



## DETECTION OF EXTENDED-SPECTRUM BETA-LACTAMASE (ESBL) AND CARBAPENEMASE GENES IN *ESCHERICHIA COLI* AND *KLEBSIELLA* SPP. FROM BUSHMEAT IN OYO STATE, NIGERIA

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### Summary

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The rise of antimicrobial resistance (AMR) in zoonotic bacteria presents a major public health concern. Bushmeat, widely consumed in many regions, may serve as a reservoir for multidrug-resistant *E. coli* and *Klebsiella* spp.. This study examines the antimicrobial resistance patterns and molecular characterisation of extended-spectrum beta-lactamase (ESBL) and carbapenemase genes in *E. coli* and *Klebsiella* spp. from bushmeat in Oyo State, Nigeria. One hundred faecal samples were collected from bushmeat at three major markets in Egbeda and Oluyole Local Government Areas. Bacterial isolation was performed using standard methods, followed by biochemical confirmation with the API 20E system. Antimicrobial susceptibility testing was conducted using the Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Polymerase chain reaction (PCR) was used to detect ESBL (*bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>CTX-M</sub>) and carbapenemase (*bla*<sub>NDM</sub>, *bla*<sub>VIM</sub>) genes in some of the selected isolates. One hundred of each *E. coli* and *Klebsiella* spp. were identified. Both exhibited high resistance to beta-lactams, with *E. coli* showing 85% resistance to ampicillin and *Klebsiella* spp. showing 100% resistance. Resistance to sulfonamides was also notable, with 62% of *E. coli* and 84% of *Klebsiella* spp. resistant to sulfamethoxazole/trimethoprim. However, both species remained fully susceptible to fluoroquinolones and aminoglycosides. PCR analysis confirmed the presence of ESBL genes in *E. coli* (*bla*<sub>TEM</sub>, 50%; *bla*<sub>CTX-M</sub>, 25%) and *Klebsiella* spp. (*bla*<sub>TEM</sub>, 85.7%; *bla*<sub>CTX-M</sub>, 14.3%). Carbapenemase genes *bla*<sub>NDM</sub> (33.3%) and *bla*<sub>VIM</sub> (42.9%) were detected in both species, highlighting potential resistance to last-line antibiotics. These findings emphasise the role of bushmeat in AMR transmission and the urgent need for enhanced surveillance, stricter antibiotic regulations, and improved hygiene in bushmeat markets. A

One-Health approach integrating human, animal, and environmental health perspectives is essential to mitigate the spread of resistant pathogens.

**Key words:** antimicrobial resistance, bushmeat, carbapenemase, ESBL, *Escherichia coli*, *Klebsiella* spp.

## INTRODUCTION

In the twenty-first century, one of the greatest threats to global public health is antimicrobial resistance (AMR) (Ahmed *et al.*, 2024). Resistance pools have been sparked by the excessive use of antibiotics in animals to treat disease and promote animals' growth. This makes it easier for MDR bacteria like *Escherichia coli* and *Klebsiella* spp. to spread across the food chain or through handling animals. Between species, resistant bacterial strains proliferate easily. AMR is also developed in wildlife as a result of indirect environmental exposures that promote further pathogen transmission (Ahmed *et al.*, 2024). Extended-spectrum  $\beta$ -lactamase (ESBL) production is a strong resistance mechanism that makes it more difficult to treat infections with antibiotics. The carbapenems are generally considered among the most effective drugs for treating severe infections caused by ESBL-producing bacteria (Shaikh *et al.*, 2015).

For many Africans, bushmeat is a significant source of income and protein, yet activities associated with bushmeat have been connected to multiple zoonotic infections and may expose people to bacteria that are resistant to antibiotics (Kurpiers *et al.*, 2015; Olaru *et al.*, 2023). Such bacteria in the environment are the main cause of AMR in wildlife. For example, rats and shrews colonised with bacteria resistant to antibiotics are at a considerably higher rate near farms than in remote regions (Olaru *et al.*, 2023). Despite the increasing evidence of AMR in foodborne bacteria, little attention has been given to the

role of bushmeat in the dissemination of MDR pathogens.

