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Antibiotypes and genetic characteristics of fluoroquinolone- and beta-lactam-resistant *Escherichia coli* isolated from food-producing animals

E. Egwu[1], C. S. Iroha[2], I. B. Moses[3], F. A. Ibiam[4], I. Orji[1], F. N. Okafor-Alu[1], C. O. Eze[5], and I. R. Iroha[3]

1. Department of Laboratory Service, Medical Laboratory Unit, Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Nigeria; 2. Department of Pharmacy, Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Nigeria; 3. Department of Applied Microbiology, Faculty of Sciences, Ebonyi State University, Abakaliki, Nigeria; 4. Department ofOtorhinolaryngology (ENT), Alex Ekwueme Federal University Ndufu-Alike Ikwo, Nigeria; 5. Department of Internal Medicine, Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Nigeria.

Corresponding author: I. B. Moses, e-mail: ben_iyke70@yahoo.com

Co-authors: EE: egwueze2@gmail.com, CSI: nmairoha@gmail.com, FAI: francisamadi461@gmail.com, IO: iykorji@gmail.com, FNO: nkem4okaforalu@gmail.com, COE: drezeconauth@yahoo.com, IRI: iriroha@yahoo.com

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Abstract

Background and Aim: Farm animals, including cattle, have been implicated as antimicrobial-resistant bacterial pathogen reservoirs. This study aimed to determine the antimicrobial resistance profiles and genetic characteristics of cattle colonized by fluoroquinolone-resistant and extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in Ebonyi state, Nigeria.

Materials and Methods: We randomly collected 100 fresh fecal samples from 100 cattle in major abattoirs and analyzed the samples using standard microbiological methods. Isolates were further characterized by polymerase chain reaction (PCR) using 16S rRNA sequence primers. Phenotypic detection of ESBL production was performed using the double disk synergy test. Antimicrobial susceptibility profiles of ESBL-producing *Escherichia coli* were determined using the disk diffusion method, whereas molecular characterization of ESBL- and fluoroquinolone-resistant genes was performed by PCR using specific primers.

Results: A total of 20 (20%) ESBL-producing *E. coli* were isolated from 100 animal fecal samples. Isolates were generally multidrug-resistant (MDR) with a resistance rate of 100% to 45% to trimethoprim-sulfamethoxazole, tetracycline, amoxicillin, cephalosporins, and ciprofloxacin. The average multiple antibiotic resistance index values of the isolates ranged from 0.5 to 0.8. *Bla*TEM (75%), followed by *bla*CTX-M (20%) and *bla*SHV (5.0%) was the most predominant ESBL gene among the isolates. The *Aac-lb-6-cr* fluoroquinolone-resistant gene was harbored by 90% of the isolates, whereas *Qnr* was absent.

Conclusion: This study showed a high frequency of MDR ESBL-producing *E. coli* harboring ESBL and fluoroquinolone-resistant genes in fecal samples of cattle with serious public health consequences if not adequately addressed.

Keywords: *Escherichia coli*, extended-spectrum beta-lactamase genes, fluoroquinolone resistance genes, cattle, multidrug-resistance.

Introduction

Escherichia coli is a Gram-negative commensal bacterium found in a wide range of ecosystems, including the intestinal tract of animals. However, extraintestinal pathogenic *E. coli* (ExPEC) has been implicated in serious diseases of animals and humans, especially ExPEC strains that cause diseases in almost any organ or anatomical site of animals [1]. Animals have been implicated as reservoirs for the spread of antimicrobial-resistant bacteria (ARB) and various resistance genes; thus, keeping livestock or the livestock profession is widely believed to be a risk factor for the dissemination of antimicrobial resistance (AMR) among various populations, including humans [2]. There is a close association between the use of antimicrobial agents for the treatment of infections in animals and the levels of resistance observed [3]. The World Health Organization recommended the prudent use of fluoroquinolones and beta-lactams in both human and veterinary medicine because of the strong correlation between their consumption and increased resistance [4]. Fluoroquinolones are antimicrobial agents of choice for the treatment of various infections caused by *E. coli* or other Gram-negative bacteria. Because fluoroquinolones and beta-lactams are extensively used for multiple clinical indications in veterinary medicine, bacterial resistance to these agents has developed over time [5].

