International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2025: 7.089

Carbapenemase Genes and Plasmid Profiles in Meropenem-Resistant Bacteria from Goats and Cattle in Ekiti State, Nigeria

Olajumoke Kemi Ekundayo[1], Isaac Iseoluwa Ajayi[2]

[1]Department of Biological Sciences, Bamidele Olumilua University of Education, Science and Technology, Ikere-Ekiti
Email: *ekundayo.olajumoke[at]bouesti.edu.ng*

[2]Department of Biological Sciences, Bamidele Olumilua University of Education, Science and Technology, Ikere-Ekiti
Corresponding Author Email: *ajayi.isaac[at]bouesti.edu.ng*

Abstract: ***Carbapenem resistance in livestock is a growing One Health concern, but data from goats and cattle in sub-Saharan Africa are limited. This study examined meropenem-resistant bacteria in 100 rectal swabs from goats and cattle in Ekiti State, Nigeria. Isolates were grown on MacConkey and blood agar and identified by biochemical tests and 16S rRNA sequencing. Meropenem MICs were measured by E-test, other antibiotics by disc diffusion, and six carbapenemase genes by multiplex PCR. Plasmids were extracted by the TENS method and sized on agarose gels. Six isolates were resistant to meropenem (MIC 4-6 µg/mL). Four came from goats (*Enterococcus casseliflavus, E. gallinarum, Enterobacter huaxiensis *and* Bacillus cereus*) and two from cattle (both* B. subtilis*). Carbapenemase genes were found in five isolates (83.3%), including every goat isolate. The genes* bla*VIM,* bla*OXA-48 and* bla*SPM were the most common (two isolates each),* bla*IMP was not detected, and two isolates carried more than one gene. Tigecycline was fully active (100%), followed by nitrofurantoin and fosfomycin (92.86% each), while polymyxin B showed the highest resistance (57.8%). Four isolates carried a single plasmid of about 10 kb, and two had none. Because genes and plasmids did not always coincide, some genes may be chromosomal. The findings give baseline evidence for including ruminants in antimicrobial resistance surveillance under a One Health framework.***

Keywords: Carbapenem Resistance, Livestock Bacteria, Antimicrobial Resistance, Carbapenemase Genes, One Health Surveillance

1. Introduction

Carbapenems, including meropenem, remain among the last dependable options for serious infections caused by multidrug-resistant Gram-negative bacteria. Carbapenem-resistant *Enterobacterales, Acinetobacter baumannii and Pseudomonas aeruginosa* are critical-priority pathogens for the World Health Organization (Tacconelli et al., 2018; WHO, 2024). Resistance arises through carbapenemases such as KPC, NDM, VIM, IMP and OXA-48-like enzymes, and through porin loss combined with extended-spectrum or AmpC β-lactamase activity (Queenan and Bush, 2007; Doumith et al., 2009; Nordmann et al., 2011).

The most clinically important carbapenemase genes are seldom confined to the chromosome. They are usually plasmid-borne and can move between unrelated species, often together with determinants for resistance to aminoglycosides, fluoroquinolones and other drug classes (Carattoli, 2013; Mathers et al., 2015). Plasmid content therefore matters as much as organism identity, because plasmids, not clonal expansion alone, frequently drive the dissemination of resistance (Carattoli, 2009; Poirel et al., 2011; Rozwandowicz et al., 2018).

Carbapenems are not licensed for routine use in food-producing animals, and livestock were long assumed to play little part in carbapenem resistance. Reports of carbapenemase-producing bacteria in pigs, poultry and cattle from Europe, Asia and, more recently, Africa have challenged this (Fischer et al., 2012, 2013; Woodford et al., 2014; Wang et al., 2017; Köck et al., 2018). Proposed explanations include selection by other β-lactams, co-selection by heavy metals or unrelated antimicrobials, and contamination from human and environmental sources (Seiler and Berendonk, 2012; Woodford et al., 2014; Köck et al., 2018; Ekundayo and Oyinloye, 2025). Whatever the origin, such organisms in food animals could reach people through direct contact, contaminated meat and milk, and manure-amended soil and water.

