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Cross sectional survey of Enterobacterales isolated from ceiling-dwelling bats sampled in Anambra and Ebonyi States of Southeast Nigeria and their antimicrobial resistance and beta-lactamase and virulence-associated genes

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Abstract

There is a growing global interest in bats as reservoirs of zoonotic and antimicrobial-resistant bacteria. However, the potential One Health risk associated with bats roosting in human dwellings in Anambra and Ebonyi States of Southeast Nigeria have not been documented. This study surveyed the Enterobacterales isolated from Nigerian free-tail bats (*Mops nigeriae*) roosting in ceilings of human dwellings in Anambra and Ebonyi States of Southeast Nigeria, and investigated their antimicrobial resistance profile and beta-lactamase and virulence-associated genes. A cross-sectional survey design was adopted for the study. Anal and oral swab samples from bats found in the ceilings of houses in six communities of Anambra and Ebonyi States were inoculated onto MacConkey agar and incubated at 37^{o}C for 18 – 24 hours. Purified colony of each morphologic type (50 from 80 bats) was identified with biochemical tests and confirmed by nucleotide sequence analysis of the 16S rRNA polymerase chain reaction (PCR). Susceptibility to seven antimicrobial agents was determined using the Kirby-Bauer disc diffusion method. Beta-lactamase genes (BLG) and virulence-associated genes (VAG) were detected by PCR. Results showed that the 50 bacterial isolates analyzed belonged to nine genera of Enterobacteriaceae, viz: *Enterobacter* spp (64.0%), *Klebsiella* spp and *Proteus* spp (10.0% each), *Erwinia* spp and *Providencia* spp (4.0% each), *E. coli, Salmonella enterica, Shigella sonnei* and *Pantoea cedenensis* (2.0% each). Resistance to cefotaxime, ceftazidime, and imipenem was exhibited by 90%, 76% and 66% of the isolates, respectively. Resistance to ciprofloxacin (66%), gentamicin (68%), chloramphenicol (20%) and trimethoprim-sulfamethoxazole (18%) was also observed. Multi-drug resistant (MDR) phenotypes were observed in 76% of the isolates. *bla*TEM (65%), *blaCTX-M-15* (40%), *blaTSO-O* (25%), *blaKPC* (25%) *and blaSHV* (10%) were detected in the 20 isolates investigated for BLG. *Hsp60* (78.8%), *CsgA* (24.2%), *CsgD* (12.1%), and *FimH* (3.0%) were detected in the 33 isolates screened for VAG. It was concluded that bats cohabiting human dwellings in Anambra and Ebonyi States of Nigeria are reservoirs of diverse beta-lactamase-producing MDR and potentially virulent Enterobacterales. This suggests a One Health risk, as these bacteria could spread to humans, other animals and the environment.

Keywords: Bats; Anambra and Ebonyi States; Southeast Nigeria; Enterobacterales; Antimicrobial resistance; Beta-lactamase and Virulence genes; One Health.

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Introduction

Bats belong to the order Chiroptera, which is further classified into two suborders: Megachiroptera and Microchiroptera (Fenton and Simmons, 2015; Wilson and Mittermeier, 2019). They are the second most varied order of mammals, accounting for over 25% of all mammalian species and having a nearly worldwide distribution (Turmelle and Olival, 2009; Álvarez-Castañeda, 2024). They are a diverse group of mammals with the ability to fly and cover long distances during seasonal migrations. Most species of bats are nocturnal and prefer to roost in large colonial populations in caves, trees, and/or human dwellings.

Many communities in Africa and other continents live in close contact with wildlife (including bats), domesticated animals and livestock. Due to their ability to fly long distances and proximity to humans, bats have been recognized as hosts capable of transmitting zoonotic pathogens (Federici *et al*., 2022; Kreuder Johnson *et al*., 2015). Bats are commonly found in human dwellings, and often interact closely with domestic animals and humans, and they can contaminate houses with their droppings and urine, as humans occasionally encroach into bat habitats (Hayman *et al*., 2013; Davy *et al*., 2023).

Undoubtedly, their role in disease epidemiology is even more important as bats are reservoirs for a multitude of different microorganisms that include viruses, bacteria, fungi and parasites (Whitaker *et al*., 2009; Wibbelt *et al*., 2009). Although the role of bats in the transmission of viruses (alphaviruses, flaviviruses, rhabdoviruses, and arenaviruses) is well established, there is growing global interest in assessing the broad range of potential infectious agents that bats harbour, with a particular emphasis on potential emerging pandemic threats (Ajayi *et al*., 2020).