Extended-spectrum beta-lactamase (ESBL) genes originally evolved from the most prevalent ß-lactamase TEM-1, TEM-2, and SHV-1 genes through mutations in the amino acids surrounding the active site [6]. This has led to the development of ESBLs with an expanded

substrate profile that allows the hydrolysis of cephalosporin, penicillin, and aztreonam [7].

However, during the past decade, the rapid and massive spread of CTX-M type ESBLs has been described, which has become the main epidemic genotype worldwide, whereas the OXA gene is the least prevalent [2, 6]. In addition, target sites for fluoroquinolone resistance in *E. coli* strains are bacterial topoisomerases, namely, DNA gyrase (topoisomerase II) as the primary site and topoisomerase IV as a secondary target [8]. Both enzymes are necessary for bacterial DNA replication. Mutations in specific domains of *gyrA*, *gyrB*, *parC*, and *parE* cause single amino acid changes in either gyrase or topoisomerase IV that contribute to quinolone resistance [8].

Although antimicrobial-resistant genes (ARG) can spread clonally [9], mobile genetic elements carrying ARG can be transferred between bacteria, notably from commensal to pathogenic *Enterobacteriaceae* [10]. The epidemic of antibiotic resistance at the global level is due to the excessive and inappropriate use of antimicrobials in veterinary practice, which strongly accelerates the development and diffusion of resistant strains. For example, intensive livestock farming, which requires farmers to rely more heavily on antibiotics has significantly increased the prevalence of ARB in farm animals and food. However, there is still a paucity of information focusing on ESBL-producing *E. coli* colonization of cattle, especially in Nigeria. Therefore, investigating and understanding the occurrence frequencies and molecular characteristics of beta-lactam- and fluoroquinolone-resistant *E. coli* from food-producing animals in Nigeria is of public health importance.

This study aimed to determine the antimicrobial resistance profiles and molecular characteristics of cattle colonized by fluoroquinolone-resistant and extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in Ebonyi state, Nigeria.

Materials and Methods

Ethical approval

Ethical approval was not required for this study because live animals were not used in this study. In this study, fecal samples of cattle were collected in abattoirs. Informed consent and permission were sought from owners of abattoirs and relevant agency/institution before fecal samples of cattle were collected.

Study period and location

This study was conducted from February 2019 to January 2021 in Afikpo and Abakaliki metropolis, Ebonyi State, Nigeria.

Sample collection

One hundred fresh fecal samples of cattle were collected from abattoirs; Abakaliki (50 samples) and Afikpo (50 samples) metropolis, Ebonyi State, Nigeria. Collected samples were immediately transported in Cary Blair transport medium (Liofilchem, Italy) to the laboratory within 1 h of collection for bacteriological analysis.

Bacteriological analysis

Swab samples were dipped into test tubes containing 5 mL of sterile brain-heart infusion broth (Oxoid, UK) and incubated at 35°C for 18–24 h. Loopfuls of tubes with turbidity were aseptically streaked onto MacConkey agar and Eosin Methylene Blue agar plates (Merck Co., Germany) and incubated at 35°C for 18–24 h. *E. coli* was identified using standard microbiological identification techniques, including colonial morphology, Gram staining, motility, and biochemical tests, as previously described by Cheesbrough [11], and John-Onwe *et al*. [12].

Detection of *E. coli* producing ESBL

ESBL production was phenotypically confirmed by the double disk synergy test technique using *E. coli* isolates that showed reduced susceptibility to third-generation cephalosporins [12, 13]. Standardized *E. coli* inoculum (adjusted to 0.5 McFarland turbidity standards) was aseptically swabbed on Mueller-Hinton (MH) agar plates, and amoxicillin/clavulanic acid disk (20/10 μg) was placed at the center of the plate, while cefotaxime (30 μg) and ceftazidime (30 μg) disks were placed adjacently at a distance of 15 mm. The plates were then incubated at 37°C for 18–24 h, and ESBL production was phenotypically inferred by the expansion of the zone of inhibition for cefotaxime or ceftazidime in the presence of amoxicillin–clavulanic acid compared with that in the absence of amoxicillin-clavulanic acid, giving a dumbbell shape [12, 14].