In Nigeria, the spread of bacteria, especially antibiotic-resistant strains, is driven by widespread poverty and harsh environmental conditions, including poor infection control practices, limited access to clean water, and substandard sanitation (Iheanacho *et al.*, 2022). The use of readily available antimicrobials, which are mainly sold over the counter, reflects Nigeria's low level of public awareness and understanding of AMR (Jemilehin *et al.*, 2024). This leads to increased exposure of microorganisms to non-lethal doses and encourages irrational use (Iheanacho *et al.*, 2022). However, few studies have examined the presence of MDR *E. coli* and *Klebsiella* spp. in wildlife and their potential public health implications. Understanding the antimicrobial resistance characteristics and genetic determinants of these pathogens in bush-meat is vital for developing effective surveillance and mitigation strategies.

This study investigated the prevalence of *Escherichia coli* and *Klebsiella* species in bush-meat samples obtained from markets in Oyo State, Nigeria. It also aimed to evaluate their antimicrobial susceptibility profiles and to detect key antibiotic resistance genes, including extended-spectrum  $\beta$ -lactamase (ESBL) genes (*bla*TEM, *bla*SHV, *bla*CTX-M) and carbapenemase genes (*bla*NDM, *bla*VIM).

## MATERIALS AND METHODS

### *Ethics statement*

Ethical approval for the research was obtained from the Animal Care Use Research and Ethics Community (ACUREC) of the University of Ibadan, with the assigned number UI-ACUREC/121-0824/23.

### *Study area*

This study was conducted in Oyo State, Nigeria, a region known for its extensive trade in bushmeat. Sample collection was carried out at three major bushmeat markets located in Egbeda and Oluyole Local Government Areas. These markets were selected based on their high sales volume and diversity of wildlife species.

### *Sample collection*

A total of 100 faecal samples were collected from freshly slaughtered bushmeat species. The sampled animals included *Tragelaphus scriptus* (antelope) – 13, *Thryonomys swinderianus* (grasscutter) – 61, *Civettictis civetta* (civet cat) – 1, *Hystrix cristata* (porcupine) – 7, *Varanus* spp. (monitor lizard) – 2, *Python sebae* (python) – 1, *Dendrohyrax arboreus* (tree hyrax) – 4, *Sciurus vulgaris* (squirrel) – 5 and *Cricetomys ansorgei* (porched rat) – 6. The samples were collected at the point of carcass preparation by bushmeat sellers.

### *Bacterial isolation and identification*

Upon arrival at the laboratory, all samples were processed following standard microbiological procedures (El-Tarabili *et al.*, 2025). The samples were streaked onto MacConkey agar (Oxoid, UK) and incubated aerobically at 37 °C for 24 hours. Lactose-fermenting colonies with distinct morphological characteristics were sub-

cultured into eosin methylene blue (EMB) agar for further differentiation. Colonies exhibiting a metallic green sheen on EMB agar were presumptively identified as *Escherichia coli*, while mucoid pink colonies were considered *Klebsiella* spp. Further biochemical identification was performed using the motility-indole-urease (MIU) test, citrate test, and urease test (Kumala, 2006). Confirmatory identification was carried out using the Analytical Profile Index 20E (API 20E) system (bioMérieux, France), which provides a rapid and reliable method for identifying *E. coli* and *Klebsiella* spp.

### *Antimicrobial susceptibility testing*

The antimicrobial susceptibility of the confirmed isolates was assessed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (Oxoid, UK) following Clinical and Laboratory Standards Institute (CLSI) guidelines (Matuschek *et al.*, 2014). The antibiotic discs used in this study included ampicillin (10 µg), amoxicillin/clavulanic acid (20/10 µg), ciprofloxacin (5 µg), pefloxacin (5 µg), ofloxacin (5 µg), sparfloxacin (10 µg), streptomycin (10 µg), chloramphenicol (30 µg) and gentamicin (10 µg). Antimicrobial susceptibility test results were interpreted according to CLSI (2024) as shown in Table 1.