The involvement of bats in the emergence of viral diseases of humans and domestic animals have attracted global attention, especially their association with diseases such as severe acute respiratory syndrome coronavirus (SARS-CoV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Hu *et al*., 2021), Middle East respiratory syndrome (MERS), disease of Nipah and Hendra (Banerjee *et al*., 2019; Ochani *et al*., 2019), rabies (Streicker *et al*., 2016), and more recently, CoV disease 2019 (COVID-19) (Zhou *et al*., 2020). Bats have also stood out as potential carriers of zoonotic bacteria, such as *Bartonella* spp. (Veikkolainen *et al*., 2014; Dhivahar *et al*., 2023), *Yersinia pestis* and *Mycobacterium tuberculosis* (Banskar *et al*., 2016; Dhivahar *et al*., 2023), *Leptospira* spp. (Silva-Ramos *et al*., 2022; Esteves *et al*., 2022; Mayer *et al*., 2017), *Brucella* spp. (Bai *et al*., 2017; Ferreira *et al*., 2024) and *Borrelia* spp. (Muñoz-Leal *et al*., 2021). However, the prevalence, diversity and associated potential public health risks of bacterial pathogens found in bats have not been adequately investigated and reported in Nigeria. The present study surveyed the Enterobacterales isolated from Nigerian free-tailed bats (*Mops nigeriae*) roosting in the ceilings of human dwellings in Southeast Nigeria, and investigated their antimicrobial resistance profile and beta-lactamase and virulence-associated genes.

Methodology

Study Area: Southeast Nigeria is located in the southern part of Nigeria, and is made up of five states: Abia, Anambra, Ebonyi, Enugu and Imo. Two states out of the five in Southeast Nigeria (Anambra and Ebonyi States) were randomly selected for the study. Anambra State is bounded by Delta State to the west, Imo State to the south, Enugu State to the east and Kogi State to the north. The state has a tropical wet and dry season or Savanna with a

yearly average temperature of 28.99°C. Anambra typically receives about 212.36 millimeters (8.36 inches) of precipitation and has 243.38 rainy days (66.68% of the time) annually (Climate-Data.org., 2023). Ebonyi State is bordered to the north and northeast by Benue State, Enugu State to the west, Cross River State to the east and southeast, and Abia State to the southwest. Ebonyi State has a humid tropical climate, with rainy and dry seasons lasting for 8 and 4 months, respectively. The temperature typically ranges from 20 to 38°C during the dry season and from 16 to 28°C during the rainy season (Hanachor, 2019).

In Anambra State, the bats were collected from households in Mmiata, Anam (6.5481°N and 6.8541°E), Anambra West Local Government area, while in Ebonyi State, the bats were captured from buildings in the local government areas of Ohaukwu; within the districts of Ezzamgbo (Izhia - 6.38780°N, 8.00283°E), Amike (6.395900°N, 7.954150°E) and Ishia Ituma (Ngbo - 6.488860°N, 7.939910°E), and Ebonyi Local Government Area; within the district of Obegu Oroke Onuoha (6.345580°N, 8.087320°E). The locations where the bats were collected are shown in Figure 1. These communities were purposively selected based on the information from key informants in the two states on the presence of ceiling-dwelling bats.

Ethical Approval: The research project protocol was reviewed and approved by the University of Nigeria, Nsukka Faculty of Veterinary Medicine Institutional Animal Care and Use Committee with Approval Reference Number FVM-UNN-IACUC-2020-1058.

Study Population and Sample Size: The research focused on populations of insectivorous bats found in areas inhabited by humans. These bats were captured from roosts located in the ceilings of human dwellings in the above-mentioned regions of Anambra and Ebonyi States. Using an online sample size calculator, at 95% confidence level, 5% margin of error and 50 population proportion, an expected sample size of 385 was arrived at. However, due to logistic constraints, a total of 80 bats were sampled in this study. This sample size has been shown to provide enough power to estimate the prevalence and diversity of bacterial agents in bat populations (Szentivanyi *et al*., 2023; Islam *et al*., 2025). The sample collections were distributed as follows: Mmiata (20), Ezzamgbo (15), Amike (15), Ishia Ituma (15), and Obegu Oroke Onuoha (15).

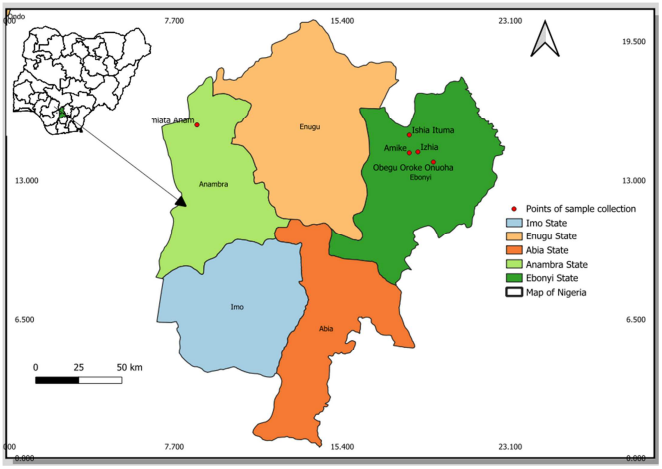

Figure 1. Map of Southeast, Nigeria showing locations where bats were collected for the study in Anambra and Ebonyi States.

Sampling and Sampling Method: A snowball sampling approach was employed, primarily based on referrals from persons inhabiting houses with the presence of bat roosts within the ceilings in the two states of the Southeast Nigeria sampled.