Antimicrobial susceptibility testing

The disk diffusion method was used according to the guidelines of Clinical and Laboratory Standards Institute [15]. A suspension was prepared from a 24-h growth of the test organisms in sterile water to match the 0.5 McFarland turbidity standard. It was seeded on the entire surface of solidified MH agar plates. The following antibiotics were tested against the isolated bacteria: Amikacin (10 μg), amoxicillin (30 μg), amoxicillin/clavulanic acid (20/10 μg), ceftazidime (30 μg), ceftriaxone (30 μg), cefotaxime (30 μg), cefuroxime (30 μg), cefoxitin (30 μg), ciprofloxacin (10 μg), imipenem (30 μg), ofloxacin (30 μg), tetracycline (30 μg), and trimethoprim-sulfamethoxazole (25 μg) (Oxoid). The inoculated MH agar plates were incubated at 35°C–37°C in an aerobic atmosphere for 18–24 h. Inhibition zone diameters were measured, recorded, and interpreted as resistant or susceptible according to established guidelines [15]. In this study, isolates with intermediate resistance were classified as "resistant."

Determination of multiple antibiotic resistance index (MARI)

MARI was calculated for each isolate based on the interpreted results of the disk diffusion method. MARI was calculated as the number of antibiotics to

which an isolate is resistant (x) divided by the total number of antibiotics tested against the isolate (y) [16].

Detection of fluoroquinolone-resistant-encoding genes by polymerase chain reaction (PCR)

A ZR fungal/bacterial DNA kit (Cat number: D6005) was used for DNA extraction. The PCR mix comprised 12.5 µL of Taq 2X Master Mix from New England Biolabs (M0270), 1 µL each of 10 M forward and reverse primers, 2 µL of DNA template, and 8.5 µL of nuclease-free water. The oligonucleotide nucleotide primers used are listed in Table-1 [17–20]. The following PCR conditions were used: initial denaturation at 94°C for 5 min, followed by 36 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 45 s. A final elongation step was performed at 72°C for 7 min and a hold temperature of 10°C. Electrophoresis was conducted at 80–150 V for approximately 1–1.5 h. Amplified PCR products were then visualized under an ultraviolet transilluminator (Cleaver Scientific, UK).

Results

Twenty (20%) ESBL-producing *E. coli* isolates were recovered from 100 cattle fecal samples collected from the selected abattoirs in Abakaliki and Afikpo, as shown in Table-2. ESB-producing *E. coli* detection rate was higher in abattoir samples collected from Afikpo (24%) than from Abakaliki (16%) (Table-2).

Isolates are generally highly resistant to trimethoprim-sulfamethoxazole, tetracycline, amoxicillin, cefotaxime, cefuroxime, ceftriaxone, ceftazidime, amoxicillin-clavulanic acid, and cefoxitin (100%–70%). A low frequency of ciprofloxacin resistance (45%) was observed, but all isolates were completely susceptible (100%) to amikacin and imipenem (Table-3).

MARI values of isolates ranged between 0.5 and 0.8.

The PCR results showed that *bla*TEM (75.0%), *bla*SHV (5.0%), and *bla*CTX-M (20.0%) were harbored by ESBL-producing *E. coli* isolates. One isolate harbored both *bla*TEM and *bla*SHV genes (Table-4). The fluoroquinolone-resistant gene *Qnr* was absent in all isolates, whereas *aac-lb-6-cr* was present in 95% of the isolates (Table-4).

Discussion

E. coli is a major member of *Enterobacteriaceae* that colonizes the digestive system of warm-blooded animals [21]. It is also considered to be an important indicator of fecal contamination in foods. Although most *E. coli* strains are non-pathogenic, some can cause a variety of intestinal and extraintestinal infections in humans and animals [22].

In this study, 20 (20%) ESBL-producing *E. coli* were isolated from 100 cattle fecal samples collected from different abattoirs in Abakaliki and Afikpo metropolis, Ebonyi State, Nigeria. ESBL-producing *E. coli* occurrence frequencies in cattle fecal samples have been reported in Turkey [23] and China [24] with frequencies of 15% and 23.5%, respectively. In contrast, Olowe *et al*. [25] reported a higher detection rate (71%) of ESBL-producing *E. coli* in cattle fecal samples from Ado-Ekiti, Nigeria. A higher detection rate (58.2%) has also been reported in a cattle farm in South Africa [26].