### *Detection of ESBL and carbapenemase genes*

Genomic DNA was extracted from 12 out of the 100 confirmed *E. coli* and 7 out of the 100 confirmed *Klebsiella* spp. isolates using the cetyltrimethylammonium bromide (CTAB) method (Wilson, 2001). The isolates were selected based on their resistance to β-lactam antibiotics from the antibiotic susceptibility test. The extracted DNA was quantified using a NanoDrop

**Table 1.** Antibiotic susceptibility test interpretive standards (CLSI, 2024)

Antibiotics disc (µg)	Diameter of inhibition zone (mm)		
	Sensitive	Intermediate	Resistant
Ampicillin (10)	≥ 17	14–16	≤ 13
Amoxicillin/clavulanic acid (20/10)	≥ 18	14–17	≤ 13
Ciprofloxacin (5)	≥ 26	22–25	≤ 21
Pefloxacin (5)	≥ 24	–	≤ 23
Ofloxacin (5)	≥ 16	13–15	≤ 12
Sparfloxacin (10)	≥ 19	16–18	≤ 15
Streptomycin (10)	≥ 15	12–14	≤ 11
Gentamicin (10)	≥ 15	13–14	≤ 12
Chloramphenicol (30)	≥ 18	13–17	≤ 12

**Table 2.** Primers sequences used for molecular detection of ESBL and carbapenemase genes

Gene	Primer	Primer sequence 5'-3'	Expected base pairs
<i>blaCTX-M</i>	<i>CTX F</i>	ATGTGCAGYACCAGTAARGTKATGGC	598 bp
	<i>CTX R</i>	TGGGTRAARTARGTSAC	
<i>blaTEM</i>	<i>Tem F</i>	GTCGCCGCATACACTATTCTCA	259 bp
	<i>Tem R</i>	CGCTCGTCGTTTGGTATGG	
<i>blaSHV</i>	<i>SHV F</i>	GCCTTGACCGCTGGGAAAC	318 bp
	<i>SHV R</i>	GGCGTATCCCGCAGATAAAAT	
<i>blaVIM</i>	<i>VIM F</i>	GGTGTGGTTCGCATATCGCAA	502 bp
	<i>VIM R</i>	ATTCAGCCAGATCGGCATCGGC	
<i>blaNDM</i>	<i>NDM F</i>	GGTTTGGCGATCTGGTTTTC	634 bp
	<i>NDM R</i>	CGGAATGGCTCATCACGATC	

2000 spectrophotometer (Thermo Scientific, USA) and stored at –20 °C until further analysis.

Polymerase chain reaction (PCR) was performed for the detection of extended-spectrum beta-lactamase (ESBL) genes (*blaTEM*, *blaSHV*, *blaCTX-M*) and carbapenemase genes (*blaNDM*, *blaVIM*). Primers used for PCR amplification were based on previously established sequences (Yue & Orbán, 2005) (Table 2). The reaction mixture for PCR was prepared in a total volume of 25 µL, which included 5×

PCR buffer (Promega, USA), 2.5 mM MgCl<sub>2</sub>, 0.2 mM dNTP mix, 0.5 µM of each forward and reverse primer, 1.25 U Taq polymerase (Promega, USA), and 2 µL of template DNA, with sterile distilled water added to reach the final volume.

The PCR conditions for each gene were optimised with an initial denaturation at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 49–50 °C for 40 seconds, and extension at 72 °C for 40

seconds. A final elongation step was conducted at 72 °C for 10 minutes.

PCR products were separated by electrophoresis on a 1.5% agarose gel stained with ethidium bromide at 100 V, 80mA for 45 minutes and visualised under UV transillumination. A 100 bp DNA ladder (Promega, USA) was used as a molecular size marker.

## RESULTS

*Escherichia coli* and *Klebsiella* spp. were isolated from all 100 samples. Biochemical confirmation using the API 20E system identified 100 *E. coli* and 100 *Klebsiella* spp. isolates.