Bat Capture and Transportation and Identification: The bats were captured using mist nets, a method described by Kunz and Kurta (1988). These nets were set up with two poles in a static arrangement, where the nets stay in one place. After capturing the bats, they were carefully placed in well-aerated and secured plastic baskets, transported to the Animal Health Antimicrobial Resistance Surveillance Sentinel Laboratory, University of Nigeria Veterinary Teaching Hospital. The bats were identified as Nigerian free-tailed bats (*Mops nigeriae*) using morphological features such as body measurements, head and ear shape, and fur colour.

Sample collection, isolation, and identification of Enterobacterales: The bats were handled using protective hard-rubber gloves. Sampling and handling were done as described by previous authors (Wimsatt *et al*., 2005). Each bat was euthanized as previously described (Obodoechi *et al*., 2021). Using sterile swab sticks, samples were collected from the oral cavity and anal orifice of each bat. Each swab sample was inoculated into nutrient broth and incubated at 37°C for 24 hours for enrichment. Loopful of each broth culture was streaked on MacConkey agar (MC) and incubated aerobically at 37°C for 24 hours. A representative colony of each morphologic type (lactose fermenting and non-fermenting colonies) was picked and sub-cultured on fresh MC. Inoculated plates were incubated at 37^{o}C for 24 hours; the purified cultures were subjected to Gram-staining and biochemical testing for phenotypic identification (Barrow and Feltham, 1993).

DNA for the molecular identification of the isolates was extracted by the phenol-chloroform method as described by Oludare *et al*. (2024). The identity of each isolate was confirmed by PCR amplification of the 16S rRNA gene using primers 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1525R (5'-AAG GAG GTG ATC CAG CC-3'), followed by sequencing of the amplified product (Oludare *et al*., 2024). To confirm amplification of the 16S rRNA gene, an integrity test was performed by electrophoresis of the amplified product on 1% agarose. After gel integrity, the amplified fragments were ethanol purified to remove the PCR reagents, and the purified products were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems based on the manufacturer's instructions, and this was performed using the BigDye terminator v3.1 cycle sequencing kit. To identify the bacteria species, the sequences were edited with Bio Edit software and compared against GenBank databases using the Basic Local Alignment Search Tool (BLAST). The sequences were deposited in the GenBank with Accession Nos.: PQ108985-PQ109021

Determination of the antimicrobial resistance phenotypes of the Enterobacterales: Susceptibility of the bacterial isolates to seven antimicrobial agents was determined using the disc diffusion method. The antimicrobial agents used included: chloramphenicol (C: 30 µg), gentamicin (GM: 10 µg), ciprofloxacin (CIP: 5 µg), imipenem (IMP: 10 µg), ceftazidime (CAZ: 30 µg), trimethoprim-sulfamethoxazole (TS: 1.25 µg–23.75 µg respectively), and cefotaxime (CTX: 30 µg), based on their regular use in the study area. Results of the antimicrobial susceptibility testing were interpreted based on the Clinical Laboratory Standard Institute (CLSI) guidelines (CLSI, 2023). The multiple antimicrobial resistance (MAR) index for each strain was calculated and interpreted according to Krumperman (1983). Any isolate resistant to at

least one agent in three or more antimicrobial classes was reported as multidrug resistant (MDR) (Magiorakos *et al*., 2012).

Detection of extended-spectrum beta-lactamase and virulence-associated genes: Selected enterobacterales were screened for the presence of beta-lactamase-coding [*blaCTX-M15, blaSHV, bla*TEM, *blaTSO-O (OXA-1, -4, -30), blaKPC* and *blaIMP]* and virulence-associated (*FimH, Hsp60, CsgA* and *CsgD*) genes, by PCR on the extracted DNA using specific primers. The primer sequences used in this study with their expected amplicon sizes as documented by previous authors are presented in Table 1. Duplex PCRs were used, and each reaction cocktail per primer set consisted of: 5X PCR SYBR green buffer (2.5 µl), $MgCl_2$ (0.75 µl), 10 pM DNTP (0.25 µl), 10 pM of each forward and backward primer (0.25 µl), 8000 U of taq DNA polymerase (0.06 µl). Sterile nuclease-free water was added to bring the master mix volume to 10.5 µl. Template DNA (2 µl) was then added to the master mix to bring the final reaction volume per primer set to 12.5 µl. Buffer control was also added to eliminate any probability of false amplification. PCR was performed in a C1000 PCR System Thermal cycler (Applied Biosystem Inc., USA) using a gradient PCR profile. Amplified fragments were visualized on ethidium bromide stained 2% agarose electrophoresis gels. A 100bp ladder marker (NEB) was used as a molecular weight standard.

Table 1. Primer sequences of the beta-lactamase and virulence genes investigated in this study.

