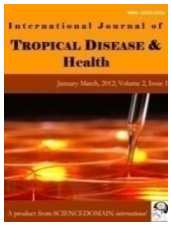

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Microbial Quality and Antibiotic Residues in Raw Beef from Selected Abattoirs in Accra, Ghana

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Authors' contributions

This work was carried out in collaboration between all authors. Authors KKA, DJS and VYA designed the study. Author VYA performed the experiment and wrote the first draft of the manuscript. Authors VYA and GIM managed the analysis of the study and author KKA edited the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the microbial quality and the presence of antibiotic residues in raw beef from four major abattoirs in Accra, Ghana.
Study Design: Cross sectional.
Place and Duration of Study: Samples were obtained from the four major abattoirs namely; Madina, Tema (GIHOC), Accra, Amasaman and the University of Ghana (UG) Farms, transferred immediately to the Bacteriology Laboratory, Noguchi Memorial Institute for Medical Research, University of Ghana, for processing. The study was carried out between June 2013 and April, 2014.
Methodology: Raw beef samples were aseptically collected from 200 cattle slaughtered for consumption. Total plate count, presence of foodborne pathogens such as *Listeria monocytogenes*, *Escherichia coli*, *E. coli* 0157:H7, *Staphylococcus aureus*, *Salmonella* and *Shigella* species were determined after culture and incubation on standard microbiological media. Both liver and kidney samples were also collected from each of the 200 carcasses and tested for antibiotic residues using

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Premi® test kit (R-Biopharm AG, Germany).
Results: The total plate counts in all the samples from the abattoirs ranged between 8.3x103 – 5.5x105 cfu/g. A total of 43 pathogens were isolated. Of this number, 30 (69.76%) were *E. coli*, 8 (18.69%) *S. aureus*, 2 (4.65%) *Salmonella* Typhimurium, 2 (4.65%) *L. monocytogenes* and 1 (2.3%) *Yersinia enterocolitica*. Fifty-nine strains from other species were also isolated: *Bacillus* spp. (21), *Enterobacter* spp. (18), *Pseudomonas aeruginosa* (1), *Aeromonas* spp. (3), coliforms (12) and *Klebsiella* spp. (4). None of the *E. coli* isolated were positive for O157: H7. Overall, 18% of both the liver and kidney samples were positive for the presence of antibiotic residues but the kidneys recorded the highest (12%) percentage of positive whilst the liver recorded (6%).
Conclusion: Beef at the abattoirs were contaminated with foodborne pathogens and antibiotic residues, however, the total aerobic counts were within the acceptable range considered safe for human consumption. Detection of pathogens and antibiotic residues in beef is of public health concern.

Keywords: Raw beef; foodborne pathogens; antibiotic residues; abattoir; Ghana.

1. INTRODUCTION

Food-borne diseases are the leading cause of illness and death in developing countries costing billions of dollars in medical care and social costs [1]. While food borne diseases remain an important public health problem worldwide, one of the most significant food safety hazards is associated with foods from animals [2]. Contaminated raw meat has been identified as one of the main sources of food-borne illness [3,4].

Consumption of meat and its products arises from reasons including high protein contents, vitamins, minerals, lipids and savoury sensation. However, meat is the most perishable of all staple foods since it contains sufficient nutrient needed to support the growth of microorganisms [5]. The source of the microorganisms can be from the hide of the animals or from the abattoirs where they are slaughtered and processed [6]. Food borne pathogens such as *Staphylococcus species, Escherichia coli, Salmonella, Shigella, Bacillus species* and *Listeria monocytogenes* have been isolated from meat [7,8].

Veterinary drugs are generally used in farm animals for therapeutic and prophylactic purposes and they include a large number of different types of compounds which can be administrated through injection and in the feed or in the drinking water [9]. However these antibiotic substances may exert other effects like growth promotion when administrated to animals. The residues occur mostly when animals are slaughtered within the withdrawal period of the drug [10]. The presence of residues of antibiotics in beef has a great public health concern. Human consumption of these beef may be associated with the development of antibiotic resistance in human pathogens that are difficult to treat with the commonly available antibiotics [11].