ESBL genes are generally located on plasmids that can spread easily among commensals and pathogenic bacteria in farm animal herds and the environment. However, the isolation rates of ESBL-producing *E. coli* from cattle fecal samples differ depending on the animal population, geographical areas under study, age of animals, and nature of feeds [27]. ESBL-producing *E. coli* is mostly reported in poultry and pigs due to the extensive use of broad-spectrum antibiotics [24]. The differences in the frequency of occurrence of ESBL-producing *E. coli* in our study compared with other reports might possibly be because we evaluated fecal samples of cattle that were readily available in the abattoirs, unlike other studies that evaluated animal herds. This difference in the evaluated cattle population may possibly influence the detection rate of ESBL-producing *E. coli* in cattle fecal samples. However, further studies are required to confirm this. The observed prevalence of ESBL producers in cattle fecal samples in the present study further supports the hypothesis that animals might become infectious sources or even reservoirs (the natural persistent source of infection) contributing to the spread of these bacterial pathogens [28]. Although the epidemiological impact and evaluation of ESBL-producing *E. coli*

Table-1: Oligonucleotide primers for ESBL and fluoroquinolone-resistant encoding genes.

Genes	Primer sequences (5′-3′)	Amplicon size (bp)	Reference
TEM	F: CATTTCCGTGTCGCCCTTATTC R: CGTTCATCCATAGTTGCCTGAC	1350	[17]
SHV	F: AGCCGCTTGAGCAAATTAAAC R: ATCCCGCAGATAAATCACCAC	700	[17]
CTX-M	F: GTTACAATGTGTGAGAAGCAG R: CCGTTTCCGCTATTACAAA	550	[18]
aac (6)-Ib-cr-	F: TTGCGATGCTCTATGAGTGGCTA R: CTCGAATGCCTGGCGTGTTT	1350	[19]
Qnr	F: ATTTCTCACGCCAGGATTTG R: GATCGGCAAAGGTTAGGTCA	200	[20]

ESBL=Extended-spectrum beta-lactamase

Table-2: Occurrence frequencies of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in fecal samples of cows in selected abattoirs.

Metropolis	Sample source	Samples collected	ESBL-positive
	Abattoir A	10	2
	Abattoir B	10	1
Abakaliki	Abattoir C	10	0
	Abattoir D	10	5
	Abattoir E	10	0
	Total	50	8 (16%)
Afikpo	Abattoir A	10	3
	Abattoir B	10	6
	Abattoir C	10	2
	Abattoir D	10	1
	Abattoir E	10	0
	Total	50	12 (24%)
	Overall total	100	20 (20%)

ESBL=Extended-spectrum beta-lactamase

Table-3: Antimicrobial resistance profiles of ESBL-producing *Escherichia coli* isolated from fecal samples of cattle in abattoirs.

Antimicrobials	Resistance (%)
Amoxicillin-clavulanic acid	15 (75)
Amoxicillin	19 (95)
Cefotaxime	19 (95)
Cefuroxime	17 (85)
Cefoxitin	14 (70)
Ceftriaxone	17 (85)
Ceftazidime	15 (75)
Ciprofloxacin	9 (45)
Tetracycline	20 (100)
Trimethoprim-sulfamethoxazole	20 (100)
Amikacin	0 (0)
Imipenem	0 (0)

ESBL=Extended-spectrum beta-lactamase

Table-4: Occurrence frequency of fluoroquinolone and beta-lactamase resistance genes in ESBL-producing *E. coli* isolates.

Antimicrobial Class	Resistance genes	Positive isolates n (%)
β-lactams	*bla*TEM	15 (75.0)
	*bla*SHV	1 (5.0)
	*bla*CTX-M	4 (20.0)
	*bla*TEM + *bla*SHV	1 (5.0)
Fluoroquinolones	*Qnr*	0 (0.0)
	aac-lb-6-cr	19 (95)

are still underestimated due to low awareness, the pathogenic traits exhibited by these bacterial pathogens colonizing food-producing animals call for gross surveillance in community settings, especially now that its spread is a growing global threat for currently available antimicrobials.

In our study, *E. coli* isolates from cattle fecal samples from abattoirs were multidrug-resistant (MDR) with MARI values ranging from 0.5 to 0.8. In general, isolates exhibited resistance to trimethoprim-sulfamethoxazole (100%), tetracycline (100%), amoxicillin (95%), cephalosporins (75%–95%), and ciprofloxacin (45%), which are the commonly used antimicrobials in our study area.