Gene	Gene sequence	Size (bp)	Reference
Beta-lactamase genes			
bla CTX-M-15	F: GTGATACCACTTCACCTC	255	Gharrah *et al*., 2017
	R: AGTAAGTGACCAGAATCAG		
bla SHV	F: ACTATCGCCAGCAGGATC	200	
	R: ATCGTCCACCATCCACTG		
bla TEM	F:GATCTCAACAGCGGTAAG	786	
	R: CAGTGAGGCACCTATCTC		
bla TSO-O (OXA-1, -4, -30)	F: GGCACCAGATTCAACTTTCAAG	564	Dallenne *et al*., 2010
	R: GACCCCAAGTTTCCTGTAAGTG		
bla- KPC	F: CATTCAAGGGCTTTCTTGCTGC	538	
	R: ACGACGGCATAGTCATTTGC		
bla- IMP	F: TTGACACTCCATTTACDG	139	
	R: GATYGAGAATTAAGCCACYCT		
Virulence Genes			
FimH	F: 5`-TGCAGAACGGATAAGCCGTGG-3	508	Abdul-Razzaq *et al*. 2013
	R: 5`-GCAGTCACCTGCCCTCCGGTA-3		
Hsp60	*F :* 5`-GGTAGAAGAAGGCGTGGTTGC-3`	341	Akbari *et al*., 2015
	R: 5`-ATGCATTCGGTGGTGATCATCAG-3		
csgD	F: 5`-TGAAARYTGGCCGCATATCAATG-3	355	
	R : 5`-ACGCCTGAGGTTATCGTTTGCC-3`		
csgA	*F :* 5`-ATTGCAGCAATCGTAGTTTCTGG-3	245	
	R : 5`-ATWGAYCTGTCATCAGAGCCCTGG-3		

Results

Enterobacterales isolated from the ceiling bats: Table 2 shows the distribution of the Enterobacterales isolated from the ceiling bats. The 50 bacterial isolates analyzed belonged to nine genera of Enterobacteriaceae, with *Enterobacter* spp (64.0%) being the dominant genus. Out of the 32 *Enterobacter* species, 27 (84.4%) were identified as *Enterobacter hormaechei*. *Salmonella enterica* (2.0%) and *Shigella sonnei* (2.0%) were also recovered from the oral and anal swabs of the bats, respectively. Other genera of bacteria isolated were: *Escherichia*, *Erwinia, Klebsiella, Pantooea, Proteus,* and *Providencia* (Table 2).

Antimicrobial resistance phenotypes and beta-lactamase genotypes of Enterobaterales isolated from the ceiling-dwelling bats: Resistance to cefotaxime, ceftazidime and gentamicin was observed in 90%, 76% and 68% of isolates, respectively, while 66% were resistant to ciprofloxacin or imipenem (Figure 2). The 50 Enterobacterales exhibited 20 resistance patterns with GM-IMI-CAZ-CTX-CIP (12%) and IMI-CAZ-CTX-CIP (12%) being the predominant patterns (Table 3). Resistance to at least one antimicrobial agent in three or more antimicrobial classes (i.e. MDR phenotype) was observed in 38 (76%) of the 50 isolates studied; the multiple antibiotic resistance indexes ranged from 0.26 to 1.0.

Table 2. Genera and species of Enterobacterales isolated from ceiling bats in Anambra and Ebonyi States of Southeast Nigeria.

Genera	Species	Isolation frequency, with percentage in bracket.
Enterobacter (n = 32)	*asburiae*	3 (6%)
	cloacae	2 (4%)
	hormaechei	27 (54%)
Erwinia (n = 2)	*typographi*	2 (4%)
Escherichia (n = 1)	*coli*	1 (2%)
Klebsiella (n = 5)	*aerogenes*	2 (4%)
	oxytoca	2 (4%)
	quasipneumoniae subsp. similipneumoniae	1 (2%)
Pantoea (n = 1)	*cedenensis*	1 (2%)
Proteus (n = 4)	*mirabilis*	3 (6%)
	vulgaris	1 (2%)
Providencia (n = 3)	*rettgeri*	1 (2%)
	vermicola	2 (4%)
Salmonella (n = 1)	*enterica*	1 (2%)
Shigella (n = 1)	*sonnei*	1 (2%)

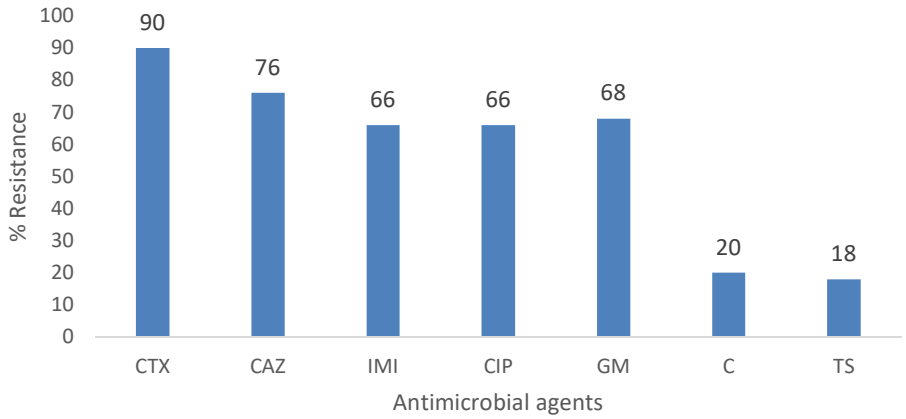

Figure 2. Antimicrobial resistance profile of enterobacterales (n = 50) isolated from ceiling-dwelling bats sampled at Anambra and Ebonyi States of Southeast Nigeria. [C = Chloramphenicol; CAZ = Ceftazidime; CIP = Ciprofloxacin; CTX = Cefotaxime; GM = Gentamicin; IMI = Imipenem; TS = Trimethoprim-sulfamethoxazole]

