represent a worldwide concern to public health [1]. About 33% of the population in developing countries is affected by foodborne sicknesses every year [2]. This high rate of illnesses recorded may be due to a large number of consumers who are utilizing the informal sector as a source of their food for consumption [3] Foods sourced from the informal markets are easy to access, cheaper and convenient to consumers on an everyday basis [4]. This is informal markets are located in congested areas, where there are large numbers of prospective customers [3,4,5]. [6] mentioned that street food vending plays a pivotal role in the livelihood of consumers. Street food vending in developing countries is presumably the single biggest source of employment and potentially one of the most significant contributors to the economies of these countries[7]. This sector may be the biggest enterprise in African countries including, South Africa [7]. However, much still needs to be done to educate vendors to improve the safety of the food they sell. These street vendors operate in areas that do not meet all food safety regulations [8]. There is also a lack of basic sanitary facilities in areas where street food vendors operate [5]. As a result, food-borne sicknesses outbreak can occur as a consequence of consumers ingesting meat products contaminated with pathogens [9]. High bacterial or microbial counts of *Salmonella spp.*, *B. cereus* and *Staphylococcus* are normally detected in street-vended foods [10,11] Contaminated vended foods can subsequently expose consumers to the risks of foodborne illnesses such as diarrhea, salmonellosis, listeriosis, and cholera [12,13]. The outbreaks of bacterial foodborne diseases can be controlled through frequent inspection by relevant personnel. The incorporation of food safety as one supporting component influencing food security and consumer nutrition may result in a healthy livelihood for the consumers [14]. Implementing food safety principles

may not be feasible without educating the public about food safety risks [15]. Even though most consumers utilize food from this sector in South Africa, not much research has been done to determine the microbial status of meat sold in the informal market. Knowledge of the microbial quality of meat sold by street vendors will help in determining its safety. Therefore, the purpose of the study was to determine the microbial quality of meat sold in the informal markets in Nkonkobe and Buffalo City Metropolitan Municipalities in the Eastern Cape Province, South Africa.

2. Materials and Methods

2.1. Ethical Consideration

The University of Fort Hare Ethics Committee (MUC161SMAZ01) approved all the protocols that were used in the experiments carried out in this study.

2.2. Description of Study Site

The study was conducted in two local municipalities, Nkonkobe and Buffalo City Metropolitan Municipalities in the Eastern Cape Province, South Africa. Within these two municipalities, two towns Alice (Nkonkobe) and King William's Town (Buffalo City) were selected for data collection. Alice is a small rural Town with a total population of approximately 15,143, of which 93% are Back African, 5.6% Coloured, 0.3% Indian/Asian , 0.6% white and others 0.5%. It lies at latitude 26° 49' 60E and 32° 45' 0S at an altitude of 572 m above sea levels with a minimum rainfall of 386mm per year and average temperatures of 29°C.

King Williams Town (KWT) is an urban Town that has a total population of approximately 34,019 in which Back African (65.3%), Coloured (25.6%), Indian/Asian (2.5%), white (5.6%) and others (1%). It lies along the latitude 32°53′S and 27°24′E at an altitude of 389 m above the sea levels and has dense and indigenous bushes which normally receive about 502mm of rain per year, with most rainfall occurring mainly during summer at average temperatures of 26.7°C.

2.3. Description of Street Vendors Operations in Alice and King Williams Town

Street vendors in Alice town are located around the taxi rank, and they are close to the road. Vendors operate in old caravans and shacks with walls, and the roofs are made of metal sheets. They purchase the meat they sell from the different butcheries in the central business district (CBD) and the rest of the food from the supermarkets. These street vendors sell basic food such as rice, samp, beans, dumplings, fat cakes, beef, mutton, pork, offal (Ulusu) and chicken. The food is served with vegetables which may consist of onions, cabbages, butternuts and beans.