Goats and cattle deserve particular attention. In much of sub-Saharan Africa they are raised under extensive or semi-intensive systems, graze communally, share water sources and live close to farming households. Informal slaughter and limited veterinary oversight add to the exposure risk (Founou et al., 2016; Kimera et al., 2020; Van et al., 2020). Even so, surveillance in ruminants lags behind that in poultry and swine, and few studies have characterized the plasmids of resistant isolates from these hosts.

Plasmid profiling, combining extraction and electrophoretic sizing with PCR-based replicon typing and, where resources permit, sequencing, allows plasmid number, size and incompatibility type to be compared across isolates, hosts and locations (Kado and Liu, 1981; Carattoli et al., 2005, 2014). This study aimed to identify meropenem-resistant bacteria from goats and cattle in Ekiti State, detect carbapenemase genes, and

determine plasmid number and size, as baseline data for surveillance under a One Health framework.

2. Materials and Methods

Sample Collection

Rectal swabs (n = 100) were collected from cattle (n = 50) and goats (n = 50) on five farms in Ekiti State, southwestern Nigeria, with the owners' consent. Swabs were inserted about 2.5 cm into the anus, placed in peptone water, transported on ice and processed within 6 h.

Isolation and identification

Samples were enriched in peptone broth and streaked onto MacConkey agar (37 °C, 24 h). Distinct colonies were sub-cultured to purity and identified by colony morphology, Gram staining and a biochemical test Cheesebrough, 2000). Genomic DNA was extracted by the CTAB method (Mushi et al., 2014). The 16S rRNA gene was amplified with universal primers 27F and 1625R (94 °C for 5 min; 30 cycles of 94, 50 and 72 °C; 72 °C for 10 min), verified on 1.5% agarose, purified by ethanol precipitation and sequenced on an ABI 3130xl Genetic Analyzer with BigDye Terminator v3.1 chemistry. Sequences were analysed in BioEdit and MEGA and identified by BLAST (Ekundayo, 2021).

Antimicrobial susceptibility and MIC testing

Disc diffusion (CLSI, 2021) was performed on Mueller–Hinton agar with fosfomycin (100 µg), tigecycline (15 µg), gentamicin (30 µg), polymyxin B (200 µg) and nitrofurantoin (300 µg) discs, and zones were read after 18–24 h at 37 °C. Meropenem MICs were determined by E-test (Liofilchem) and read at the ellipse–strip intersection (CLSI, 2024). *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were the quality control strains.

Detection of carbapenemase genes

Carbapenemase genes were detected by three multiplex PCR assays: multiplex 1 (blaNDM, 624 bp; blaVIM, 502 bp), multiplex 2 (blaKPC, 498 bp; blaOXA-48, 238 bp) and multiplex 3 (blaIMP, 568 bp; blaSPM, 271 bp), using primers from Shoja et al. (2013), Mushi et al. (2014) and Mahmoud et al. (2020) (Table S1). Cycling comprised denaturation at 94 °C for 5 min, 35 cycles of denaturation, annealing (50, 46 and 52 °C for multiplexes 1, 2 and 3) and extension at 72 °C, and a final extension at 72 °C for 10 min.

Plasmid extraction and profiling

Plasmid DNA was extracted from the six resistant isolates by the TENS rapid alkaline lysis method (Kado and Liu, 1981), with minor modifications. Cells from 18–24 h nutrient agar cultures were suspended in TE buffer, pelleted and lysed in alkaline SDS solution, and the lysate was deproteinised with phenol:chloroform. The aqueous phase was used directly or stored at −20 °C. Extracts were resolved on 0.8% (w/v) agarose gels in TAE buffer, stained and run at 80–100 V for 60–90 min alongside a HindIII-digested lambda marker. Gels were visualised under UV, and plasmid sizes were estimated from relative migration against the marker on a semi-log plot.

