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Research Article

Prevalence of Extended Spectrum Beta-Lactamases Producing *Enterobacteriaceae* in Sheep and Goats from Selected Markets in Ibadan, Oyo-State, Nigeria.

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Abstract

Antimicrobial misuse, unhygienic husbandry practices, close interaction between humans and animal as obtained in livestock market facilitate the emergence, dissemination and transmission of resistant *Enterobacteriaceae*. These organisms are responsible for various intestinal and extra-intestinal infections in human and animals. According to this report, the prevalence of Extended Spectrum Beta-Lactamase (ESBL)-producing *Enterobacteriaceae* in sheep and goats from selected markets in Ibadan. Three hundred and four (304) samples were collected for a cross-sectional survey among the sheep and goat markets in Ibadan. From the same sheep and goats, 152 milk samples and 152 faeces samples were collected. In order to identify any antibiotic-resistant *Enterobacteriaceae*, these samples underwent bacteriological analysis. On MacConkey agar plates with 1 mg/L cefotaxime added, all samples were cultivated. Utilizing a biochemical test kit (Oxoid Microbact GNB 24E®), the isolates were identified. ESBL products were evaluated utilizing a double disc diffusion test with discs impregnated with cefpodoxime and cefpodoxime-clavulanic acid. By using the disc diffusion approach, antibiotic resistance was identified. Data was analysed using descriptive statistics. Eighty-eight ampicillin resistant *Enterobacteriaceae* strains were isolated from 304 samples collected and 23 (26.1%) of the isolates were cefotaxime- resistant *Enterobacteriaceae* isolates. Only 9(10.2%) were confirmed phenotypic ESBL-producers and they were all from faeces. All ESBL-producing *Enterobacteriaceae* were *E. coli* strain and showed 100% resistance to tetracycline and ceftazidime, 77.8% resistance to amoxicillin and sulphamethoxazole but susceptible to gentamycin and ciprofloxacin in this investigation. The ESBL-producing *E. coli* isolates showed different antibiotic resistance patterns. In this study, 100% of ESBL-producing *E. coli* were multidrug- resistant, showing resistance to at least three separate classes of antibiotics. Public awareness of the significance of stringent hygiene in animal husbandry needs to be raised in light of the possible threat that the existence of multidrug resistant ESBL-producing *E. coli* in small ruminants poses to public health.

Key Words: Caprine, Ovine, *Escherichia coli*, *Enterobacteriaceae*, Extended Spectrum Beta Lactamase

INTRODUCTION

Antimicrobial resistance (AMR) is now understood to be a serious danger to public health and is defined as the ability of a microbe to resist an antimicrobial medicine to which it was previously sensitive, rendering treatment ineffective, allowing infections to persist, and ultimately spreading infections (WHO, 2017). Antimicrobial resistance is a very complex problem and not a specific disease as it is a global response of bacteria world as a whole to enormous global usage of antibiotics in human, animal and in agriculture (WHO, 2019). *Enterobacteriaceae* are diverse and ubiquitous microorganisms that can readily cross between human, animal and the environment in a cyclic reciprocating manner. One of the most important mechanisms for resistance among the food borne pathogens includes production of extended spectrum β-lactamases (ESBLs) by the *Enterobacteriaceae* (Geser *et al.*, 2012). Beta-lactam antibiotics, such as penicillin and oxyimino-cephalosporins, are broken down by a class of enzymes known as ESBLs, rendering them useless, the majority of transmissible β-lactamases, or ESBLs, are inhibited by the antibiotics clavulanic acid, tazobactam, and sulbactam. ESBLs are commonly transmitted β-lactamases that are encoded and expressed by genes that can be exchanged between bacteria (Rawat *et al.*, 2010). In any context, beta-lactamase genes are highly transmissible to other bacterial species and can be expressed in higher quantities (Gelinski *et al.*, 2014). According to Chisimba *et al.*, (2016), ESBL-producing microorganisms have a complicated epidemiology and are most frequently found in members of the *Enterobacteriaceae* family, such E. coli, whose reservoirs are animals (farm and food). According to reports, the main source of zoonotic foodborne infections, including bacteria that are resistant to antibiotics, is animals that are raised for food (Nguyen *et al.*, 2019). Antibiotic resistance genes can spread among bacteria in different taxonomic groups, and it is