Street vendors in KWT town are scattered around taxi ranks and bus stations. Most of these vendors are not sheltered while a few have permanent shelters including caravans and shacks made up of metal sheets or small tents. Workers, scholars and public transport drivers purchase food from these vendors. The street vendors who participated in the study had shelter in the form of either a caravan or a shack. Most of them had wooden tables which they covered with tablecloths. On top of the tables, they placed buckets of water which were used as reservoirs, stainless steel pots for cooking and the rest of the cutlery for easy access when required.

2.4. Assessment of Hazard Identification and Exposure

The preparation, handling and serving of meat after cooking was assessed to identify incidences of cross contamination. The temperature of the raw and cooked meat (at holding) was measured using a portable probe thermometer to determine the potential growth of bacteria. Samples were collected from raw and cooked meat to determine potential contamination of the meat in these two different states. Reservoir water was collected and surface contacts were plated for microbial identification and counting.

2.5. Sample Collection

A total of 48 beef, pork, and mutton meat samples were collected from 8 street vendors in Alice (n=4) and King Williams Town (n=4). The meat samples (cooked, n=24 and raw, n=24) were aseptically collected into sterile polyethylene bags, sealed and immediately transported in an ice box to prevent microbial growth during sample transportation. A total of 48 Contact plate samples were also taken from the table, cutlery, and hands of vendors. A total of 3 meat samples and 3 contact plates were taken from each vendor in each town. Also, a total of 40 samples were aseptically collected from the street vendor (5 water samples from each vendor) water reservoirs using 500ml sterile glass bottles for microbial determination. After collection, all the samples were immediately transported Grahamstown veterinary diagnosis center for microbial analysis.

2.6. Microbial Determination

2.6.1. *Salmonella spp*.

Samples were weighed, and ±25 g of each sample was used for the biochemical tests according to international standards (ISO) methods [16] (ISO 6579:2002). The weight was multiplied by 9 to calculate the volume of the buffer to be added to the sample to give a 10^{-1} dilution. The samples were emptied into a stomacher bag (Bag mixer®DOA 20550) and buffered peptone water was added. The sample was placed in a stomacher and mashed for three minutes. The stomacher bag was emptied into a 250ml flat-bottom flask, which was marked for identification. The flask was placed in 37°C incubator for 18 h. 0.1 ml of the pre enriched broth was added to 10ml of Modified semisolid Rappaport-Vassiliadis (MSRV) agar and the plates were inoculated with the culture at 41.5°C for 24h. The negative plates were further inoculated for 24h. The sample from MSRV was

streaked into Xylose lysine deoxycholate (XLD) and β-galactosidase (BGS) agar plates then inoculated at 37°C for 24h. After the complete incubation period, plates were colony counted according to the ISO methods [17] (ISO 6579:2002). The presumptive positive bacterial isolates obtained from the culture were confirmed using media from Oxid.

2.6.2. Aerobic Plate Counts

According to the international standards [17] (ISO 21528-2, 2004), ±25 g of samples was used for the biochemical tests. The weight was multiplied by 9 to calculate the volume of the buffer to be added to the sample to give a 10^{-1} dilution. The samples were emptied into a stomacher bag (Bag mixer®DOA 20550) and buffered peptone water was added into the stomacher bag with the sample. The stomacher bag with the sample was placed in a stomacher and mashed for three minutes. 1 ml of the test sample was transferred into two Petri dishes using a sterile pipette. 15 ml of plate count agar at 44°C to 4°C was added into each petri dish. The inoculum was carefully mixed with the medium by rotating the Petri dishes and the mixture was left in a cool horizontal surface to allow it to solidify. An overlaying layer medium of 4 ml at 44°C to 47°C was added into the surface of the inoculated medium. The layer was allowed to solidify by putting it in a cool horizontal surface. The prepared dishes were inverted and placed in an incubator at 30°C ±1°C for 72 h ± 3 h. After the complete incubation period, plates were colony counted.

