Characterization of Extended-Spectrum Beta-Lactamase-Producing *Escherichia Coli* From Domestic Free-Range Poultry In Agogo, Ghana

Charity Wiafe Akenten (✉ danquah@kccr.de)

Kumasi Centre for Collaborative Research in Tropical Medicine in Tropical Medicine

Linda Aurelia Ofori

Kwame Nkrumah University of Science and Technology

Joyce Mbwana

Institute for Medical Research (NIMR)

Nimako Sarpong

Kumasi Centre for Collaborative Research in Tropical Medicine in Tropical Medicine

Jürgen May

Bernhard Nocht Institute for Tropical Medicine

Thorsten Thye

Bernhard Nocht Institute for Tropical Medicine

Kwasi Obiri-Danso

Kwame Nkrumah University of Science and Technology

Ellis Kobina Paintsil

Kumasi Centre for Collaborative Research in Tropical Medicine in Tropical Medicine

Richard Odame Philipps

Kumasi Centre for Collaborative Research in Tropical Medicine in Tropical Medicine

Daniel Eibach

Bernhard Nocht Institute for Tropical Medicine

Ralf Krumkamp

Bernhard Nocht Institute for Tropical Medicine

Denise Dekker

Institute for Medical Research (NIMR)

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Abstract

Background: Extended-spectrum beta-lactamase (ESBLs) producing bacteria in poultry meat has been suggested as one of the sources of resistant genes, that can lead to difficult-to treat infections in humans. This study aims at determining the frequency of ESBL-producing *E. coli*, the genetic characterization and antibiotic profile among domestic free-range poultry in Agogo, Ghana. Faecal samples were collected from domestic free-roaming chicken and cultured on ESBL screening agar. Strain identification and antibiotic susceptibility were performed using the VITEK 2 compact system. ESBL-producing *E. coli* were confirmed using the Double Disk Synergy (DDS) test. Molecular detection and further sequencing of *bla*TEM, *bla*SHV and *bla*CTX-M genes was performed using standardized methods.

Results: 56.2% (n/N=81/144) of collected faecal samples were positive for ESBL-producing *E. coli*. All (n/N=81/81) strains were resistant to ampicillin, ceftazidime, cefpodoxime, cefotaxime and cefuroxime. High rates of resistance were also observed for tetracycline (93.8%, n/N=76/81) and trimethoprim-sulfamethoxazole (66.67; n/N=54/81). Resistance to Carbapenems was not found. The majority of ESBL producing *E. coli* carried *bla*CTX-M genes with *bla*CTX-M-15, 95.1% (n/N=77/81) being the dominant genotype.

Conclusions:

We report high ESBL rates, which is a potential infection source of animals as well as humans, and that a control of antibiotic overuse and animal hygiene/sanitation measures are important from a one health perspective.

Background

The rapid increase of resistance to commonly used antibiotics in human medicine and animal husbandry has become one of the leading global health concerns (1). Besides, overuse of antibiotics in both humans and animal farming has been considered as the driving force for the increase in antimicrobial resistance (AMR) worldwide. Currently, the World health organization (WHO) considers AMR as a 'One Health issue' as well as a "One World issue" (2). Without preventive measures in place, by 2050 the annual deaths due to infections with AMR bacteria are expected to have reached 10 million (3).

AMR infections, including infections caused by extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in sub-Saharan Africa (SSA), are a major concern. *E. coli* are gram-negative bacteria found as commensals in the gut of humans and animals but are also implicated in a variety of infections, including life-threatening sepsis (4).

A recent study from Ghana indicated poultry as a likely source for ESBL-producing bacteria leading to difficult-to-treat infections in humans (5). This has also been shown in a global systemic review (6). ESBL- producing pathogens can be acquired by direct contact with animals or by consumption of meat products. In addition, ESBL-producing bacteria are often linked to concomitant resistance to other classes

of antibiotic agents leading to multidrug resistance (MDR) (7). Therefore, there are global ongoing efforts to address this issue and the issue of AMR as a whole (8, 9).

In Ghana, poultry farming is one of the predominant animal businesses with an estimated number of 74.5 million birds per year (10). Of these, approximately 25 million are kept on household level, freely roaming within communities (11). Amongst all meat products, poultry is the most popular, largely consumed in all regions of the country. In the quest to meet this demand, poultry keepers employ antimicrobials as growth promoter, and to prevent and treat infections (12).