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well known that resistant microorganisms can spread from animals to humans (Malini *et al.*, 2016). Sheep and goats' milk are alternative sources of animal protein and constituent of human food chain especially for those with allergy to cattle milk (Alexandratos and Bruinsma 2012). Due to unclean milking and storage procedures, milk has a high nutritional content and is a perfect medium for the rapid proliferation of germs (OECD 2005). Numerous tons of manure from sheep, goats, and other animals is used to pastures for the production of silage and feed, as well as agricultural fields as fertilizer to supply nutrients to crops and enhance soil quality (OECD 2005). Antibiotic residues in the treated soil may make it easier for microbes to develop resistance through ongoing selective pressure.

According to Akagha *et al.* (2015), the use of antibiotics in animal husbandry to produce food of animal origin is a significant element that results in antibiotic residues in these animals and selection pressure that leads to the evolution of resistance bacteria. In animal husbandry, antibiotics are used for therapeutic, preventative, and food additive purposes. Since their introduction, antibiotics have helped cure pathogenic bacteria in animals, prevent disease, and boost livestock production yield (Akagha *et al.*, 2015). Antibiotics are improperly used in animal husbandry, which puts pressure on bacteria to evolve drug resistance genes over time and reduces the effectiveness of some antibiotics (Ejikeugwu *et al.*, 2017). Antibiotics can be administered orally and some of them have low level of absorption in the gastrointestinal tract resulting in bioavailability and exposure of microbes of normal intestinal flora to antibiotics and antibiotics metabolites acquiring resistance.

The detection of ESBL positive bacteria in animal food products like milk and animal waste (rectal swabs) samples is a significant epidemiological tool for containing potential disease outbreaks caused by these pathogens (Ejikeugwu *et al.*, 2017). Numerous research on the antimicrobial resistance of commensal and pathogenic bacteria from foods derived from animals, such as chicken, swine, and cattle, have been conducted (Adelowo *et al.*, 2014; Olowe *et al.*, 2015) accompanied by a report indicating a high frequency of antimicrobial resistance in commensal bacteria to a number of therapeutically significant drugs. The prevalence of antibiotic resistance in small ruminants, however, has received scant attention (Scott *et al.*, 2011). This investigation examines the distribution and frequency of *Enterobacteriaceae* isolates from milk and rectal swabs of sheep and goats in Ibadan, Nigeria, that exhibit extended spectrum beta-lactamases.

MATERIALS AND METHODS

Sample collection: Samples were collected for the detection of ESBL producing *Enterobacteriaceae* in three animal markets located in Ibadan, Oyo State. Two types of samples were collected: milk sample and feces from the same sheep and goats. 152 each of milk and feces were collected at three locations. Samples were collected aseptically avoiding cross-contamination, labeled appropriately and transported in ice pack to the Laboratory for immediate microbiological analysis.

Table 1: Showing the breakdown of the total samples collected and location.

LOCATIONS	FAECAL SAMPLES	MILK SAMPLES
Akinyele Kraal market	132	132
Oranmiyan market	13	13
Bodija Kraal market	7	7
TOTAL	152	152

Isolation and identification of ampicillin resistant isolates: Pre-enrichment was achieved by incubating the sample in buffer peptone water (BPW) for an overnight period at 37°C. To isolate ampicillin-resistant bacteria, a loop of the pre-enrichment broth culture was streaked over MacConkey Agar that had been treated with ampicillin at a concentration of 100 g/L. The bacteria were then incubated at 37° C for 24 hours.

Isolation and identification of cefotaxime resistant isolates: To isolate C-R strains, one colony from each MAC-AMP-100 plate was inoculated onto a different MacConkey Agar plate that had 1 g/L of supplement (MAC- CTX-1) and allowed to grow for 24 hours at 37° C. From each MAC CTX-1 plate, a unique colony of the C-R isolate was selected. To preserve the selected isolate for later analysis, it was placed on nutrient Agar slant.

