Antimicrobial Resistance of Escherichia coli and Salmonella isolated from Raw Retail Broiler Chickens in Zambia

Elizabeth Muligisa Muonga (elizabethmuligisa@gmail.com)
Eden University Zambia, School of Health Sciences

Geoffrey Mainda
Ministry of Fisheries and Livestock, Department of Veterinary Services-Public Health Unit, Lusaka, Zambia

Mercy Mukuma
Department of Food Science and Nutrition, School of Agricultural Sciences, University of Zambia, Lusaka, Zambia

Geoffrey Kwenda
Department of Biomedical Sciences, School of Health Sciences, University of Zambia, Lusaka, Zambia

Bernard Hang'ombe
Department of Paraclinical Studies, School of Veterinary Medicine, University of Zambia, Lusaka, Zambia

Nelson Phiri
Department of Environmental Health, School of Health Sciences, Eden University, Lusaka, Zambia

Mwaba Mwansa
The Copperbelt University School of Medicine

Musso Munyeme
University of Zambia School of Veterinary Medicine

John Bwalya Muma
University of Zambia School of Veterinary Medicine

Research article

Keywords: Antimicrobial resistance, E. coli, Salmonella, Poultry, Zambia

Posted Date: September 7th, 2019

DOI: https://doi.org/10.21203/rs.2.14062/v1

Version of Record: A version of this preprint was published at Journal of Epidemiological Research on May 21st, 2021. See the published version at https://doi.org/10.5430/jer.v6n1p35.

Abstract

Background Antimicrobial resistance (AMR) of foodborne pathogens is of public health concern, especially in developing countries like Zambia. This study was undertaken to determine the resistance profiles of Escherichia coli (E. coli) and Salmonella isolated from dressed broiler chickens purchased from open markets and supermarkets in Zambia.

Results A total of 189 E. coli and five Salmonella isolates were isolated. Identification and confirmation of the isolates was done using Analytical Profile Index (API 20E) (Biomerieux ®) and 16S rRNA sequencing. Antimicrobial susceptibility tests (AST) were performed using the Kirby Bauer disk diffusion technique using a panel of 10 different antibiotics and multiplex PCR was used to determine the presence of three target genes encoding for resistance: tetA, Sul1 and CTXM. AST results were entered and analyzed in WHONET 2018 software. A total of 189 E. coli and five Salmonella isolates were identified. Among the E. coli isolates, Tetracycline recorded the highest resistance of 79.4%, followed by Ampicillin 51.9%, Trimethoprim/Sulfamethoxazole 49.7%, Nalidixic Acid 24.3%, Chloramphenicol 16.4%, Cefotaxime 16.4%, Ciprofloxacin 10.1%, Colistin 7.4%, Amoxicillin/Clavulanic acid 6.9%, and Imipenem 1.1%. Two of the five Salmonella isolates were resistant to at least one antibiotic. Forty- seven (45.2%) of the isolates possessed at least one of the targeted resistance genes.

Conclusion This study has demonstrated the presence of AMR E. coli and Salmonella on raw broiler chickens from both open markets and supermarkets. Such resistance is of public health concern and measures need to be put in place to regulate the use of these antimicrobials in poultry production.

Background

Poultry meat forms an integral part of the diet, especially in developing countries. The popularity of poultry meat is because it is a cheaper source of protein compared to other meat products and also because it is easier to produce (Musaba and Mseteka, 2014). However, this high demand for poultry meat puts a strain on producers, who have to meet the ever-growing demand and also realize profits in a competitive market environment (Ahuja and Sen, 2007). One of the strategies producers often resort to is the use of antibiotics to prevent and treat diseases of poultry in order to optimize growth (Apata, 2009). Antibiotics are also used for growth promotion. However, if they are misused, they can lead to the development of resistance in organisms found in the chickens. In the absence of a national surveillance system on the use of antibiotics, it is impossible to know whether they are being used appropriately (WHO Global Report, 2014).

In monitoring development of AMR in bacteria, *Escherichia coli* (*E. coli*) is commonly used because it is part of the gut microbiota that have been shown to be a reservoir for antimicrobial resistance genes (Van Schaik, 2015; Yassin *et al.*, 2017). Despite *E. coli* being an innocuous resident of the digestive system, it can also be pathogenic and cause severe intestinal and extra-intestinal diseases (Diarrassouba *et al.*, 2007). It is estimated that every year, about 48 million people get sick from foodborne illness, the majority

of these cases are hospitalized and some of these die (Torgerson *et al.*, 2015). Pathogenic forms of *E. coli* such as *Enterotoxigenic E. coli* (ETEC) also cause significant diarrheal illness. It is the leading cause of travelers' diarrhea and other diarrheal illnesses in developing countries, especially among children (Nataro and Kaper, 1998).

E. coli infections can be treated using relevant antibiotics, but there is also accumulating evidence of the consequences of drug resistance. These consequences have a lot to do with the reduction in the efficiency of treatment with first-line drugs and limited choices after microbiological diagnosis (Clarke *et al.*, 2012; Mshana *et al*, 2013).

Non-typhoidal *Salmonella* species are responsible for causing gastroenteritis and bacteremia, eventually leading to secondary infection. These bacteria are a problem in immune-compromised individuals such as patients with malignancy, human immunodeficiency virus, diabetes, and those receiving medication for anti-inflammatory diseases (Gordon, 2008).

In Zambia, recent findings showed that *E. coli* was among the most detected pathogens causing bacterial diarrheal disease in children between the ages of 0-59 months at the University Teaching Hospital (Chiyangi *et al.*, 2017). This suggests that foodborne pathogens, poor hygiene and sanitation and other food safety risks such as the emergence of antimicrobial resistance (AMR) in foodborne pathogens are having a negative impact on public health (Mainda *et al.*, 2015). There is currently limited data on AMR occurrence on food borne pathogens, in developing countries, including. Study of AMR in developing countries is important because information from such studies could enhance correct and controlled use of antibiotics in food production (Mshana *et al*, 2013; Chishimba *et al.*, 2016).

