Detection of extended-spectrum β-lactamase producing *Escherichia coli* in retail chicken meat and humans in Bulawayo, Zimbabwe

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Abstract

Introduction: Infections due to extended spectrum β-lactamase (ESBL) strains pose a major challenge in the management of infections worldwide.

Aim: This study was conducted to detect ESBL producing *Escherichia coli* associated with retail chicken meat and humans in Bulawayo, Zimbabwe.

Materials & methods: A total of 120 *E.coli* isolates obtained from poultry samples and 64 isolates associated with urinary tract infections in humans were collected and analyzed for ESBL production. ESBL producers were determined using antimicrobial susceptibility tests and the polymerase chain reaction was used to detect ESBL genes (Bla_{TEM}, Bla_{SHV}, Bla_{CTX-M} and Bla_{OXA}). A *Klebsiella pneumonia* isolate which was positive for the Bla_{TEM} (Genbank accession number KT818790), Bla_{SHV} (KT818791) & Bla_{CTX-M} (KT818792) was used as the positive control.

Results: Fifty nine (49%) of the isolates (chicken meat) and 40 (63%) (UTI) were found to be ESBL producers. The TEM, CTX-M and SHV genes were successfully detected in both meat and UTI samples. No OXA genes were detected. The prevalences of the ESBL genes were; TEM 23.3% (chicken isolates) and 29.7% (UTI isolates), CTX-M, 5 and 20,3%, SHV 4.2 and 10.9%. Typing of the *E.coli* isolates using Amplified Ribosomal DNA Restriction Analysis (ARDRA) showed a high degree of similarity between human and meat isolates.

Conclusion: Presence of ESBL genes in poultry destined for human consumption points to a potential reservoir for transmission of these genes to humans.

Key words: Extended spectrum β-lactamase, *Escherichia coli,* poultry, Zimbabwe

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Introduction

Extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae are on the increase worldwide (BIOHAZ, 2011; Cantón et al., 2008). The abundant presence of ESBL genes in the food chain may have a profound effect on future treatment options for a wide range of infections caused by Gram negative bacteria (Overdevest et al.,2011). The relatively narrow spectrum β-lactamases are often called penicillinases or cephalosporinases. Others have a much broader spectrum, and can hydrolyze many different β lactams including 3rd- and 4th-generation cephalosporins and aztreonam but not carbapenems (Poole, 2004). The majority of the ESBLs are mainly composed of the TEM (Temoniera), SHV (Sulphydryl Variable), CTX-M (Cefotaxime-Munich) and OXA enzymes (Johann et al., 2007). ESBL production has been reported in *Escherichia coli* associated with urinary tract infections (Pitout and Laupland, 2008), as well as commensal *E. coli* strains isolated from humans and food-producing animals (Trott , 2013; Mevius et al., 2012). Surveillance of antimicrobial resistance in commensal bacteria from food producing animals is considered as one of the main priorities of the World Health Organization (WHO) and the European Commission to better control the spread of antimicrobial resistance from food animal products to humans through the food chain (Cantón et al., 2008). In many cases, the origin of *E. coli* that causes infection in humans remains unknown, and the significance of the animal reservoir of antimicrobial-resistant *E. coli* has not been quantified. However, treatment options in humans

are compromised if the causative bacteria are already resistant to commonly used antimicrobial agents. Due to heavy use of antimicrobial agents in food animal production, bacteria originating from food animals frequently carry resistance to a range of antimicrobial agents, including those commonly used in humans (Hammerum and Heuer, 2009). Resistant *E.coli* can be transmitted to humans from animals. A large proportion of resistant isolates causing human infections are derived from food animals (Collignon et al., 2013). *E. coli* from meat has mostly been associated with intestinal pathogenic *E. coli* but recently *E. coli* of animal origin has been shown to also be associated with extra-intestinal infections, such as urinary tract infections. In the Netherlands, ESBL-producing *E. coli* was found to be highly prevalent in poultry (Dierikx et al., 2013) and an overlap between ESBL genotypes from chicken meat and clinical *E. coli* isolates has been established in previous studies, (Overdevest et al.,2011; Leverstein-van Hall et al.,2011) implying that chicken meat may be a source of ESBL-producing *E. coli* (Leverstein-van Hall et al.,2011; Campos et al.,2014) Previous studies from Europe have reported high ESBL contamination rates of chicken meat notably those from the Netherlands (Overdevest et al.,2011), Sweden (Börjesson et al., 2013), and Germany (Kola et al., 2012).

In Africa studies on ESBL producing Enterobacteriaceae in both human isolates (Abdelaziz et al., 2013; Obeng-Nkrumah et al., 2012) and retail chicken meat (Chishimba et al.,2016; Abdallah et al., 2015) have revealed that ESBL producing Enterobacteriaceae are a growing problem on the continent. In Zimbabwe no reports have been published as yet on the occurrence of ESBL producing organisms in humans or in retail chicken meat, this study therefore sought to determine the carriage of ESBL producing *E.coli* in retail chicken meat and in humans.