Phenotypic detection of ESBL producing *Enterobacteriaceae*: Using the combination disc kits (Oxoid, Basingstoke) containing cefpodoxime (CPD, 10g) and cefpodoxime-clavulanic acid (CD 01,10/1g), all C-R isolates were examined for the formation of ESBL. A fresh culture of the test organism on nutrient Agar was emulsified on normal saline. This was spread evenly on Mueller Hinton Agar (MAH) and the disk introduced firmly on the agar. The Inoculated MAH was incubated at 37° C for 18 hours. The difference in the zone of inhibition around the two disks was determined. Phenotypic ESBL producers were identified as isolates that cause variations in the diameter of the zone of inhibition between cefpodoxime and cefpodoxime-clavulanic acid discs that are equal to or more than five millimeters. (CLSI, 2018).

Biochemical characterization and Identification of *Enterobacteriaceae*: The colonial characteristics of the organism was recorded. Suspected *Enterobacteriaceae* Gram staining, oxidase production, and catalase production were evaluated for isolates. Utilizing a biochemical test kit (Oxoid Microbact GNB 24E®) for isolate identification, the isolates were further determined based on substrate usage as shown by color change. The test was performed in accordance with the manufacturer's instructions, and the reaction was determined using a color chart included in the kit. The data were decoded using computer software (Oxoid Microbact® 2000 version 2.03) created for the test kit after reading the color change reaction.

Testing for Antimicrobial Susceptibility: According to the recommendations of the Clinical Laboratory Standard Institute (CLSI, 2019), C-R isolates were evaluated using the Kirby Bauer disk diffusion method for susceptibility to a number of different antibiotics. The following antimicrobials were used: Tetracycline (TET 30 µg), sulfamethoxazole (SXT 25 µg), Ceftazidime (CAZ, 30 µg), Chloramphenicol (CHL, 30 µg), Gentamicin (GEN 120 µg), Ciprofloxacin (CIP 5 µg), Amoxicillin (AMX 30 µg). A suspension of test organism was prepared in normal saline. A sterile cotton swab was used to evenly distribute the suspension on Mueller Hinton agar. A 16-hour incubation period at 37° C was completed once the antimicrobial disk had firmly set on the inoculated agar. CLSI (2018) was used to measure and analyze the diameter of the zone of inhibition surrounding each disk.

Statistical Analysis: Data were expressed in absolute values and in percentages. Data from faecal and milk samples were compared with the Chi square test. (Significant values at P< 0.05).

RESULTS

Description of sample collected: A total of three hundred and four samples were collected, 152 faecal and milk samples of sheep and goats. 132 of each sample from Akinyele Kraal Market, 13 of each sample from Oranmiyan market and 7 of each sample from Bodija Kraal market.

Ampicillin- resistant *Enterobacteriaceae* from sheep and goats: Eighty- eight 88 (28.9%) of the 304-sample yielded AMP- resistant isolates as follows; 29 (32.9%) from milk and 59 (67.1%) from faeces.

Cefotaxime- resistant *Enterobacteriaceae* from sheep and goats: Twenty- three (26.1%) of the ampicillin resistant isolates were cefotaxime-resistant and 22 (25%) are from fecal samples, 1(1.1%) from milk sample. C-R isolates were identified as *Escherichia coli* (n=16), klebsiella pneumoniae (n=4), Acinetobacter species (3).

Table 2:
Distribution of ampicillin resistance *Enterobacteriaceae* isolates from samples collected

LOCATION	*ENTEROBACTERIA* ISOLATES		TOTAL	
	FAECES	MILK		P value
Akinyele Kraal market	48	27	75	
Oranmiyan market	7	1	8	
Bodija Kraal market	4	1	5	
TOTAL	**59 (67.1%)**	**29 (32.9%)**	**88 (100%)**	**< 0.001**

Table 3:
The distribution of cefotaxime resistance *Enterobacteriaceae* isolates across different locations and samples

LOCATION	ENTEROBACTERIA ISOLATE		TOTAL
	FAECES	MILK	
Akinyele Kraal market	19	1	20
Oranmiyan market	1	0	1
Bodija Kraal market	2	0	2
TOTAL	22 (25%)	1 (1.1%)	23 (26.1%)