3. Results

Isolates and meropenem resistance

Six meropenem-resistant isolates were recovered, four from goats and two from cattle (Table 1). The goat isolates were *B. cereus, E. huaxiensis, E. casseliflavus and E. gallinarum* (one each), and both cattle isolates were *B. subtilis.* All six were resistant by E-test, with MICs of 4 µg/mL (*B. subtilis* Cf13 and Cf18, *E. huaxiensis* Fg30) or 6 µg/mL (*B. cereus* Fg33, *E. casseliflavus* Gf15b, *E. gallinarum* Gf16).

Table 1: Meropenem-resistant isolates, sources and MICs (all resistant)

Code	Source	Isolate	MIC (µg/mL)
Gf15b	Goat	Enterococcus casseliflavus	6
Gf16	Goat	Enterococcus gallinarum	6
Fg30	Goat	Enterobacter huaxiensis	4
Fg33	Goat	Bacillus cereus	6
Cf13	Cattle	Bacillus subtilis	4
Cf18	Cattle	Bacillus subtilis	4

Susceptibility to other antibiotics

Susceptibility was highest for tigecycline (100%), nitrofurantoin (92.86%) and fosfomycin (92.86%), followed by gentamicin (83.3%), whereas polymyxin B showed the highest resistance (Table 2).

Table 2: Susceptibility of the isolates to other antibiotics

Antibiotic	Susceptible (%)	Resistant (%)
Polymyxin B	42.2	57.8
Gentamicin	83.3	16.7
Nitrofurantoin	92.86	7.14
Fosfomycin	92.86	7.14
Tigecycline	100	0

Carbapenemase genes

At least one carbapenemase gene was detected in five of six isolates (83.3%): all four goat isolates (100%) and one of two cattle isolates (50%) (Table 3; Plates 1–6). In total, eight gene detections were recorded. blaVIM, blaOXA-48 and blaSPM were most frequent (n = 2 each), followed by blaNDM and blaKPC (n = 1 each); blaIMP was not detected. Two isolates carried more than one gene: *E. gallinarum* Gf16 (blaNDM, blaOXA-48, blaSPM) and *B. cereus* Fg33 (blaKPC, blaOXA-48). *B. subtilis* Cf18 was negative for all six genes despite a MIC of 4 µg/mL.

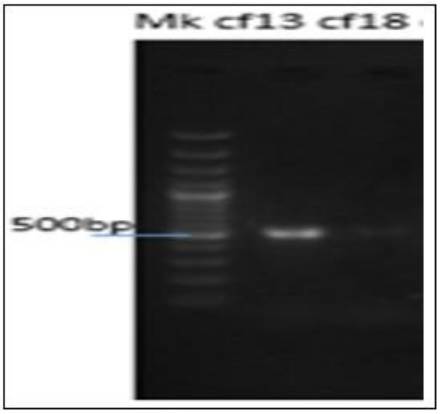


Plate 1: Multiplex PCR of *bla*$_{NDM}$ (624) and *bla*$_{VIM}$(502) genes .Cf13 is positive for *bla*$_{VIM}$ (502) while cf18 is negative.

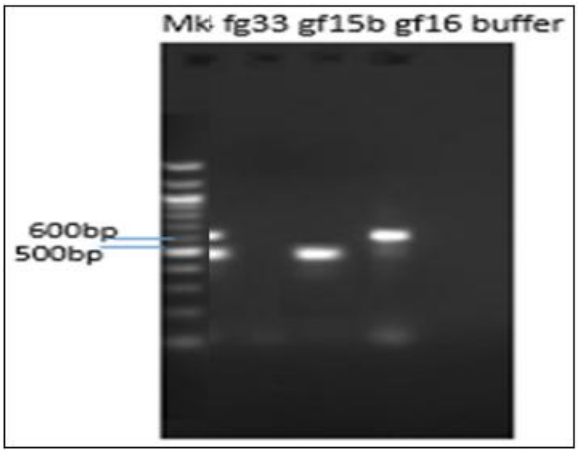


Plate 2: Multiplex PCR for amplification of *bla*$_{NDM}$ (624) and *bla*$_{VIM}$ (502) genes on 2% agarose gel electrophoresis: gf16 are positive for *bla*$_{NDM}$ gf15b is positive for *bla*$_{VIM}$. Fg33 no gene seen.