Majority of abattoirs in Ghana have no Hazard Analysis Critical Control Point (HACCP) systems in place to ensure safety of the meat. Hence poorly designed facilities for the slaughtering and processing of beef can easily result in contamination of meat and food products and lead to food-poisoning incidents. Again, antibiotic are administered to beef cattle when they are sick or as prophylaxis by cattle farmers. It is expected that a withdrawal period is allowed after which the animal can be slaughtered [12]. However, some farmers for fear of losing their animals slaughter them within the withdrawal period. Unfortunately most of the abattoirs do not routinely check for antibiotic residues in the carcasses before they are introduced into the market. As a result the quality and safety of beef sold to the public from abattoirs in Accra is not known.

The study was therefore designed to determine the microbial quality and the presence of antibiotic residues in beef from four major abattoirs that sell beef to most communities in Accra, the capital city of Ghana.

2. MATERIALS AND METHODS

2.1 Sample Collection and Isolation of Foodborne Pathogens

Per sampling day, samples of about 250 g of raw beef muscle, kidney and liver from the same animal were aseptically collected in a sterile stomacher bag using the excision method [13]. Each sample was, labelled and stored in an ice box and was immediately transported to the Bacteriology laboratory, Noguchi Memorial

Institute for Medical Research. In the laboratory, 10 grams of each sample was cut and put in stomacher bags. 90 ml of sterile phosphate buffered saline (PBS) was added and homogenized into a homogeneous mixture. The homogenates was used for all the microbiological analyses. One mL of the homogenate was serially diluted in an aseptic condition and used for the enumeration of microorganisms. Total aerobic counts were performed using Plate Count Agar (Oxoid CM 463). The presence of *L. monocytogenes* was investigated on PALCAM Agar (Oxoid), after pre-enriched on Half Frazer and Frazer broths at 37ºC for 48-72 hours. MacConkey Agar (Oxoid) was also inoculated for isolation of Gram negative rods at 37ºC for 18-24 hours. Rappaport Vassiliadis Broth (Merck 1. 07700), was used for enrichment for isolation of *Salmonella* spp. on Salmonella-Shigella Agar (Merck 1.07667). Bacteria colonies were further sub cultured on Nutrient agar (Oxoid CM003), identified and confirmed using Gram staining and biochemical tests such as catalase, oxidase and triple sugar iron in addition to API 20 E (Biomérieux, France). For detection of *S. aureus,* 0.1ml of the homogenate was cultured on Baird Parker agar (Oxoid, CM275). Black shiny convex colonies surrounded by zone of clearing formed after 18 hours of incubation, suspected to be *S. aureus* were confirmed using Staphyloslide Latex Test (BD 240952). Serotyping of confirmed *Salmonella* spp. (by API) was performed using Salmonella antisera (Polyvalent O and H). Confirmed *E. coli* isolates were also sub cultured on Sorbitol MacConkey for 24 h. Colourless colonies which are non-sorbitol fermenters, (characteristic of *E. coli* 0157) were confirmed for *E. coli* 0157:H7 using the latex slide agglutination kit (DR0622M, Oxoid). Colonies of *Y. enterocolitica* were confirmed using API 20E.

2.2 Detection of Antibiotic/ Antimicrobial Residues

The presence of antibiotic residues in the meat samples was detected by Premi® test kit (R-Biopharm AG, Germany) [14]. 250µl of the fluid from the kidney and liver was obtained by freeze thawing the meat. 100µl of the fluid was pipetted onto the agar of the ampoule and was allowed to pre- incubate at room temperature for 20 minutes. The meat juice was washed off by gently filling and emptying the test ampoule twice with demineralised water. The ampoule was covered with a foil and incubated in a water bath at 64ºC for 3 hours. A clear colour change in the ampoule from purple towards yellow indicated the absence of antibiotics/ sulphonamides and no clear colour change indicated the presence of antibiotics and/ sulphonamides.