Table 3. Antimicrobial resistance patterns and multiple antibiotic resistance indices (MARI) of enterobacterales (n=50) isolated from ceiling-dwelling bats sampled at Anambra and Ebonyi States of Southeast Nigeria.

S/N	Resistance pattern	MARI	Frequency, with percentage in brackets
1	CAZ-CTX-CIP	0.43	2 (4%)
2	CAZ-CTX-TS	0.43	3 (6%)
3	C-CAZ-CTX-CIP	0.57	1 (2%)
4	C-CAZ-CTX-TS-CIP	0.74	1 (2%)
5	C-GM-CTX-TS-CIP	0.74	1 (2%)
6	C-GM-IMI-CAZ-CTX-CIP	0.86	3 (6%)
7	C-GM-IMI-CAZ-CTX-TS-CIP	1.0	3 (6%)
8	C-GM-IMI-CTX-CIP	0.74	1 (2%)
9	GM-CAZ-CTX	0.43	4 (8%)
10	GM-CAZ-CTX-CIP	0.57	1 (2%)
11	GM-CTX-CIP	0.43	2 (4%)
12	GM-IMI	0.26	2 (4%)
13	GM-IMI-CAZ	0.43	1 (4%)
14	GM-IMI-CAZ-CTX	0.57	5 (10%)
15	GM-IMI-CAZ-CTX-CIP	0.74	6 (12%)
16	GM-IMI-CAZ-CTX-TS-CIP	0.86	1 (2%)
17	GM-IMI-CTX-CIP	0.57	4 (8%)
18	IMI-CAZ-CTX	0.43	1 (2%)
19	IMI-CAZ-CTX-CIP	0.57	6 (12%)
20	IMI-CIP	0.26	2 (4%)

Figure 3 shows the representative PCR products of the beta-lactamase genes detected in 20 Enterobacterales investigated for beta-lactamase genes. *bla*TEM, *blaCTX-M-15, blaTSO-O, blaKPC and blaSHV* were detected in 65, 40, 25, 25 and 10%, respectively, of the 20 isolates tested while one of the isolates was negative for the investigated genes. Figure 4 shows the beta-lactamase gene combinations observed in the 20 Enterobacterales. Nine (45%) of the 20 isolates harbored only one beta-lactamase gene type, while 6 (30%) and 4 (20%) contained two and three beta-lactamase gene types, respectively.

Virulence-associated gene profile of Enterobacterales isolated from the ceiling bats: Thirty-three Enterobacterales were investigated for the presence of four virulence-associated genes (VGs), and 29 (87.9%) of them harboured at least one of the genes. Figure 5 shows the gel image of the VGs detected in the bacterial isolates. Hsp60 gene was detected in 26 (78.8%) of the 33 isolates while CsgA, CsgD, and FimH were observed in 8 (24.2%), 4 (12.1%), and 1 (3.0%), of the isolates, respectively. Figure 6 shows the VGs coexistence observed in 33 Enterobacterales. Hsp60 gene was found coexisting with *CsgA, CsgD,* and *FimH* in 5 (15.2%), 4 (12.1%), and 1 (3.0%) of the Enterobacterales, respectively, of the 33 isolates.

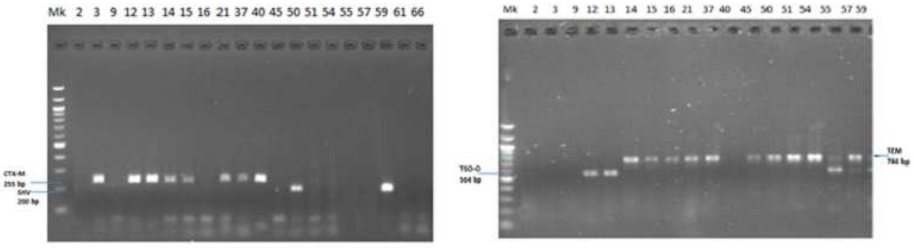

Figure 3. Gel electrophoresis image of PCR products of blaCTX-M15 (255 bp), blaSHV (200 bp), blaTEM (786 bp), and blaTSO-O [OXA-1, -4, -30] (564 bp) ESBL genes in enterobacterales from ceiling bats sampled at Anambra and Ebonyi States of Southeast Nigeria. Mk = 100 bp molecular weight marker.

