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An Assessment of the Microbial Quality of Sausage and Chicken Sold in Formal and Informal Markets at a Taxi Rank in Bulawayo, Zimbabwe.

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ABSTRACT

A study was conducted to determine the microbial quality and safety of meat sold in formal and informal markets around Egodini Taxi Rank in Bulawayo. Sausage and chicken samples randomly selected from each outlet were screened for bacterial contamination. *E.coli* was the predominant bacteria in sausage samples (43% in butchery samples and 85% in vendor samples), whilst *S. aureus* was abundant in chicken samples (43%). Contamination of meat with *Klebsiella spp* and *Streptococcus spp* was minimum. The mean Total bacterial counts (TBCs) for sausage and chicken in vendor samples were 7.66×10^5; 2.41×10^5 cfu/ml respectively whilst the TBCs for butchery samples were 1.14×10^4; 5.45×10^2 cfu/ml respectively. Overally, vendor meat was more contaminated than butchery meat which poses a serious health hazard to the public. The type of meat and outlet had no significant influence on the bacterial load ($P=0.298$ and $P=0.061$ at $\alpha=0.05$).

Introduction

Meat contamination by bacteria is a challenge in controlling food safety worldwide (Schelin *et al*., 2011). In Zimbabwe, chicken and sausage are common products of meat markets and when contaminated, bacteria can grow to levels that affect appearance, taste and smell and subsequently cause diseases (Betts and Everis, 2007). Microorganisms either infect an animal while it is still alive (endogenous disease), or can be as a result of poor hygiene while handling the meat during slaughter, processing and retail (exogenous disease) (Lawrie and Ledward, 2006).

Chicken is a general term used to describe the flesh from poultry of the *Gallus gallus* spp. Contamination of chicken with bacteria is from the skin, gut and the feet during slaughter, especially through using unsterilized equipment, processing with unsafe water or during packaging. Poultry skin is moist hence it allows for microorganisms to reproduce quickly. Stale chicken looks slimy and has an unpleasant odour (Russell, 2009). The rate of reproduction of microorganisms on the skin surface is reduced by immediately storing the carcass at low temperatures (0-5˚C) but however psychrotrophic bacteria like the *Pseudomonas spp*. and *E.coli* 0157:H7 will survive and cause spoilage. Other factors like pH and packaging material affect the rate of chicken spoilage (Moyer and Morita, 2007).

Sausages are prepared from adult animal flesh or muscle, mostly beef and pork. UK Essays, (2015) define sausages as a mixture of mostly ground meat, fatty tissue, curing agents, salt, nitrite, sugar and spices filled into casings and left for a fermentation process to take place before drying. The preparation involves a lot of handling as the ingredients are mixed together before being put into casings then fermented. According to Adebayo *et al*., (2014) fermented foods are more susceptible to attack by microorganisms than unfermented foods. The beef or pork is ground and this increases surface area for microbial growth as more of the meat surface is exposed to bacterial contamination. Mixing the ground meat with other ingredients during sausage production results in the pathogens being mixed throughout the whole meat. Stale sausage may be seen by being slimy or the presence of grey-white mould (USDA, 2015).

Food poisoning bacteria that are commonly associated with chicken spoilage include *Campylobacter jejuni*, *Salmonella spp*. (*S.typhi, S.dysenteria*), *Clostridium perfringens*, *Pseudomonas spp. (P. lundensis, P. flourescens, P.putida, P. fragi*) and *Staphylococcus aureus*. Pathogenic bacteria that are commonly associated with sausages include *Salmonella spp (S. typhi, S. dysenteria*), shiga-toxin producing *Escherichia coli, Campylobacter jejuni, Listeria monocytogenes* and *Staphylococcus aureus* (Mboto *et al*., 2012). *Campylobacter jejuni, Escherichia coli* and *Salmonella spp* are usually found in the intestines of aves and other animals, especially mammals. These can be transmitted to other body parts of the animal during slaughter when the contents of the intestines come into contact with other tissues of the animal or into the environment through faeces. *Staphylococcus aureus* is usually found in the nose, body fluids and skin of animals and humans and is transmitted through unhygienic handling of food. *Clostridium perfringens* and *Listeria monocytogenes* are generally found in the environment and are transmitted through unhygienic handling during slaughter, packaging and retail (Todar, 2014).

When these microbes infect the chicken or sausage, they proliferate and eventually decompose the meat, leaving behind toxins that cause food poisoning. Proliferation of microorganisms is prompted when the meat is exposed to

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warmer temperatures (4.4°C - 60°C) and damp air. Generally, raw meat is an ultimate medium for bacterial growth because it has high water content, high protein content and contains fermentable carbohydrates. Raw chicken or sausage are thus an ecological niche for many genera of bacteria (Mboto *et al*., 2012). Most bacteria are killed during thorough cooking but those that survive multiply in the human intestines and cause food poisoning. Food poisoning is a life threatening and possibly fatal problem. Symptoms of food poisoning include diarrhoea, vomiting, nausea, abdominal cramps, fever, and inflammation but vary depending on the causative pathogen (Sanone, 2014).

