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Antibiogram and Diversity of Extended-Spectrum Beta-Lactamase Genes in Scavenging Local Chicken in Morogoro Municipality, Tanzania

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A B S T R A C T

The poultry industry, especially chicken production has in recent times faced a major set-back due to devastating effects of APEC Extended Spectrum Beta-Lactases (ESBL) producing organisms. This research aimed at investigating antimicrobial susceptibility profile and diversity of ESBL producing avian pathogenic *Escherichia coli*(APEC) among fecal cloacal swaps of scavenging local chickens based on the various housing systems. The APEC isolates were determined by virulence factor profiling and by Kirby-Baeur disc diffusion, 42% of the APEC isolates were found to be ESBL producers. Of the ESBL isolates, 87.5% were resistant to nalidixc acid, 37.5% were resistant to cefotaxime, Trimethroprim-Sulfamethoxazole, augmentnin and cephalothin, 25% were resistant to Ceftriaxone whiles no isolate was resistant to Imipenem, gentamycin and ciprofloxacin. On screening, a total of 32 beta-lactamase genes were found amongst these isolates, all of these isolates harbored the *bla*TEM gene.Semi-intensively kept chickens harbored more ESBL genes and in more diverse forms than the extensively kept ones.

Introduction

Avian pathogenic *Escherichia coli* is responsible for the annual million-dollar loss in the poultry industry worldwide. APEC and other Extended spectrum beta-Lactamase (ESBL)-producing organisms pose major health and economic threats to livestock production, especially in the poultry industry Selective pressure exerted by antimicrobials leads to the spread of multidrug resistance among avian *Escherichia coli*(Johnson *et al.*, 2004). ESBLs have been widely identified in *Escherichia coli*(*E. coli*) from both healthy and diseased animals and hence considered epidemiologically important (Leigue *et al.*, 2013). It was reported that apparently-healthy poultry could harbor multidrug resistance of

extra-intestinal *E. coli* (Lima-Filhi *et al.*, 2013). This presents a health risk to the main consumers, the human populace. Needless is to say, the increasing incidences of ESBL-infections among humans is attributable to the contamination of retail chicken by bacteria that carry them (Cohen-Stuart *et al.*, 2012).

Scavenging local chickens makes over 70% of the entire chicken population in Tanzania (Minga *et al.,* 2001).Ironically, studies on ESBLs have shunned away from these birds, although they stand a high chance of being the main source of transfer to humans than any poultry. Majority of these birds are kept mainly as free-ranged. A few of them, however, are housed as semi-intensive and a far lesser percentage of them are kept entirely intensive. This is hypothesized to have effect on the control of disease amongst them (Fotsa, 2011).

The aim of this research is to access the diversity of ESBL genes in avian pathogenic *Escherichia coli* amongst scavenging local chickens in selected areas of Morogoro Municipality, Tanzania.

Materials and Methods

Study area and sample collection

The Morogoro municipality. It has a total area of about 531.6 km^2 and a population growth rate of 4.7% per annum. The percentage of the populace thatis engaged in livestock keeping and subsistence farmingis 33% (National Bureau of Statistics, (2011)). The municipal is subdivided 29 administrative wards, out of which six vicinities were randomly selected. These are: Vibandani, Mazimbu, Kididimo, Misufini, Magadu and the farm at the department of Animal Science and Production at the Sokoine University of Agriculture (figure 1). A total of 400 swabs were collected from six different locations in municipality. The swabs were collected and kept in transport media and transferred into the laboratory on ice.

Isolation of *Escherichia coli* and DNA extraction

Procedures used were as described in the Bacterial Analytical Manual BAM 2007. The organisms were grown on MacConkey and Blood Agar media media to detect positive *Escherichia coli.* Samples were suspected to be *E.coli* based on morphological appearance. The suspected samples were confirmed by biochemical tests.