### Antimicrobial susceptibility pattern

Antimicrobial susceptibility testing revealed that both *E. coli* and *Klebsiella* spp. exhibited multidrug resistance (MDR). High levels of resistance were observed against  $\beta$ -lactam antibiotics, particularly ampicillin (Table 3). Resistance to sulfonamides and sparfloxacin was moderate, while susceptibility to fluoroquinolones

(pefloxacin, ciprofloxacin, and ofloxacin) and aminoglycosides remained high.

*E. coli* isolates exhibited high resistance to ampicillin (85%) and sulfonamides (62%). However, they were fully susceptible (100%) to fluoroquinolones (ciprofloxacin, ofloxacin, pefloxacin) and aminoglycosides (streptomycin, gentamicin), indicating that these classes of antibiotics remain effective against *E. coli* isolates (Table 3). Out of the 100 *E. coli* isolates tested, 78% were sensitive to chloramphenicol. Sensitivity to amoxicillin/clavulanic acid was observed in 88% of the isolates. Gentamicin showed 98% sensitivity. All the isolates (100%) were sensitive to pefloxacin, ofloxacin, streptomycin, and ciprofloxacin. Sparfloxacin exhibited 80% sensitivity. Only 38% of the isolates were sensitive to sulfamethoxazole/trimethoprim. Ampicillin had the lowest sensitivity, with just 15% of the isolates susceptible.

*Klebsiella* spp. displayed complete resistance (100%) to ampicillin and high resistance to sulfonamides (84%). However, they were highly susceptible (100%)

**Table 3.** Antimicrobial susceptibility pattern of *Escherichia coli* isolates

Antimicrobial class	Antimicrobial agent	<i>Escherichia coli</i>		<i>Klebsiella</i> sp	
		Sensitive n (%)	Resistant n (%)	Sensitive n (%)	Resistant n (%)
Phenicol	Chloramphenicol	78 (78)	22 (22)	50 (50)	50 (50)
Penicillins	Amoxicillin/clavulanic acid	88 (88)	12(12)	66 (66)	34 (34)
Penicillins	Ampicillin	15 (15)	85 (85)	–	100 (100)
Aminoglycosides	Gentamicin	98 (98)	2 (2)	100 (100)	–
Aminoglycosides	Streptomycin	100 (100)	–	100 (100)	–
Fluoroquinolones	Ciprofloxacin	100 (100)	–	100 (100)	–
Fluoroquinolones	Pefloxacin	100 (100)	–	100 (100)	–
Fluoroquinolones	Ofloxacin	100 (100)	–	100 (100)	–
Fluoroquinolones	Sparfloxacin	80 (80)	20 (20)	51 (51)	49 (49)
Sulfonamides	Sulfamethoxazole/trimethoprim	38 (38)	62 (62)	16 (16)	84 (84)

to fluoroquinolones and aminoglycosides, suggesting these antibiotic classes remain viable treatment options (Table 3). Out of the 100 *Klebsiella* spp. isolates tested, 50% were sensitive to chloramphenicol. For amoxicillin/clavulanic acid, 66% of the isolates were sensitive. All isolates (100%) were sensitive to gentamicin, pefloxacin, ofloxacin, streptomycin, and ciprofloxacin. Sensitivity to sparfloxacin was observed in 51% of the isolates. Only 16% of the isolates were sensitive to sulfamethoxazole/trimethoprim. All isolates (100%) were resistant to ampicillin (Table 3).

The multidrug resistance profile showed that both *E. coli* and *Klebsiella* spp. isolates exhibited resistance to antibiotics belonging to four different classes: phenicols, penicillins, fluoroquinolones (specifically sparfloxacin), and sulfonamides (Table 4).