2.6.3. *Staphylococcus aureus*

According to the international standards [18] (ISO 4833:2003), samples were weighed and ±25 g was used for the biochemical tests. The weight was multiplied by 9 to calculate the volume of the buffer to be added to the sample in order to give a 10^{-1} dilution according to ISO methods. The samples were emptied in a stomacher bag (Bag mixer®DOA 20550) and buffered peptone water was added into the stomacher bag with the sample. The stomacher bag with the sample was placed in a stomacher and mashed for three minutes. 1 ml of the test sample was transferred into two Petri dishes by means of a sterile pipette. 15 ml of *staphylococcus aureus* baired parker agar at 44°C to 47°C was added into each petri dish. The inoculum was carefully mixed with the medium by rotating the Petri dishes and the mixture was left in a cool horizontal surface to allow it to solidify. An overlaying layer medium of 4 ml at 44°C to 47°C was added into the surface of the inoculated medium. The layer was allowed to solidify by putting it in a cool horizontal surface. The prepared dishes were inverted and placed in an incubator at 37°C ±1°C for 24 h ± 3 h. After the complete incubation period, plates were colony counted according to the ISO methods [19] (ISO 4833:2003).

2.6.4. *Escherichia coli*

The most probable number technique [19] (ISO 16649-2, 2003) was used for identification of *E. coli* according to the international standards guidelines. The weight was multiplied by 9 to calculate the volume of the buffer to be added to the sample in order to give a 10^{-1} dilution. The samples were emptied in a stomacher bag (Bag mixer®DOA 20550) and buffered peptone water was added into the stomacher bag with the sample. The stomacher bag with the sample was placed in a stomacher and mashed for three minutes. 1 ml of the test sample was transferred into two Petri dishes by means of a sterile pipette then after the mixture incubated at 44°C for 48 hours. The Agriculture Research Council [20] reference table was used to determine the most probable number of *E. coli* per milliliter. The positive results were streaked onto McConkey agar medium in order to isolate and confirm *E. coli.* For 24 hours at 37°C, the plates were incubated and examined for any pink colonies.

2.6.5. Water Analysis

Total viable counts (CFU/ml, 35°C 48h), total coliform counts (MAC/100ml, 35°C 24h), *Escherichia coli* (MAC/100ml, 35°C 24h and 44°C 24h) and *Enterococci* counts (MAC/100ml, 37°C 48h) were determined from the water samples collected.

A water sample of 100mls was emptied onto a filter paper (pore size 0.44µm) to trap bacteria and isolate total viable organisms. After filtration, the filter paper was placed in a Petri dish containing the Plate Count Agar and incubated at 35°C for 48 hours. Coliform bacteria and *E. coli* enumeration were done simultaneously; water sample of 100ml was filtered and then the filter paper was placed in Petri dishes with EMB agar. The Petri dishes were incubated for a period of 24 hours at 35°C. Dark-blue to violet colonies in EMB were counted as presumptive *E. coli* and salmon to red as coliforms. Confirmation of *E. coli* was done using the Indole test with Kovac's reagent. Enumeration of *Enterococci* was done by placing filter paper in Bile Esculin agar and then incubated at 37°C for 48 hours. Black colonies after incubation were counted as presumptive *Enterococci* [19,20]. (ISO 4833, 2003, ISO 21528-2, 2004).

2.6.6, Contact Surface Analysis

2.6.6.1. Agar Contact Plate Method

Street vendor hands and equipment such as cutlery and tables used in vendor stalls may provide an indication of the hygiene and microbiological quality of the meat produced. Agar contact plates which had an internal diameter of 5.0cm were used and the dishes had a contact surface of $20cm^2$. The dishes were filled with violet red bile glucose agar and the other dishes with plate count agar. They were hard-pressed onto each sampling site for a minimum of 10 seconds and correctly sealed. The plates were immediately transported to the laboratory in a cooler bag and aerobically incubated at 37°C for 24 hours for evidence of microbial growth (ISO, 6579, 2002) [17].