Virulence factor profiling to detect APEC strains

The positive *E.coli* were investigated for various virulence genes by multiplex PCR, with protocol based on Ewers *et al.,* (2007). Isolates that contain four or more virulence genes were considered APEC isolates (Lima-Filho *et al.*, 2013). The virulence genes that were screened include were in 5 categories; Iron Acquisition (*Chu A, Iro N, Irp 2, Iuc D, Sit chr,sit ep.*), Serum resistance (*Cvi/Cva, Iss, Omp A, Tra t*) Adhesins (*Pap C, Tsh*), Toxins (*Ast A, vat*) and Invasins (*Gim B,Ibe A*). The procedures were performed in 25µlreaction mixture. This includes: 12.5 µl ofTaq polymerase (Dream Tag PCR Master mix, Inqaba Biotec East Africa Ltd), 0.5 µl of each 100Mm dNTP, 0.1µl(100pmol) oligonucleotide primer pair, 6.9 µl of nuclease-free water and 4µl of template DNA. Primer concentration is 0.4 M. Conditions of the reaction mixtures include: 5mins at 95ºC initial denaturation,94ºC of denaturation for 30s, annealing at 56ºC for 30s, elongation at72ºC for 3minutes at 25 cycles, a final elongation at 72ºC for 10 minutes and a hold at 4ºC. List of primers used is shown in the appendix 3.

Kirby-Bauer disc diffusion test on APEC strians: to detect potential ESBL producers

The Kirby-Bauer antimicrobial sensitivity test method was used to determine the isolates that were susceptible to cephalosporins and betalactams, potential ESBL producers. However, susceptibility test were carried on other drugs as well, thus a total of ten antimicrobial drugs were used. These include; augmentinin (30µg), imipenem (10µg), cephalothin (30µg), cefotaxime (30µg), ceftazadime (30µg), ceftriaxone (30µg).The zones of inhibition were measured and the resistance was recorded based on Clinical and Laboratory Standards Institute (CLSI).

Double disc synergy test: to confirm of ESBL producers

As described by Ravi *et al.*(2011), the double disk synergy was performed on the suspected ESBL producers for confirmation. The disks of ceftazadime (30µg), ceftriaxone (30µg) and cefotaxime (30µg) were place around an augmentin disk (30µg), 20mm apart, on a Mueller-Hinton Agar plated swabbed with the test isolate. Enhancement of the inhibition zone of the cephalosporin toward the augmentin disc was interpreted as positive for ESBL production (Ravi *et al.*, 2011).

Screening of ESBL genes

Isolates that were positive for ESBL were screened to know the types of beta-lactamase (*bla*) genes they harbored. A total of eight samples were screened for the presence of 12 *bla* genes. The protocol was by Kiiru *et al.*, (2012). The reactions were carried out in a 25 µl reaction volume. This consist of 12.5 µl of taq polymerase, 1 µl each of the primer sequence, 5.5 µl of the Nuclease free water and 5 µl of the DNA template. The primer concentration was 0.4M.

The PCR reactions included the following:5 minutes of initial denaturation at 95ºC,94ºC of denaturation for 30 seconds, annealing for 30 seconds, elongation at72ºC for 30 seconds at 30 cycles, a final elongation at 72ºC for 10 minutes and a hold at 4ºC.The annealing temperatures were different for the different primers. The primers used for this and the corresponding primers sequences, as well as the annealing temperatures have been listed in Appendix 1.

Analysis

Statistical analysis was done by use of Microsoft excel 2003/7 and Epi. Info. Proportions of various characteristics were tested by use of the chi-square test (x^2). The threshold for statistical significance was indicated in the table with a $P < 0.05$ reflected statistical significance. In biological analysis; the following software were employed: MEGA 7, Sequencing products were analyzed on the National Committee for Biotechnology Information (NCBI) using the basic alignment search tool BLAST.

Results and Discussion

Antibiogram of ESBL isolates

PCR amplification to detect the virulence genes showed that 19 out of 192 samples, (9.8%) were APEC positive. Thus, these isolate had at least 5 virulence factors, 42% of the APEC isolates were found to be ESBL producers.