Food and Food Standards Act in Zimbabwe requires meat companies and abattoirs to inspect meat and perform bacteria enumerating tests like Total bacteria count, Total Coliform counts and other counts specific to certain bacteria to ensure that infected meat is completely eliminated. Public Health Inspectors from the City councils and Veterinary Services departments also inspect meat from different meat markets periodically. The permissible total bacterial count in any kind of meat for human consumption in Zimbabwe should be less than 1×10^6cfu/g (Nhari, 2013).

Zimbabwe has suffered numerous outbreaks of diseases like cholera, which are attributed to poor sanitary conditions (water and food contamination) resulting in death of many patients. An outbreak of Cholera in the year 2008-2009 affected about 55 districts of the country, infecting 99 704 people and killing 4 420 people (NHS, 2009-2013). According to Bangure *et al*., 2013, writing in the Chronicle newspaper of Zimbabwe more than 300 census enumerators in Gokwe fell ill with gastrointestinal illnesses in 2012 after consuming infected meat from a local restaurant.

Due to the current economic challenges in Zimbabwe, a lot of informal meat markets have emerged. The area around Egodini taxi rank has many formal butcheries and informal meat vendors. However, sanitation is generally poor in the area. Chicken and sausages are mainly sold in open markets because chicken is easy to rear and sausages are usually affordable.

Meat is an important source of protein in the human diet but is however a potential vehicle for the transmission of food-borne diseases. Kariuki *et al*., 2013 asserts that many food related diseases have been historically attributed to pathogenic bacteria found in meat. This study intended to assess the extent of food safety in Zimbabwe specifically raw meat in both formal and informal markets. Studies have been done worldwide on different kinds of raw meat and most of these investigations have indicated the presence of pathogenic bacteria in meat. The main aim of the study was thus to assess the microbial quality of chicken and sausages sold in formal and informal markets around Egodini taxi rank in Bulawayo. Food safety was assessed by culturing, isolation and identification of pathogenic bacteria in randomly selected meat samples. The TBCs were also determined in all samples to assess and compare the bacterial load between chicken and sausage samples in both markets. The current study also aimed at informing the public of the possible health hazards associated with purchasing food products from open markets and therefore encourage implementation of appropriate food safety measures.

Materials and methods

Study Site

The study was carried out around Egodini Taxi Rank. The area is generally hyperactive and has poor sanitation. The public toilets in the rank are often filthy, dirty and uncollected garbage is strewn everywhere.

Sample collection

Seven butcheries and seven meat vendors were selected at random in and around Egodini taxi rank using a number generator. From each selected butchery, 100g chicken cuts and 100g sausages were purchased and placed into labelled sterile plastic bags. From each selected meat vendor, every 3rd pack on each sample type was purchased and placed into labelled sterile plastic bags. This gave a total of 28 samples. The samples were then transported to Bulawayo Provincial Veterinary Laboratory for laboratory analysis and stored at -20˚C in a freezer until tests were conducted.

Sample Enrichment

Universal bottles with 10 ml peptone water were labelled with the sample type and sample number. One gram of each sample was cut using a sterile blade and placed in the peptone water. The samples were homogenized using a sterilized homogenizer at 15000 rpm until the suspension was homologous then incubated at 37˚C for 24 hours.

Universal bottles with Rappaport Vassiliadis (RV) Broth were also labelled with sample type and sample number. One gram of each sample was cut using a sterile blade and placed in the RV broth. The samples were homogenized using a sterilized homogenizer at 15000 rpm until the suspension was homologous then incubated at 37˚C for 24 hours.

Bacterial Isolation

The enriched samples were removed from the incubator for culturing of bacteria using the steak-plate method on Blood Agar (BA), MacConkey Agar (MAC), Xylose Lysine Deoxycholate (XLD) Agar, and Mannitol Salt Agar (MSA).

Peptone water-sample suspensions were inoculated into relevant labelled petri dishes with BA, MAC, and MSA using the streak plate method. The plates were incubated at 37˚C for 24 hours. RV-sample suspensions were inoculated into relevant labelled petri dishes with XLD agar using streak plate method. The plates were also incubated at 37˚C for 24 hours. Bacterial colonies on the agar plates were isolated and identified microscopically and subjected to the following biochemical tests for confirmation: gram staining, catalase reaction, indole production, motility test, and citrate test (Quinn, 1994).