2.3 Statistical Analysis

Data was recorded and analysed using Microsoft office Excel 2013 (Microsoft, Palisade Corp, Newfield, NY, USA).

3. RESULTS AND DISCUSSION

The quality of beef samples were determined using total aerobic count and the presence of food borne pathogens such as *E. coli (*O157:H7) *Salmonella, Shigella, S. aureus* and *L. monocytogenes.* Table 1 shows the type and number of pathogens isolated from the abattoirs. A total of 43 (21.50%) isolates, of different food borne pathogens species, were isolated from the 200 samples that were cultured. Of this number, 30 (69.76%) were *E.coli*, 8 (18.69%) *S. aureus,* 2 (4.65%) *S. typhimurium,* 2 (4.65%) *L. monocytogenes* and 1 (2.30%) *Y. enterocolitica.* Fifty-nine other organisms isolated included *Bacillus* spp. (21), *Enterobacter* spp. (18), *Pseudomonas aeruginosa* (1), *Aeromonas* spp (3), coliforms (12) and *Klebsiella* spp. (4) as shown in Table 2. However, none of the 30 *E. coli* isolates was positive for O157: H7.

The GIHOC abattoir recorded the highest number of pathogens isolated (32.55%). Of the 14 pathogens, 10 (23.8%) were *E. coli*, 2 (4.7%) *S. aureus*, 1 (2.4%) *S. typhimirium* and 1 (2.4%) *L. monocytogenes.* Madina and Accra abattoirs recorded the second highest number of pathogens isolated. 10 pathogens each were isolated from these sites. From Accra abattoir, 8 (22.85%) of the pathogens were *E. coli*, 1 (2.85%) *S.* Typhimirium and 1(2.85%) *S. aureus*. From the Madina abattoir however, 6 (9.23%) of the pathogens were *E. coli* and 3 (4.6%) *S. aureus* and *1 Y. enterocolitica* out of the 65 samples examined. 9 (18.78%) pathogens were recorded at Amasaman abattoir; 6 (12.5%) were *E. coli, 2* (4.16%) *S. aureus* and *1 (2.08%) L. monocytogenes. A*t the U.G farm, a total of 10 meat samples were collected and analysed. No food borne pathogen was isolated.

E. coli, *S. typhimurium, L. monocytogenes, S. aureus* and *Y. enterocolitica* were the potential foodborne pathogens isolated in this study which is in agreement with Soyiri et al. [15] who reported similar findings in beef sold at retail outlets in Ghana. *Campylobacter* and *Clostridium species* which are also foodborne pathogens were not sort after in this study.

Table 1. Type and number of pathogens isolated from the abattoirs

Abattoir	No. of samples	No. of pathogens	*E. coli*	*S. aureus*	*S.* typhimirium	*L. monocytogenes*	*Y. enterocolitica*
Accra	35	10	8	1	1	0	0
Amasaman	48	9	6	2	0	1	0
GIHOC	42	14	10	2	1	1	0
Madina	65	10	6	3	0	0	1
U.G farms	10	0	0	0	0	0	0
Total	200	43	30	8	2	2	1

Table 2. Other bacteria isolated

Abattoir	No. of samples	*Enterobacter* spp	*Bacillus* spp	*Aeromonas* spp	*Pseudomonas* spp	Coliforms	*Klebsiella* spp
Accra	35	4	2	0	0	2	0
Amasaman	48	3	3	1	0	0	1
GIHOC	42	2	3	2	0	3	2
Madina	65	8	11	0	1	7	1
UG farms	10	1	2	0	0	2	0
Total	200	18	21	3	1	12	4

The presence of the isolated bacteria species from the meat has been reported in other parts of the world and their presence is mainly due to unhygienic handling and processing. The internal tissues of healthy slaughtered animals is free of bacteria at the time of slaughtering however the muscle tissues are easily contaminated with both pathogenic and non-pathogenic microorganisms at the time of slaughter under poor processing conditions [11]. Knives, cutting surfaces, aprons and unhygienic practices by the butchers such as coughing and sneezing during processing results in contamination of meat [12].