3. Statistical Analysis

All microbial counts were converted to a $\log_{10}$ colony forming a unit (CFU) per gram values to confirm to normality. The mean and variances between the bacterial counts of meat, contact plates and water from sheltered vendors were analyzed using analysis of variance (ANOVA) in SAS [21] (2003). The means were compared using Tukey's test and were considered to be significant at

P<0.05. Correlations between APC, *Staphylococcus aureus*, *E. coli*, Contact plates for *Enterobacteriaceae* (cENT) ,contact plates for total viable counts (cTVC), Total viable counts from water (wTVC) and Temperature (Temp) were performed using Pearson's analyses procedure of SAS [22] (2003). The results were considered to be significant at P<0.05, P<0.01 and P<0.001 respectively.

4. Results and Discussion

4.1. Aerobic Plate, *Staphylococcus aureus* and *Escherichia coli* counts of Meat from Street Vendors in Alice and King Williams Town

Figure 1, represents APC, *Staphylococcus aureus* and *Escherichia coli* counts on the meat sold by street vendors from Alice and King Williams Town. There were no significant differences in the overall APC found in meat sold by street vendors between the two towns (P> 0.05). A similar trend was also observed for *Staphylococcus aureus* and *Escherichia coli* counts from the meat sold by street vendors (Figure 1). This may be due to the similarity in the mode of operation of the vendors between the two study sites. Furthermore, microbial loads were relatively low in all the samples collected as compared to the standards set by the Commission Regulation [22] for determining the microbiological quality of ready-to-eat food. This could also be because these vendors did no re-sell leftovers the next day, so their ready-eat-foods were fresh and were not contaminated by different bacteria. A lot still needs to be done to improve the quality of meat sold by street vendors as it is a source of food for thousands of consumers.

4.2. Microbial Counts and Temperature in Raw and Cooked Meat Sold by Street Vendors

This study did no record the presence of *Salmonella spp*. in all the meat samples, this is similar to what [11] found in their study in Johannesburg, South Africa where they discovered that there were no species of *salmonella* on meat sold by street vendors. On the other hand [23] found *salmonella spp.* in cooked food and identified improper hygiene, sanitation and storage facilities as the main cause of food contamination. Table 1 represents the least square means (log) ±standard errors of microbial counts and temperature in raw and cooked meat sold by street vendors. The results revealed that there were no significant differences between the Aerobic Place Counts of raw beef (4.8 Log CFU/g), mutton (3.7 Log CFU/g) and pork (2.8 Log CFU/g). Also, no significant differences between cooked beef (1.5 Log CFU/g), mutton (1.3 Log CFU/g) and pork (1.9 Log CFU/g) sample tested for microbial quality. This is because most of the pathogens that tested positive in meat samples were aerobic bacteria such *Staphylococcus aureus*. Also, most of the vendor chopped their meat with the same instrument without in between disinfection. [24] earlier reported that similarities in raw meat microbial counts may be because the vendors use the same knife during chopping of meat without in between disinfection. The Meat Safety Act No. 40 of 2000 [25], however, states that equipment must be sterilized and not contaminate meat with greases to control such pathogens. This can be linked to lack of knowledge about meat safety amongst street vendors. There were significant differences in the values of Aerobic Plate Counts of cooked and raw beef, mutton and pork meat used in the study (P<0.05). Cooking might have played a role in the reduction in the Aerobic counts differences between cooked and raw meat because high temperature kills most of the pathogens.