This study, therefore, aimed at characterizing the phenotypes and genotypes of antimicrobial resistant *E. coli* and *Salmonella* in retail broiler chickens in Zambia.

Methods

Study Design

A cross-sectional study involving seven districts in Zambia that included Lusaka, Chilanga, Chongwe, Kafue, Choma, Kabwe and Kitwe was undertaken to investigate AMR *E. coli* and *Salmonella* in market-ready broiler meat. The study was conducted between August 2017 and May 2018. Choma, Lusaka, Kabwe and Kitwe districts were purposely selected because as provincial headquarters, they are retail destinations for many poultry products from other districts while Chilanga, Chongwe and Kafue districts were included due to their proximity to Lusaka. The primary sampling units were the markets (broadly classified as Open markets and Supermarkets) and the secondary sampling units were individual dressed broiler chickens. An open market was defined as an unrestricted competitive market not housed in a building, where foodstuffs are sold often exposed and in which any buyer and seller was free to participate, while a supermarket was defined as a market housed in a closed building with modernized facilities. Proportion stratified random sampling was employed where Open markets and Supermarkets

were the strata. For Lusaka province, from the information collected from Lusaka city council, there were 47 supermarkets and 33 open markets at the time of the study; Choma had five open-markets and four supermarkets; Kitwe had six open markets and nine supermarkets; Kabwe had nine open markets and six supermarkets, Kafue had three open markets and two supermarkets, Chilanga had three open markets and one supermarket, Chongwe had one open market and no supermarket. This formed the sampling frame from which a study population was drawn.

Sample size calculation

The sample size for estimation of a single proportion was calculated using Epi tools software (www.epitools.ausvet.com). The sample size was estimated using the following assumptions: assumed prevalence of AMR *E. coli* on dressed poultry =25%; confidence level=95%; level of precision=5%. Using the above assumptions, the total number of supermarkets and open markets to be included in the study was calculated to be 63 markets, proportionally distributed as follows: 58.8% = Supermarkets; 41.2% = Open markets. For Lusaka districts, the estimated study population was adjusted according to the finite population (n=80), resulting in a sample size of 63 (37 supermarkets and 26 open markets). For other districts, because of the fewer number of open markets and supermarkets, all markets that were trading in dressed broiler chickens were included in the study, bringing the total number of markets to 92 markets (Table 6).

Samples from open markets

Forty-two open markets from Chilanga, Choma, Chongwe, Lusaka, Kafue, and Kitwe districts that had shops and/or tables trading in dressed broiler chickens were selected and included in the study. From each open market within Lusaka district that was sampled (n=24), three shops trading in dressed broiler chicken carcasses were selected and from each shop two-dressed broiler carcasses purchased (22×2×2=88). Also at the markets, three stands that traded in dressed broiler chickens, where available, were included in the study (one dressed broiler carcass from each stand/table). For other districts, all markets that traded in dressed broiler chickens were included in the study, bringing the total number of samples from open markets to 178.

Samples from the Supermarket

Fifty (50) supermarkets that traded in dressed broiler chickens from Chilanga, Choma, Chongwe, Lusaka, Kafue and Kitwe districts were selected. From each supermarket in all districts, a maximum of three different brands of dressed broiler chickens were sampled (one of each brand) were available. Therefore, the number of broiler carcasses from supermarkets in all supermarket in this study were 154.

Upon purchase, all samples were transported in a cooler box containing ice packs to the laboratory and processed within 8 hours. Laboratory isolation included a whole carcass rinse in buffered peptone water (Oxoid), pre-enrichment and subsequent incubation at 37^{O}C overnight that was done within eight hours in

the laboratories within the area of sampling. Processing of pre-enriched broths was undertaken in the Public Health laboratory, School of Veterinary Medicine.

Laboratory Analysis

The methods proposed by The Food and Drug Administration's Bacteriological Analytical Manual (U.S. Food and Drug Administration, 2001) were used with a few modifications for the isolation of *Salmonella* and *E. coli*. Laboratory isolation included a whole carcass rinse in buffered peptone water (Oxoid) (Figure 1a-c), pre-enrichment and subsequent incubation at 37^{O}C overnight. During the carcass rinse technique, 450mL of sterile buffered peptone water was also poured into an empty bag that did not contain a carcass to act as a control. The rinsate was incubated overnight and later streaked onto MacConkey agar plate (Oxoid, UK) to ensure that the batch of bags was sterile and that the organisms isolated were indeed from the chicken carcasses and not the bags used for rinsing. 10µL of the incubated broth was then transferred to MacConkey agar (Oxoid UK) and resulting colonies were gram stained for detection of Gram-negative short rods, which were subsequently sub-cultured onto Eosin Methylene Blue (EMB) agar (Oxoid UK). Colonies that showed a metallic green sheen (Figure 2) were subjected to biochemical tests for identification for *E. coli* isolates while 1ml of the incubated pre-enrichment broth was also transferred to Rappaport Vassiliadis (Oxoid UK) and later subcultured on Xylose-Lysine Deoxycholate agar (Oxoid UK). Pink and black colonies on XLD agar (Figure 3) were then Gram stained and subjected to biochemical tests for identification of *Salmonella* using Analytical Profile Index (API 20E) (Biomerieux®). Further confirmation of the isolates was done using 16S rRNA sequencing (Weisburg *et al.*, 1991). The Kirby-Bauer disk diffusion technique for AST was used on all confirmed *Salmonella* and *E. coli* isolates (Figure 4) using a panel of 10 different antibiotics (Kirby-bauer, 1961). The isolates were prepared by sub-culturing onto Blood agar (Oxoid UK) overnight at 37^{o}C. A Gram's stain was then done to identify the organisms and to check for purity. One or two colonies were then suspended in 4mL of 0.9% sodium chloride solution and their turbidity compared to that of a 0.5 McFarland's turbidity standard. An inoculum of the suspension was then spread on two Muellar Hinton agar (4 ml thickness) plates (Oxoid UK) until the entire surfaces of the plates were covered and 5 antibiotic wafers (Oxoid) placed on the surface of each of 2 plates using the applicator (Oxoid). Two plates were used for each isolate to accommodate the 10 antibiotics (Table 7) The list of antibiotics was prioritized based on the most frequently used in the poultry industry in Zambia and also based on the priority list by the WHO and OIE list of critical antibiotics (WHO, 2017). The plates were then incubated at 37^{o}C for 24hrs and the diameters of the zones of inhibition entered and analyzed in WHONET 2018 software.