Several studies on ESBL-producing bacteria, particularly *E. coli*, in commercial poultry farming have been published (5, 13, 14). However, to our knowledge, such studies have not been conducted on free-range poultry (household) farming-level in Ghana. Nevertheless, due to the animals' proximity to humans, such studies are essential to identify possible ESBL-transmission reservoirs within communities. This study aims at determining the frequency of ESBL-producing *E. coli*, their genetic characterization and antibiotic profile among domestic free-range poultry in Agogo located in the Ashanti Region of Ghana.

Results

In total, 144 faecal samples were collected from six households from three communities in Agogo. From the 144 faecal samples investigated, 81 (56.3%, n/N=81/144) were phenotypically positive for ESBL-producing *E. coli*. Frequencies detected within the communities were 76.1%, (n/N=35/46) in Sukuumu, 52.9% (n/N=37/70) in Freetown and 32.1%, (n/N=9/28) in Bontodiase (Figure 1).

Genotype identification of ESBL-producing *E. coli*

Genotype characterization identified two different beta-lactamase-encoding genes (*bla*CTX-M and *bla*TEM) out of the three tested (*bla*CTX-M, *bla*SHV, *bla*TEM). The majority were *bla*CTX-M-15 (95.1%, n/N=77/81), followed in frequency by *bla*TEM1-b (2.5%, n/N=2/81) and *bla*CTX-M-15/TEM 1b (1.2%, n/N=1/81). None of the ESBL-producing *E. coli* isolates carried the SHV gene. However, in one of the phenotypic confirmed ESBL *E. coli* isolates neither *bla*CTX-M, *bla*SHV, nor *bla*TEM were identified.

2.2 Antibiotic Susceptibility of ESBL-producing *E. coli*

All *E. coli* were sensitive to meropenem, imipenem, ertapenem and tigecycline (100%, 81/81) (Figure 2). Aside 100% resistance to cephalosporins, the highest rate of resistance was observed for tetracycline (93.8%, n/N=76/81), followed by trimethoprim-sulfamethoxazole (66.7%, n/N=54/81) and ciprofloxacin (35.8%, n/N=29/81) as shown in Figure 2. MDR was found among the ESBL producing *E. coli*. 11.1% (n/N=9/81) of the ESBL producing *E. coli* were resistant to four classes of antibiotics (i.e., fluoroquinolone, tetracycline, aminoglycoside and sulphonamides), while 19.75% (n/N=16/81) isolates were resistant to three classes of antibiotics (i.e., fluoroquinolone, tetracycline and sulphonamides).

Discussion

The study examined ESBL-producing *E. coli* among domestic free-range poultry in a rural community of Ghana. The considerably high rate of ESBL-producing *E. coli* exceeds what was found in commercial poultry farming in Ghana (5). This is not surprising, as Paintsil and colleagues demonstrated that 43% of domestic farmers from the same study area use antibiotics in poultry farming (15), which in turn increases the risk of higher ESBL acquisition in the normal gut flora of the animal (16). In general, not exclusively on household farm-level, frequencies of ESBL-producing bacteria found in chicken seem high, on the African continent. For example, a study showed 20% ESBL-producing *E. coli* in poultry from Zambia (17). A review by Alonso and colleagues has reported 42.0% and 55.5% ESBL-producing *E. coli* among chicken faeces from Tunisia and Algeria respectively, as well as 61.6% from chicken meat in Egypt (18).

Generally, in resource limited countries, inadequate sanitation aside the overuse of antibiotics are likely to play significant roles in the selection and transmission of antibiotic resistance (19). This is probably particularly true for areas where free-range (domestic) chicken is raised in communities with close contact to their owners. In sub-Saharan African countries, the potential transmission risk of antimicrobial resistant bacteria from animals or animal products to humans has been demonstrated before (20).

Besides, high level of resistance to beta-lactam antibiotics, the degree of resistance seen for other classes of antibiotics such as tetracycline and trimethoprim-sulfamethoxazole but also the much lower levels as seen for gentamycin and piperacillin is in line with what was found in other studies around the globe (21–23). Resistance in this study was reflected by the overall usage and availability of drugs in Ghana (24, 25). Also fluoroquinolones, such as ciprofloxacin are important drugs in Ghana for the treatment of infections (26, 27). The level of ciprofloxacin resistance (35.8%, n/N=29/81) seen was in line with 35.7% that was reported from a study in poultry from Nigeria (14) but lower than 59% that was reported from a previous study in Ghana on poultry meat (13). The difference seen might be attributed to the fact that ciprofloxacin usage in farming on household level is not as common in this study area (15).