Table 4:
Illustrative of the impact of the high prevalence of ESBL-producing *E. coli*

MARKET LOCATION	Number of Samples		Ampicillin Resistance *E. coli* Isolates		Cefotaxime Resistance *E. coli* Isolates		Esbl-Producing *E. coli*		P value
	Faeces	Milk	Faeces	Milk	Faeces	Milk	Faeces	Milk	
Akinyele Kraal market	132	132	48	27	19	1	8	0	
Oranyan market	13	13	7	1	1	0	1	0	
Bodija Kraal market	7	7	4	1	2	0	0	0	
TOTAL	152 304	152	59 88 (28.9%)	29	22 23(26.1%)	1	9 9 (10.2%)	0	< 0.001

Nine (10.2%) out of the 23 C-R isolates who were found positive for phenotypic ESBL production. The detection rate of ESBL producing bacteria in all 304 samples was 2.9%. The ESBL producing isolates were all E. coli. Antimicrobial susceptibility tests revealed that isolates of ESBL-producing *E. coli* were extremely resistant to most of the tested antibiotics. The nine (9) *E. coli* strains that produce ESB, showed 100% resistance to tetracycline and ceftazidime, 77.8% showed resistance to sulfamethoxazole and amoxicillin while, 11.1% resistance to chloramphenicol and ciprofloxacin were obtained (Table 5). All the isolates showed 100% sensitivity to gentamicin.

Each and every one of the isolates exhibited multidrug resistance, meaning that they were resistant to at least one member of each of the six tested classes of antimicrobials. (Beta-lactam, Aminoglycosides, Phenicol, Fluoroquinolones, Sulfonamide, and Tetracycline).

Table 5:
Showing Antibiotics sensitivity test for ESBL-producing *E. coli*

S/N	Antimicrobial class	Antimicrobial agent	Disc Potency (µg)	Number of isolates T= 9		
				Sensitive N (%)	Intermediate N (%)	Resistance N (%)
1	Tetracycline	Tetracycline	30	0 (0.0%)	0 (0.0%)	9 (100%)
2	Sulphonamide	Sulphamethoxazole	25	1 (11.1%)	1 (11.1%)	7 (77.8%)
3	Cephalosporin	Ceftazidime	30	0 (0.0%)	0 (0.0%)	9 (100%)
4	Phenicol	Chloramphenicol	30	7 (77.8%)	1 (11.1%)	1 (11.1%)
5	Aminoglycoside	Gentamycin	120	9 (100%)	0 (0.0%)	0 (0.0%)
6	Floroquinolone	Ciprofloxacin	5	8 (88.9%)	0 (0.0%)	1 (11.1%)
7	Penicillin	Amoxicillin	30	2 (22.2%)	0 (0.0%)	7 (77.8%)

Table 6:
Antimicrobial Multi-drug resistance pattern for ESBL-producing *E.coli*

Resistance Antibiotics	Number of resistant isolates (%)
TE-CAZ	9 (100%)
TE-CAZ-STX-AMC	7 (77.8%)
TE-CAZ-STX-AMC-CIP	1 (11.1%)

CAZ-ceftazidine TE-Tetracycline STX-Sulphamethaxazole AMC-Amoxicillin C-Chloramphenicol

DISCUSSION

Food producing animals serve as reservoir for spread of antibiotics resistance bacteria in the community through the food chain (Ejikeugwe *et al.*, 2017). The 10.2% prevalence of ESBL-producing E.coli isolated in the study area suggests that the small ruminants are important in the epidemiology of Extended spectrum β-lactamase (ESBL) and represent direct risk to public health through environmental contamination with faecal droppings (Bitrus *et al.*, 2019). The prevalence rate in the study is lower than that reported by Ugwu *et al.*, 2018 from Anambra state who observed prevalence rate of 12% also lower than 14.8% prevalence of ESBLs producing *E. coli* reported by Ogefere *et al.*, (2017) from Edo state.