As much as 87.5% of ESBL isolates were resistant to nalidixc acid, 37.5% were resistant to cefotaxime, Trimethroprim-Sulfamethoxazole, augmentnin and cephalothin, 25% were resistant to Ceftriaxone whiles no isolate was resistant to Imipenem, gentamycin and ciprofloxacin

Prevalence and diversity of ESBL isolates

The phenotypically positive ESBL isolates were screened for the presence of twelve beta-latamase genes, out of which seven different genes were found to be present in various percentages. The highest was *bla* TEM which recorded a 100% prevalence. This was followed by bla OXA-1 with 75%. Then *bla* CMY-2 group, CTX-M group III, with 62.5% and 50% respectively. CTX-M group1 and *bla* SHV recorded the least prevalence of 12.5% each (Table 2). PCR detection of blaCMY-2 gene (758bp) showed in figure 2

Forty percent of the semi-intensive isolates were observed to be multi-drug resistant, while none of the extensively kept isolates were. The former harbored all the various types of genes including CTX-M group I and *bla* SHV genes which were absent in the later. Each of the CTX-M group IV, III and *bla* CMY were harbored by 40% of samples from semi-intensive chicken. In the extensive ones they were harbored by 33.33%, 66.67% and 100% respectively. Whiles all samples of extensive harbored *bla* OXA 1, *bla* TEM and *bla* CMY-2, they were harbored by 60%, 100% and 40% respectively in semi-intensive isolates (Table 3).

PCR products of the APEC isolates were sequenced using the Sanger sequencing method. The sequencing products were blasted on the NCBI website and various samples revealed identities to various genes at different identities as shown below. When blasted the *bla*SHV gene detected a LEN-2 gene with an identity of 92% and the bla CTX-M 9 gene detected a CTX-M-15 gene with an identity of 92%. (Table 4)

The *bla*TEM and CTX-M genes have been observed to be the most abundant ESBL genes as reported by other researchers and seen here (Mshana *et al.*, 2013). In a cross-sectional study to identify ESBL producing *Enterobacteriaceae* from rectal and cloacae swabs of 600 companion and domestic animals in Mwanza, Tanzania,(Schaumberg *et al.*, 2014), the TEM and CTX-M (specifically CTX-M-15) genes were the most prevalent ESBL genes. In that study, *bla*TEM was harbored by 60% of the isolates. In this study, the TEM gene was the highest gene recorded and was harbored by all isolates (Table 2). Their study also revealed that the CTX-M-15 was present among all isolates. The CTX-M-15 gene was detected with an identity of 92% to the CTX-M 9(group IV) gene when blasted (Table 4), in this present study. It is one of the most prevalent variants in the world and are mainly associated with the FII plasmids (Nagano *et al.*, 2009). The CTX-M-15 is related to phylogenetic group B2, a virulent extra-intestinal strain (NCBI-BLAST). Its association with apparently healthy chicken is somewhat alarming.Two years before the Tanzanian study, there was an assessment of the risk of importing ESBL producing *Enterobacteriaceae* and *S. aureus* through chicken meat in Gabon (Sambo *et al.*, 2015). It was also realized that CTX-M-1 and CTX-M-14 were predominant in ESBL *E. coli* from chicken. In this present study, the CTX-M1, together with other genes in the CTX-M group I was detected by the primer CTX-M1. On the other hand, the CTX-M-14 belonging to CTX-M group IV was detected by the CTX-M914 primer (Table 4). Together, these genes were harbored by 50% of *E. coli* in this study (Table 2). In effect, the CTX-M and TEM genes of ESBL are still predominant among *E. coli* in chicken. The is the most common plasmid mediated AmpC beta-lactamase (Shaheen *et al.*, (2011). The *bla*CMY-2 gene is knownto offer strong resistance against oxyimino-cephalosporins (cefotaxime, ceftriaxone and ceftaxidime) and as such, 80% of isolates harboring this gene showed resistance to the oxyimino-cephalosporins (Table 1). Shaheen *et al.*,