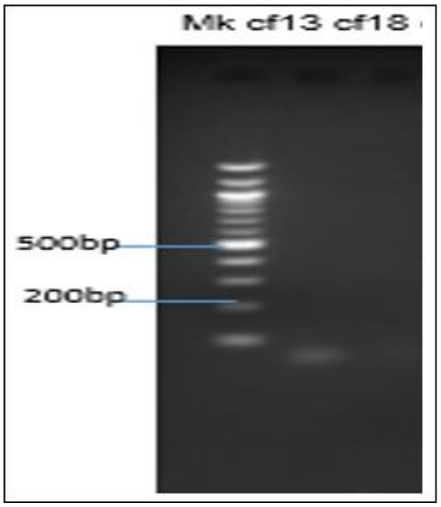


Plate 3: Multiplex PCR of *bla*$_{KPC}$ (498) and *bla*$_{OXA-48}$(238) genes. None were positive

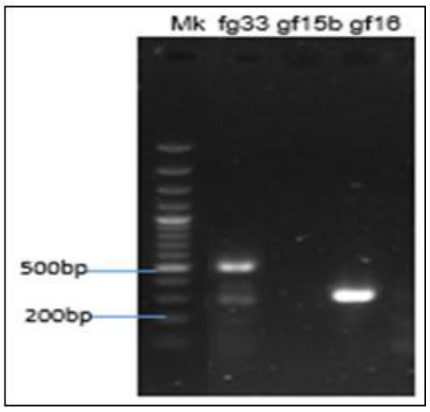


Plate 4: Multiplex PCR of *bla*$_{KPC}$ (498) and *bla*$_{OXA-48}$(238) genes, fg33 (second band) and gf16 are positive for *bla*$_{OXA-48}$ while fg 33(first band) is positive for *bla*KPC genes and gf15 has no result for both genes.

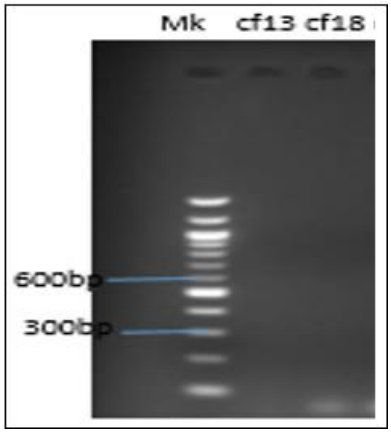


Plate 5: Multiplex PCR for amplification of *bla*$_{SPM}$ and *bla*$_{IPM}$ genes. None of the isolates on this plate is positive for *bla*$_{SPM}$ and *bla*$_{IPM}$ genes.

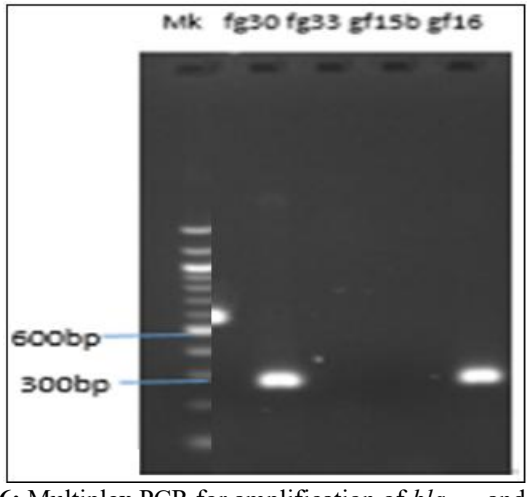


Plate 6: Multiplex PCR for amplification of *bla*$_{SPM}$ and *bla*$_{IPM}$ genes. fg30 and gf 16 are positive for *bla*$_{SPM}$ and none was positive for *bla*$_{IPM}$ genes.