E. coli was the most frequently isolated pathogen in this study and was recovered from 15% of the 200 samples examined. This number was higher than the 8% reported by Iroha et al. [16], in Nigeria and lower than the 17.8% reported [17] in Australia. In our study no *E. coli* 0157: H7 was identified though it is associated with carcasses [18], as it was recovered from 0.3% of meat in Australia. The absence of this pathogen is reassuring as it is able to infect humans at very low doses and form non-culturable forms. This finding also supports the fact that no foodborne outbreak attributed to *E. coli* 0157:H7 has been reported in Ghana.

*L. monocytogenes (*1%), *S.* Typhimurium (1%), *Y. enterocolitica* (0.5%) and *S. aureus* (4%) were isolated from the beef carcasses examined. Similar organisms were isolated elsewhere [12,17,19,20]. The presence of *L. monocytogenes* and *Salmonella* spp. particularly in meat makes it unfit for consumption [21] and also give an indication of poor and unhygienic condition the meat is exposed to. It is worth noting that these 2 types of pathogens were isolated from Amasaman, Accra and GIHOC abattoirs where carcasses are cleaned and handled mostly on floors and also lacked modern equipment. The presence of *E. coli* in the meat samples is as a result of contamination with faecal matter from the environment, air and materials used including water [7] and that of *S. aureus* could also be attributed to excessive handling by the butchers [12]. Other bacteria isolated in the study included *Bacillus spp.*, *Enterobacter* spp., *Pseudomonas aeruginosa*, *Aeromonas spp*, coliforms and *Klebsiella* spp. These bacteria are generally considered non-pathogenic in a healthy adult and also not associated with foodborne disease. However, the presence of *Pseudomonas spp*, which is meat spoilage bacteria, would decrease the shelf life of meat when not stored properly. Again the presence of these pathogens in beef can cause serious diseases in an immunocompromised person.

From Table 3 it is observed that aerobic plate count for all the samples ranging from 8.3×10^3 – 5.5×10^5 cfu/g were within the standard requirements of the International Commission on Microbiological Specification (ICMS, 1982) (< 1.0×10^6 cfu/g).

Mean bacterial count of 5.5×10^5 cfu/g was recorded at GIHOC abattoir. Though the count was the highest it was below 10^7-10^8 for which spoilage of meat is apparent [14].

Table 3. Total aerobic bacterial count from the various abattoirs

Abattoir	No. of samples	Mean bacterial count cfu/g
Accra	35	4.5 x 104
Amasaman	48	4.7 x 104
GIHOC	42	5.5 x 105
Madina	65	5.1 X104
U.G farm	10	8.3 X 103

The mean aerobic count between 8.3 x 10^3 – 5.5 x 10^5 cfu/g observed in this study showed that all the beef from the abattoirs were not spoiled since counts were below 10^6 - 10^7 cfu/g [22] however, the presence of food borne pathogens could lead to foodborne infection or intoxication. Total aerobic count of 5.5 x 10^5 cfu/g was recorded at the GIHOC abattoir where the environmental conditions are below standard. Least count of 8.3 x 10^3 cfu/g was observed at the UG farm. This could be attributed to the small number of sample (10) examined at this site, however, it agrees with the 1.9 x 102 -2.3 x 104 cfu/g reported in Ghana [7].

It should however be noted that the meat samples were obtained early in the morning immediately after slaughter from the abattoirs hence the relatively low bacterial count. When meat is taken to the market for retail under ambient temperatures and unhygienic environmental conditions, these pathogens will proliferate to an unacceptable level and cause foodborne infection or intoxication.

A total of 400 liver and kidney samples (200 liver and 200 kidney from 200 carcasses) were tested for antimicrobial residues using the Premi® test. Out of the 200 cattle tested, 36 (18%) were positive for the presence of antibiotic residues in one or more organs as shown in Table 4.