Total Bacterial Count

Serial Dilutions

One gram of each sample was weighed and placed in a universal bottle with 9 ml Phosphate Buffered Saline (PBS), labelled 10^{-1}, sample type and number. The samples were homogenized using a homogenizer at 15000 rpm until the suspension was homologous. For each sample, three additional universal bottles with 9 ml PBS were labelled with the sample number and 10^{-2}, 10^{-3} and 10^{-4} respectively. For each sample, 1ml of the 10^{-1} dilution was transferred into the 10^{-2} bottle using a micropipette and mixed. 1ml of the 10^{-2} dilution was then transferred into the 10^{-3} bottle and mixed. 1 ml of the 10^{-3} dilution was then transferred into the 10^{-4} bottle and mixed. 1 ml of the 10^{-4} dilution was then discarded.

Inoculation

For each sample, five sterile petri dishes were labelled$10a^{-1}$, $10b^{-1}$, 10^{-2}, 10^{-3} and 10^{-4} respectively. Two more plates were labelled as the positive and negative control. 1 ml of each dilution was inoculated into the relevant petri dish using a micropipette. 1 ml of sterile PBS was then pipetted into the negative control plate and 1ml of positive control (*E. coli, S. aureus* and *S. tryphimurium* mixed in peptone water)

into the positive control plate. About 15 ml of Plate Count Agar (PCA) was poured into each petri dish and was mixed with the sample dilution by slow rotations. The PCA was left to set for 30 minutes at room temperature then incubated at 30˚c for 72 hours.

Enumeration

The plates were removed from the incubator and examined. For each sample, the highest dilution which had growth of between 30-300 colonies was selected and the colonies were counted. A marker pen was used to indicate those already counted. For samples which had less than 30 colonies in higher dilutions, the $10a^{-1}$ and $10b^{-1}$ plates were counted and the average was calculated. The results were recorded and calculations of colony forming units per millilitre (cfu/ml) were done as follows;

Cfu/ml= number of colonies x inverse of dilution factor

Data analysis

The results were analyzed using Two Way Analysis of Variance (Two Way Anova), SPSS Package version 21. The two way anova was done to test effects of 2 factors. Factor 1 was the effect of the type of meat (sausage or chicken) and factor 2 was the type of outlet (vendor or butcher). The responsible variable was the bacterial load.

Results

Bacterial isolation

Four types of bacteria were isolated from vendor chicken (*E. coli, S. aureus, Klebsiella and Streptococcus spp*.) as shown in "Table 1" below. *S. aureus* and *E. coli* were common bacterial isolates found in meat samples from all outlets. No *Klebsiella spp* were found in butchery meat.

Table 1. Distribution of bacteria isolates in food samples from different locations

Bacterial isolate	Butchery Sausage	Butchery chicken	Vendor Sausage	Vendor Chicken
Escherichia coli	+	+	+	+
Staphylococcus aureus	+	+	+	+
Klebsiella	-	-	+	+
Streptococcus	+	-	-	+

Key (+) indicates presence of bacteria, (-) indicates absence of bacteria

Prevalence of bacteria in meat samples from different outlets

The prevalence of identified bacteria was expressed as percentage prevalence of bacteria on each type of outlet and type of sample i.e. Butchery-Chicken, Butchery-Sausage, Vendor-Chicken and Vendor-Sausage as shown in "Fig. 1" below. *E. coli* was the most prevalent bacteria isolated from butchery (43%) and vendor (85%) sausage samples. *S. aureus* was more abundant in chicken samples from both butcheries (43%) and vendors (43%). *Streptococcus spp* were only isolated from butchery sausage and vendor chicken samples whilst *Klebsiella spp* were present in vendor meat samples only.

Mean Total Bacterial counts of meat samples from butcher and vendor outlets

Vendor chicken and sausage samples (2.41×10^5 cfu/ml and 7.66×10^5 cfu/ml) were the most contaminated samples as shown by the high mean TBCs summarized in "Table 2". However, significant contamination was also observed in butchery sausage (1.14×10^4 cfu/ml).

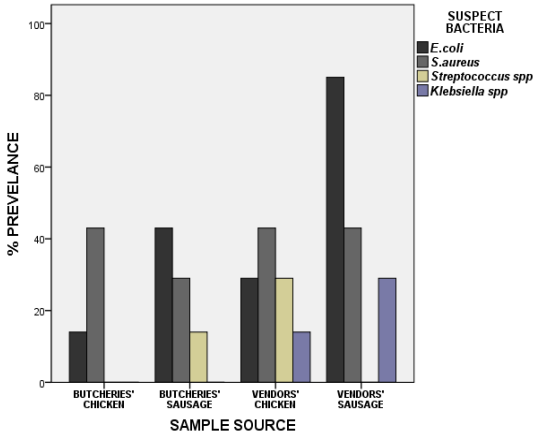

Figure 1. The prevalence of bacterial isolates

Table 2. Mean Total Bacteria Counts of meat samples in cfu/ml

Type of Outlet	Butcher	Vendor
Chicken	5.45×10^2	2.41×10^5
Sausage	1.14×10^4	7.66×10^5

Effect of type of meat and type of outlet on the bacterial load

At α=0.05, there was evidence that type of meat had no significant effect on the bacterial load (P=0.298) and type of outlet had no significant effect on the bacterial load (P=0.061). There was evidence that type of meat and type of outlet did not interact in influencing bacterial load P=0.317).