Plasmid profiles

A single band of approximately 10 kb was seen in *B. subtilis* Cf18, *E. casseliflavus* Gf15b, *E. huaxiensis* Fg30 and *B. cereus*

Fg33. No plasmid band was detected in *B. subtilis* Cf13 or *E. gallinarum* Gf16 (Plates 7 and 8). Table 3 summarizes plasmid carriage alongside gene content.

Table 3: Carbapenemase genes and plasmid carriage by isolate.

Isolate	Organism	Source	Carbapenemase genes	Genes (n)	Plasmid
Cf13	B. subtilis	Cattle	BlaVIM	1	None detected
Cf18	B. subtilis	Cattle	None	0	~10 kb
Fg33	B. cereus	Goat	blaKPC, blaOXA-48	2	~10 kb
Gf15b	E. casseliflavus	Goat	BlaVIM	1	~10 kb
Gf16	E. gallinarum	Goat	blaNDM, blaOXA-48, blaSPM	3	None detected
Fg30	E. huaxiensis	Goat	BlaSPM	1	~10 kb

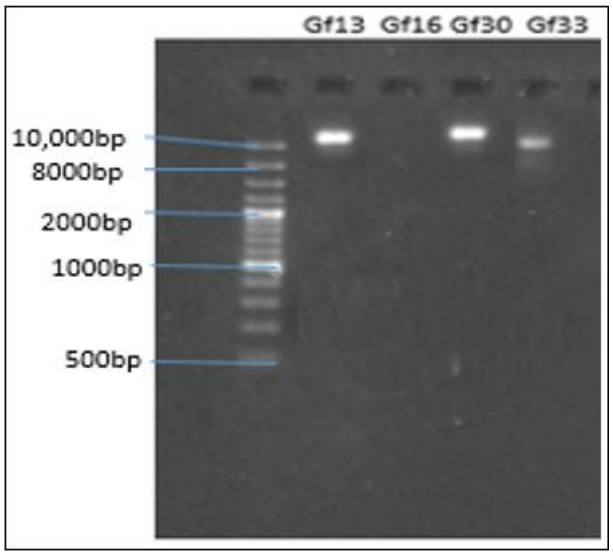


Plate 7: Plasmid profile analysis of bacteria isolated from goats (female) Gf 13, Fg 30 and Fg 33 has single band with a molecular weight of 10,000basepair. Gf 16 has no plasmid band, Plasmid DNA on 0.8g Agarose, M-Molecular marker Hinda lll lambda.

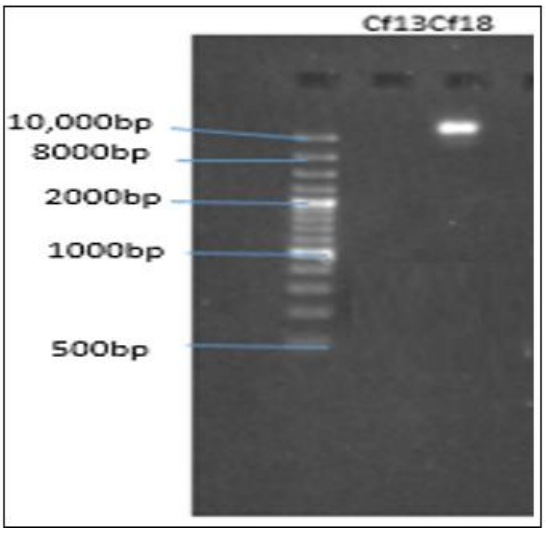


Plate 8: Plasmid profile analysis of bacteria isolated from goats (female) Gf 13, Fg 30 and Fg 33 has single band with a molecular weight of 10,000basepair. Gf 16 has no plasmid band, Plasmid DNA on 0.8g Agarose, M-Molecular marker Hinda lll lambda.

4. Discussion

This study recovered six meropenem-resistant isolates from 100 rectal swabs of goats and cattle in Ekiti State. Carbapenem resistance in livestock is usually discussed in terms of Enterobacterales (Köck et al., 2018; Woodford et al., 2014), but only one isolate here, *E. huaxiensis*, belongs to that order. The others were Gram-positive Enterococcus and Bacillus species. These genera show reduced β-lactam susceptibility, and *B. cereus* can carry chromosomal metallo-β-lactamases, so MICs of 4–6 µg/mL should be interpreted with care. The breakpoint source used for these non-Enterobacterales isolates should be stated explicitly.