Antibiotics preparations containing β-lactams are widely used for prophylaxis and chemotherapy in small ruminants and heavy usage of β-lactams antibiotic exerts considerable selection for resistance to this class of antibiotic agents (Livermore, 2003). In this study 88(28.9%) of the 304 samples collected were ampicillin resistant *Enterobacteriaceae* strains an indication of β-lactamase producers. This observation supports previous report (Chah *et al.*, 2007) that resistance to β-lactams in *Enterobacteriaceae* is mainly due to production of β-lactamases. Although 23 (26.1%) of the β-lactamase producing *E. coli* isolates were predicted as ESBL producers by cefotaxime, only 9(10.2%) were phenotypically confirmed as ESBL-producers. These is similar to the observations of previous works (Saleem *et al.*, 2017; Iroha *et al.*, 2017). This underscores the need for phenotypic confirmation of ESBL production using Oxoid Double Disc Diffusion method or other sensitive methods. As it has been reported from previous research works ESBLs mediates resistance to third generation cephalosporin (Ogbolu *et al.*, 2011; Snow *et al.*, 2012). All the ESBL producing *E. coli* isolates detected in this study were from fecal samples, showing a high occurrence of ESBL isolates from faeces of sheep and goats in the studied areas. This may be because these locations are commercial hub of small ruminants in south- western part of Nigeria.

The antibiogram of ESBL-resistant *E. coli* isolates from sheep and goats in the Ibadan market was evaluated in this study. All of the isolates tested positive for resistance to 6 antimicrobial agents, including beta-lactam and non-beta-lactam antibiotics, with varying levels of resistance noted with different antimicrobial drug groups used in this study. Different antibiotic resistance rates and patterns were displayed by the ESBL-producing *E. coli* isolates.

High rates of resistance to first-line antibiotics such tetracycline, ceftazidime, sulphamethoxazole, and amoxicillin were found among ESBL-producing E. coli, which is comparable to a recent report by Kwoji *et al.*, (2019). According to Bitrus *et al.*, (2019), the rise of ESBL-producing *E. coli* in small ruminants may be linked to the overuse of antimicrobial drugs, notably third generation cephalosporin. According to Ugwu *et al.*, (2015), Nigeria has lax regulations on the use of antibiotics in veterinary medicine. *E. coli* has developed antibiotic resistance due to the indiscriminate use

of beta -lactams, especially 3rd and 4th generation cephalosporins (Geser *et al.*, 2011).

All eight of the ESBL-producing isolates showed evidence of multi-drug resistance to at least three antimicrobials from various antimicrobial classes. This can be due to feed addictive antimicrobial usage as an integral part of animal production, as a lot of antibiotics produced are used as feed additives in form of supplements and premixes (Dibner *et al.*,2005). Commensal bacteria like *E. coli* species develop resistance as a result of antibiotic consumption. According to Paterson (2000), antibiotic use patterns appear to be correlated with resistance. Of all the *E. coli* isolates that produce ESBLs, gentamycin has the highest sensitivity, whereas ceftazidime and tetracycline have the highest rates of resistance. This can be attributed to the availability of the drug and the fact that these drugs are cheap and easily accessible.

CONCLUSION

This study demonstrates the existence of multi-drug resistant food-borne infections in sheep and goats, and the presence of these organisms in our study, together with the high profile of antibiotic resistance, suggests excessive use of antibiotics in the production of these animals. Due to the enormous public health implications, fecal shedding in market settings is a new epidemiological issue that calls for the implementation of revised control strategies. Further research is needed, including molecular investigations, to identify the reservoirs and means of ESBL dissemination within the community, in order to confirm the presence of community-based ESBL transmission. In order to provide the proper care and promote infection management, it is crucial to monitor and identify *Enterobacteriaceae* that produce ESBLs in the laboratory. Additionally, detecting and monitoring these resistant bacteria in community surveillance systems depends on it. Programs to educate livestock workers on hygiene standards are also suggested as a preventative approach.

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Authors' contributions

The study's concept and design were created by EAA and OEO. The document was written by FEA. EAA oversaw the data collection, processing, and interpretation processes as well as the paper revision. FEA took part in the collection of samples and the microbiological examination. IOO gave the manuscript a critical evaluation. The finished manuscript has been read and approved by all authors.