Discussion

Bacterial isolation

Four pathogenic bacteria were isolated from the chicken and sausage samples; *Staphylococcus aureus, Escherichia coli, Klebsiella* and *Streptococcus*. *E. coli* and *Klebsiella* are coliforms and their presence in any food sample reflects faecal contamination hence they should not be isolated in food samples (Stiles and La, 1981). Presence of these bacteria could be attributed to the location of some of the retail outlets which were within the same proximity as public toilets at the commuter terminus. These were generally of poor hygienic standards with no adequate water supply and could therefore be suspected to be the source of contamination of the meat. In Ibadan, Nigeria, water was considered to be the major source of food contamination (Yah *et al*., 2009). Another possible source of contamination could be improper and unhygienic slaughter of the animals.

Singh *et al* (2014) emphasizes the need for a microbial check to be carried out on regular basis and meat hygiene practices to be observed by proper examination of animals before slaughter and the meat after slaughter to check the spread of diseases which have a definite life cycle. *E. coli* was the most prevalent bacteria isolated from sausage samples (43% in butcher and 85% in vendor meat). According to USDA (2015), *E. coli* is of particular concern in ground meat, especially the shiga-toxin producing strain *E. coli* 0157:H7. Grinding of meat and mixing of ingredients increases the surface area for contamination (USDA, 2015). It is also psychrotrophic and will survive even if the meat is refrigerated (Moyer and Morita, 2007). In some samples *E. coli* was observed to be β haemolytic on blood agar. This *E. coli* was suspected to be the shiga-toxin producing strain (E. coli 0157:H7) which is pathogenic and fatal in serious cases (Clark, 2015). However this could not be confirmed due to limitations in laboratory resources.

Staphylococcus aureus and *Streptococcus* bacteria are usually found on the skin, infected cuts, pimples, wounds and on the nose together with associated body fluids such as mucus and sputum (Todar, 2014). The presence of these pathogens in retail meat is an indicator of unhygienic handling of the meat either during slaughter, packaging or retail. These pathogens produce toxins when they multiply which are the causes of the gastro-intestinal illnesses (CDC, 2015). *Staphylococcus aureus* was the most prevalent bacteria isolated in chicken samples (43% in butchery chicken and in vendor chicken samples), therefore indicating unhygienic handling of poultry carcasses after slaughter.

Bacterial enumeration

The bacterial load in all the samples was generally lower in butchery samples than in vendor samples. This indicates that vendors are at a greater risk of posing a health hazard to the public due to poor sanitary conditions in the vending sites. Most vendors handle the meat without any protective clothing or washing their hands and the meat they sell is not from reliable sources. Gitahi *et al* (2012) reported that most street food vendors are not trained on food hygiene and safety and consequently consumption of street food can lead to food poisoning and food borne illnesses. The mean TBCs were higher in sausage samples (1.14×10^4; 7.66×10^5) than in chicken samples (5.45×10^2; 2.41×10^5) because ground meat is more susceptible to contamination than chicken since most of the meat surface is exposed (USDA, 2015). However, analysis of data showed evidence that the type of meat and the type of outlet had no significant effect in influencing the bacterial load ($P=0.298$ and $P=0.061$). Therefore, it is assumed that the sanitary level of the study area generally influenced the bacterial loads regardless of the sample type or the type of outlet.

Proper sanitation needs to be observed in the formal markets by keeping retail outlets as clean as possible, disinfection of hands at all times as well as wearing protective clothing. An adequate and clean water supply must be provided for butcheries and meat suppliers so as to avoid contamination of the meat at packaging and retail level and work surfaces must be disinfected at regular intervals. Thorough cooking of meat is also encouraged as most pathogens are eliminated by heat and as such, food poisoning incidents can be reduced.

In conclusion, the study enlightened on the safety of meat sold in and around Egodini taxi rank in Bulawayo. The majority of meat considered in the study had a high microbial load and pathogens were also isolated. This proved that food safety practices are not being fully observed in Bulawayo. *E. coli*, a coliform which reflects faecal contamination should not be present in food but was however found to be a major contaminant of raw meat. Training and inspections are important in the food sector. Moreover, provision of basic infrastructures and establishment of code of practice for the sector are also recommended.

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