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A PILOT MICROBIAL ASSESSMENT OF BEEF SOLD IN THE ASHAIMAN MARKET, A SUBURB OF ACCRA, GHANA

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ABSTRACT

Food safety is a matter of great public health concern worldwide and particularly crucial if the environment in which the food is handled is heavily contaminated. Most fresh foods particularly that of animal origin like beef is highly susceptible to microbial invasion and food poisoning. In poorly managed market environment particularly in Ghana, unhygienic practice is the major cause for food contamination. This study observed the hygienic practices and microbiological food safety standards of butchers who specifically sold beef in the Ashaiman market in Accra, Ghana. Hygienic practices of sixteen (16) butchers were randomly selected in a cross sectional study using an eight point scale checklist weekly over a period of four weeks. The microbial quality of one hundred and twenty-eight (128) fresh beef samples were aseptically collected and analysed using standard microbiological techniques. It was observed that majority of the butchers did not practice safe hygiene standards as recommended by the Ghana Food and Drugs Board and the Ghana Standards Board. The beef samples were contaminated with Aerobic mesophiles (189-23000 cfu/g), *Staphylococcus aureus* (22-59 cfu/g), *Bacillus cereus* (17-41 cfu/g), *Clostridium perfringens* (21-48 cfu/g) and *Escherichia coli* (31-2200 cfu/g). The pH of the beef samples were between 6.50 and 6.90. The butchers in Ashaiman market supplied fairly contaminated beef to the general public. *Escherichia coli,* which is a sign of faecal contamination, was the predominant microbial contaminant in the samples examined. The result of unhygienic practices and poor handling of beef by butchers in the Ashaiman market is the major cause of contaminated beef. There are chances that other meat sold by virtually the same group of persons could equally or even more be contaminated by food borne pathogens. Hence food industry and consumers should be made aware of the potential risk of food borne pathogens in beef sold by butchers in Ashaiman market.

Key words: Hygienic practices, meat, food safety.

INTRODUCTION

Microbial food poisoning or infections are a matter of grave public health concern. Beef is a high protein food which is widely consumed by majority of the urban populace and it is a very delicate product which is susceptible to microbial invasion and subsequent deterioration. Raw retail meats have been identified as potential vehicles for transmitting food-borne diseases, and hence the need for increased implementation of hazard analysis of critical control point (HACCP) and consumer food safety education efforts [1].

Up to 60,000 individuals sell an estimated $100 million worth of food annually on the streets of Accra in Ghana and this market makes an important contribution to the well-being of the poor but this is not without its hazards [2]. Majority of these individuals depend on butchers all over the metropolis for their supply of beef [3]. In 2004, the out-patient morbidity data of cases reported from only public hospitals and clinics showed that food related infections were considerably high with diarrhoea (331,998), typhoid fever (65,333) and cholera (2,216). Recently, the demand for beef has risen sharply as a result of a drastic fall in the consumption of chicken products nationwide. Despite the commitment and dedication of the Ghana Food and Drugs Board, improved food safety systems have not been widely implemented because of their limiting capacity. Concerns have already been raised in the past about the role of meat and meat products in food poisoning. Records indicate that more than 74% of incidents of food poisoning are due to meat dishes [4].

Bacterial food poisoning is widely spread and occurs when our environments are untidy and the foods are not hygienically maintained. Fresh meats are sometimes contaminated with bacteria [5, 6], which can be harmful to the human body [7]. The major bacterial pathogens include: *Salmonella, Staphylococcus aureus, Clostridium botulinum, Clostridium perfringens, Bacillus cereus* and *Escherichia coli* [4]. The sources of these microbes in meat could be inherent micro-flora in normal tissues of animals, air, environment, or contamination due to unhygienic slaughtering, handling and processing conditions [8].