The results of our study reiterate the findings of other studies that have reported an increasing and alarming rate of antibiotic resistance among bacteria, particularly *E. coli* from cattle and other animals [25]. According to some previous studies, the resistance frequencies of ESBL-producing *E. coli* from fecal samples of cattle to cephalosporins ranged from 58% to 95% [29, 30]. The frequencies of resistance to cephalosporins reported by other researchers are similar to those observed in our study (75%–95%). Resistance to tetracycline, a commonly used first-line antimicrobial for domestic animals [31], has also been reported. All isolates in our study were completely resistant (100%) to tetracycline and trimethoprim-sulfamethoxazole. This is supported by the excessive and long-term use of these antimicrobials for disease treatment, prophylaxis, or livestock growth promotion in our study area. Resistance to ciprofloxacin and beta-lactams (38.9%–100%) has also been reported elsewhere [18, 22, 30, 32].

All isolates in this study were completely susceptible to imipenem and amikacin. This is supported by existing trends in other studies [23, 33]. The high activity of amikacin and imipenem in our study area can be attributed to their very low usage due to the strict restrictions imposed on them, as well as the stress associated with parental preparation.

In Nigeria, beta-lactams, aminoglycosides, fluoroquinolones, folate pathway inhibitors, and tetracyclines are widely used for the treatment of a variety of infections (e.g., enteritis, mastitis, pneumonia, and septicemia), which may lead to selective pressure, thus favoring the emergence of MDR bacteria.

In the present study, *bla*TEM was the most predominant ESBL gene harbored by ESBL-producing *E. coli* isolates with a frequency of 75%. This was followed by *bla*CTX-M (20.0%) and *bla*SHV (5.0%). Interestingly, the isolate also co-harbored ESBL genes (*bla*TEM + *bla*SHV). ESBL-producing *E. coli* harboring the *bla*TEM gene has also been reported in fecal samples of cattle in Nigeria [17, 25] and other regions, especially in Europe [34]. The *bla*CTX-M gene has also been reported in fecal samples of cattle [23, 35]. The quinolone resistance rate in *E. coli* is unusually high, especially in ESBL-producing strains [36]. Moreover, plasmids carrying plasmid-mediated quinolone resistance genes may harbor genes that confer resistance to extended-spectrum cephalosporin and/or resistance to other classes of antimicrobial agents [37]. This is evident in the resistance traits of the isolates in our study as 95% of these MDR ESBL-producing *E. coli* harbored the *aac-lb-6-cr* gene, a fluoroquinonlone-resistant gene. In China, *aac(6)-Ib-cr* genes have been detected in 8.0% ESBL-producing *E. coli* harboring *bla*CTX-M-14 and TEM-1 [38]. On the other hand, *Qnr* was not harbored by any of the isolates in this study. Previous studies have reported

E. coli isolates from fecal samples of farm animals harboring the *aac(6')-Ib-cr* fluoroquinolone-resistant gene [1, 39].

Although MDR ESBL-producing *E. coli* from the fecal samples of cows in abattoirs in our study was reported, further studies are required to comprehensively understand the prevalence, pathogenic arsenals, clonal relatedness/diversities, and epidemiological identities of bacterial pathogens colonizing food-producing animals, particularly cattle.

Conclusion

This study reports a high frequency of MDR ESBL-producing *E. coli* from cow fecal samples collected from abattoirs in our study region. Isolates harbored *bla*TEM (75%), *bla*CTX-M (20.0%), *bla*SHV (5.0%), and *aac-lb-6-cr* (95%) genes. The high frequency of ESBL- and fluoroquinolone-resistant genes harbored by ESBL-producing *E. coli* isolates in the present study is worrisome and has a significant public health impact. Therefore, there is a need to establish more surveillance studies to further monitor AMR trends in food-producing animals (including their by-products) and to formulate strong policies to prevent the misuse of antimicrobials, especially in veterinary medicine.