Globally also in most SSA countries, *bla*CTX-M positive *E. coli* have been shown to be the most common genotype found in clinical isolates from humans and animals (28, 29) with *bla*CTX-M-15 being the most prevalent gene associated with human infections (30). This was also true for the present study, where *bla*CTX-M-15 was found to be the dominant genotype amongst the ESBL-positive isolates. This is in line with data from a previous study analysing chicken meat in Ghana (5). *bla*CTX-M-15 as predominant genotype among the ESBL-producing strains is of particular interest, as it has been associated with clinical *E. coli* strains carrying several virulence factors (31, 32). *bla*CTX-M-15 is often found on incompatibility group FII plasmids (33), known as “epidemic resistance plasmids” (34) due to their tendency to acquire other resistance genes, and their high potential to be transmitted through horizontal gene transfer (35).

Notably, the coexistence of *bla*CTX-M-15 and *bla*TEM-1b observed in this study was considerably lower, with only 1.2% of isolates harbouring both resistance genes, than what was reported from Nigeria (8.1%) (14). As in the present study ESBL genes itself were analysed only, no assumption can be made on

whether these were located on the chromosome or the plasmid. Discrimination between chromosomal or plasmid location of ESBL genes in *E. coli* was previously analysed by Falgenhauer and colleagues (5) including isolates collected in the same area in which this study was conducted. The study revealed chromosomal and plasmid-mediated ESBL genes in the study area. Thus, we can assume that for some of our isolates carrying ESBL, the resistance can be located on the plasmids.

Our study has limitations, which have to be considered when interpreting the results. Faecal samples were collected from only three communities in the study area and from seven households. This may not be representative for other parts of Ghana. Also, the sample size was rather small. Therefore, to generalize the interpretation of this study, faecal samples from additional communities should be evaluated to cover a representative study area. Seasonality was not captured within this study as households were visited once within the 6 months study period only.

Conclusion

This study demonstrates a high frequencies of ESBL-producing *E. coli* in free-range chicken in rural Ghana. It also shows a high carriage level of CTX-M-15 producing *E. coli* isolates in household chicken. Hence, poultry, even when bred on household level, might be an important source for ESBL-producing bacteria in this part of the world.

Therefore, monitoring of antibiotic use and restrictions in poultry farming must be encouraged as well as the implementation of surveillance systems that monitor antimicrobial use and inform on emerging antibiotic resistant bacterial strains.

Methods

Study site and sample collection

Fresh faecal samples from poultry were randomly collected from six households in three different communities in Agogo in the Asante Akyem North Municipality within the Ashanti Region of Ghana (Fig. 1). Sample collection took place between June 2019 to December 2019. Faecal samples were collected from households where chickens were kept at the backyard. Each household possessed approximately 25 chicken and was visited once during the study period. Samples were transported at 2°C – 8°C in a cool box to the microbiology laboratory at the Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR) within 2-4 hours after sampling for further analyses.

Identification And Antibiotic Susceptibility Testing

On arrival to the laboratory, samples were cultured on two MacConkey agars containing 1mg/L Ceftazidime and 1mg/L Cefotaxime, respectively. Plates were incubated at 35°–37°C for 18–24h at normal atmosphere. All morphological different lactose fermenters colonies (not exceeding 3 colonies)

were subsequently sub-cultured on Columbia blood agar. Isolate identification and antibiotic susceptibility were tested using the VITEK 2 compact system (bioMérieux, Marcy L'Etoile, France). Confirmed *E. coli* were classified as S (Susceptible, standard dosing regimen) I (Susceptible, increased exposure) or R (Resistant) to commonly used antibiotics according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (version 10.0, 2020; <http://www.eucast.org/breakpoints/>). Antibiotics tested included: ampicillin, ceftazidime, cefpodoxime, cefotaxime, cefuroxime, tetracycline, imipenem, meropenem, gentamicin, ciprofloxacin, ampicillin-sulbactam, trimethoprim/sulfamethoxazole, ertapenem, piperacillin-tazobactam and tigecycline and ESBL screening. MDR was defined as resistance to more than 2 classes of antibiotics.

Production of ESBLs was confirmed by the combined double-disk test with ceftazidime and cefotaxime alone and in combination with clavulanic acid (Becton, Dickinson Company, Sparks, MD, USA) as defined by EUCAST (EUCAST guidelines, version 10.0, 2020; <http://www.eucast.org/breakpoints/>).

Confirmed strains were saved in microbanks (Pro-lab Diagnostics, Richmond Hill, ON, Canada) and stored at -80°C for further analysis. Quality control of cephalosporin-containing agar was performed using the ATCC 25922 and a *bla*CTX-M-15 positive *E. coli*.