**Table 4.** Multidrug resistance profile of *E. coli* and *Klebsiella* isolates

Isolate	Classes of resistant antibiotics
<i>E. coli</i> (n=100)	Phenicols (22%) Penicillins (85%) Fluoroquinolones (sparfloxacin) (20%) Sulfonamide (62%)
<i>Klebsiella</i> spp. (n=100)	Phenicols (50%) Penicillins (34 -100%) Fluoroquinolones (sparfloxacin) (49%) Sulfonamides (84%)

#### Molecular detection of ESBL and carbapenemase genes

The results of the PCR analysis of ESBL (*blaTEM*, *blaSHV*, *blaCTX-M*) and carbapenemase (*blaNDM*, *blaVIM*) genes in the

*E. coli* and *Klebsiella* spp. isolates are shown in Table 5 and Fig. S1, S2 and S3<sup>1</sup>.

**Table 5.** Prevalence of ESBL and carbapenemase genes in *E. coli* (n=12) and *Klebsiella* spp. (n=7)

Gene	<i>E. coli</i> n (%)	<i>Klebsiella</i> spp. n (%)
<i>blaTEM</i>	7 (58.3)	5 (71.4)
<i>blaSHV</i>	1 (8.3)	1 (14.3)
<i>blaCTX-M</i>	3 (25)	1 (14.3)
<i>blaNDM</i>	4 (33.3)	4 (57.1)
<i>blaVIM</i>	2 (16.7)	2 (28.6)

The most common ESBL gene detected was *blaTEM*, with *E. coli* carrying it in 58.3% of isolates and *Klebsiella* spp. in 71.7% (Table 5). In *E. coli* isolates, *blaTEM* was the most prevalent gene (58.3% of samples), followed by *blaNDM* (33.3%), *blaCTX-M* (25%) and *blaVIM* (16.7%). The *blaSHV* gene showed the lowest prevalence at 8.3%. Among *Klebsiella* species, *blaTEM* was predominantly detected in 71.7% of isolates, followed by *blaNDM* (57.1%), *blaVIM* (28.6%). Similar to *E. coli*, the *blaSHV* gene showed the lowest prevalence in *Klebsiella* spp. at 14.3% (Table 5).

#### DISCUSSION

This study investigated the prevalence, antimicrobial resistance patterns, and molecular characteristics of *Escherichia coli* and *Klebsiella* spp. isolated from bushmeat samples obtained from markets in Oyo State, Nigeria. Our findings revealed a high prevalence of multidrug-resistant (MDR) isolates, with significant resistance observed against  $\beta$ -lactam antibiot-

<sup>1</sup> Fig. S1–S3 are available at <https://trakia-uni.bg/wp-content/uploads/2026/03/2025-0099-supplementary-figures.pdf>

ics, particularly ampicillin, and against sulfonamides. Molecular screening further confirmed the presence of extended-spectrum  $\beta$ -lactamase (ESBL) and carbapenemase genes, with *bla*TEM and *bla*NDM being the most commonly detected among both species.

To our knowledge, this is one of the few studies in southwestern Nigeria to concurrently evaluate both phenotypic resistance and molecular detection of ESBL and carbapenemase genes in *E. coli* and *Klebsiella* spp. from bush-meat. This integrated approach provides valuable insight into the potential role of bush-meat as a reservoir of clinically relevant antimicrobial resistance genes.

The high prevalence of antimicrobial-resistant *E. coli* observed in our study is consistent with a previous one, reporting *E. coli* as the most commonly encountered antimicrobial-resistant Gram-negative organism in bush-meat (Devnath *et al.*, 2022). Similarly, some authors have documented the occurrence of *E. coli* (18.6%) and *Klebsiella pneumoniae* (6.8%) in smoked bushmeat in Nigeria and *E. coli* (19.2%) from antelope and 25% from cane rat samples in Rivers State, Nigeria (Kayode & Kolawole, 2008; Ikeh *et al.*, 2021). The ubiquity of *E. coli* in wildlife was also emphasised in a study from the Democratic Republic of Congo, in which a 100% prevalence was reported in bushmeat samples (Mpalang *et al.*, 2013).

Beyond prevalence, our molecular analysis revealed the presence of ESBL-producing and carbapenem-resistant strains. These findings align with earlier results of Obodoechi *et al.* (2021), who reported ESBL-producing *E. coli* with *bla*CTX-M-15 in bats in Nigeria and Ojo *et al.* (2022) detectingg enterohaemorrhagic *E. coli* (EHEC) in a range of wildlife species, including cane rats and royal

antelope, with high resistance rates to ampicillin (99.2%), ceftiofur (90.6%), and tetracycline (90.0%).