At each stage of beef processing after slaughtering, different microbes get introduced and these tend to contaminate the meat [9, 10]. The microbes cause biochemical and microbiological changes in the meat which may lead to the production of noxious substances resulting in the incidence of illnesses such as cholera, typhoid fever and other fatal diseases. Besides the chemical composition, meat culinary and technological value is determined by its physicochemical properties and one such main indicator is pH [11]. The pH of food is critical because at low levels, it favors the growth of moulds and yeasts. In neutral or alkaline pH foods such as meat and meat products, bacteria are more dominant in spoilage process. The high protein content of meat makes the pH approximately neutral and it leads to a high level of spoilage in the meat [12] and this is further explained by the breakdown of muscle glycogen leading to the production of lactic acid in the muscle fibres.

The implementation of appropriate risk mitigation strategies which includes the design of public health policies and appropriate food safety measures is urgently needed. This will require food borne disease surveillance data from food monitoring systems in mandated institutions as well as independent institutions that have no personal interests at stake. To date, there is no literature on the level of contamination of fresh meat sold in the market at Ashaiman. Therefore, information generated in this study will be of significance in policy formulation as well as for the Microbiological Risk Assessment of Foods in Ghana.

Majority of the slaughter houses in Ghana have no HACCP systems in place. Hence poorly designed facilities for the slaughtering and processing of beef can easily result in contamination of food products and lead to food-poisoning incidents. Sanitation in the entire Ashaiman suburb of Accra as well as other urban centres in Ghana is very poor. Meanwhile this market happens to be the main point of source supplying many food vendors and retailers in most of the suburbs of Accra with fresh meat. This study was designed to observe the hygienic practices of butchers and assess the microbiological quality of beef sold in the Ashaiman market in Accra, Ghana.

MATERIALS AND METHODS

The field observation was guided by a simple eight point checklist on items and facilities required for good hygienic practices in the handling of raw meat by butchers. These were 1. Wash hand basin in service area, 2. Sink with running water for hand washing, 3. Wash hand basin in back preparation area, 4. Soap for washing hands, 5. Towels for drying hands, 6. Other sanitary facilities (local detergent), 7. Used aprons and/or head cover, and 8. Use a screen to protect meat from flies.

Samples

In this study four butchers who had a supply of fresh beef at the time of visit (between the hours of 7:00am and 8:00am) to the Ashaiman market were randomly sampled. Freshly cut beefsteaks, all arising from the same meat piece (flank abdominal wall, fore and hind limb areas) were consistently sampled. Eight samples (one from each butcher weighing 1.0 kg) were aseptically collected in sterile polythene pouches, sealed and transported in ice packs to the laboratory for microbiological analysis within one hour of collection. This exercise was repeated weekly for a month. A total of one hundred and twenty-eight (128) fresh meat (beef) samples, were thus obtained from selected slaughterhouses / beef vendors in the Ashaiman market.

pH determination

A pH meter (PHM 92, Radiometric, Copenhagen, Denmark) was initially calibrated using standard buffers. A 1.0 g of beef was minced in 10 ml of distilled water and the pH measured. Duplicate measurements were determined and the average taken.

Test for Food Borne Pathogens

The methods described by the Nordic Committee on Food Analysis (NMKL) was adopted and modified slightly to suit our local conditions as described below.

Aerobic plate count (APC)

The method was adapted according to the NMKL no.86, 1999. A 10.0g sample of meat was weighed into 90.0 ml of saline peptone solution (SPS) and homogenized. Serial dilutions of the stock solutions were made and 1.0 ml of each dilution was pipetted into a Petri dish to which about 15.0 ml of molten plate count agar (PCA) was added. The contents were swirled clockwisely and anticlockwise to thoroughly mix the media with the sample. The plates were inverted and then incubated at 30ºC for 48 to 72 hours.

Identification of Colonies

Colonies on selected plates were counted using a colony counter. The morphological characteristics of each colony were examined to indicate the shape, size elevation and pigmentation to facilitate the process of grouping and identification.

Detection of *Staphylococcus* spp.