Materials and Methods

Chicken meat survey

Chicken meat samples were collected from 9 different retail butcheries and 2 chicken abbatoirs and 1 backyard processing industry in Bulawayo, Zimbabwe. A total of 120 chicken samples consisting of chicken pieces were collected and analysed. Ten samples were collected from each of the 12 sampling sites at different time intervals, over a six month period. Randomly chosen packages of chicken meat from retail butcheries were used, each sample contained raw and unspiced meat. Pre-packaged chicken samples were immediately transported at 4° C to the laboratory for bacterial isolation within 24 hrs. The pre-packaged samples were aseptically opened using a flamed scissors and the meat was swabbed with sterile cotton swabs which were then transferred into sterile tubes containing 0.8% peptone water. For back yard and abbatoir chickens; samples were swabbed on site. The swabs were transferred onto sterile tubes containing 0.8% peptone water and transported to the laboratory. A loop full of the peptone broth was streaked on MacConkey agar plates (Oxoid, Basingstoke, UK) and incubated at 37 ^{o}C for 24 hours. Confirmatory tests were carried out on the isolates and the identification of *E. coli* was performed according to methods described by Barrow and Feltham (1993) Biochemical tests included the Gram stain and the catalase, oxidase, indole and citrate tests.

For preliminary screening of ESBL producers the Double Disc Synergy Test (DDST) was carried out on Mueller-Hinton agar (New England Biolabs, Midrand, South Africa) using the Kirby-Bauer disc diffusion method. The plates were inoculated with a swab dipped into bacterial suspension which was prepared by inoculating 3 to 4 colonies into peptone water. The bacterial suspension was compared to a 0.5 McFarland's standard. The antibiotic discs used were Aztreonam (30 μg), Cefpodoxime (10 μg), Cefotaxime (30 μg) and Piperacillin-tazobactam (110 μg) (Mast Diagnostics, UK). The plates were incubated at 37 ^{o}C for 24 hours and the inhibition zones were measured. The test organism was said to be ESBL

producing if it had the following zones of inhibition; aztreonam ≤ 27 mm; cefotaxime ≤ 27 mm; ceftazidime ≤ 22 mm and cefpodoxime ≤17 mm (CLSI, 2011).

Uropathogenic *E. coli* samples

Sixty four (64) residual *E. coli* isolates were collected from two major diagnostics laboratories in Bulawayo. Only *E. coli* cultured from urine samples from symptomatic patients being tested for urinary tract infections (UTIs) were used in this study. On arrival at the National University of Science and Technology (NUST) microbiology laboratory, isolates were sub-cultured on MacConkey agar (New England Biolabs, Midrand, South Africa), and plates were incubated at 37°C for 24 hrs. Pink colonies with a darker centre appearing on the MacConkey agar plate, were then subjected to biochemical tests. Biochemical tests included the Gram stain and the catalase, oxidase, indole and citrate tests. Preliminary screening for ESBL producers was done using the DDST as described for the chicken meat samples.

Genetic detection of antibiotic resistance genes

Bacterial cells were sub cultured in Luria Bertani (LB) broth (Oxoid, Basingstoke, Hampshire, UK) and incubated overnight at 37° C. Genomic deoxyribonucleic acid (DNA) was then extracted using a standard phenol-chloroform method (Sambrook & Russell, 2001). PCR was performed to screen for the presence of four major ESBL genes OXA, TEM, SHV and CTX-M. The primers used in PCR for each gene have been described in previous studies (Sharma et al., 2013). All primers used were obtained from Inqaba Biotech, Pretoria, South Africa. One microlitre of each of the DNA samples was mixed with all the necessary components for amplification in a 0.2ml PCR tube (Perkin-Elmer Waltman, Massachusetts, USA) in a 10µl reaction. The reaction mixture included 1µl Dream Taq buffer (10X concentration) (Thermo Scientific, Waltman, Massachusetts, USA), 0.2µl of deoxyribonucleotide trisphosphate (dNTP) mix, 10mM, 0.16µl of each of the forward and reverse primers, 0.4µM (Inqaba Biotech, South Africa) and 0.08 µl of Dream Taq DNA