Isolates that showed resistance to tetracyclines, sulphonamides and beta lactam antibiotics were then forwarded for molecular analysis that involved extraction of Deoxyribonucleic Acid (DNA) and checking for presence of target resistance genes. The process of DNA extraction involved the suspension of a few bacterial colonies in 100µL of nuclease free water and heating of the vials at 80^{O}C for 10 minutes. The suspension was then centrifuged at 60000G with temperature of 4°C for 3 minutes. Multiplex polymerase chain reaction (PCR) was performed to check presence of resistant genes of interest according to the

method described by (Adesiji *et al*, (2014). The mastermix volumes and PCR reaction were as outlined in Table 8 and Table 9 below.
The target genes were selected based on the antimicrobial susceptibility results and the genes that were outlined as genes of importance for antimicrobial susceptibility. The 3 target genes were TetA (for tetracycline resistance), Sul1 (for sulfonamide resistance) and CtxM (for beta lactam resistance) (Table 10; Figures 5**).** Every batch of samples was processed along with a positive and negative control using *E. coli* 25922 (ATCC) and *Salmonella typhimurium* 14028 (ATCC).

Results

Descriptive statistics

A total of 332 dressed broiler carcasses where sampled from both supermarkets and open markets (Table 1). Majority of the samples were obtained from Lusaka district because it had the highest number of both market types.

Antimicrobial susceptibility

A total of 189 *E. coli* and five *Salmonella* isolates were isolated and identified (Table 2). For *E. coli* isolates, Tetracycline, Trimethoprim-Sulfamethoxazole and Ampicillin recorded the highest resistance of 79.4% (n=150 isolates), 51.9% (n=98 isolates) and 49.7% (n=94 isolates), respectively (Table 3) while only Ampicillin and Tetracycline recorded resistance among the *Salmonella* isolates (Table 4).

E. coli isolates from broiler carcasses obtained from open markets had a higher resistance of 91.7% (n=88) while supermarkets recorded 83.9% (n=78). The overall resistance for both the open markets and supermarkets was 88% (n=166). Four out of five *Salmonella* isolates were from supermarkets (n=4).

Determination of Antimicrobial resistance genes

One hundred and four isolates were analyzed for the presence of resistant genes and 45.2% (n=47) showed the presence of at least one of the targeted genes. The Beta-lactam gene (CtxM) was the most detected in most of the isolates from supermarkets while the Tetracycline gene (TetA) was the most detected in isolates from open markets (Table 5).

Discussion

The resistance to tetracyclines, sulfonamides and beta-lactam antibiotics was generally high. This could be attributed to the use of antibiotics in both livestock and humans which is not well regulated and is subject to misuse and abuse, especially among small poultry producers. In Zambia, there is poor regulation of veterinary drugs and antibiotics, whereby farmers are able to purchase antibiotics over the counter without a prescription (Mainda, 2016; Manyi-Loh *et al*., 2018). Further, the poor hygienic processing methods that are employed by small and medium scale producers may facilitate the

contamination of the carcasses with AMR organisms. The handling of the carcasses during slaughter, rinsing, transportation and sale may all also introduce resistant organisms from humans and the environment. Broiler carcasses that originate from commercial abattoirs, however, may get contaminated mostly from the abattoir bench surfaces and intestines of the broilers during processing (Voidarou *et al.*, 2011). Some isolates from carcasses from supermarkets were highly resistant to Nalidixic acid, in addition to Tetracycline and Trimethoprim/sulfamethoxazole. This could be as a result of the use of fluoroquinolone antibiotics such as Enrofloxacin and Ciprofloxacin during poultry production at commercial level, which are currently on the Zambian market. Similar trends have been noticed in other parts of the world (Donado-Godoy *et al.*, 2012). Open markets that were surrounded by over-populated areas recorded the highest number of resistant isolates from dressed chickens. These were also the areas where some of the broiler carcasses were sold mostly on tables and thus subject to contamination and proliferation of intrinsic bacteria in the absence of the cold chain. Moreover, most traders in the open markets sourced their birds from small producers who probably abused or misused antibiotics (Apata, 2009). It has also been documented that Tetracycline and Sulfadimidine are among the most commonly used antibiotics for therapy, especially at small-scale production (Mainda *et al.*, 2015). Over-time, farmers have learnt about these drugs and tend to self-prescribe whenever they have a disease situation when raising the birds. This overuse and misuse of antibiotics in livestock production has been reported to cause antimicrobial resistance (Lowe, 1982; Ngoma *et al.*, 1993; Koluman and Dikici, 2013; Kalonda *et al.*, 2015; Ayukekbong, Ntemgwa and Atabe, 2017). Of the three resistant genes that were targeted, the most detected was that of the beta-lactams (CtxM gene). The phenotypic and genotypic results of resistance profiles were similar, confirming the efficiency of the Kirby-bauer disk diffusion method. This implies that the beta-lactam gene of interest that was targeted is similar to the one that was found in other countries were similar studies were undertaken (Adesiji, Deekshit and Karunasagar, 2014; Chishimba *et al.*, 2016; Ramachandran, Bhanumathi and Singh, 2017). Though the other two genes for resistance to sulfonamides and tetracyclines (Sul1 and TetA) were also detected, the detection rates were not as high as that of the beta-lactam gene of interest. These discrepancies could be attributed to differences in target sequences of the resistant genes that were being targeted.