Authors' Contributions

IRI, EE, and IBM: Conceptualized and designed the study. CSI, FAI, IO, FNO, and COE: Methodology and retrieval of data. EE and IBM: Writing-original draft, methodology, retrieval, analysis, and interpretation of the data. IRI and IBM: Edited and revised the manuscript. All authors have read, reviewed, and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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References

1. Röderova, M., Halova, D., Papousek, I., Dolejska, M., Masarikova, M., Hanulik, V., Pudova, V., Broz, P., Htoutou-Sedlakova, M., Sauer, P., Bardon, J., Cizek, A., Kolar, M. and Literak, I. (2017) Characteristics of quinolone resistance in *Escherichia coli* isolates from humans, animals and the environment in the Czech Republic. *Front. Microbiol*., 7: 2147.
2. Salman, R.A. and Ghaima, K.K. (2018) Prevalence of ESBL genes in ESBL-producing *Klebsiella pneumonia* isolated from patients with urinary tract infections in Baghdad, Iraq. *J. Biosci. Res*., 15(3): 2049–2059.
3. Schwarz, S. and Chaslus-Dancla, E. (2001) Use of antimicrobials in veterinary medicine and mechanisms of resistance. *Vet. Res*., 32(3–4): 201–225.
4. World Health Organization. Global Priority List of Antibiotic-resistant Bacteria to Guide Research, Discovery and Development of New Antibiotics. WHO, Geneva, p17–21.
5. Andriole, V.T. (2005) The quinolones: Past, present and future. *Clin. J. Infect. Dis*., 15(2): 113–119.
6. Newire, E.A., Ahmed, S.F., House, B., Valiente, E. and Pimentel, G. (2013) Detection of new SHV-12, SHV-5 and SHV-2a variants of extended-spectrum beta-lactamase in *Klebsiella pneumoniae* in Egypt. *Ann. Clin. Microbiol. Antimicrob. Agent*, 12: 16–340.
7. Marchandin, H., Carriere, C., Sirot, D., Pierre, H.J. and Darbas, H. (1999) TEM-24 produced by four different species of *Enterobacteriaceae*, including *Providencia rettgeri*, in a single patient. *Antimicrob. Agents Chemother*., 43(8): 2069–2073.
8. Dupouy, V., Abdelli, M., Moyano, G., Arpaillange, N., Bibbal, D., Cadiergues, M.C., Lopez-Pulin, D., Sayah-Jeanne, S., de Gunzburg, J., Saint-Lu, N., Gonzalez-Zorn, B., Andremont, A. and Bousquet-Mélou, A. (2019) Prevalence of Beta-lactam and quinolone/fluoroquinolone resistance in *Enterobacteriaceae* from dogs in France and Spain-characterization of ESBL/pAmpC isolates, genes and conjugative plasmids. *Front. Vet. Sci.*, 6: 279.
9. Ewers, C., Grobbel, M., Stamm, I., Kopp, P.A., Diehl, I., Semmler, T., Fruth, A., Beutlich, J., Guerra, B., Wieler, L.H. and Guenther, S. (2010) Emergence of human pandemic O25: H4-ST131 CTX-M-15 extended-spectrum-β-lactamase-producing *Escherichia coli* among companion animals. *J. Antimicrob. Chemother*., 65(4): 651–660.
10. Blake, D.P., Hillman, K., Fenlon, D.R. and Low, J.C. Transfer of antibiotic resistance between commensal and pathogenic members of the *Enterobacteriaceae* under ileal conditions. *J. Appl. Microbiol*., 95(3): 428–436.
11. Cheesbrough, M. (2006) District Laboratory Practice in Tropical Countries (Part II). Cambridge University, Cambridge, p19–110.
12. John-Onwe, B.N., Iroha, I.R., Moses, I.B., Onuora, A.L., Nwigwe, J.O., Adimora, E.E., Okolo, I.O., Uzoeto, H.O., Ngwu, J.N., Mohammed, I.D. and Oladimeji, A.S. (2022) Prevalence and multidrug-resistant ESBL-producing *e. coli* in urinary tract infection cases of HIV patients in Abakaliki, Nigeria. *Afr. J. Microbiol. Res*., 16(5): 196–201.
13. Iroha, I.R., Onyia, U., Moses, I.B., Ejikeugwu, C.P., Nwakaeze, A.E. and Ugbo, E.N. (2019) Prevalence and antibiotic resistance profiles of extended spectrum β-lactamase-producing *Escherichia coli* among paediatric patients with urinary tract infection in St. Patricks' Hospital, Mile Four, Abakaliki, Ebonyi State, Nigeria. *Afr. J. Clin. Exp. Microbiol*., 20(4): 332–336.
14. Ovia, K., Ugbo, B., Iroha, I.R. and Chika, E. (2018) Extended-spectrum β-lactamase (ESBL) expression in *Escherichia coli* isolates from anal swabs of donkeys in a local donkey Abattoir in Abakaliki, Nigeria. *Acta Sci. Microbiol*., 1(8): 2–6.
15. Clinical and Laboratory Standards Institute (CLSI). (2021) M100-Performance Standards for Antimicrobial Susceptibility Testing, 31st ed. Clinical and Laboratory Standards Institute, USA.
16. Moses, I.B., Esimone, C.O., Iroha, I.R., Ugbo, E.N., Orji, J.O., Nwuzo, A.C., Orji, J.O., Nwakaeze, E.A., Agumah, N.B., Emioye, A.A., Ukpai, E.G. and Ogene, L.N. (2020) First report on the antibiotic resistance profiles and virulence genes of *Staphylococcus pseudintermedius* colonizing dogs and humans in Abakaliki, South-East Nigeria. *Res. J. Microbiol.*, 15(1): 22–34.