polymerase (Thermo Scientific, Waltman, Massachusetts, USA), 5U/µL and made up to 10µl with nuclease free water. Negative controls comprised of a water control. A *Klebsiella pneumonia* isolate positive for the bla_{TEM} (Genbank accession number KT818790), bla_{SHV} (KT818791) & $bla_{CTX\text{-}M}$ (KT818792) was used as the positive control. This was obtained courtesy of Mr J. Mbanga (NUST). An Applied Biosystems GeneAmp® PCR System 9700 was used for the PCR thermal cycling conditions. The PCR profile was as follows: for bla_{SHV}, $bla_{CTX\text{-}M}$ and bla_{TEM}; denaturation 95˚C for 2 minutes, 35 cycles (denaturation 95˚C for 30 seconds, annealing at 60˚C for 30 seconds, extension 72˚C for 1 minute) and then final extension at 72˚C for 10 minutes. The PCR profile for OXA was as follows: denaturation 95˚C for 2 minutes, 35 cycles (denaturation 95˚C for 30 seconds, annealing at 62˚C for 50 seconds, extension 72˚C for 1 minute) and final extension at 72˚C for 10 minutes. The amplified products were then run along a 1% ethidium bromide– stained agarose gel with a 100 bp DNA ladder (Thermo Scientific, Waltman, Massachusetts, USA) in TBE buffer for 1 hr at 100 V and then viewed using the Uvipro Silver Gel Documentation System (Uvitec, Cambridge, UK).

Amplified Ribosomal DNA Restriction Analysis (ARDRA)

ARDRA was used to evaluate the relationship between ESBL producing *E.coli* infecting humans and retail chicken meat. A total of 20 ESBL producing *E.coli* isolates were used in this part of the study. The isolates consisted of 10 uropathogenic *E.coli* (UPEC) and 10 retail chicken meat isolates. The 16S rDNA fragments were amplified by PCR using universal primers 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT) that were synthesized at Inqaba Biotech (Pretoria, South Africa). Five microlitres of each of the DNA samples were mixed with all the necessary components for amplification in a 0.2 ml PCR tube (Perkin-Elmer Waltman, Massachusetts, USA) in a 25 µl reaction. The reaction mixture included 2.5 µL of ×10 PCR Dream Taq buffer (Thermo Scientific, Waltman,

Massachusetts, USA), 2 µL of deoxynucleotide triphosphates (dNTPs) 10 mM; 0.5 µl of each of the forward and reverse primers (0.5 µM) (Inqaba Biotech, Pretoria, South Africa), 0.25 µL of Dream Taq polymerase (Thermo Scientific, Waltman, Massachusetts, USA), 5U/µL nuclease-free water to maintain a total volume of 25 µL. Negative control comprised of a water control. The PCR thermal profile used was an initial denaturation at 95 °C for 30 sec, 30 cycles of denaturation at 95°C for 30 sec, annealing at 60°C for 30 sec and extension for 72°C for 1min 30 sec, final extension at 72°C for 7min. The presence and yield of specific PCR products was viewed on an ethidium bromide stained 1% agarose gel after gel electrophoresis.

The enzymatic digestions were performed in a total volume of 30 µL using 10 µL of amplified PCR product. A volume of 2 µL fast digest 10X green buffer (Thermo Fisher Scientific, Waltman, Massachusetts, USA) was used for 1 µL of the enzyme *Taq* 1 (Thermo Fisher Scientific, Waltman, Massachusetts, USA) and topped up to 30 µL with nuclease free water. Digestion conditions were 5 minutes at 65°C for *Taq* 1. Restricted DNA was analysed by gel electrophoresis in an ethidium bromide stained 1.5% agarose gel. Electrophoreses were carried out at 100 V for 1hr 30min and the gel was viewed under a Uvipro-Silver Gel Documentation system (Uvitec, UK).

Results

Retail chicken meat survey

E. coli was isolated from retail chicken samples and identified through culturing and biochemical tests. A total of 120 *E.coli* isolates were obtained from 12 sampling points used in the study and these comprised, 9 retail butcheries, 2 chicken abattoirs and 1 backyard market. The double disc synergy test was used to screen for ESBL producers and 59 (49.2%)

of the isolates were found to be ESBL producers (Table 1). The ESBL producing *E.coli* isolates from retail chicken meat carried the *bla*$_{TEM}$, bla$_{SHV}$ and *bla*$_{CTX-M}$ genes or a combination. The *bla*$_{OXA}$ gene was not detected in all of the isolates. A total of 28 (23.3%) were positive for the *bla*$_{TEM}$ gene, followed by 6 (5%) *bla*$_{CTX-M}$, 5 (4.2%) for *bla*$_{SHV}$ (Table1). Of the 12 retail outlets sampled, 10 (83.3%) were confirmed to have ESBL genes, only 2 butcheries (16.7%) did not have *E.coli* harboring ESBL genes (Table 2).