Conclusions

Tetracyclines, beta-lactams, sulfonamide and fluoroquinolone antibiotics recorded the highest resistance. This could be attributed to both the overuse of these drugs for therapeutic reasons at both commercial and small-scale levels of production. The presence of these resistant organisms both in open markets and supermarkets is a major public health concern because this could lead to the spread of resistance to humans in households where these carcasses end up. The spread of this resistance to pathogenic bacteria is a major public health concern. There is need to regulate the use of these antibiotics during production. There is also need to do more molecular work that can give a complete understanding of the actual genes that are conferring resistance in Zambia. An understanding of the genes will be beneficial in the event that there is need for new drug formulation or combinations.

Declarations

Acknowledgements

I would like to thank Mr. Joseph Ndebe; Mr. Penjani Kapila; Mr. Patrick Katemangwe; Mrs. Lweendo Hachamba-Sachikolo and Miss. Yambilani Nyirenda for the help rendered during sample processing. I would also like to thank the WHO-funded Advisory Group for Integrated Surveillance of Antimicrobial Resistance (AGISAR) project at the University of Zambia and the World Bank-funded African Centre of Excellence in Infectious Diseases of Humans and Animals (ACEIDHA) programme for the provision of funds and research materials required for laboratory sample analysis. My sincere gratitude also goes out to the technical staff at the Department of Disease Control, and Department of Paraclinical Studies at the University of Zambia, School of veterinary medicine for their technical support and guidance they rendered to me during my study.

Availability of Data and Supporting Materials

The datasets supporting the conclusions of this article are available within the article (Tables) and separate data files uploaded (Figures). Any additional datasets required are available from the corresponding author on request.

Conflict of Interest

The authors declare that there is no conflict of interest regarding this publication.

The author(s) declare that there

References

Adesiji, Y. O., Deekshit, V. K. and Karunasagar, I. (2014) 'Antimicrobial-resistant genes associated with Salmonella spp . isolated from human , poultry , and seafood sources'. doi: 10.1002/fsn3.119.

Ahuja, V. and Sen, A. (2007) 'Scope and Space for small scale poultry production in developing countries', *Management*. doi: 10.1080/00045608.2011.652888.

Apata, D. F. (2009) 'Antibiotic resistance in poultry', *International Journal of Poultry Science*. doi: 10.3923/ijps.2009.404.408.

Ayukekbong, J. A., Ntemgwa, M. and Atabe, A. N. (2017) 'The threat of antimicrobial resistance in developing countries: Causes and control strategies', *Antimicrobial Resistance and Infection Control*. doi: 10.1186/s13756-017-0208-x.

Chishimba, K. *et al.* (2016) 'Detection of Extended-Spectrum Beta-Lactamase-Producing Escherichia coli in Market-Ready Chickens in Zambia', *International Journal of Microbiology*. doi: 10.1155/2016/5275724.

Chiyangi, H. *et al.* (2017) 'Identification and antimicrobial resistance patterns of bacterial enteropathogens from children aged 0-59 months at the University Teaching Hospital, Lusaka, Zambia: A prospective cross sectional study', *BMC Infectious Diseases*. doi: 10.1186/s12879-017-2232-0.

Clarke, K. R. *et al.* (2012) 'Outbreak of multi-drug resistant salmonella Typhi, lusaka, zambia 2011-2012', *American Journal of Tropical Medicine and Hygiene. Conference: 61st Annual Meeting of the American Society of Tropical Medicine and Hygiene, ASTMH*.

Diarrassouba, F. *et al.* (2007) 'Antibiotic Resistance and Virulence Genes in Commensal Escherichia coli and Salmonella Isolates from Commercial Broiler Chicken Farms', *Journal of Food Protection*. doi: 10.4315/0362-028X-70.6.1316.

DONADO-GODOY, P. *et al.* (2012) 'Prevalence of Salmonella on Retail Broiler Chicken Meat Carcasses in Colombia', *Journal of Food Protection*. doi: 10.4315/0362-028X.JFP-11-513.

Gordon, M. A. (2008) 'Salmonella infections in immunocompromised adults', *Journal of Infection*. doi: 10.1016/j.jinf.2008.03.012.

Kalonda, A. *et al.* (2015) 'Characterization of Antimicrobial Resistance in Salmonella enterica Serovars Typhi and Paratyphi B in Zambia', *Jour of Med Sc & Tech J Med. Sci. Tech*.

Kirby-bauer, T. (1961) 'KIRBY-BAUER TEST FOR ANTIBIOTIC SUSCEPTIBILITY'.

Koluman, A. and Dikici, A. (2013) 'Antimicrobial resistance of emerging foodborne pathogens: Status quo and global trends', *Critical Reviews in Microbiology*. doi: 10.3109/1040841X.2012.691458.

Lowe, J. (1982) 'Mechanisms of Antibiotic Resistance', *Annual Reports in Medicinal Chemistry*. doi: 10.1016/S0065-7743(08)60495-9.

