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Fruit bats (Eidolon helvum) as sentinels of antimicrobial resistance and clinically relevant bacteria in Ghana: A One Health perspective

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Abstract

Antimicrobial resistance (AMR) poses a critical global public health threat, but surveillance remains heavily skewed toward clinical settings, creating a significant gap regarding the role of synanthropic wildlife. This cross-sectional study investigated the bacteriological diversity, phenotypic resistance profiles, and molecular resistance determinants of bacteria harbored by the straw-colored fruit bat (*Eidolon helvum*) at a high-density human-wildlife interface in Sunyani, Ghana. Between April and June 2025, a total of 253 bacterial isolates were recovered, non-invasively, from naturally shed, fresh bat guano stratified across locations categorized by roosting densities. The most prevalent, clinically relevant bacteria recovered was *Bacillus* spp. (62.5%), *Staphylococcus aureus* (9.5%), *Lactobacillus* spp. (8.7%), and *Salmonella* spp. (7.1%). Phenotypic susceptibility testing revealed widespread resistance to foundational antibiotics. Of the 105 resistant Gram-negative isolates, ampicillin and cefuroxime exhibited the highest resistance rates (21.9% each), followed by ceftriaxone (19.0%) and meropenem (11.4%), a last-resort antibiotic. Gram-positive isolates displayed high resistance to cloxacillin (19.5%), penicillin (19.5%), and ampicillin (18.6%). Interestingly, most Gram-positive isolates (63.6%) demonstrated Multidrug-Resistant (MDR) phenotypes. Molecular screening via Polymerase Chain Reaction verified the presence of the beta-lactamase determinant bla_{TEM} in 69.2% of the screened resistant Gram-negative isolates. But bla_{CTX-M}, bla_{SHV}, bla_{NDM}, *mecA*, and *mecC* genes were not detected. These findings indicate that synanthropic fruit bats act as environmental reservoirs and sentinels for clinically relevant, drug-resistant bacteria. Integrating wildlife monitoring into national policy and strategies is essential to achieve effective One Health surveillance and mitigate bi-directional transmission risks in these shared ecosystems.

INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the most critical global One Health challenges of the 21st century, threatening public health systems worldwide [1]. The rapid evolution and global dissemination of resistant microorganisms are driven by complex, interconnected biological and anthropogenic pressures which span human, animal, and environmental interfaces [2]. Epidemiological models indicate that in 2019 alone, approximately 1.27 million deaths globally were directly attributable to bacterial AMR, with an additional 4.95 million deaths associated with drug-resistant infections [3]. In sub-Saharan Africa, this burden is disproportionately severe, compounding existing public health vulnerabilities and inflicting substantial annual economic and societal costs through prolonged hospitalizations and treatment failures [4].

Historically, mitigation strategies have focused heavily on clinical settings and livestock production, but recent evidence highlights the critical role of wildlife in the maintenance and transmission of AMR [5]. Wildlife species, particularly those adapted to urbanized landscapes, act as vital environmental sentinels and potential reservoirs for antibiotic-resistant bacteria and mobile genetic elements [6]. Among wild mammals, bats (Order: Chiroptera) represent a unique evolutionary and ecological niche and are the only mammals capable of sustained flight [7]. They display remarkable mobility, long lifespans, and complex

migratory behaviors that expose them to diverse microbial communities across vast geographical ranges [8]. Bats provide essential ecosystem services like pollination and insect control, whilst their unique physiology and dense roosting behaviors allow them to harbor diverse bacterial assemblages, including clinically relevant pathogens [9].

Despite their potential to disseminate AMR, wildlife surveillance remains critically underdeveloped, particularly in low- and middle-income countries like Ghana [10]. Rapid urbanization and agricultural encroachment have accelerated habitat fragmentation, forcing synanthropic species like the straw-colored fruit bat (*E. helvum*) into close, continuous proximity with human populations [11]. *E. helvum* is a highly gregarious, migratory species that frequently establishes large colonies in urban centers across sub-Saharan Africa, roosting in public parks, university campuses, and residential areas [12]. This close coexistence facilitates frequent human-wildlife interactions through exposure to bat droppings, saliva, and the handling of bushmeat, creating direct pathways for bidirectional microbial transmission [13].

In Ghana, while the potential risk of zoonotic spillover from chiropteran reservoirs is recognized, empirical data regarding wildlife resistomes remain highly fragmented [14]. Most national AMR surveillance data are derived from hospital laboratories or livestock operations, leaving a critical gap concerning environmental circulation [15]. Urban bat colonies roosting near major academic and municipal centers present an immediate public health concern. This is because these populations forage across agricultural lands, open markets, and urban waste streams, potentially acquiring and redistributing clinical resistance traits [16].

To address this critical knowledge gap, this study evaluates the prevalence, phenotypic resistance profiles, and molecular resistance determinants of clinically relevant bacteria harbored by *E. helvum* within a high-density anthropogenic interface in Sunyani, Ghana. It integrates bacteriological isolation with genotypic validation, thereby establishing a vital baseline for environmental AMR surveillance in West Africa. Thus, it contributes directly to the operationalization of national One Health frameworks.