Table 1: Distribution of TEM, SHV, and CTX-M genes in retail chicken *E. coli* isolates

Detected Gene(s)	**Number of *E.coli* isolates**	**% *E. coli* isolates** (n = 120)[a]
CTX-M	6	5
SHV	5	4.2
TEM	28	23.3
CTX-M & SHV	0	0
CTX-M & TEM	5	4.2
SHV & TEM	2	1.7
CTX-M, TEM & SHV	1	0.8
None[b]	20	33.9
Proven ESBL producers	59	49.2

[a]120 suspected ESBL producers were assayed
[b]negative for all assayed ESBL genes

Table 2: Distribution of TEM, SHV, CTX-M and OXA genes in *E.coli* in the Bulawayo market

Retail outlet	TEM	SHV	CTX-M	OXA
BUT1	✓	✓	-	-
BUT2	-	-	-	-
BUT3	-	-	-	-
BUT4	✓	-	-	-
BUT5	✓	✓	✓	-
BUT6	✓	-	-	-
BUT7	✓	-	✓	-
BUT8	✓	✓	-	-
BUT9	-	-	-	-
AB1	✓	✓	✓	-
AB2	✓	-	-	-
BYD	✓	-	✓	-

✓ Present - absent BUT- Butchery AB – Abbatoir BYD – Backyard industry

Human UTI survey

A total of 64 residual *E. coli* isolates obtained from urine samples of individuals suffering from UTIs were also screened for ESBL production. Phenotypic screening revealed that the majority 40/64 (62.5%) of the tested isolates were ESBL producers (Table 3). The ESBL producing *E. coli* isolates from humans carried the bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$ genes or a combination. The bla_{TEM} was detected in 19 (29.7%), $bla_{CTX\text{-}M}$ in 13 (20.3%) and the bla_{SHV} in 7 isolates (10.9%) (Table 3). Four isolates (6.3%) had a combination of the bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$ clusters, 5 (7.8%) isolates had the bla_{TEM} and $bla_{CTX\text{-}M}$ cluster, and 3 (4.7%)

isolates had both bla_{SHV} and $bla_{CTX\text{-}M}$ (Table 3). The most commonly detected ESBL gene in both chicken and human isolates was the bla_{TEM} cluster.

Table 3: Distribution of TEM, SHV, and CTX-M genes in Human *E. coli* isolates

Detected Gene(s)	Number of *E.coli* isolates	% *E. coli* isolates (n = 64)[a]
CTX-M	13	20.3
SHV	7	10.9
TEM	19	29.7
CTX-M & SHV	3	4.7
CTX-M & TEM	5	7.8
SHV & TEM	0	0
CTX-M, TEM & SHV	4	6.3
None[b]	1	1.6
Proven ESBL producers	40	62.5

[a]64 suspected ESBL producers were assayed
[b]negative for all assayed ESBL genes

Amplified Ribosomal DNA Restriction Analysis (ARDRA)

The relationship between ESBL producers from the chicken and human isolates was investigated using ARDRA. A total of 20 *E. coli* isolates were used in this part of the study, 10 uropathogenic *E. coli* (UPEC) and 10 isolates from retail chicken meat.

Genomic DNA was isolated from all the isolates using the phenol/chloroform method and the 16S rDNA gene was amplified (Figure 1). Restriction digestion analysis was carried out using *Taq* 1 endonuclease (Figure 2). Similar banding patterns were generated between the UPEC and retail meat isolates.

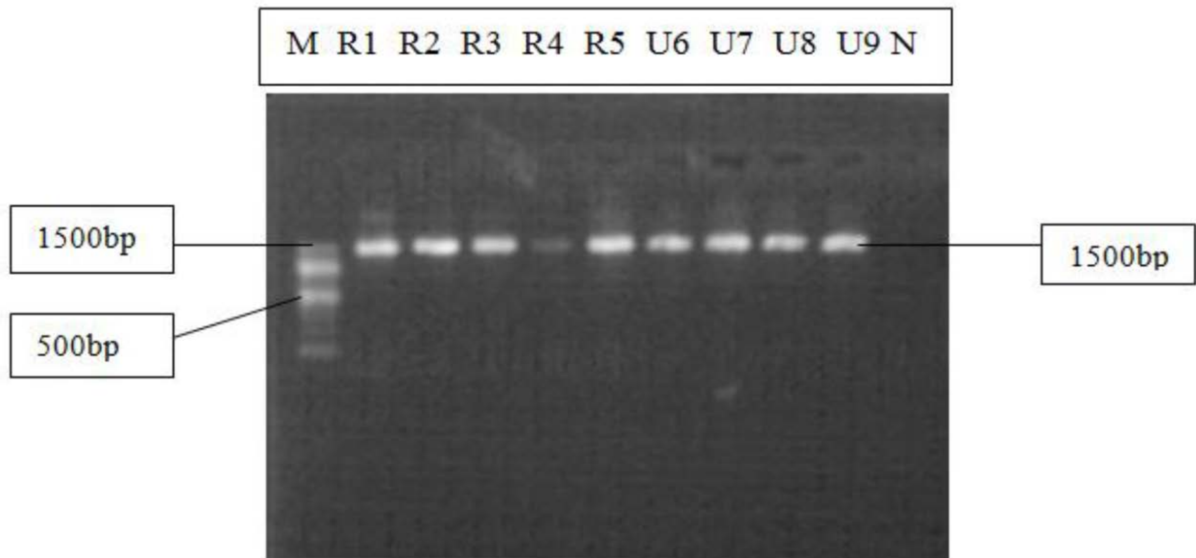

Figure 1:16S rDNA PCR products. Lanes R1-R5 retail chicken meat isolates and lanes U6-U9 UTI isolates. Lane M, 100bp DNA Ladder (New England Biolabs, USA) and lane N negative water control.