The highest number of positives was recorded from 24 (12%) out of the 200 kidney samples analysed. The liver samples recorded the least with 12 (6%) positives out of the 200 liver samples examined for antibiotic residues. At the abattoirs the highest number of positives, (6.5%) was recorded at Amasaman followed by Madina and GIHOC with 9 (4.5%) and 8(4%) positives respectively. Whilst no antibiotic residue was detected in beef from UG farms, 6 (3%) of the carcasses from the Accra abattoir were positive for antibiotic residue.

Antibiotic residues or it's metabolites in meat and other animal products may cause adverse toxic effects on consumer's health. This current study detected antibiotics in beef that could be hazardous for human consumption. Out of 200 cattle tested for antibiotic residues, 36 (18%) were found to be positive, which indicated the misuse of antibiotics by cattle owners. This finding agrees with the 16.8% and 17.33% observed in Sudan [11,21]. Similarly in Nigeria, Ezenduka and Ugwumba [23], reported that, 30% (of 40) pigs and 25% (of 40) goats screened tested positive for antibiotic residues. Of the two organ tested for antibiotic residues in the current study, kidneys had the highest percentage of positive 24 (12%) than the livers 12 (6%).

The high percentage of antibiotic residues obtained in this study could be attributed to the fact that Premi® Test is very sensitive to aminoglycosides such as gentamycin, neomycin and diphydrostreptomycin [14] which are common antibiotics administered to animals. Another possible explanation to the high percentage of positives observed in the kidney could be that the kidney is the organ for excretion for most antibiotics. Recent study in the United Kingdom by Pikkemaat et al. [14] also confirmed our results that the kidney which recorded the highest percentage (12%) of antibiotic residue is the best organ or matrix to test for antibiotic residue in meat since the liver recorded 6% of positives.

Table 4. Number of samples positive for antibiotic residues

Abattoir	No. of liver and kidney samples	Negative (liver)	Negative (Kidney)	No. of positives. (Liver)	No. of positives (Kidney)	Total positives (%)
Accra	70	35	29	0	6	6(3)
Amasaman	96	42	41	6	7	13(6.5)
GIHOC	84	39	37	3	5	8(4)
Madina	130	62	59	3	6	9(4.5)
UG farms	20	10	10	0	0	0(0)
Total	400	188	176	12	24	36(18)

Almost all the sites involved in the study had at least one cattle positive for antibiotic residue. The highest was observed at Amasaman (6.5%). This results obtained is not surprising because most butchers prefer to slaughter their animals there because of cheaper fees compared to other places. It is possible that the animals were within the withdrawal period when they were slaughtered. Though a large number of samples was obtained from Madina abattoir 4.5% positives were observed. The possible explanation could be that the withdrawal period could have passed before the animals were slaughtered. The UG farms recorded no antibiotic residues and this could be attributed to the small sample size.

The high percentage observed in our study is an indication that butchers do not adhere to the withdrawal period for antibiotic treatments in livestock.

Consumption of beef contaminated with antibiotic residues maybe associated with the development of antibiotic resistance in human pathogens that are difficult to treat with the commonly available antibiotics [11].

4. CONCLUSION

The results of the study show that beef carcasses offered for sale in Accra to the various markets were grossly contaminated with pathogenic bacteria such as *S. typhimirium, E. coli, S. aureus, Bacillus* spp., *L. monocytogenes* and *Y. enterocolitica* Though the total aerobic counts were within the acceptable range deemed safe for human consumption, the number of cells could increase to an unacceptable level if the meat is not properly stored. The current work indicates a problem of misuse of antibiotics in cattle for meat production and the percentage of positive samples was relatively high compared to studies in other countries. The presence of residues of antibiotics in beef as observed in the present study has a great public health concern. Human consumption of beef containing antibiotic residue may be associated with the development of antibiotic resistance in human pathogen that are difficult to treat with the commonly available antibiotics.

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CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interest exists.

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