Carbapenemase genes were found in five of six isolates (83.3%), including all four goat isolates. VIM-producing bacteria have been reported from livestock in Europe (Fischer et al., 2012, 2013), and the multiple genes in Gf16 and Fg33 suggest acquisition of more than one resistance determinant. However, several of these genes are classically described in Gram-negative bacteria, and their detection in Bacillus and Enterococcus is unusual. Sequencing the amplicons would rule out non-specific amplification. *B. subtilis* Cf18 was resistant but negative for all six genes, so non-enzymatic mechanisms such as altered penicillin-binding proteins, reduced permeability or efflux, or a carbapenemase gene outside the panel, may be responsible.

Plasmid profiling showed a single band of about 10 kb in four isolates, while Gf16 and Cf13 had no visible band. No isolate carried more than one plasmid, so plasmid diversity was low. Carbapenemase-encoding plasmids described in Enterobacterales are often considerably larger than 10 kb (Poirel et al., 2011; Carattoli, 2013; Mathers et al., 2015), so the band may be a small cryptic plasmid or a mobile element carrying a single gene, and gel mobility alone cannot settle this. Supercoiled plasmids also migrate faster than linear markers, so the size is approximate.

The match between gene and plasmid was imperfect. Gf16 carried three genes but no visible plasmid, and Cf13 carried blaVIM without one, which points to a chromosomal location or to large or low-copy plasmids missed by the TENS method (Kado and Liu, 1981). Conversely, Cf18 had a plasmid but no detectable gene. The same band size in different species and hosts hints at a shared mobile element, but restriction digestion

or replicon typing is needed to confirm it. Because plasmids drive the spread of resistance between species (Rozwandowicz et al., 2018), these steps are the priority for future work.

Susceptibility to tigecycline (100%), nitrofurantoin and fosfomycin (92.86% each) was high, so these agents remain options in this setting. Polymyxin B resistance (57.8%) is concerning because the drug is a last-resort agent, although Gram-positive isolates are typically not susceptible to polymyxins, which probably inflates this figure.

Goats and cattle in the region graze communally, share water and live close to households, and informal slaughter and limited veterinary oversight add further exposure routes (Founou et al., 2016; Kimera et al., 2020; Van et al., 2020). Carbapenemase genes in animal-associated bacteria therefore carry potential public health risk through direct contact, meat and milk, and manure-contaminated soil and water. The study is limited by its small number of resistant isolates (six of 100 swabs), a single state, gel-based plasmid sizing, no replicon typing or sequencing, and no human or environmental comparison. The results are best read as baseline surveillance data.

5. Conclusion

Meropenem-resistant bacteria carrying carbapenemase genes (blaVIM, blaOXA-48, blaSPM, blaNDM and blaKPC) were present in goats and cattle in Ekiti State, with genes detected in all goat isolates and one of two cattle isolates. Most isolates carried a single ~10 kb plasmid, but gene carriage and plasmid presence did not consistently match, so some determinants may be chromosomal. Tigecycline, nitrofurantoin and fosfomycin remained highly active. Ruminants may serve as reservoirs of carbapenem resistance and should be included in surveillance under a One Health framework. Larger sampling, replicon typing and whole-genome sequencing are needed to establish whether the genes are plasmid-borne and whether related elements circulate among animals, people and the environment.

Acknowledgment
The authors acknowledge funding from the Institution-Based Research (IBR) fund of Bamidele Olumilua University of Education, Science and Technology, Ikere-Ekiti.

Conflict of interest
The authors declare no conflict of interest.