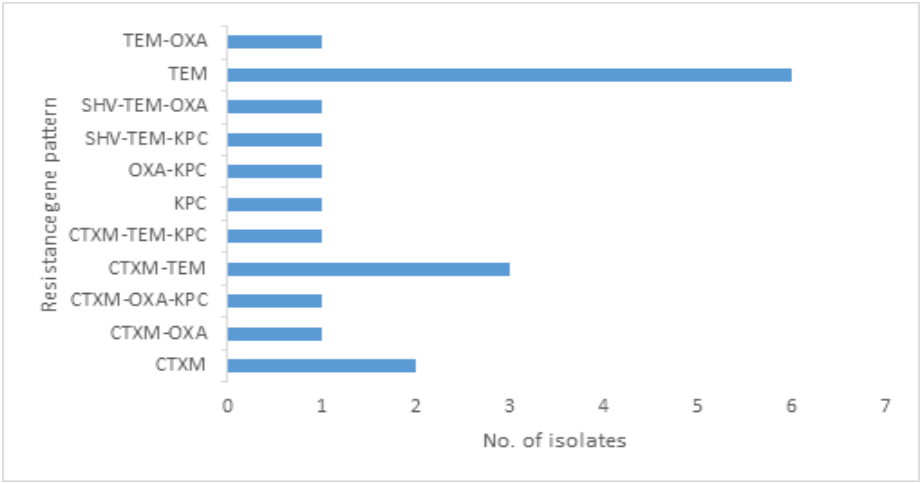

Figure 4. Beta-lactamase gene patterns in enterobacterales from ceiling bats sampled at Anambra and Ebonyi States of Southeast Nigeria.

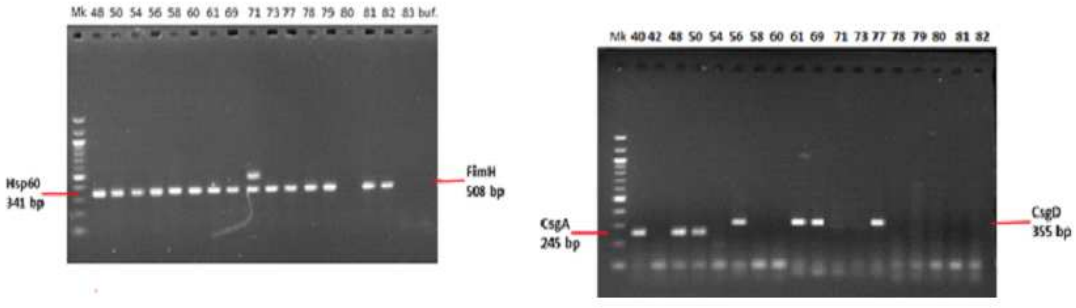

Figure 5. Gel electrophoresis image of PCR products of FimH (508 bp), Hsp60 (341 bp), CsgA (245 bp) and CsgD (355 bp) virulence-associated genes in enterobacterales from ceiling bats sampled at Anambra and Ebonyi States of Southeast Nigeria. Mk = 100 bp molecular weight marker.

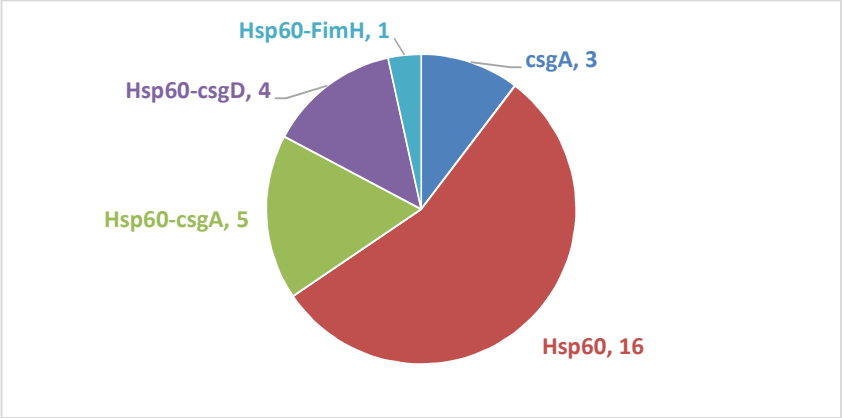

Figure 6. Virulence-associated gene combinations in Enterobacterales isolated from ceiling-dwelling bats sampled at Anambra and Ebonyi States of Southeast Nigeria

Table 4 shows the distribution of beta-lactamase and virulence-associated genes in Enterobacterales isolated from the ceiling bats. As presented in Table 4, 14 (73.7%) and 21 (72.4%) of the 19 ESBL- and 29 VG gene-harboring isolates, respectively, were *Enterobacter* species; particularly *E. hormaechei*.

Discussion

A remarkable diversity of enteric bacteria (9 genera and 14 species) were isolated from the bats surveyed. *Enterobacter spp.*, particularly *Enterobacter hormaechei*, accounted for the majority of the isolates encountered, suggesting it may be a dominant colonizer in these bats. It is a member of the *Enterobacter cloacae* complex and is widely distributed in the environment (Sekyere and Reta, 2021). Although it is commonly considered a pathogen of nosocomial infections (Mshana *et al*., 2011; Ding *et al*., 2021), *Enterobacter hormaechei* has been implicated in respiratory disease in unweaned calves in China (Wang *et al*., 2020) and septic arthritis in free-living green turtle in Brazil (Goldberg *et al*., 2019). It has been associated with bloodstream infections in tertiary healthcare hospitals in Southeast Nigeria (Oni *et al*., 2025).