(2011) reported that the *bla*CMY- gene was widely distributed and exhibited resistance against oxyimino-cephalosporins. Although they employed companion animals (as against chicken in this study) and worked in the United States (as against Africa), similar results could only imply that regardless of location and kind of isolates, CMY-gene exhibits high resistance against oxyimino-cephalosporins. On the blast, the *bla*CMY-2 gene detected an OXY-1 gene with an identity of 92% (Table.4), which was previously known to be associated *Klebsiella oxytoca.* (González-López *et al.*, 2009).

The *bla*SHV- gene was one of the least detected (12.5%) (Table2). Derivatives of the SHV-1 gene, unlike their projenitor, is known to confer resistance to broad-spectrum penicillinas well asoxyimino-cephalosporins (Peirano *et al.,* 2010). In line with this, the only *bla*SHV-harbored isolate was resistant to all oxyimino-cephalosporins (Table 1).When blasted the *bla*SHV gene detected a LEN-2 gene with an identity of 92% (Table 4). This gene was hitherto known to be only associated to *K. pneuminiae* and does not hydrolyze extended-spectrum cephalosporins (Shayan and Bokaeian, 2015).

Generally, beta-lactamase genes were found to be widely distributed and more diverse among isolates from the semi-intensively kept birds than the extensive ones. Thus, the beta-lactamase genes thrived better amongst semi-intensively kept birds that the extensive ones.

Table.1 Antimicrobial resistance profile of ESBL producers (n=8)

	Susceptible isolates		**Intermediate isolates**		**Resistant isolates**	
Antimicrobial	**Number**	**Percent**	**Number**	**Percent**	**Number**	**Percent**
CRO(30µg)	4	50	2	25	2	25
CTX(30µg)	1	12.5	4	50	3	37.5
CAZ(30µg)	4	50	3	37.5	1	12.5
CN(10µg)	8	100	0	0	0	0
STX(25µg)	5	62.5	0	0	3	37.5
AUG(30µg)	5	62.5	0	0	3	37.5
NA(30µg)	1	12.5	0	0	7	87.5
CIP(5µg)	3	37.5	5	62.5	0	0
KF(30µg)	3	37.5	2	25	3	37.5
IMI(10µg)	8	100	0	0	0	0

Note: Antimicrobial resistance profiles of APEC. CRO: ceftriaxone, CTX: cefotaxime, CAZ: ceftazadime, CN: gentamycin, STX: Trimethroprim-Sulfamethoxazole, AUG: augmentin, NA: nalidixic acid, CIP: ciprofloxacin KF: cephalothin, IMI: imipenem.

Table.2 Prevalence of beta-lactamase genes amongst ESBL producing isolates

Primer name	Target gene	Number (n=8)	Percentage
TEM	*bla* TEM	8	100
CTXM 1	CTX-M group I	1	12.50
CTXM 914	CTX-M group IV	3	37.50
CTXM 825	CTX-M group III	4	50.00
CMY 2	*bla* CMY-2 group	5	62.50
OXA 1	*bla* OXA-1	6	75.00
SHV	*bla* SHV	1	12.50

Note: Among the beta-lactamase genes, the highest recorded was *bla* TEM, followed by OXA-and CMY-2 while SHV was the least.