The method was adapted from the NMKL No. 66, 3.ed. 1999. A sample of the beef, (10.0g) was well minced in a 90 ml suitable diluent, SPS (Saline peptone containing, g/L Distilled water; 1.0 g, Peptone; 8.5 g, NaCl; pH 7.2 ± 0.2 and autoclaved at 121 ^{o}C for 15 min.). An amount of 0.1 ml of each dilution of the sample was transferred to the surface of Baird-Parker agar plates (g/L Distilled water; 63 g, Baird-Parker Medium (Oxoid CM275); 50 ml, SR54; pH 6.8 ± 0.2). The inoculum was evenly spread with a sterile bent rod and allowed to dry for 15 min. at room temperature. The plates were inverted and incubated for 24 ± 3 h and re-incubated to a total of 48 ± 4 h at 37.0 ± 1.0 ^{o}C. Typical colonies of *Staphylococcus* spp (black or grey, shining and convex), diameter 1.0 – 1.5 mm after 24 h incubation and 1.5 – 2.5 mm after 48 h incubation and with each colony surrounded by a clear zone were isolated and tested for coagulase positive as a confirmatory test. After incubation for at least 24 h typical colonies were observed as having an opalescent ring immediately in contact with the colony and appearing in a clear zone. Atypical colonies on the other hand lacked the clear and the opaque zones.

Detection of *Bacillus cereus*

Bacillus cereus was tested for using the NMKL No. 67 4th ed. 1997. As a pretreatment, 1.0 g of beef /9.0 ml of SPS was incubated in a water bath for 30 min. at 30.0 ± 1.0 ^{o}C prior to inoculation. Serial dilutions were made and 0.1 ml of each dilution of the sample plated out on the surface of a Bacillus Cereus Agar Base plate containing g/L, Distilled water; 20.5 g, Bacillus Cereus Agar Base (Oxoid CM617); 2 ml, SR99 and

25 ml, SR47 at pH 7.2 ± 0.2. The plates were incubated in an inverted position at 30.0 ± 1.0 ^{o}C for 24 ± 3 h. Colonies numbering 10 – 100, large irregular and greyish–white, surrounded by a well–defined zone of precipitation were identified. Those which microscopically have ellipsoidal or cylindrical pores that are centrally or terminally positioned in the sporangium were recognized as typical of *Bacillus cereus*.

Detection of *Clostridium perfringens*

The NMKL No. 95 3rd ed. 1997 was used in the test for *Clostridium perfringens*. A sample of beef (10.0 g) was well mixed in 90 ml diluent (Saline peptone containing, g/L Distilled water; 1.0 g, Peptone; 8.5 g, NaCl; pH 7.0 ± 0.2 and autoclaved at 121 ^{o}C for 15 min.) and prepared into suitable dilutions. About 5 ml of Tryptose Sulphite Cycloserine (TSC) agar containing g/L, distilled water; 46 g, TSC (Oxoid CM587); pH 7.6 ± 0.2, was poured into a sterile petri dish and evenly distributed. After the agar had solidified, 1 ml of each dilution of the sample was pipetted on the agar surface in triplicates, then about 15 ml of TSC agar previously held at 45 ± 1 ^{o}C was poured and mixed thoroughly before agar solidified. The petri dishes were incubated anaerobically in an inverted position using Anaerocult® (A. 1.13829. Microbiologie, Merck KgaA, Darmstadt, Germany) at 37.0 ± 1.0 ^{o}C for 24 ± 3 h. Dishes containing 10 – 100 black colonies were read and further identified by spreading 3 – 10 black colonies on Blood – Free Pyruvate Clostridium Perfringens (BCP) agar (Hood *et al.,* 1990). Alternatively Perfringens Agar Base containing g/500ml, distilled water; 23g, BCP (Oxoid CM587); 25 ml, SR47; 1 vial, SR93; pH 7.6 ± 0.2 was used to double check the outcome. The plates were incubated anaerobically for 24 ± 3 h at 37.0 ± 1.0 ^{o}C. The pure culture was then stab-inoculated into the lactose medium (g/L, distilled water; 8.0 g, Brain Heart Infusion; 2.5 g, Na_2HPO_4; 5.0 g, Galactose; pH 7.3 ± 0.2 autoclaved at 121 ^{o}C for 15 min.), incubated anaerobically for 24 ± 3 h at 37.0 ± 1.0 ^{o}C.