Mainda, G. *et al.* (2015) 'Prevalence and patterns of antimicrobial resistance among Escherichia coli isolated from Zambian dairy cattle across different production systems', *Scientific Reports*. doi: 10.1038/srep12439.

Mainda, G. (2016) *Molecular epidemiology of antimicrobial resistance (amr) and shiga toxin producing e coli (stec) in dairy herds of central zambia*, *PQDT - UK & Ireland*.

Manyi-Loh, C. *et al.* (2018) 'Antibiotic use in agriculture and its consequential resistance in environmental sources: Potential public health implications', *Molecules*. doi: 10.3390/molecules23040795.

Mshana, S. E., Matee, M. and Rweyemamu, M. (2013) 'Antimicrobial resistance in human and animal pathogens in Zambia, Democratic Republic of Congo, Mozambique and Tanzania: An urgent need of a sustainable surveillance system', *Annals of Clinical Microbiology and Antimicrobials*. doi: 10.1186/1476-0711-12-28.

Musaba, E. C. and Mseteka, M. (2014) 'Cost efficiency of small-scale commercial broiler production in Zambia: A stochastic cost frontier approach', *Developing Country Studies*.

Nataro, J. P. and K. J. B. J. and Kaper, J. (1998) 'Diarrheagenic Escherichia coli Strains', *Clinical Microbiology Reviews*. doi: file://Z:\References\Text Files\00000004472.txt.

Ngoma, M. *et al.* (1993) 'Antibiotic resistance of Escherichia coli and Salmonella from apparently healthy slaughtered cattle and pigs, and diseased animals in Zambia', *Japanese Journal of Veterinary Research*.

Ramachandran, D., Bhanumathi, R. and Singh, D. V (2017) 'Multiplex PCR for detection of antibiotic resistance genes and the SXT element : application in the characterization of Vibrio cholerae', (2007), pp. 346–351. doi: 10.1099/jmm.0.46655-0.

van Schaik, W. (2015) 'The human gut resistome', *Philosophical Transactions of the Royal Society B: Biological Sciences*. doi: 10.1098/rstb.2014.0087.

Torgerson, P. R. *et al.* (2015) 'World Health Organization Estimates of the Global and Regional Disease Burden of 11 Foodborne Parasitic Diseases, 2010: A Data Synthesis', *PLoS Medicine*. doi: 10.1371/journal.pmed.1001920.

U.S. Food and Drug Administration (FDA) (2001) 'Microbiological Methods & Bacteriological Analytical Manual (BAM)', *U.S. Department of Health and Human Services*.

Voidarou, C. *et al.* (2011) 'Microbial challenges of poultry meat production', *Anaerobe*. doi: 10.1016/j.anaerobe.2011.05.018.

Weisburg, W. G. *et al.* (1991) '16S ribosomal DNA amplification for phylogenetic study', *Journal of Bacteriology*. doi: 10.1128/jb.173.2.697-703.1991.

WHO (2017) *WHO updates Essential Medicines List with new advice on use of antibiotics, and adds medicines for hepatitis C, HIV, tuberculosis and cancer.*, *World Health Organization*.

WHO Global Report (2014) 'Antimicrobial resistance'.

Yassin, A. K. *et al.* (2017) 'Antimicrobial resistance in clinical Escherichia coli isolates from poultry and livestock, China', *PLoS ONE*. doi: 10.1371/journal.pone.0185326.

Tables

Table 1: Summary distribution of broiler samples collected by market type and district

District	No. of open markets	No. of Super market	Total No. of markets	Samples from Open Markets	Samples from Supermarkets	Total No. of samples
Chilanga	2	1	3	8	4	12
Chongwe	1	0	1	11	0	11
Kafue	1	2	3	2	6	8
Lusaka	22	34	56	113	106	219
Kitwe	7	8	15	15	18	33
Choma	4	2	6	14	7	21
Kabwe	5	3	8	15	13	28
Total	**42**	**50**	**92**	**178**	**154**	**332**

Table 2: Distribution of samples and isolates

Province	Number of samples	Number of *E. coli* isolates	Number of *Salmonella* isolates
Lusaka	250	148	5
Southern	21	12	0
Central	28	6	0
Copperbelt	33	23	0
Total	**332**	**189**	**5**

Table 3: Resistance profiles for E. coli isolates

Antibiotic name	Breakpoints	Number	%R	%I	%S	%R 95%C.I.
Ampicillin	14 – 16	189	51.9	4.8	43.4	44.5-59.2
Amoxicillin/Clavulanic acid	14 – 17	189	6.9	5.8	87.3	3.9-11.8
Cefotaxime	23 – 25	189	16.4	6.3	77.2	11.6-22.6
Imipenem	20 – 22	189	1.1	6.3	92.6	0.2-4.2
Nalidixic acid	14 – 18	189	24.3	9.5	66.1	18.5-31.2
Ciprofloxacin	16 – 20	189	10.1	4.2	85.7	6.4-15.5
Trimethoprim/Sulfamethoxazole	11 – 15	189	49.7	0.5	49.7	42.4-57.0
Colistin	S >= 11	189	7.4	0	92.6	4.3-12.4
Chloramphenicol	13 – 17	189	16.4	4.8	78.8	11.6-22.6
Tetracycline	12 – 14	189	79.4	2.1	18.5	72.8-84.8

Table 4: Resistance profiles for Salmonella isolates

Antibiotic name	Breakpoints	Number	%R	%I	%S	%R 95%C.I.
Ampicillin	14 – 16	5	60	0	40	17.0-92.7
Amoxicillin/Clavulanic acid	14 – 17	5	0	20	80	0.0-53.7
Cefotaxime	23 – 25	5	0	20	80	0.0-53.7
Imipenem	20 – 22	5	0	0	100	0.0-53.7
Nalidixic acid	14 – 18	5	0	20	80	0.0-53.7
Ciprofloxacin	21 – 30	5	0	20	80	0.0-53.7
Trimethoprim/Sulfamethoxazole	11 – 15	5	0	0	100	0.0-53.7
Colistin	S >= 11	5	0	0	100	0.0-53.7
Chloramphenicol	13 – 17	5	0	0	100	0.0-53.7
Tetracycline	12 – 14	5	40	20	40	7.3-83.0