REFERENCES

Adelowo, O.O., O.F., Fagade, and Y., Agersø. 2014. Antibiotic resistance and resistance genes in *Escherichia coli* from poultry farms, Southwest Nigeria. *J. Infect Develop Count.* 8:1103-1112

Akagha, T.N., H.T., Gugu, E.C., Enemor, P.C., Ejikeugwu, and B.C., Ugwu. 2015. Prevalence and antibiogram in retail meats sold in Awka Metropolis, Southeast Nigeria. *Int J Biol Pharm Res*. 6: 924-929.

Alexandratos, N., and J., Bruinsma. 2012. World agriculture towards 2030/2050: the 2012 revision. ESA Working paper No. 12-03. Rome, FAO. Hudzicki J. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. 2009.

Bitrus, A.A., P.A., Mshelia, I.D., Kwoji, M.D., Goni, and S.M., Jajere. 2019. Extended-spectrum beta-lactamase and ampicillin Class C beta-lactamase-producing *Escherichia coli* from food animals: A review. *Int J One Health*. 5:65-75.

Chah, K. F., and S.I., Oboegbulem. 2007. Extended – spectrum β-lactamase production among ampicillin-resistant *Escherichia coli* strains from chicken in Enugu State, Nigeria. *Braz J Microbiol*. 38:681-686

Chishimba, K., B.M., Hang'ombe, K., Muzandu, S.E., Mshana, M.I., Matee, C., Nakajima, and Y., Suzuki Y. 2016. Detection of Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* in Market-Ready Chickens in Zambia. *Int J Microbiol*. 2016:5275724. doi: 10.1155/2016/5275724.

Clinical and Laboratory Standards Institute (CLSI). 2018. Performance Standards for Antimicrobial Susceptibility Testing. CLSI Approved Standard M100-S15.

Clinical and Laboratory Standards Institute (CLSI). 2019. Performance standards for antimicrobial susceptibility testing. CLSI supplement M100, 29th ed. Clinical and Laboratory Standards Institute, Wayne, PA.

Dibner, J.J., and J.D., Richards. 2005. Antibiotics growth promoters in agriculture: history and mode of action. *Poult. Sci.* 84:634-643.

Ejikeugwu, C., C., Duru, I., Iroha, B., Oguejiofor, and L., Okoro. 2017. Detection of *Escherichia coli* strains producing AmpC enzymes using Ceftazidime-Imipenem Antagonism Test (CIAT*). Int. J of Res Stud Bioscie*. 5:41-45.

Gelinski, J., A., Bombassaro, C.M., Baratto, and A.V., Vania. 2014. Resistance to Extended-Spectrum β-Lactamases in from a Broiler Supply Chain. *Int J Environ Res Public Health* 11: 11719-11726.

Geser, N., R., Stephan, R., Zbinden, U., Kaeppeli, and H., Haechler. 2011. Faecal carriage of extended-spectrum beta-lactamase producing *Enterobacteriaceae* in swine and cattle at slaughter in Switzerland. J Food Protect. 74: 446-449.

Geser, N., R., Stephan, and H., Haechler. 2012. Occurrence and characteristics of extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* in food producing animals, minced meat and raw milk. *BMC Veter Res*. 8:21.

Iroha, I. R., E., Okoye, C.A., Osigwe, I.B.,Moses, and C.P.,Ejikeugwu. 2017. Isolation, Phenotypic Characterization and Prevalence of ESBL-Producing *Escherichia coli* and Klebsiella species from Orthopedic Wounds in National Orthopedic Hospital Enugu (NOHE), South East Nigeria. *J Pharm Care Health Sys*. 4: 184. doi:10.4172/2376-0419.1000184.

Kwoji, I. D., J.A., Musa, N., Daniel, D. L., Mohzo, A.A., Bitrus, A. A., Ojo, and K. U., Ezema. 2019. Extended-spectrum β-lactamase-producing *Escherichia coli* in chickens from small-scale (backyard) poultry farms in Maiduguri, Nigeria. *Int J One Health*; 5; 26-30

Livermore, D.M. 2003. Bacterial resistance; Origins, epidemiology and impact. *Clin. Infect. Dis*., 36: 11-23.