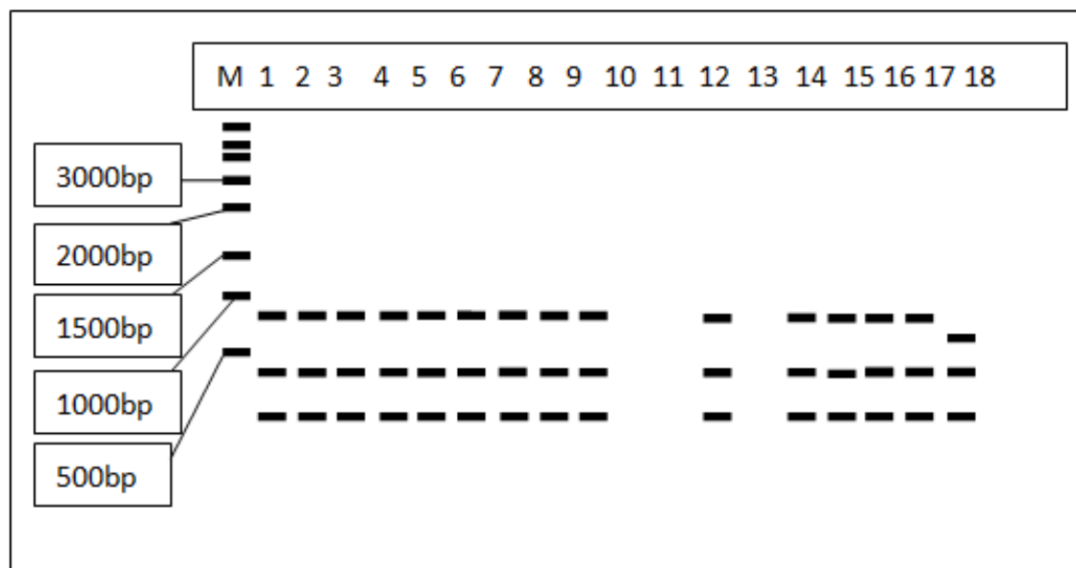

Figure 2: Schematic representation for *Taq*1 digestion patterns. Lanes 1-10 retail Meat isolates; lanes 11-18, UPEC.

Discussion

This study shows the presence of ESBL producing *E. coli* which is associated UTIs in Bulawayo. ESBL detection is not routinely carried out in many microbiology units in developing countries, Zimbabwe included. This could be attributed to lack of awareness, resources and facilities to conduct ESBL screening. The high rate of resistance mediated by ESBL production noted among the *E. coli* isolates in the present study is of serious concern. Of the 64 isolates tested, 40 (62.5%) were found to be ESBL producers using the Double Disc diffusion method (Table 3). This was similar to the prevalence of ESBL producing *E. coli* associated with UTIs in Ghana (56.1%) (Obeng-Nkrumah et al., 2013). Slightly different results were obtained for the retail chicken meat samples with 59 out of 120 isolates (49.2%) testing positive for ESBL production using the DDST (Table 1). The prevalence of ESBL producing *E. coli* in chicken meat is consistent with findings of other investigators. Slightly higher results were found in Spain, where 67% of the chicken meat was reported to be contaminated with ESBL or ESBL-like resistance genes (Doi et al., 2010). A higher prevalence was also observed in Egypt were 66% of chicken meat was contaminated with ESBL producing *E. coli* (Abdallah et al., 2015). On the other hand, lower rates of ESBL producing *E.coli* in chicken meat have been reported in Zambia (Chishimba et al.,2015) and Gabon (Schaumburg et al., 2014). Phenotypic screening of ESBLs using the double disc diffusion method provides a quick and inexpensive means of ESBL detection which can be conducted in many settings including, rural settings (Harwalkar et al., 2013) where there are no structures, equipment or funding for molecular detection of this overgrowing threat of antibiotic resistance due to ESBL production. The major drawback of the double disc synergy in ESBL screening is that the sensitivity of the DDST strongly depends on the precise placement of the discs interpretation of the results is more biased and confusing at times ((Harwalkar et al., 2013). Detection of ESBL production is important, because it is recommended that any organism that is confirmed for ESBL production according to CLSI

criteria should be reported as resistant to all extended spectrum beta –lactam antibiotics, regardless of their susceptibility test results (Romanus et al., 2009).