*Klebsiella* spp. were also prominently represented in our study. In Côte d'Ivoire, *Klebsiella pneumoniae* was found to be the dominant *Enterobacteriaceae* species in marsh cane rats, further supporting the significance of this pathogen in bushmeat microbiomes (Yéboué *et al.*, 2022). The isolates were sensitive to the fluoroquinolone antibiotics except for sparfloxacin; the *Klebsiella* isolates had a very high level of resistance to sparfloxacin. It has been reported that some bacterial isolates, especially the Gram-negative bacteria susceptible to most of the fluoroquinolones, can show enhanced resistance to sparfloxacin, as seen in this case (Hooper & Jacoby, 2015).

The detection of ESBL (*bla*TEM, *bla*SHV, *bla*CTX-M) and carbapenemase (*bla*NDM, *bla*VIM) genes in isolates from bushmeat is particularly concerning. These genes confer resistance to critically important antibiotics, including carbapenems, which are considered last-resort treatments for MDR infections (Mancuso *et al.*, 2023).

The identification of *bla*NDM and *bla*VIM in bushmeat-associated isolates are typically linked to hospital-acquired infections, particularly in bacteria such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. These genes encode metallo- $\beta$ -lactamases, enzymes that confer resistance to carbapenems and other  $\beta$ -lactam antibiotics, posing significant therapeutic challenges in healthcare settings (Kumari *et al.*, 2021). This development raises concerns about the potential transmission of resistance genes from wildlife to humans, possibly through plasmid-mediated horizontal gene transfer. This represents an emerging

and underexplored public health threat with implications for both rural and urban populations. These findings reinforce the importance of a One Health approach to AMR surveillance, which integrates human, animal, and environmental health perspectives. Bushmeat markets, where humans and wild animals frequently interact under unhygienic conditions, represent hotspots for the spillover of zoonotic and resistant pathogens. Several factors may underlie the high levels of antimicrobial resistance observed. First, the widespread and unregulated use of antibiotics in the veterinary and agricultural sectors in Nigeria is well-documented and may have created environmental reservoirs of resistance (Manyi-Loh *et al.*, 2018). Second, the poor sanitary conditions under which bushmeat is processed and sold – often lacking basic hygiene facilities – can facilitate the transmission and genetic exchange of resistant bacteria. Third, the proximity and interactions among humans, domestic animals, and wildlife in these settings heighten the risk of zoonotic transmission of MDR organisms.

This study is not without limitations. The geographic scope was limited to bushmeat markets in Oyo State, which may restrict the generalisability of the findings to other regions of Nigeria or West Africa. In addition, the absence of plasmid profiling or whole-genome sequencing limits our understanding of the genetic mobility and evolutionary relationships of the resistance determinants detected.

## CONCLUSIONS

The findings of this study underscore the need for improved surveillance of antimicrobial-resistant bacteria in the bushmeat trade. Given the potential public health

risks, there is an urgent need for routine surveillance of antimicrobial resistance in wildlife and food animals. Regulatory authorities should prioritise hygiene monitoring in bushmeat markets, enforce prudent antibiotic use policies in livestock production, and raise awareness among traders and consumers regarding the risks associated with handling and consuming bushmeat.

Future studies should explore the role of wildlife in the transmission of AMR using larger sample sizes and advanced genomic approaches such as whole-genome sequencing and metagenomics. Additionally, there is a need for targeted public health interventions to improve hygiene in bushmeat markets and regulate antibiotic use in both veterinary and human medicine. Strengthening One Health initiatives that integrate human, animal, and environmental health perspectives will be crucial in mitigating the spread of resistant pathogens from wildlife to humans. Regulatory authorities should prioritise hygiene monitoring in bushmeat markets, enforce prudent antibiotic use policies in livestock production, and raise awareness among traders and consumers regarding the risks associated with handling and consuming bushmeat.

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