Table.3 Occurrence of *bla* genes among housing systems of scavenging local chicken

ESBL gene	Extensive(n=3)		Semi-Intensive(n=5)	
	Number	Percent	Number	Percent
***bla*TEM**	3	100	5	100
CTX-M group I	0	0	1	20
CTX-M group IV	2	33.33	2	40
CTX-M group III	1	66.67	2	40
***bla*CMY- 2**	3	100	2	40
***bla*OXA1**	3	100	3	60
***bla*SHV**	0	0	1	20

Identity of Bla genes found and local scavenging chicken

Table.4 Description of beta-lactamase genes on basic local alignment sequence tool (BLAST)

Gene	Housing system	Description gene	Identity	Accession
***bla*OXA 1**	Semi-Intensive	OXA-1	99%	NG 049392.1
	Extensive	OXA-1	99%	NG 049392.1
***bla*TEM**	Semi-Intensive	TEM 171	99%	NG 050214.1
	Extensive	TEM 171	99%	NG 050214.1
***bla*CTX-M 9**	Semi-Intensive	CTX-M-15	92%	FJ997866.1
	Extensive	OXY-1-6	92%	NG 049845.1
***bla*CMY-2**	Semi-Intensive	CMY-71	98%	NG 048859.1
	Extensive	CMY-71	98%	NG 048859.1
***bla*SHV**	Semi-Intensive	LEN-2	92%	NG 049274.1

Appendix.1 List of Betalactamase Genes

	Screening beta-lactamase genes		
Target Gene	**Primer**	**5'-3' sequence**	**Size**
***bla*TEM**	TEM-F	ATGAGTATTCAACAT TTC CG	840
	TEM-R	CCAATGCTTAATCAG TGA GG	
***bla*SHV**	SHV-F	TTCGCCTGTGTATTATCTCCCTG	854
	SHV-R	TTAGCGTTGCCAGTGYTCG	
***bla*CTX-M concensus**	MA1	ATGTGCAGYACCAGTAARGTKATGGC	593
	MA2	TGGGTRAARTARGTSACCAGAAYCAGCGG	
CTX-M group I	CTXM1-F3	GAC GAT GTC ACT GGC TGA GC	499
	CTXM1-R2	AGC CG C CGA CGC TAA TAC A	
CTX-M group II	TOHO1-2 F	GCG ACC TGG TTA ACT ACA ATC C	351
	TOHO1-1R	CGG TAG TAT TGC CCT TAA GCC	
CTX-M group III	CTXM825F	CGC TTT GCC ATG TGC AGC ACC	307
	CTXM825R	GCT CAG TAC GAT CGA GCC	
CTX-M group IV	CTXM914F	GCT GGA GAA AAG CAG CGG AG	474
	CTXM914R	GTA AGC TGA CGC AAC GTC TG	
***bla*CMY (consensus)**	CF1	ATGATGAAAAAATCGTTATGC	1200
	CF2	TTGCAGCTTTTCAAGAATGCGC	
***bla*CMY-1 group**	CMY-1 F	GTGGTGGATGCCAGCATCC	915
	CMY-1R	GGTCGAGCCGGTCTTGTTGAA	
***bla*CMY-2 group**	CMY-2 F	GCACTTAGCCACCTATACGGCAG	758
	CMY-2R	GCTTTTCAAGAATGCGCCAGG	
***bla*OXA-1**	OXA-1 F	ATGAAAAACACAATACATATCAACTTCGC	820
	OXA-1R	GTGTGTTTAGAATGGTGATCGCATT	
***bla*OXA-2**	OXA-2 F	ACGATAGTTGTGGCAGACGAAC	602
	OXA-2R	ATYCTGTTTGGCGTATCRATATTC	