Table 4. Distribution of beta-lactamase and virulence-associated genes among Enterobacterales isolated from bats in Ebonyi and Anambra States, Southeast Nigeria.

Genes detected	Enterobacterale species (no of isolates) positive for specified gene	Bat sampling site (State)
Beta-lactamase		
CTX-M	*Enterobacter hormaechei* (7), *Proteus vulgaris* (1)	Ebonyi and Anambra
TSO-O (OXA-1, -4, -30)	*Enterobacter hormaechei* (3), *Proteus mirabilis* (1), *Klebsiella quasipneumoniae subsp. similipneumoniae* (1)	Ebonyi and Anambra
KPC	*Enterobacter hormaechei* (3), *Erwinia topographi* (1), *Proteus mirabilis* (1)	Ebonyi and Anambra
TEM	*Enterobacter hormaechei* (9), *Enterobacter cloacae* (1), *Klebsiella oxytoca* (1), *Klebsiella quasipneumoniae subsp. similipneumoniae* (1), *Klebsiella aerogenes* (1)	Ebonyi and Anambra
SHV	*Enterobacter hormaechei* (2)	Anambra
Virulence associated genes		
Hsp60	*Enterobacter hormaechei* (13), *Enterobacter asburiae* (1), *Proteus mirabilis* (2), *Proteus vulgaris* (1), *Providencia vermicola* (2), *Providencia rettgeri* (1), *Klebsiella oxytoca* (1), *Klebsiella quasipneumoniae subsp. similipneumoniae* (1), *Klebsiella aerogenes* (1), *E. coli* (1), *Salmonella enterica* (1), *Shigella sonne*i (1)	Ebonyi and Anambra
CsgA	*Enterobacter hormaechei* (4), *Enterobacter asburiae* (1), *Enterobacter cloacae* (1), *Proteus mirabilis* (1), *Proteus vulgaris* (1)	Ebonyi and Anambra
CsgD	*Providencia vermicola* (1), *Shigella sonne*i (1), *Klebsiella oxytoca* (1), *Klebsiella quasipneumoniae subsp. similipneumoniae* (1)	Ebonyi and Anambra
FimH	*Providencia vermicola* (1)	Ebonyi

Other enteric bacteria isolated from bats in this study, which included *E. coli, Proteus mirabilis, P. vulgaris, Klebsiella aerogenes, K. similipneumoniae* (a member of the *K. pneumonia* complex), *K. oxytoca, Providencia rettgeri, P. vermicola, Salmonella enterica, Pantoea cedenensis, Shigella sonnei,* and *Erwinia typographi*, have been reported in various bat species in many parts of the world, as documented in several reviews (Federici *et al*., 2022; Devnath *et al*., 2023; Dhivahar *et al*., 2023; Soto-López *et al*., 2025). Apart from *Erwinia typographi*, the other bacterial species have been reported to have clinical or zoonotic significance. For instance, *K. oxytoca* is a significant cause of health-care associated

infections in ICU patients (Singh *et al*., 2016), and it is among the major pathogens causing infection in neonatal ICU patients and a frequent cause of Gram-negative neonatal bacteremia (Liébana-Rodríguez *et al*., 2024; Neog *et al*., 2021). *Providencia vermicola* is associated with urinary tract infections and gastroenteritis in humans (Rajni *et al*., 2022). Serovars of *Salmonella enterica* cause a wide range of disease conditions in man and animals. *Shigella sonnei* is a human pathogen associated with dysentery.

The detection of a plethora of bacteria of clinical and zoonotic significance from the Nigerian free-tailed bats (*Mops nigeriae*) in the present study is a public health concern considering the fact that these bats live in the same buildings with humans and are therefore potential disseminators of these bacterial agents to humans. The public health risk is heightened by the unhygienic handling of bats and their excretions, especially the reports of the common use of bat guano for manure in the Southeast and other parts of Nigeria (Ojobo, 2020; Ajuzieogu *et al*., 2024)

The isolates obtained in this study demonstrated high resistance towards cefotaxime, ceftazidime, gentamicin, ciprofloxacin, and imipenem. Several studies have reported similar findings in enterobacterales from various bat species (Aladejana *et al*., 2024; Mustika *et al*., 2024; Obodoechi *et al*., 2021; Mbehang Nguema *et al*., 2020; Nowakiewicz *et al*., 2020). Enterobacter species, being the dominant bacteria in the present study, have been reported to be highly resistant to antimicrobial agents (Huang *et al*., 2023; Hiba Abdel *et al*., 2022; Nirbhavane and Bagde, 2017). This may explain the high resistance rates recorded in our study. Thirty-eight of the 50 isolates were found to be multidrug resistant, with multiple antibiotic resistance indices (MARI) ranging from 0.26 to 1.0, an indication that the isolates originate from a high-risk contamination source (Krumperman, 1983). The presence of MDR enterobacterales and isolates with MARI > 0.2 in bats with no history of direct exposure to antimicrobial agents is quite surprising. The source of the MDR bacteria in the ceiling-dwelling bats in this study is not easily discernible. However, as previously suggested (Obodoechi *et al*., 2021), it is plausible that these insectivorous bats acquired the MDR bacteria through ingestion of flies contaminated with antimicrobial resistant bacteria found in human and animal wastes in the environment. Being migratory mammals capable of flying long distances, these bats are therefore potential disseminators of bacteria of public health importance.