The ESBL-producing *E. coli* isolates from both retail chicken meat and humans carried the β-lactamase genes of either $la_{\text{CTX-M}}$, bla_{SHV}, and bla_{TEM} or a combination. All the isolates were however negative for $bla_{\text{OXA.}}$ This may be due to the fact that most ESBLs are found in *E.coli, Klebsiella* species and other enterobacteria, but the OXA-type ESBLs are mostly found in *Pseudomonas aeruginosa* (Bradford, 2001). Our results for the detection of ESBLs by PCR, tally with those obtained by Bali *et al.* (2010). Their results revealed that most of the ESBLs were derivatives of bla_{SHV}, bla_{TEM} and $bla_{\text{CTX-M}}$ and none of the bla_{OXA}.

The major gene detected by PCR in both retail chicken meat and humans was the bla_{TEM} cluster , followed by $bla_{\text{CTX-M}}$ and bla_{SHV}. This differs from other studies where the $bla_{\text{CTX-M}}$ has been found to be the most common gene (Chishimba et al.,2016; Abdallah et al., 2015; Sharma et al., 2013; Doi et al., 2010). A $bla_{\text{CTX-M}}$ + bla_{TEM} cluster combination was the most common in both human (7.8%) and retail chicken meat (4.2%) (Table 1 & Table 3). This implies a close genetic similarity among extended-spectrum β-lactamase-producing *E. coli* isolated from chicken meat and humans as the concurrent presence of $\text{bla}_{\text{CTX-M}}$ and bla_{TEM} genes implies the carriage of these on the same plasmids (Leverstein-van Hall et al.,2011; Kluytmans et al., 2013).

Out of the 12 sampling points, 10 places were confirmed to have ESBL genes (Table 2). The frequent recovery of ESBL producing *E.coli* from retail chickens from different commercial sites, confirms the role of animals as possible reservoirs for dissemination of resistance genes in the community. Although there are extensive campaigns promoting safe handling of chicken meat during processing, enteric pathogens are frequently transferred to humans and pose a continuous public health threat (Overdevest et al., 2011). Gregova et al. (2012) showed that the occurrence of ESBLs in poultry meat could be related to the environmental

microbes of the slaughterhouse, to the processes of scalding, defeathering and evisceration and to that of bacteria transferred from chickens because of contact through water and improper cleaning and disinfecting. Furthermore, the meat products are cooked and then the colonisation in humans by antibiotic resistant *E.coli* takes place in the course of preparing and eating cooked products at home.

The high restriction band similarity between 16S rDNA genes of isolates that originated from ESBL *E. coli* from humans and chickens (Figure 2) suggested that there is transmission of ESBL *E. coli* from chickens to humans. An obvious limitation of this part of the study was that a few isolates were analysed using ARDRA, however the results (Figure2) were in agreement with a study done in the Netherlands in 2009 (Willemsen et al.,2009) that confirmed a high degree of similarity between drug resistance genes in retail chicken meat and human *E. coli*. The similarity of *E. coli* strains and predominant drug resistance genes in meat and humans provides circumstantial evidence for an animal reservoir for a substantial part of ESBL genes found in humans.

Conclusion

This is the first study from Zimbabwe showing high rates of ESBL producing *E. coli* isolated from retail chicken meat and humans. These results raise serious concerns about public health and food safety as retail chicken meat could serve as a reservoir for these resistant bacteria which could be potentially transferred to humans through the food chain. Analysis of *E.coli* isolates using ARDRA revealed that there is most likely a close relationship between ESBL *E.coli* from retail chicken meat and humans.

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Competing interests

The authors declare that they have no financial or personal relationship(s) which may have inappropriately influenced them in writing this paper.

Authors' contributions

This work was conducted by J.M, A.M and V.N at the NUST, Bulawayo. J.M (NUST) was responsible for the project design, was the principle supervisor responsible for the management of the project and the guidance of A.M & V.N in all aspects of the project. A.M & V.N (NUST) were involved in the experimental design and did experimental work in the project. All three authors contributed to the writing of this article.

References

1. EFSA Panel on Biological Hazards (BIOHAZ). Scientific opinion on the public health risks of bacterial strains producing extended-spectrum β-lactamases and/or AmpC β-lactamases in food and food-producing animals. EFSA J 2011; 9: 2322.

2. R. Cantón , A. Novais, A. Valverde, E. Machado, L. Peixe, F. Baquero, T.M. Coque. (2008). Prevalence and spread of extended-spectrum ßlactamase-producing Enterobacteriaceae in Europe, *Clin. Microbiol. Infect*. 14 (1):144–153. http://dx.doi.org/10.1111/j.1469-0691.2007.01850.x.