The medium was examined for gas production (Durham tubes) and for change of colour from red to yellow; indicated by the fermentation of lactose with gas production.

Detection of *Escherichia coli*

The method specific for the identification of *E. coli* in food samples was adapted from the NMKL no. 125, 1996 specifications. The pour plate technique (with TSA) was used with an over layer of Violet Red Bile Lactose Agar before incubation at 44ºC for 24 hours. Suspected colonies of the *E coli* were confirmed in E.C Broth at 44ºC for 24 hours with the production of gas, after which 1 ml of the broth was transferred into Tryptone water and incubated at 44ºC for 48 hours. Kovac's reagent was added to the test culture and observed for any reaction. Formation of red colour indicated a positive reaction, thereby confirming the presence of *Escherichia coli*.

RESULTS

The study observed that about 13% of butchers in Ashaiman market have a wash basin in their service area whilst at least 25% had some form of wash basin not too far away from their area of operation (Table 1). None had a sink with running water for hand washing. Only 17% could display soap for washing hands and nearly 19% possessed some other sanitary facilities like local detergents. We did not observe any towels for drying hands. Of all the butchers visited 13% wore aprons or overcoats and 38% had some form of screens to protect the meat from flies.

The total aerobic plate counts on Plate Count Agar (PCA) for the samples handled ranged between 189 – 23000 cfu/g (Table 2). *Escherichia coli* counts (180-540 cfu/g) were very high in majority of the samples analysed. In the case of *Staphylococcus aureus* the counts were least in some samples of week IV (22 cfu/g) and highest in week I (59 cfu/g). The counts for *Bacillus cereus* and *Clostridium perfringens* were 17-41 cfu/g and 21-48 cfu/g respectively (Table 2).

The pH values measured were in the range of 6.50 - 6.90 and this is within the norm for meat (5.6-7.0).

DISCUSSION

The study on the microbiology of fresh beef sold in the Ashaiman market was conducted mainly to determine the state of hygiene (microbial load) and also to observe hygienic practices which may reduce incidences of cross contamination of beef in the market arena. The major findings have been summarised and illustrated in Tables 1 and 2.

The practice of good hygiene in food handling translates to the hygienic quality of the produce. It was observed in this study that majority (Table 1) of butchers did not give priority to good hygienic practices in their business. With the maximum exposure that the meat had to contaminants, in an environment charged with so much filth and poor sanitation, cross contamination was very highly inevitable. Supervision, improved process control and training may be necessary to improve behavioural changes among butchers since this has been proven successful [13, 14].

It was observed that aerobic plate count for all the samples ranging from about $1.9x10^2$ – $2.3x10^4$ cfu/g were within the standard requirements of both the Ghana Standards Board (GSB) ($<1.0x10^4$cfu/g) and the International Commission on Microbiological Specification (ICMS, 1982) ($<1.0x10^6$cfu/g). Usually butchers in the Ashaiman market slaughter and prepare their meat for sale first thing in the morning. It is worth noting that this just represents an early morning sample which is actually expected to be of the best quality as the beef is freshly processed. The cause for public health concern is that as the product stays on shelf the microbial load of the beef builds up, and this could lead to its deterioration rendering it unsafe for consumption.

Even in cold storage, it has been proven that isolates of food borne pathogens have survived on meat over a period of time [15].