Malini, S., and S., Aditi. 2016. Antimicrobial resistance: challenges and the way forward. *Indian J Chest Dis Allied Sci* 58: 157-159.

Nguyen, V.T., D., Jamrozy, S., Matamoros, J. J., Carrique-Mas, H.M., Ho, and Q.H., Thai, 2019. Limited contribution of non-intensive chicken farming to ESBL-producing *Escherichia coli* colonization in humans in Vietnam: an epidemiological and genomic analysis. *J Antimicrob Chemother.* 74: 561–70.

OECD. 2005. Dairy policy reform and trade liberalization. Directorate for Food, Agriculture and Fisheries, Paris.

Ogbolu, D.O., O.A., Daini, A., Ogunledun, A.O., Alli, and M.A., Webber. 2011. High levels of multidrug resistance in clinical isolates of Gramnegative pathogens from Nigeria. *International Journal of Antimicrobial Agents* 37:62-66

Ogefere, H.O., S.A., Agbe, and E.E., Ibadin. 2017. Detection of extended spectrum beta-lactamases among Gram negative bacilli recovered from cattle feces in Benin city, Nigeria. *Nigeria. notulae scientia biologicae*. 177-181. 10.15835/nsb9210005.

Olowe, O.A., O., Adewumi, G., Odewale, O., Ojurongbe, and O.J., Adefioye. 2015. Phenotypic and molecular characterization of extended-spectrum beta-lactamase producing *Escherichia coli* obtained from animal fecal samples in Ado Ekiti, Nigeria. *J. Environ. Public Health*.2015;2015:497980.

Paterson, D.L. 2000. Recommendation for treatment of severe infections caused by *Enterobacteriaceae* producing extended-spectrum beta-lactamases (ESBLs). *Clin. Microbiol. Infect.* 6:460–463.

Rawat, D., and, D., Nair. 2010. Extended – spectrum βlactamases in Gram Negative bacteria. J Glob Infect Dis. 2010; 2(3): 263 – 274. Doi:10.4103/0974-777x.68531.

Snow, L.C., R.G., Warner, T.R.G., Cheney, H., Wearing, M., Stokes, K., Harris, C.J., Teale, and N.G., Coldham. 2012. Risk factors associated with extended spectrum beta-lactamase *Escherichia coli* (CTX-M) on dairy farms in North West England and North Wales. Prevent. *Vet. Med.* 106 (3-4):225-234.

Saleem, R., H., Ejaz, A., Zafar. S., Younas, and A.W., Rathore. 2017. Phenotypic characterization of extended-spectrum-beta-lactamase producing *E. coli* from healthy individuals, patients, sewage sludge, cattle, chickens and raw meat. *Pak J Med Sci.* vol. 33(4):886-890.

Scott, H. M., B., Norby, and G.H., Loneragan. 2011. Antimicrobial resistance surveillance: Bacterial prevalence estimates are not enough. *Epidemiol*. Sante Anim. 59–60:182–184.

Ugwu, I. C., M.W., Anyanwu, C.U., Chidozie, and W.O., Ugwuanyi. 2015. Prevalence and antibiogram of generic extended-spectrum β-lactam resistant *Enterobacteriaceae* in healthy pigs. *Notul. Scie. Biolo*. 7(3):272-280.

Ugwu, M.C., J.O., Igbokwe, U., Okezie, U, Eze, and C.P., Ejikeugwu. 2018. Prevalence of ESBLs and MBLs among *Escherichia coli* and Klebsiella pneumoniae Isolates from a Nigerian Abattoir. *J Trop Dis 6*: 261. doi:10.4172/2329-891X.1000261.

World Health Organization. 2017. Critically important antimicrobials for human medicine – 5th rev. Geneva: World Health Organization, Geneva, Switzerland

World Health Organization. 2019. Food and Agriculture Organization of the United Nations & World Organization for Animal Health. Monitoring and evaluation of the global action plan on antimicrobial resistance: framework and recommended indicators. World Health Organization.