17. Ugwu, M.C., Shariff, M., Nnajide, C., Beri, M., Okezie, U.M., Iroha, I.R. and Esimone, C.O. (2020) Phenotypic and molecular characterization of *β*-lactamases among enterobacterial uropathogens in Southeastern Nigeria. *Can. J. Infect. Dis. Med. Microbiol*., 2020: 5843904.
18. Kpalap, J.A., Nwokah, E.G. and Alo, M.N. (2019) Distribution of some antibiotics resistance genes in multi-drug-resistant *E. coli* Isolates from the urogenitals of women in Port Harcourt, Nigeria. *Asian J. Res. Infect. Dis*., 2(1): 1–7.
19. Al-Agamy, M.H., Aljallal, A., Radwan, H.H. and Shibl, A.M. (2018) Characterization of carbapenemases, ESBLs, and plasmid-mediated quinolone determinants in carbapenem-insensitive *Escherichia coli* and *Klebsiella pneumoniae* in Riyadh hospitals. *J. Infect. Public Health*, 11(1): 64–68.
20. Ranjbar, R., Tolon, S.S., Sami, M. and Golmohammadi, R. (2018) Detection of plasmid-mediated *qnr* genes among the clinical quinolone-resistant *Escherichia coli* strains isolated in Tehran, Iran. *Open Microbiol. J*., 12: 248-253.
21. Barzan, M., Gharibi, D., Ghorbanpoor, M., Hajikolaei, M.H. and Pourmehdi-Boroujeni, M. Phylogenetic grouping and phenotypic detection of extended-spectrum β-lactamases among *Escherichia coli* from calves and dairy cows in Khuzestan, Iran. *Int. J. Enteric Pathol*., 5(1): 24–29.
22. Mahmud, S., Nazir, K.H.M. and Rahman, M.T. (2018) Prevalence and molecular detection of fluoroquinolone-resistant genes (*qnrA* and *qnrS*) in *Escherichia coli* isolated from healthy broiler chickens. *Vet. World*, 11(12): 1720–1724.
23. Pehlivanoglu, F., Turutoglu, H., Ozturk, D. and Yardimci, H. (2016) Molecular characterization of ESBL-producing *Escherichia coli* isolated from healthy cattle and sheep. *Acta Vet. Beograd*, 66(4): 520–533.
24. Ali, T., Rahman, S., Zhang, L., Shahid, M., Zhang, S., Liu, G., Gao, J. and Han, B. (2016) ESBL-producing *Escherichia coli* from cows suffering mastitis in China contain clinical class 1 integrons with CTX-M linked to *ISCR1*. *Front. Microbiol*., 7: 19–31.
25. Olowe, O.A., Adewumi, O., Odewale, G., Ojurongbe, O. and Adefioye, O.J. (2015) Phenotypic and molecular characterisation of extended-spectrum beta-lactamase producing *Escherichia coli* obtained from animal fecal samples in Ado Ekiti, Nigeria. *J. Environ. Public Health*, 2015: 497980.
26. Montso, K.P., Dlamini, S.B., Kumar, A. and Ateba, C.N. (2019) Antimicrobial resistance factors of extended-spectrum beta-lactamases producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from cattle farms and raw beef in North-West Province, South Africa. *Biomed. Res. Int*., 2019: 4318306.
27. Reid, C.A., Small, A., Avery, S.M. and Buncic, S. (2002) Presence of foodborne pathogens on cattle hides. *J. Food Control*, 13(6–7): 411–415.
28. Carattoli, A. (2008) Animal reservoirs for extended-spectrum *β*-lactamase producers. *Clin. Microbiol. Infect*., 14(1): 117–123.