METHODOLOGY

Study area, environmental context and design

This study was conducted at the bat sanctuary of the University of Energy and Natural Resources (UENR) in Sunyani, Ghana (7°20'N, 2°20'W). This site presents a unique One Health interface where an estimated population of over 200,000 straw-colored fruit bats (*E. helvum*) co-exist with over 18,000 humans. The bat colony is not restricted to an isolated or cordoned-off sanctuary tract. Instead, the bats actively roost within the abundant tree canopy distributed across the active, open public university campus, bringing them into immediate proximity with daily institutional activities. This facilitates frequent human-wildlife interactions through unavoidable exposure to falling bat droppings, saliva, and aerosols directly beneath major roosting sites along campus walkways and communal areas. The local canopy is dominated by *Newbouldia laevis*, while *Triplochiton scleroxylon* represents the least abundant tree species. Because the

sanctuary layout is integrated into an open public campus where academic field research is standard practice, specialized institutional access permits were not required for sample collection. A cross-sectional study design was implemented between April and June 2025 using entirely non-invasive field protocols to prevent animal stress and eliminate physical handling risks. Specifically, sampling relied exclusively on the gathering of naturally shed environmental guano caught using clean polythene sheets elevated on four-stick corner mounts placed beneath the roosting trees. Thus, the fieldwork did not require wild animal research permits but adhered strictly to standard institutional ecological surveillance guidelines.

Sample collection and stratification

Freshly shed bat guano samples were collected weekly across three distinct zones stratified by host roosting density: Low Density (LD: <800 bats), Medium Density (MD: 800–1500 bats), and High Density (HD: >1500 bats). Sterile plastic sheets were deployed beneath active roosting trees between 05:00 AM and 07:00 AM to collect guano. Using sterile spatula, a total of 30–50 g per pool of fresh guano was transferred immediately into sterile stool containers and immediately transported under cold chain (4°C) to the Consortium for Neglected Tropical Diseases and One Health (NeTroDisOH) Laboratory for immediate processing.

Bacterial isolation and phenotypic identification

Samples were homogenized in normal saline (0.85%) and inoculated onto a panel of basal, selective and differential media, including Cysteine Lactose Electrolyte Deficient (CLED) agar, MacConkey agar, Salmonella-Shigella (SS) agar, and Nutrient agar [14]. Plates were incubated under aerobic conditions at 37°C for 18–24 hours. Presumptive colonies were purified via sub-culturing and identified using a combination of culture morphology, Gram staining, and standard biochemical profiling (citrate utilization, urea hydrolysis, and Triple Iron Sugar-TSI).

Antimicrobial Susceptibility Testing (AST)

Phenotypic susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar in strict accordance with the Clinical and Laboratory Standards Institute (CLSI, 2025) guidelines [15]. Isolates were evaluated against a comprehensive panel of clinically relevant antibiotics across major classes. The Gram-positive panel included penicillin (1.5 µg), ampicillin (10 µg), cloxacillin (5 µg), tetracycline (10 µg), gentamicin (10 µg), vancomycin (30 µg), cotrimoxazole (25 µg), and ciprofloxacin (5 µg). The Gram-negative panel consisted of ampicillin (10 µg), tetracycline (10 µg), cotrimoxazole (25 µg), gentamicin (10 µg), cefuroxime (30 µg), chloramphenicol (10 µg), ceftriaxone (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), amikacin (30 µg), and meropenem (10 µg). Zone diameters were measured and interpreted as susceptible, intermediate, or resistant based on CLSI breakpoints [15].

Multidrug resistance (MDR) was defined as resistance to three or more distinct antimicrobial classes [15].

Quality assurance protocols

Sterility and batch performance were verified by incubating 5% of each freshly prepared media batch overnight at 37°C without bacterial inoculants and batches showing growth were rejected. Quality control of performance and disk potency was maintained using reference strains including *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922). All downstream molecular assays were implemented thrice to confirm initial results.

Molecular screening of resistance determinants

Genotypic validation was conducted via Polymerase Chain Reaction (PCR) across recovered bacterial isolates displaying phenotypic resistance. PCR assays targeted beta-lactamase genes (bla_{TEM}, bla_{SHV}, bla_{CTX-M}, and bla_{NDM}) and staphylococcal methicillin-resistance structural determinants (*mecA* and *mecC*) using established primer sequences (Table 1). Amplifications were executed in a 96-well thermal cycler using a 25 µL final reaction setup containing 12.5 µL of OneTaq® Hot Start Quick-Load® 2X Master Mix, 0.2 µM of each forward and reverse primer, 10.5 µL of nuclease-free water, and 1 µL of extracted DNA template. Cycling parameters for bla_{TEM}, bla_{SHV}, and bla_{CTX-M} comprised initial denaturation at 95°C for 3 min; 30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min; and a final extension at 72°C for 7 min. For bla_{NDM}, parameters were initial denaturation at 94°C for 45 s; 30 cycles of 94°C for 45 s, 52°C for 1 min, and 72°C for 1 min; followed by final elongation at 72°C for 10 min. Thermal profiles for *mecA* and *mecC* utilized initial denaturation at 94–95°C followed by 30 cycles of denaturation, primer annealing (60°C for *mecA*, 55°C for *mecC*), and extension at 72°C. PCR products were resolved via electrophoresis on a 1.5% agarose gel alongside a 1 kb DNA ladder, stained with SafeView dye, and visualized under blue-light transillumination.