Genotyping Of Esbl Genes By Polymerase Chain Reaction

DNA extraction for *E. coli* was performed using the boiling method as described by Tellevik and colleagues (36). DNA was subject to molecular characterization by polymerase chain reaction (PCR) for the detection of the resistance genes *bla*TEM (temoneira), SHV (sulphydryl variable enzyme) and *bla*CTX-M, (cefotaximase-Munich) respectively as described elsewhere (37). To further differentiate the *bla*CTX-M groups, specific target primers (Table 1) were used for amplification and sequencing of the PCR amplicons (37). The PCR amplicon was sent to Microsynth SeqLab (Göttingen, Germany) for Sanger sequencing. The resulting sequences were aligned and identified by comparison with known sequences using CLC Sequence Viewer 8.0 (<http://www.clcbio.com>) and Resfinder 4.1 (<https://cge.cbs.dtu.dk/services/ResFinder>)

Descriptive statistics were applied to analyse study data. All analysis were done using R studio software (<https://www.rstudio.com>).

Table 1
Primers for ESBL Genes

Target gene	Primer group	Sequences	Amplicon size (bp)
bla _{SHV}	SHV-F	5'-GCCGGGTTATTCTTATTTGTCCG-3'	1007
	SHV-R	5'-ATGCCGCCGCCAGTCA -3'	
bla _{TEM}	TEM-F	5'-GTATCCGCTCATGAGACAATA-3'	966
	TEM-R	5'-TCTAAAGTATATATGAGTAAAC-3'	
bla _{CTX-M}	CTX-M-F	5'-TTTGCGATGTGCAGTACCAGTAA-3'	544
	CTX-M-R	5'-CGATATCGTTGGTGGTGCCATA-3'	
bla _{CTX-M-type}	CTX-M-1_F	5'-TCTTCCAGAATAAGGAATCCC-3'	909 bp
	CTX-M-1_R	5'-CCGTTTCCGCTATTACAAAC-3'	
bla _{CTX-M-type}	CTX-M-2_F	5'-ATGATGACTCAGAGCATT-3'	884 bp
	CTX-M-2_R	5'-TTATTGCATCAGAAACCGTG-3'	
bla _{CTX-M-type}	CTX-M-8_F	5'-TGATGAGACATCGCGTTAAG-3'	871 bp
	CTX-M-8_R	5'-TAACCGTCGGTGACGATTTT-3'	
bla _{CTX-M-type}	CTX-M-9_F	5'-ATGGTGACAAAGAGARTGCAA-3'	873 bp
	CTX-M-9_R	5'-CAGCCCTTCGGCGATGAT-3'	
bla _{CTX-M-type}	CTX-M-14_F	5'-ATTCAACAAAACCAGTTACAGCCC-3'	897 bp
	CTX-M-14_R	5'-TTTGAGATGGTGACAAAGAGA-3'	

Abbreviations

WHO——World Health Organization

ESBL——Extended-Spectrum Beta-Lactamase

DDS——Double Disk Synergy

AMR——Antimicrobial Resistance

MDR——Multidrug Resistance

TEM——Temoneira

SHV——Sulphydryl Variable Enzyme

CTX-M——Cefotaximase-Munich

PCR——Polymerase Chain reaction

S——Susceptible, standard dosing regimen

I——Susceptible, increased exposure

R——Resistant

Declarations

Additional Files Legends; NOT APPLICABLE

- **Ethics approval and consent to participate**

The ethical approval was obtained from the Committee on Human Research Publication and Ethics (CHRPE) of the School of Medical Sciences, KNUST, Kumasi.

The owner of each household poultry farm was informed of the study purpose and oral permission was obtained before sampling.

- **Consent for publication:**

Not applicable

- **Availability of data and materials**

All data generated or analysed during this study are included in this published article [and its supplementary information files].

- **Competing interests;** The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results
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The funding body did not play any role in design, analysis and reporting of this study.

- **Authors' contributions**

CWA, LAO, DD designed and coordinated the study, N.S conducted and supervised fieldwork. CWA, and EKP conducted laboratory work. CWA, JM and RK, performed data analysis. CWA and TT performed the gene characterization, CWA wrote the first draft of the paper. ROP, DE, JM, KOD LAO, DD, supervised and validate the work. All authors read and approved the final manuscript. LAO and DD acquired the funds

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- **Authors' information (optional)**

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Figures

Figure 1

Geographical Location of Domestic (Household) farms in Agogo, Ghana

Figure 2

Antibiotic resistance among ESBL *E. coli* isolated from domestic poultry.

Supplementary Files

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