References

[1] Carattoli, A. Resistance plasmid families in Enterobacteriaceae. Antimicrobial Agents and Chemotherapy, 2009; 53(6): 2227–2238.
[2] Carattoli, A. Plasmids and the spread of resistance. International Journal of Medical Microbiology, 2013 303(6–7): 298–304.
[3] Carattoli, A. Identification of plasmids by PCR-based replicon typing. Journal of Microbiological Methods, 2005; 63(3): 219–228.
[4] Carattoli, A., et al. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrobial Agents and Chemotherapy, 2014; 58(7): 3895–3903.
[5] CLSI. Performance Standards for Antimicrobial Susceptibility Testing.2024; 35th ed. CLSI supplement M100.
[6] Doumith, M. (2009). Molecular mechanisms disrupting porin expression in ertapenem-resistant Klebsiella and Enterobacter spp. Journal of Antimicrobial Chemotherapy, 2009; 63(4): 659–667.
[7] Ekundayo, O.K., and Oyinloye, J.M.A. Plasmid profiling analysis of some meropenem-resistant Enterobacteriaceae isolates from poultry farms in Ado Ekiti, Nigeria. Microbes and Infectious Diseases. 2025; DOI:10.21608/mid.2024.220023.1558.
[8] Fischer, J., et al. Escherichia coli producing VIM-1 carbapenemase isolated on a pig farm. Journal of Antimicrobial Chemotherapy, 2012; 67(7): 1793–1795.
[9] Fischer, J., et al. Salmonella enterica subsp. enterica producing VIM-1 carbapenemase isolated from livestock farms. Journal of Antimicrobial Chemotherapy, 2013; 68(2): 478–480.
[10] Founou, L.L., Founou, R.C., and Essack, S.Y. Antibiotic resistance in the food chain: a developing country-perspective. Frontiers in Microbiology, 2016; 7, 1881.
[11] Kado, C.I., and Liu, S.T. Rapid procedure for detection and isolation of large and small plasmids. Journal of Bacteriology, 1981; 145(3): 1365–1373.
[12] Kimera, Z.I., et al. Antimicrobial use and resistance in food-producing animals and the environment: an African perspective. Antimicrobial Resistance and Infection Control, 2020; 9, 37.
[13] Köck, R., et al. Carbapenem-resistant Enterobacteriaceae in wildlife, food-producing, and companion animals: a systematic review. Clinical Microbiology and Infection, 2018; 24(12):1241–1250.
[14] Mathers, A.J., Peirano, G., and Pitout, J.D.D. The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant Enterobacteriaceae. Clinical Microbiology Reviews, 2015; 28(3): 565–591.
[15] Nordmann, P., Naas, T., and Poirel, L. Global spread of carbapenemase-producing Enterobacteriaceae. Emerging Infectious Diseases, 2011: 17(10): 1791–1798.
[16] Poirel, L., Bonnin, R.A., and Nordmann, P. Genetic features of the widespread plasmid coding for the carbapenemase OXA-48. Antimicrobial Agents and Chemotherapy, 2011; 56(1): 559–562.
[17] Queenan, A.M., and Bush, K. Carbapenemases: the versatile β-lactamases. Clinical Microbiology Reviews, 2007; 20(3): 440–458.
[18] Rozwandowicz, M., et al. Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. Journal of Antimicrobial Chemotherapy, 2018; 73(5): 1121–1137.
[19] Seiler, C., and Berendonk, T.U. Heavy-metal-driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. Frontiers in Microbiology, 2012; 3, 399.

[20] Tacconelli, E., et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. The Lancet Infectious Diseases, 2018; 18(3): 318–327.
[21] Van, T.T.H., et al. Antibiotic use in food animals worldwide, with a focus on Africa: pluses and minuses. Journal of Global Antimicrobial Resistance, 2020; 20: 170–177.
[22] Wang, Y., et al. Comprehensive resistome analysis reveals the prevalence of NDM and MCR-1 in Chinese poultry production. Nature Microbiology, 2017: 2, 16260.
[23] WHO. WHO Bacterial Priority Pathogens List, 2024. Geneva: World Health Organization.
[24] Woodford, N., et al. Carbapenemase-producing Enterobacteriaceae and non-Enterobacteriaceae from animals and the environment: an emerging public health risk of our own making? Journal of Antimicrobial Chemotherapy, 69(2), 2014; 287–291.