Table 5: Summary of resistant genes according to strata

Target Gene	Open Markets		Supermarkets	
	E. coli	*Salmonella*	*E. coli*	*Salmonella*
TetA, Sul1, CTXM	4	0	1	0
TetA, Sul1	2	0	1	0
Sul1, CTXM	0	0	0	0
TetA, CTXM	3	0	2	1
TetA	7	0	4	0
Sul1	0	0	0	0
CTXM	9	0	13	0

Table 6: Distribution of Open Markets and Supermarkets included in the study

District	Number of Markets available		Number of markets included in the study	
	Open markets	Supermarkets	Open Markets	Supermarkets
Chilanga	5	1	2	1
Choma	4	4	4	2
Chongwe	1	0	1	0
Kabwe	9	6	5	3
Kafue	3	2	1	2
Kitwe	7	8	7	8
Lusaka	33	47	22	34
Total	**62**	**68**	**42**	**50**

Table 2: List of antibiotics and their concentrations

Antibiotic	Concentration (µg)
Amoxicillin-Clavulanic Acid	30
Ampicillin	10
Cefotaxime	30
Chloramphenicol	30
Ciprofloxacin	5
Colistin Sulphate	10
Imipenem	10
Nalidixic Acid	30
Tetracycline	30
Trimethoprim-Sulfamethoxazole	25

Table 8: PCR mastermix volumes

Item	Volume (µL)
10X ExTaq Buffer	2
DNTPs	1.6
Forward Primer	0.8
Reverse Primer	0.8
ExTaq HS Enzyme	0.1
Nuclease-Free Water	13.7
Template	1
Total Volume	20

Table 9: PCR conditions

Stage	Temperature (°C)	Time (Mins)	Number of cycles
Initial Denaturation	95	5	
Denaturation	95	1	35
Annealing	58	1	
Extension	72	1	
Final Extension	72	1	
Hold	4		

Table 10: Primer sequences for the 3 target genes encoding resistance

Primers	Sequence (5’-3’)	Annealing Temp	Expected Band Size
TetA (Forward)	GTAATTCTGAGCACTGTCGC	58	494
TetA (Reverse)	CTGCCTGGACAACATTGCTT	58	
Sul1 (Forward)	TGAGATCAGACGTATTGCGC	58	793
Sul2 (Reverse)	TTGAAGGTTCGACAGCACGT	58	
CtxM (Forward)	CGATGTGCAGTACCAGTAA	55	585
CtxM (Reverse)	TAAGTGACCAGAATCAGCGG	58	