In the present study a high proportion of the isolates harbored extended-spectrum β-lactamase (ESBL) genes, particularly *blaTEM* (65%) and *blaCTX-M* (40%), while 10% of the isolates were positive for *blaSHV*. These ESBL genes are plasmid-mediated (Chaudhary *et al*., 2023), thus allowing for horizontal gene transfer thereby facilitating the spread of resistance genes. The *blaCTX-M, blaTEM* and *blaSHV* are the predominant ESBL genes reported in isolates from humans and animals worldwide (Olorunleke *et al*., 2024; Ejaz *et al*., 2021; Abrar *et al*., 2019; Pishtiwan and Khadija, 2019; Gundran *et al*., 2019). They have been detected in enterobacterales, particularly *E. coli*, *Enterobacter* species and *Klebsiella* species from bats in Peru (Benavides *et al*., 2018), Makokou, Garbon (Mbehang Nguema *et al*., 2020), Enugu State, Nigeria (Obodoechi *et al*., 2021), and Lombok Island, Indonesia (Mustika *et al*., 2024). *blaTSO-O (OXA-1,-4,-30)* and *blaKPC* detected in *Proteus mirabilis*, *Klebsiella quasipneumoniae*, and *Erwinia topographi* in the present study have previously been reported in *Klebsiella* species and *E. coli* isolated from bat guano in Algeria (Alima *et al*., 2019).

The virulence-associated genes, Hsp60, CsgA, CsgD, and FimH, detected in our study, are among the genes that play significant roles in

the establishment of bacterial infection. The *Hsp60*, the most abundant of the VGs in this study, is a heat shock protein and a reliable marker for bacteria species identification (Ganbold *et al*., 2023; Rebecca *et al*., 2002). However, as a VG, it enhances bacterial survival within macrophages, cell adhesion and bacterial survival under stressful conditions, and it modulates host immune system (Baj *et al*., 2020; Kamiya *et al*., 1998; Yamaguchi *et al*., 1997). The **c**urli fimbriae-associated genes (*CsgA* and *CsgD*)**,** majority of which co-existed with *Hsp60* in our study, are known to play crucial roles in cell adhesion, aggregation, and biofilm formation, thereby enhancing the ability of bacteria to colonize, persist in host tissues, and resist environmental stress, including antimicrobial agents (Al-Saadi *et al*., 2024; Anchana *et al*., 2021; Uhlich *et al*., 2009). In the present study, *FimH*, a type 1 fimbrial adhesin enoding gene, was found only in *Providencia vermicola*. This gene, though well documented in *E. coli* as a key adhesion-encoding gene, particularly in uropathogenic *E. coli* (UPEC) strains (Whelan *et al*., 2023; Baldiris-Avila *et al*., 2020), where it enhances urothelial colonization, is reported for the first time in *Providencia* species in our study. Although the VGs detected in the present study have been reported in enterobacterales from human, animal and environmental samples (Al-Saadi *et al*., 2024; Malesa *et al*., 2024; Lozano-Villegas *et al*., 2022; Dyer *et al*., 2006), our study appears to be the first report of their detection in enterobacterales from bats. In Osun State, Nigeria, Modupe *et al*. (2022) found eaeA, PapC, and iss virulence genes but no biofilm formation genes, in *E. coli* from fruit bats. The Nigerian free-tailed bats can therefore act as reservoirs of enterobacterales with zoonotic spillover potential.

Conclusion: The Nigerian free-tailed bats (*Mops nigeriae*) sharing human dwellings in Southeast Nigeria harbor potentially virulent multidrug-resistant Enterobacterales. *Enterobacter hormaechei* was the most prevalent species among the Enterobacterales isolated, and it was also the most dominant carrier of ESBL genes such as *blaTEM* and *blaCTX-M*. A significant proportion of the Enterobacterales harbored virulence-associated genes, particularly *Hsp60* and CsgA, suggesting a potential for pathogenicity and public health concern. The co-existence of multiple beta-lactamase genes and virulence factors in Enterobacterales from ceiling-dwelling bats in Nigeria indicate possibility of enhanced fitness and potential risk of environmental persistence, colonization of animal hosts and zoonotic transmission from the bat populations – a potential One Health risk. These findings underscore the critical need for an integrated active AMR surveillance across human, animals (wildlife like bats), and the environment to provide data to guide development of strategies to curb the spread of AMR and virulence-associated genes.

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Conflict of interest

The authors declare no conflict of interest

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