3. I. Overdevest, I. Willemsen, M. Rijnsburger, A., Xu, P. Hawkey, M. Heck P. Savelkoul, C. Vandenbroucke-Grauls, K. van der Zwaluw, X. Huijsdens and J. Kluytmans. (2011). Extended-spectrum beta-lactamase genes of *Escherichia coli* in chicken meat and humans, The Netherlands, *Emerg. Infect. Dis*. 17:1216–1222. http://dx.doi.org/10.3201/eid1707 .110209.

4. K. Poole. 2004. Resistance to Beta-lactam antibiotics. *Cell Mol Life Sci*. 61: 2200–23. doi: 10.1007/s00018-004-4060-9 PMID: 15338052.

5. D. D. Johann, M.D. Pitout, F. F. Path.(2007). Enterobacteriaceae Producing ESBLs in the Community: Are They a Real Threat. *Infect Med*.24: 57-65.

6. J.D.D. Pitout and K.B. Laupland. (2008). Extended-spectrum b-lactamase producing Enterobacteriaceae: an emerging public-health concern. *Lancet Infect Dis*, 8: 159-66.

7. D. Trott**.** (2013).Beta-lactam resistance in gram-negative pathogens isolated from animals, *Curr. Pharm. Des*.19: 239–249. http://dx.doi.org/10.2174/1381612138040703 39.

8. D.J. Mevius, M.G.J. Koene, B. Wit, W. van Pelt and N. Bondt. (2012). Monitoring of antimicrobial resistance and antibiotic usage in animals in the Netherlands in 2010/2011. Nethmap_MARAN report. http://www.uu.nl/SiteCollection Images/Fac_DGK/Nieuwsplaatjes/Nieuws/2012/NethmapMaran_Web.pdf.

9. Hammerum and O.E. Heuer. (2009). Human health hazards from antimicrobial-resistant Escherichia coli of animal origin**.** *Clin Infect Dis.* 48 (7): 916-21. doi: 10.1086/597292.009.

10. P. Collignon, F.M. Aarestrup, R. Irwin, and S. McEwen. (2013). Human Deaths and Third-Generation Cephalosporin use in Poultry Europe. Emerging Infectious Diseases, 19 (8). DOI: http://dx.doi.org/10.3201/eid1908.120681

11. C. Dierikx, J. van der Goot, T. Fabri, A. van Essen-Zandbergen, H. Smith and D. Mevius.(2013). Extended-spectrum-beta-lactamase- and AmpC-betalactamase- producing *Escherichia coli* in Dutch broilers and broiler farmers. *J. Antimicrob. Chemother*. 68:.60–67, http://dx.doi.org/10.1093/jac/dks349.

12. M.A. Leverstein-van Hall, C.M. Dierikx, J. Cohen Stuart, G.M. Voets, M.P. van den Munckhof, A. van Essen-Zandbergen, T. Platteel, A.C. Fluit, N. van de Sande-Bruinsma, J, Scharinga M.J. Bonten and D.J Mevius**.** (2011). Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. *Clin. Microbiol. Infect.* 17: 873–880. http://dx.doi.org /10.1111/j.1469-0691.2011.03497.x.

13. C.B. Campos, I. Fenner, N. Wiese, C. Lensing, M Christner, H. Rohde, et al. (2014). Prevalence and genotypes of extended spectrum beta-lactamases in Enterobacteriaceae isolated from human stool and chicken meat in Hamburg, Germany. *Int J Med Microbiol*. doi: 10.1016/j.ijmm.2014.04.012.

14. H. Blaak, RA. Hamidjaja, A.H.A.M. van Hoek, L. de Heer, A.M. de Roda Husman and F.M. Schets. (2014). Detection of extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli on flies at poultry farms. *Appl Environ Microbiol*. 80:239–46. doi: 10.1128/AEM.02616-13 PMID: 24162567

15. S. Börjesson, M. Egervärn, M. Lindblad, S. Englund. (2013). Frequent occurrence of extended-spectrum beta-lactamase-and transferable ampc beta-lactamase-producing Escherichia *coli* on domestic chicken meat in Sweden. *Appl Environ Microbiol*.79: 2463–6. doi: 10.1128/AEM.03893-12 PMID: 23354705

16. A. Kola, C. Kohler, Y. Pfeifer, F. Schwab, K. Kühn, K. Schulz, et al. (2012). High prevalence of extended-spectrum-β-lactamase-producing Enterobacteriaceae in organic and

conventional retail chicken meat, Germany. *J Antimicrob Chemother*. 67: 2631–4. doi: 10.1093/jac/dks295 PMID: 22868643.

17. M.O. Abdelaziz, C. Bonura, A. Aleo, T. Fasciana and C. Mammina. (2013). NDM-1- and OXA-163-producing *Klebsiella pneumoniae* isolates in Cairo, Egypt. *J Glob Antimicrob Resist*. 1:213–215.. doi: 10.1016/jjgar.2013.06.003.