Figures

A B C

Figure 1

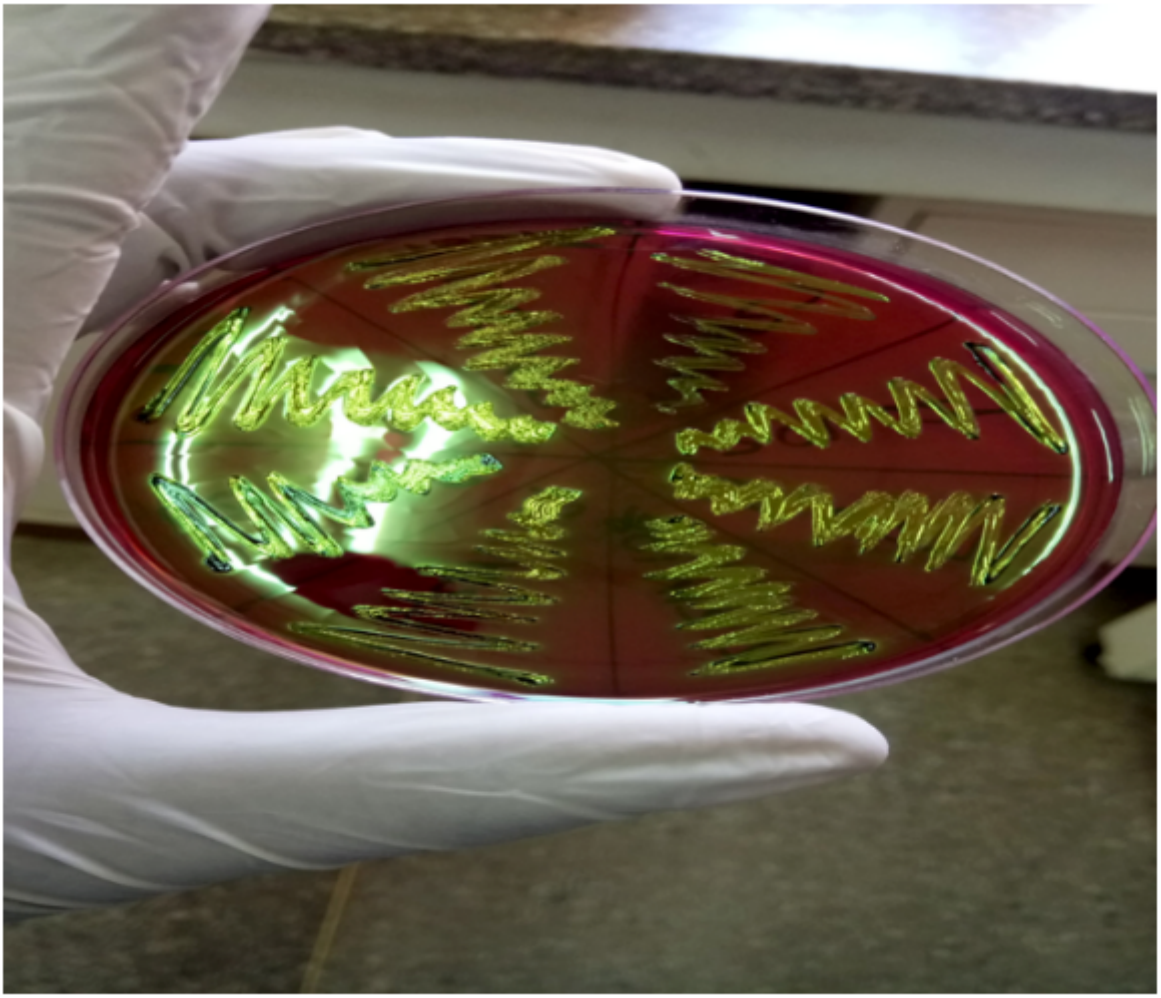

Figure 2

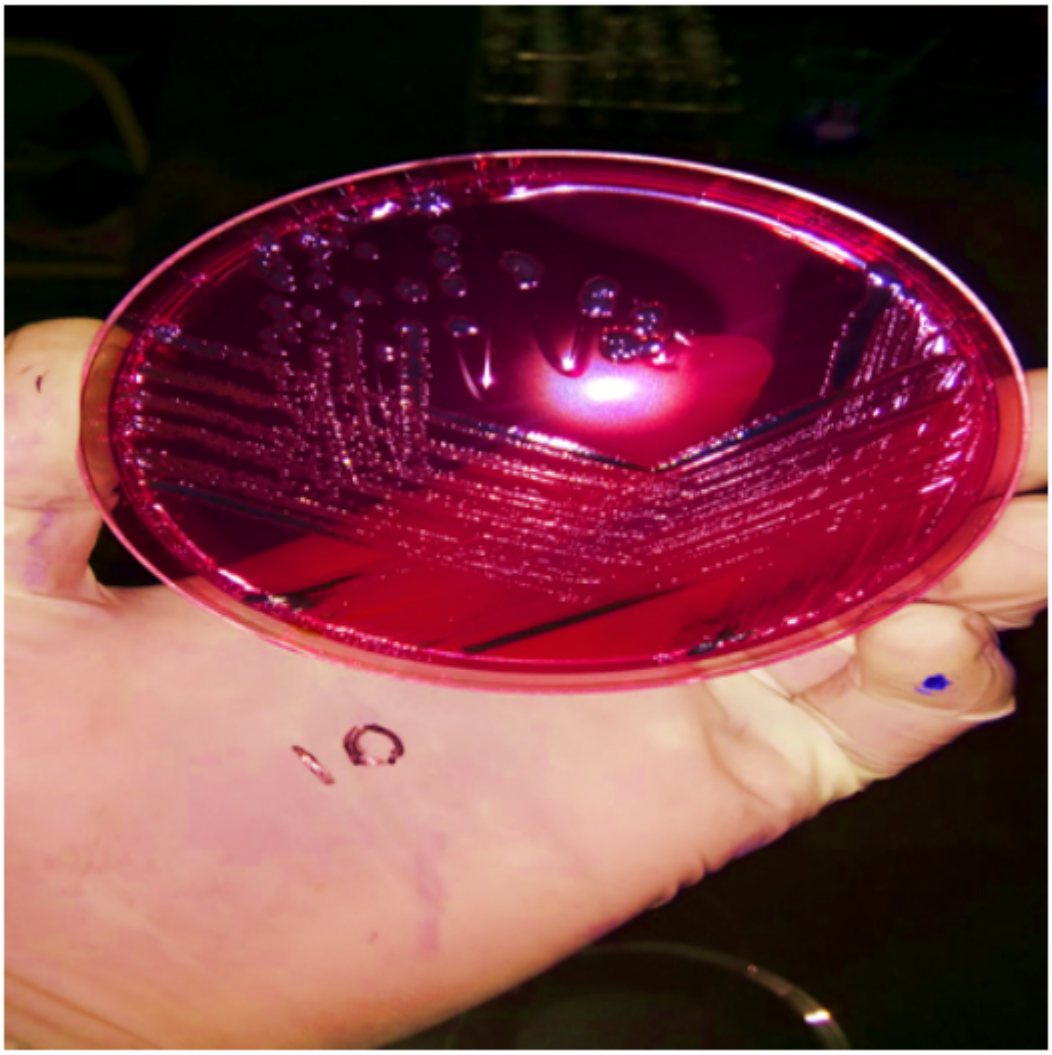