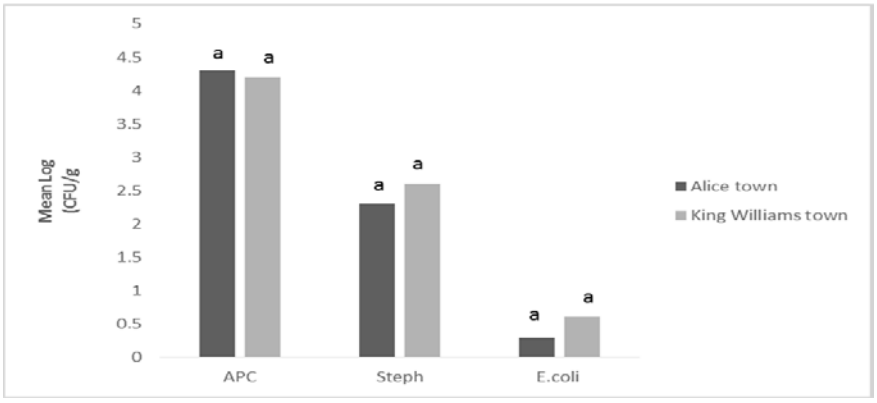
Furthermore, the result of *Staphylococcus aureus* count did not reveal any significant differences in raw and cooked meat samples. However, the raw mutton had the highest *staphylococcus* count (3.7 Log CFU/g), followed by beef (3.3 Log CFU/g), and least in pork (2.8 Log CFU/g) (Table 1). Whereas in cooked meat, pork had the highest the *staphylococcus* count (1.9 Log CFU/g), followed by beef (1.5 Log CFU/g) and least in mutton (1.3 Log CFU/g), (Table 1). This is similar to the findings of [25] who observed that cross contamination influenced the microbial quality of meat from street vendors. Also, the higher counts of pathogens in raw meat may be due to unsterilized vendor's hand. As it is common for vendors to seek for help from colleagues when cutting large difficult chunk of meat. This could potentially bridge hygiene and lead to meat contamination. Furthermore, Staphyloccocal organisms are common the respiratory tract of human, hence the possibility of its introduction if meat is handled by too many workers. [26] reported that hands are the major source for most cross contaminations in most foods. Cross-contamination may increase in cases where the vendors handle money from customers with the same hands that handle food [27]. Cross contamination through hands observed in the present study is almost similar in some other countries as well. [28] reported that about 60% of the people who are involved in food services in Spain neglected proper hand hygiene. In this study, *Staphylococcus aureus* counts were lower than the standard >105 CFU/g, and thus potrays the food as acceptable. There were no significant differences in *Escherichia coli* counts across all the meat samples used in the study (Table 1). There were generally lower counts of *Escherichia coli* in both cooked and raw meat. This result is in contrast with the findings of [11] who reported higher levels of *Escherichia coli* in meat collected from street vendors. Similarly, [24] in their study also reported coliforms of *Escherichia coli* in 50% of the beef sampled in Bloemfontein, South Africa while Ahmed and [29] detected about 2.6% *E. coli* O157: H7 in beef samples in a study done in Egypt. More importantly, the levels of *Escherichia coli* were much less than the standard counts ≥100CFU/g set for determining the microbiological quality of ready-to-eat food [23].

Table 1 below shows that there were significant differences between the temperature of raw beef, mutton, and pork. The differences in the temperatures of the meat samples may be due to the different temperatures at which different meat samples were prepared. This may have resulted in the decrease in the bacterial counts in different meat types. Also, the temperature at which food is kept

during holding and display may influence microbial growth. Foods that are prepared should be kept at least 60°C if kept for more than ±5 hours a day [30]. Total coliform counts (MAC/100ml, 35°C 24h), *Escherichia coli* (MAC/100ml, 35°C 24h and 44°C 24h) and *Enterococci* counts (MAC/100ml, 37°C 48h) in water samples all tested negative. This might be because water was taken in the morning when the environmental temperatures were low however the water was used throughout and kept in closed containers. Water and soap were the only solutions that were commonly used to wipe the surfaces. Furthermore, water was used to clean the utensils that were used for cooking, cutting, and storage, due to cross contamination the water contact surfaces may exhibit the same counts as the water stored. [24] reported that few vendors practice the basics of using water and soap to wipe their equipment. Contaminated water spreads pathogens around thus increasing their proliferation [31]. The quality of water is vital as water plays a pivotal role during preparation of these ready-to-eat foods. Access to good quality water may be elusive for other street vendors, due to water problems facing Africa as a whole.