However, *S. aureus* and *E. coli*, could not pass the test of a Zero cfu/g which the Ghana Standards Board sets for fresh beef. *B. cereus* and *C. perfringens* were detected in all the samples examined. The presence of *E. coli* in the meat samples is as a result of contamination with faecal matter which could be from the environment, air, materials used including water. The hands of the handlers or even the contents of portions of the meat like the intestines which appear to be the very immediate sources could also be implicated. From preliminary investigation conducted, the environments in which the meat was processed and sold were not hygienically maintained, thus the presence of the *E. coli*. The consistent levels of *Staphylococcus aureus* were $<1.0x10^2$ cfu/g in all the samples from the various vendors during the period of the study. The standard recommended by the GSB and ICMS is $<1.0x10^2$ cfu/g. Normally, pathogens in general should have a zero or no count in all ready to eat foods. Reference to the GSB and ICMS criteria may suggest that the pathogen levels in the beef are acceptable since they would have been destroyed after processing at high temperatures. This notwithstanding there is a risk of infection if virulent forms of this bacterium are present and the beef which is not well processed before consumption.

Bacillus cereus counts were comparatively low because this pathogen thrives very well in media rich in starch [16]. The counts of *Clostridium perfringens* were $<1.0x10^2$ cfu/g in the various meat samples from the various vendors.

The pH measures of the various samples of meat were between 6.50 and 6.90. According to Ronald [17], glycogen, the stored carbohydrate in animal tissue is converted into lactic acid in the aging process of meat. This lactic acid produced tends to lower the pH of the muscle from about 7.0 in the living animal to 5.6 in the dead animal after a period of time. The protein units in the meat however tends to make the pH neutral [7] and this condition favour the growth and survival of bacteria. It is known [18, 19] that at a certain minimum pH the growth of the following bacteria is limited *E. coli* (5.0), *Salmonella spp*. (4.6), *S. aureus* (4.9), and *B. cereus* (5.0). This implies fresh meat and beef in particular have a high risk of harbouring a lot of bacterial pathogens.

The management of microbial risk in beef and other meat products in the event of contamination and growth can present a difficult situation to butchers. Strict hygiene and/or the implementation of decontamination technologies are recommended as a means to assure the safety of meat with respect to food borne pathogens. There is the need for useful data to be generated for assessing food safety issues and analysis. Predictive modelling tools can be employed to determine the survival of some common pathogens in beef and meat products [20, 21].

CONCLUSION

Hygienic practices are not rigidly enforced or practiced by butchers in the Ashaiman market and the beef supplied to the public is fairly contaminated. The food industry and consumers should be made aware of the potential risk of food borne pathogens in beef sold by butchers in Ashaiman market. The study looked at beef only, but there are chances that other meat sold by virtually the same group of persons could equally or even more be contaminated with food borne pathogens. There is the need for further studies to look at the microbiological quality of various meat products sold by these butchers as well as the diversity and resistance of some commonly found pathogens to cleaning agents. Also, knowledge, attitude and practice study of butchers with respect to hygiene is highly recommended to understand their behaviour.

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Table1: Hygiene facilities in 16 Butchers Shops observed in a period of four weeks at Ashaiman Market, Accra, Ghana

Facilities in 16 shops	Percentage of Butchers' Shops
Wash hand basin in service area	13
Sink with running water for hand washing	0
Wash hand basin in back preparation area	25
Availability of soap for washing hands	17
Towels for drying hands	0
Other sanitary facilities (local detergent)	19
Used aprons and or head cover	13
Use a screen to protect meat from flies	38

Table 2: Microbial counts (cfu/g) on Beef observed in four weeks from the Ashaiman Market, Accra, Ghana

Microbial Counts	Week			
	I	II	III	IV
Aerobic microorganisms	224*±21#	17167±3889	9675±3163	4827±4623
Total *Colifiorms*	31±18	33±12	15±3	217±178
Escherichia coli	200±20	377±38	430±93	335±45
Staphylococcus aureus	47±9	40±5	37±5	36±8
Bacillus cereus	24±3	29±9	25±6	23±3
Clostridium perfringens	32±4	35±10	28±4	33±8

*All mean values of four butcheries; #Average deviation from mean values

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