Figure 3

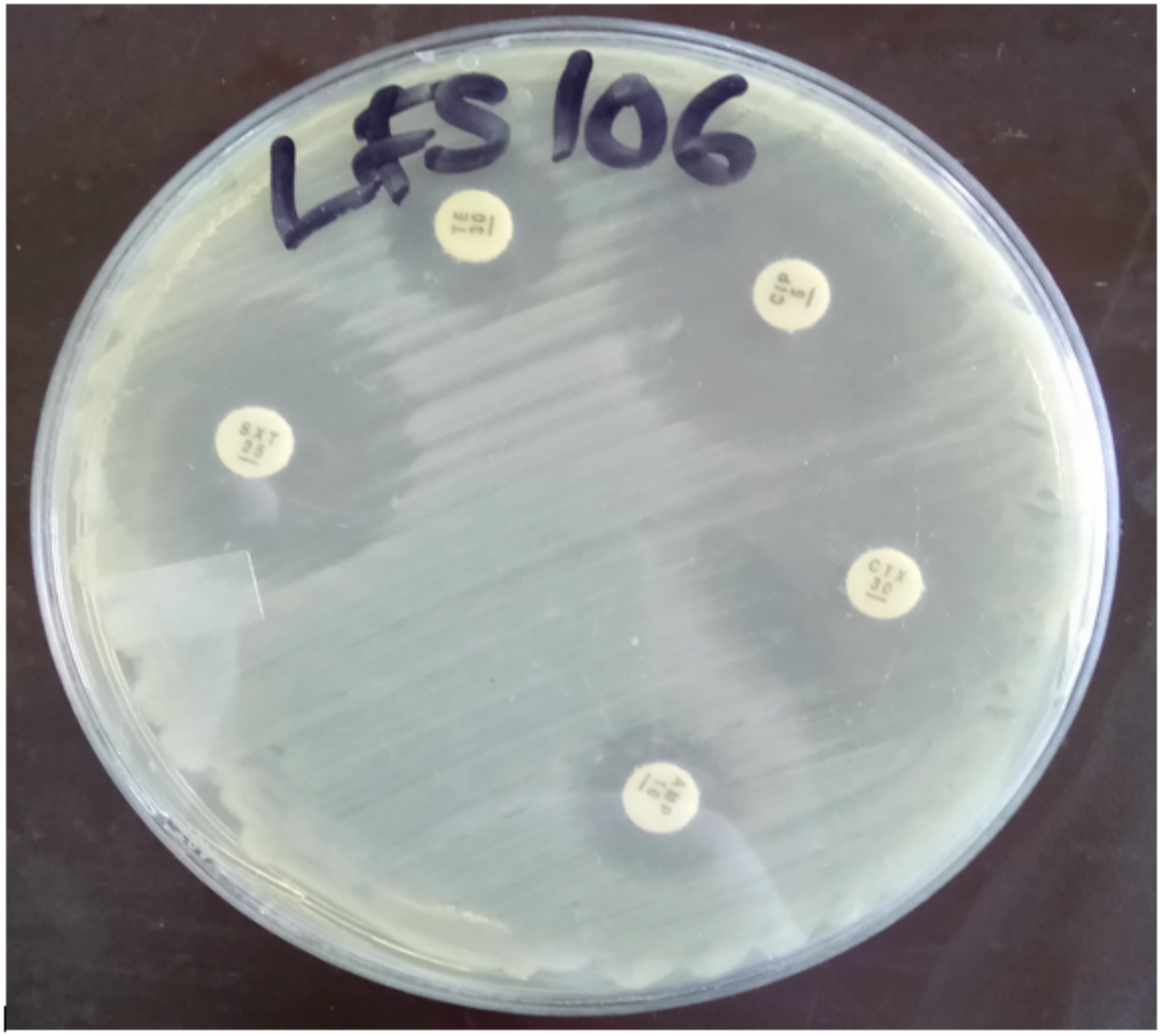

Figure 4

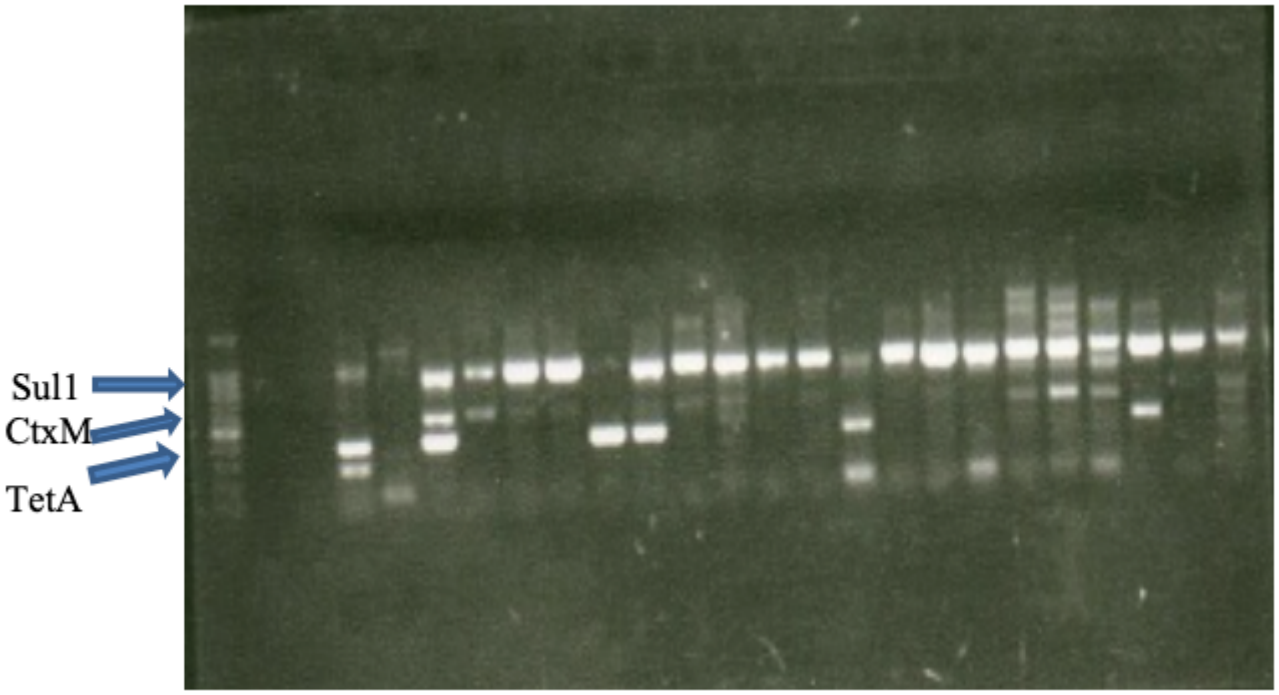

Figure 5