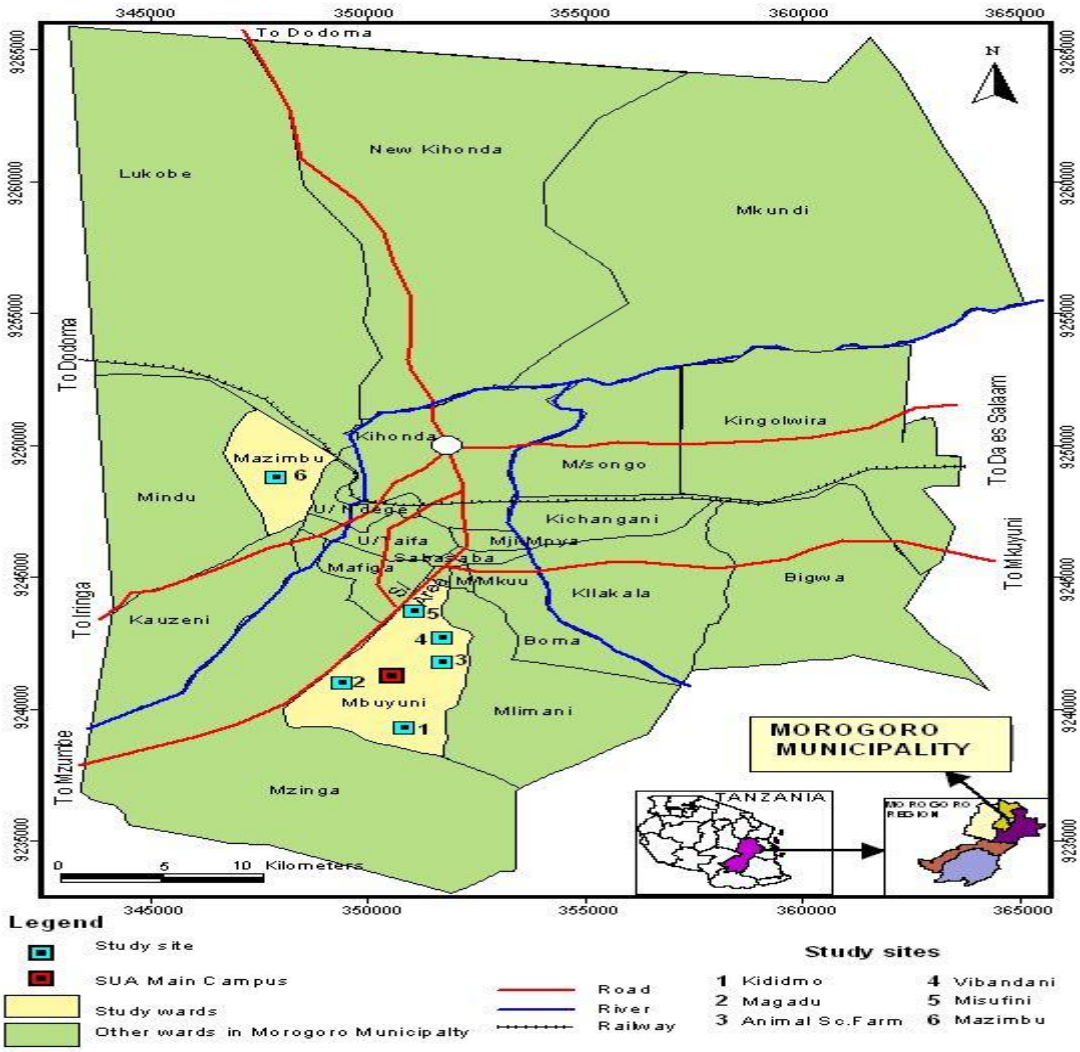

Figure.1 Map of Morogoro municipality wards (map constructed using Arc view GIS)