29. Ajayi, A.O., Oluyege, A.O., Olowe, O.A. and Famurewa, O. (2001) Antibiotic resistance amongst commensal *Escherichia coli* isolated from feces of cattle in Ado-Ekiti, Nigeria. *J. Anim. Vet. Adv*., 10(2): 174–179.
30. Atnafie, B., Paulos, D., Abera, M., Tefera, G., Hailu, D., Kasaye, S. and Amenu, K. (2017) Occurrence of *Escherichia coli* O157:H7 in cattle feces and contamination of carcass and various contact surfaces in abattoir and butcher shops of Hawassa, Ethiopia. *BMC Microbiol*., 17(1): 24.
31. Liu, X., Liu, H., Wang, L., Peng, Q., Li, Y., Zhou, H. and Li, Q. (2018) Molecular characterization of extended-spectrum beta-lactamase-producing multidrug-resistant *Escherichia coli* from swine in Northwest China. *Front. Microbiol*., 9: 1756.
32. Umeda, K., Hase, A., Fukuda, A., Matsuo, M., Horimoto, T. and Ogasawara, J. (2020) Prevalence and mechanisms of fluoroquinolone-resistant *Escherichia coli* among sheltered companion animals. *Access Microbiol*., 2(1): acmi000077.
33. Alegría, A., Arias-Temprano, A., Fernández-Natal, I., Rodríguez-Calleja, J.M., García-López, M., Santos, J.A. (2020) Molecular diversity of ESBL-producing *Escherichia coli* from foods of animal origin and human patients. *Int. J. Environ. Res. Public Health*, 17(4): 1312–1367.
34. Ceccarelli, D., Kant, A., van Essen-Zandbergen, A., Dierikx, C., Hordijk, J., Wit, B., Mevius, D.J. and Veldman, K.T. (2019) Diversity of plasmids and genes encoding resistance to extended-spectrum cephalosporins in commensal *Escherichia coli* from Dutch Livestock in 2007–2017. *Front. Microbiol*., 10(3): 234–456.
35. Zheng, B., Feng, C., Xu, H., Yu, X., Guo, L., Jiang, X. and Song, X. (2019) Detection and characterization of ESBL-producing *Escherichia coli* expressing mcr-1 from dairy cows in China. *J. Antimicrob. Chemother*., 74(2): 321–325.
36. Mahamat, O.O., Lounnas, M., Hide, M., Dumont, Y., Tidjani, A., Kamougam, K., Abderrahmane, M., Benavides, J., Solassol, J., Bañuls, A.L., Jean-Pierre, H., Carrière, C. and Godreuil, S. (2019) High prevalence and characterization of extended-spectrum ß-lactamase-producing *Enterobacteriaceae* in Chadian hospitals. *BMC Infect. Dis*., 19(1): 205–210.
37. Silva-Sanchez, J., Cruz-Trujillo, E., Barrios, H., Reyna-Flores, F., Sanchez-Perez, A. and Garza-Ramos, U. (2013) Characterization of plasmid-mediated quinolone resistance (PMQR) genes in extended-spectrum beta-lactamase-producing *Enterobacteriaceae* pediatric Clinical Isolates in Mexico. *PLoS One*, 8(10): e77968.
38. Jiang, Y., Zhou, Z., Qian, Y., Wei, Z., Yu, Y., Hu, S. and Li, L. (2008) Plasmid-mediated quinolone resistance determinants *qnr* and *aac(60)- Ib-cr* in extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in China. *J. Antimicrob. Chemother*., 61(5): 1003–1006.
39. de Jong, A., Muggeo, A., El Garch, F., Moyaert, H. and de Champs, C. (2016) Characterization of quinolone resistance mechanisms in *Enterobacteriaceae* isolated from companion animals in Europe (ComPath II study). *Vet. Microbiol*., 216: 159–167.

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