4.3. Correlations among Microbial Counts and Temperature of the Meat Sold by Street Vendors

Aerobic plate counts (APC) are counts of aerobic bacteria that may be found in raw and cooked food. APC may be a good indicator of low-quality food including meat which is contaminated by numerous microorganisms. Table 2 represents Pearson correlation analysis between microbial counts and temperature on the meat from street vendors. The results revealed a positive correlation between Aerobic plate counts (APC) and *Staphylococcus aureus* (Staph) (P<0.001), as well as *Staphylococcus aureus* and *Escherichia coli*, count n (P<0.001). *Staphylococcus aureus* is a common commensal found in the human and animal skin and respiratory tract and can in cases of poor hygiene management is found in human foods such as raw meat [32]. Improper handling of food before and after processing contributes to the presence of pathogens in foods after processing [33]. There was a positive correlation between cTVC and wTVC in the samples (Table 2). The corelation is possibly due to cross contamination from water used for cleaning surfaces. More so, the results also revealed a negative correlation between APC and meat temperature and between *Staphylococcus aureus* and meat temperature (P<0.001). The negative relationship between APC and meat temperature may be because high temperature destroy most of the aerobic bacteria. [11] reported that cooking temperatures over 80°C kill all the vegetative forms of pathogens. However, cooking at high temperature is also known to denature some of the essential nutrients in foods. On the other hand, *Staphylococcus aureus* is abundant is its ability to grow over a wide range of temperatures [34] from 7° to 48.5°C. However, [35] mentioned that food that is not going to be consumed imminently must be kept at least 7°C to avoid contamination. There was a negative correlation between contact plates of *Enterobacteriaceae* (cENT) and contact plates for total viable counts (cTVC) and a negative correlation between wTVC and *Escherichia coli* in the samples that were used (P<0.01).



[abc]Means with the same superscripts are not significantly different at P < 0.05

Figure 1. Mean log10 of Aerobic plate, *Staphylococcus eureus*(staph) and *Escherichia coli* counts from street vendors from Alice and King Williams Town, at α= 0.05

Table 1. least square means (log) ±standard errors of microbial counts and temperature in raw and cooked meat sold by street vendors

Raw	Meat sample	APC	Staph	*E. coli*	Temp
	Beef	4.8±0.38	3.3±0.57	1.0±0.41	1.0±0.02
	Mutton	3.7±0.63	3.7±0.63	0.6±0.34	0.8±0.04
	Pork	2.8±0.81	2.8±0.81	0.3±0.30	0.7±0.04
	P-value	0.93	0.65	0.39	<0.001
Cooked	**Beef**	1.5±0.37	1.5±0.37	0.4±0.30	1.8±0.12
	Mutton	1.3±0.36	1.3±0.36	NF	1.7±0.21
	Pork	1.9±0.31	1.9±0.31	NF	1.3±0.4
	P-value	0.13	0.40	0.12	0.002

NF=not found
APC= Aerobic plate counts, Steph=*Staphylococcus eureus* counts, *E. coli=Escherichia coli* counts and Temp=meat temperature.

Table 2. correlations among microbial counts and Temperature of meat sold by street vendors

	APC	Staph	*E. coli*	cENT	cTVC	wTVC	Temp
APC	-	0.56***	0.37**	-0.02ns	-0.08ns	0.00ns	-0.44***
Staph		-	0.53***	-0.10ns	-0.15ns	-0.14ns	-0.46***
E. coli			-	-0.21ns	-0.19ns	-0.29*	-0.27ns
cENT				-	-0.33*	0.14ns	-0.02ns
cTVC					-	0.30*	-0.03ns
wTVC						-	0.04ns
Temp							-

5. Conclusion

There were no differences in the microbial quality of meat sold by street vendors in Alice and King Williams's Town. The results obtained from the samples revealed fewer chances of consumers contracting illnesses from the meat sold by street vendors. However, much still needs to be done to make sure that their produce is fit for consumption even for the next generation. The pathogenic microorganisms, which were found in the cooked meat sold by street vendors, were lower when compared to the standards set for determining the microbiological quality of ready-to-eat food. However, improved sanitation facilities, hygiene tools, and training will promote the safe food produced by the street vendors.

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