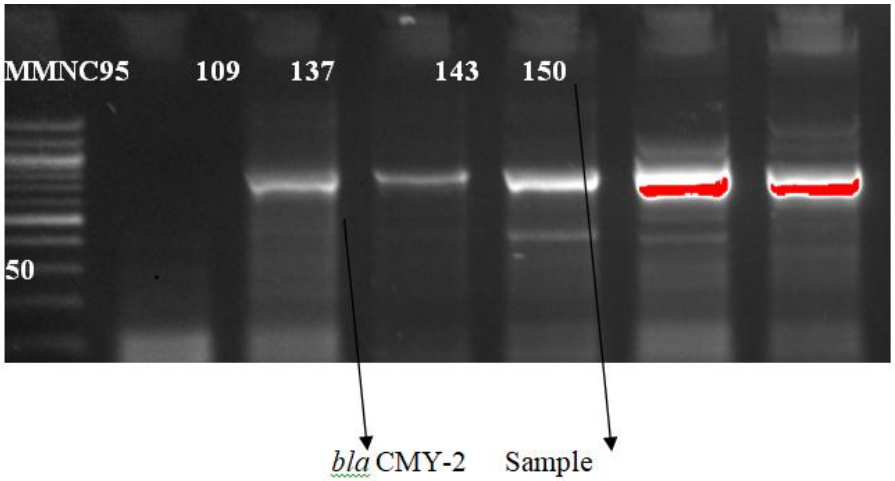

Figure.2 PCR detection of *bla*CMY-2 gene. Five samples (95, 109, 137, 143 and 150) were positive for the *bla*CMY-2(758bp); samples.NC is negative control. M is marker (100bp)

In reviewing the characteristics of scavenging local chickens in Africa, it was stated that, extensively kept chickens have adequate immuno-competence. This is because they roam extensively in the environment for their nutritional requirements and are free from vaccines and antimicrobials (Fotsa, 2011).Semi-intensive system of poultry rearing is characterized by farm in put supplies like drugs, feed and vaccine (Katakweba *et al.*, 2012). In supporting evidence, only 2 out of 3, (66.67%) of isolates from extensively kept birds were resistant to at least one of the antimicrobials used, whiles all isolates from the birds in the semi-intensive (100%) showed resistance at various levels. In reference to multi-drug resistance (resistance to more than two categories of drugs), none of the isolates from the extensively kept birds proved to be multi-drug resistant. On the other hand, 40% of isolates from the semi-intensively kept birds were multi-drug resistant.

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References

Bauer, A. W., Kirby, W. M., Sherris, J. C. and Turk, M. (1966). Antibiotic susceptibility testing by a standard single disc method. *American Journal of Clinical Pathology* 45: 493-496.

Center for Food Safety and Applied Nutrition (2007). United States Food and Drug Administration. *Bacteriological Analytical Manual*. 80pp.

Clinical Laboratory Standards Institute, (2014). *Updates and Recommendations for Antimicrobial Susceptibility Testing and Reporting*. Medialab.20pp.

Cohen-Stuart, J., Van den Munckhof, T., Voets, G., Scharringa, J., Fluit, A. and Hall, M. L. (2012). Comparison of ESBL contamination in organic and conventional retail chicken meat. *International Journal of Food Microbiology* 154(3): 212-214.

Ewers, C., Li, G., Wilking, H and Wieler, L. H. (2007). Avian pathogenic, uropathogenic and newborn meningitis-causing *Escherichia coli*: How closely related are they? International journal of medical microbiology 297(3):163-176.

Fotsa, J. C. (2011). Opportunities of poultry breeding programmes for family production in developing countries : The bird for the poor. Proceedings of FAO conference. Rome, Italy, 24January -18February, 2011.126pp.

González-López, J. J., Coelho, A., Larrosa, M. N., Lavilla, S., Bartolomé, R. and Prats, G. (2009). First detection of plasmid-encoded blaOXY β-Lactamase. *Antimicrobial Agents and Chemotherapy* 53(7): 3143-3146.

Johnson, J. R., McCabe, J. S., White, D. G., Johnston, B., Kuskowski, M. A. and McDermott, P. (2009). Molecular Analysis of *Escherichia coli* from retail meats (2002-2004) from the United States National Antimicrobial Resistance Monitoring System. *Clinical Infectious Diseases* 49(2): 195-201.

Katakweba, A. A. S., Mtambo, M. M. A., Olsen, J. E., and Muhairwa, A. P. (2012). Awareness of human health risks associated with the use of antibiotics among livestock keepers and factors that contribute to selection

of antibiotic resistance bacteria within livestock in Tanzania. *Livestock Research for Rural Development* 24(10): 9.