18. N. Obeng-Nkrumah , K. Twum-Danso , K,A Krogfelt , M.J Newman.(2013). High levels of extended-spectrum beta-lactamases in a major teaching hospital in Ghana: the need for regular monitoring and evaluation of antibiotic resistance. *Am J Trop Med Hyg*. 89 (5):960-4. doi: 10.4269/ajtmh.12-0642..

19. K. Chishimba, B. M. Hang'ombe, K. Muzandu, S. E. Mshana, M. I. Matee, C. Nakajima, and Y. Suzuki. (2016). Detection of Extended-Spectrum Beta-Lactamase-Producing Escherichia coli in Market-Ready Chickens in Zambia. International Journal of Microbiology Volume 2016 Article ID 5275724, 5 pageshttp://dx.doi.org/10.1155/2016/5275724

20. H.M. Abdallah, E.A. Reuland, B.B. Wintermans, N. al Naiemi, A. Koek, A.M. Abdelwahab, et al.(2015). Extended-Spectrum β-Lactamases and/or Carbapenemases-Producing Enterobacteriaceae Isolated from Retail Chicken Meat in Zagazig, Egypt,"PLoS ONE.10 (8): e0136052. doi:10.1371/journal.pone.0136052.

21. G.I. Barrow and R.K.A. Feltham. (1993). Steel's manual for the identification of medical bacteria,3rd,edn.CambridgeUniversityPress,Cambridge.1993.http://dx.doi.org/10.1017/CBO9780511527104.

22. J. Sambrook and D.W.Russell. (2001). Molecular cloning: A laboratory manual, 3rd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

23. M. Sharma, S. Pathak and P. Sriva Stava. (2013). Prevalence and antibiogram of Extended Spectrum β-Lactamase (ESBL) producing Gram negative bacilli and further molecular characterization of ESBL producing Escherichia coli and Klebsiella spp. *J. Clin.Diag. Res*. 10: 2173-2177.

24. Y. Doi, D.L .P, Egea, A. Pascual, L. López-Cerero, M.D. Navarro, et al. (2010). Extended-spectrum and CMY-type beta-lactamase-producing *Escherichia coli* in clinical samples and retail meat from Pittsburgh, USA and Seville, Spain. *Clin Microbiol Infect*. 16:33–8.. doi: 10.1111/j.1469-0691.2009.03001.x PMID: 19681957.

25. F. Schaumburg, A.S. Alabi, L. Frielinghaus, M.P R, Köck, K. Becker, et al. (2014).The risk to import ESBL-producing Enterobacteriaceae and *Staphylococcus aureus* through chicken meat trade in Gabon. *BMC Microbiol*.14: pp.286. doi: 10.1186/s12866-014-0286-3 PMID: 25406798.

26. A. Harwalkar, J. Sataraddi, S. Gupta, R. Yoganand, A. Rao and H. Srinivasa. (2013). The detection of ESBL- producing *E.coli* in patients with symptomatic urinary tract infections using different diffusion methods in a rural setting. *J. Infect. Publ. Health*, 6:108-114.

27. I.I. Romanus, O.A. Egwu, A.T. Ngozi, N.A. Chidiebube and E.P. Chika. (2009). Extended spectrum beta - lactamase (ESBL) mediated resistance to antibiotics among *Klebsiella pneumoniae* in Enugu Metropolis. *Macedonian J. Med. Sci*. 2 (3):.1-4.

28. P. A. Bradford. (2001). Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clin. Microbiol. Rev*. 14:933–951. 10.1128/CMR.14.4.933-951.

29. E.B. Bali, K.L. Ac and N. Sultan. (2010). Phenotypic and molecular characterization of SHV, TEM, CTX-M and extended-spectrum-lactamase produced by *Escherichia coli*, *Acinobacter* baumannii and *Klebsiella isolates*in a Turkish hospital. *Afr. J. Microbiol. Res*. 4, (8):650–54.

30. J.A.J.W. Kluytmans, I.T.M.A. Overdevest, I. Willemsen, M.F.Q. Kluytmans-van den Bergh, K. van der Zwaluw, M. Heck, et al. “Extended-spectrum β-lactamase-producing Escherichia coli from retail chicken meat and humans: comparison of strains, plasmids, resistance genes, and virulence factors.” *Clin Infect Dis*. Vol. 56 pp. 478–87. 2013 doi: 10.1093/cid/cis929 PMID: 23243181.

31. G. Gregova, M. Kmetova, V. Kmet, J. Venglovsky and A. Feher. (2012). Antibiotic resistance of *Escherichia coli* isolated from a poultry slaughterhouse. *Annals. Agric. Env. Med*. 19: 75–77.

32.I. Willemsen, M. Mooij, M. van der Wiel, D. Bogaers, M. van der Bijl,. and S. Savelkoul.(2009). Highly resistant microorganisms in a teaching hospital: the role of horizontal spread in a setting of endemicity. *Infect. Control. Hosp. Epidemiol*. 29: 1110–7. doi:10.1086/592408.