Kiiru, J., Kiriuki, S and Goddeeris, B. M. (2012). Analysis of beta-lactamase phenotype and carriage of selected beta-lactamase genes among *Escherichia coli* obtained from Kenyan patients during an 18-year peroid. *BiodMed Central Microbiology* 12(1): 155.

Leigue, L., Moura, R., Aguilar, P. and Lincopan, N. (2013).Current status of extended-spectrum beta-lactamase producing. *Enterobacteriaceae* in Animlas. *Microbial Pathogens and Strategies for Combating them: Science, Technology And Education* 3:1600-1607.

Lima-Filho, J. V., Martins, L. V., Ventura, R. F. and Evencio-Neto, J. (2013). Zoonotic potential of multidrug resistant extra-intestinal pathogenic Escherichia coli obtained from healthy poultry carcasses in Salvador, Brazil. *Journal Of Infectious Diseases* 17(1):54-61.

Minga, U., Mtambo, M. and Katule, A. (2001). Improving the health and productivity of the rural chicken in Africa: research and development efforts in Tanzania. *Australian Centre for International Agricultural* 2(1): 134–139.

Mshana S. E, M. Matee, and M. Rweyemamu, "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*, vol. 12, no. 1, article 28, 2013.View at: Publisher Site | Google Scholar

Nagano, Y. I., Nagano, N., Wachino, J., Ishikawa, K. and Arakawa, Y. (2009). Novel chimeric beta-lactamase CTX-M-64, a hybrid of CTX-M-15-like and CTX-M-14 beta-lactamase, found in a *Shigella sonnei* strain resistant to various oxyimino-cwphalosporins, including ceftazidime. *Antimicrobial Agents Chemotherapy* 53(1): 69-74.

National Bureau of Statistics, Ministry of Planning, Economic and Empowerment, (2011). *Morogoro Regional and District Projections*, volume XII, Dar es Salaam, Tanzania. 194pp.

National Committee for Biotechnology Information, Basic Local Alignment Sequence Tool (NCBI-BLAST)

Peirano, G. and Pitout, J. D. D. (2010). Molecular epidemiology of *Escherichia coli* producing CTX-M beta-lactamase: the worldwide emergence of clone ST131 O25:H4. *International Journal of Antimicrobial Agents* 35(4): 316-321.

Ravi, S. G., Namratha, W. N., Krishna, B. V. S., Asha, B. P. and Chandrasekhar, M. R. (2011). Comparison of disc diffusion methods for the detection of extended-spectrum beta lactamase-producing Enterobacteriaceae. *Journal of Laboratory Physician* 3(1): 33-36.

Sambo, E., Bettridge, J., Dessie, T., Amare, A., Habte, T., Wigley, P. and Christley, R. M. (2015). Participatory evaluation of chicken health and production constraints in Ethiopia. *Preventive Veterinary Medicine* 118(1): 117-127.

Schaumburg, F., Alabi, A. S., Frielinghaus, L., Grobusch, M. P., Kock, R., Becker, K., Issifou, S., Kremsner, P. G., Peters, G. and Mellmann, A. (2014). The risk to import ESBL-producing Enterobacteriaceae and Staphylococcus aureus through chicken meat trade in Gabon. *BMC*

Microbiol 14: 286pp.

Shaheen, B. W., Nayak, R., Foley, S. L., Kweon, O., Deck, J., Park, M., Rafii, F. and Boothe, D. M. (2011). Molecular characterization of resistance to extended-spectrum cephalosporins in clinical *Escherichia coli* isolates from companion animals in the United States. *Antimicrobial Agents and Chemotherapy* 55(12): 5666-5677.

Shayan, S. and Bokaeian, M. (2015). Detection of ESBL- and AmpC-producing *E.coli* isolates from urinary tract infections. *Advanced Biomedical Research* 4: 220.

Tamura, K., Stecher, G., Peterson, D., Filipski, A.,Sudir, K. (2013) MEGA: Molecular Evolutonary Genetics Analysis version 7.0. *Molecular Biology and Evolution:* 302725-2729.

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