Table 1
Primer sequences and amplicon sizes for optimized PCR assays

Target Gene	Primer Direction	Sequence (5' → 3')	Amplicon Size (bp)	Reference
bla$_{SHV}$	Forward	TGCTTTGTTATTCGGGCCAA	747	[16]
	Reverse	ATGCGTTATATTCGCTGTG		
bla$_{CTX-M}$	Forward	ATGTGAGYACCAGTAARGTGAT	593	[16]
	Reverse	TGGGTRAARTARGTSACCAGAAT		
bla$_{TEM}$	Forward	ACGCTCACCGGCTCCAGATTAT	455	[16]
	Reverse	TCGCCGCATACACTATTCTCAGA		
bla$_{NDM}$	Forward	GGTTTGGCGATCTGGTTTTC	624	[17]
	Reverse	CGGAATGGCTCATCACGATC		
mecA	Forward	CCTAGTAAAGCTCCGGAA	533	[18]
	Reverse	CTAGTC-CATTCGGTCCA		
mecC	Forward	TGTTGTAGCAATGTTCACAC	304	[19]
	Reverse	CAAGCACTTAATATCAACGC		

RESULTS

Bacterial isolation and density distribution

A total of 253 bacterial isolates were successfully recovered from the freshly shed guano samples across the three stratified sampling zones (Table 2). The High Density (HD) site recorded the highest recovery rate with 92 isolates (36.4%), followed by the Mid Density (MD) site with 87 isolates (34.4%), and the Low Density (LD) site with 74 isolates (29.2%).

Across all sites, the predominant genus identified was *Bacillus* spp., accounting for 62.5% (158/253) of the total collection. This was followed by *Staphylococcus aureus* (9.5%; 24/253), *Lactobacillus* spp. (8.7%; 22/253), and *Salmonella* spp. (7.1%; 18/253). Other recovered taxa included *Enterobacter* spp. (5.9%), *Klebsiella* spp. (2.8%), *Serratia* spp. (1.2%), *E. coli* (1.2%), *Proteus* spp. (0.8%), and Coagulase-Negative Staphylococci (CONS; 0.4%).

Table 2
Bacterial distribution across the three sampling sites

Organisms	HD (n, %)	LD (n, %)	MD (n, %)	Total (n, %)
Bacillus spp.	63 (24.9%)	44 (17.4%)	51 (20.2%)	158 (62.5%)
CONS	0 (0.0%)	1 (0.4%)	0 (0.0%)	1 (0.4%)
E. coli	1 (0.4%)	0 (0.0%)	2 (0.8%)	3 (1.2%)
Enterobacter spp.	3 (1.2%)	7 (2.8%)	5 (2.0%)	15 (5.9%)
Klebsiella spp.	0 (0.0%)	5 (2.0%)	2 (0.8%)	7 (2.8%)
Lactobacillus spp.	9 (3.6%)	11 (4.3%)	2 (0.8%)	22 (8.7%)
Proteus spp.	1 (0.4%)	0 (0.0%)	1 (0.4%)	2 (0.8%)
S. aureus	3 (1.2%)	5 (2.0%)	16 (6.3%)	24 (9.5%)
Salmonella spp.	9 (3.6%)	1 (0.4%)	8 (3.2%)	18 (7.1%)
Serratia spp.	3 (1.2%)	0 (0.0%)	0 (0.0%)	3 (1.2%)
Total	**92 (36.4%)**	**74 (29.2%)**	**87 (34.4%)**	**253 (100.0%)**

Phenotypic antimicrobial resistance profiles

Phenotypic profiling revealed substantial resistance against tested antibiotics (Table 3). Among the Gram-negative isolates, ampicillin (AMP) and cefuroxime (CRX) tied for the highest resistance rates, each accounting for 21.9% (n = 23) of the total resistant isolates. High frequencies were also observed for the third-generation cephalosporin ceftriaxone (CTR) at 19.0% (n = 20) and cefotaxime (CTX) at 12.4% (n = 13). Notably, phenotypic resistance to the last line carbapenem meropenem (MEM) was identified in 11.4% (n = 12) of isolates. Low resistance rates were recorded for co-trimoxazole (6.7%), ciprofloxacin (1.9%), and tetracycline (1.9%), while amikacin, chloramphenicol, and gentamicin each exhibited the lowest frequency at 1.0% (n = 1).

For the Gram-positive isolates, the highest resistance frequencies were observed for cloxacillin (COX) and penicillin (PEN), each representing 19.5% (n = 22) of the resistance profile. This was followed closely by ampicillin (AMP) and co-trimoxazole (COT), both presenting at 18.6% (n = 21). Moderate resistance was recorded for vancomycin (VAN) at 10.6% (n = 12) and tetracycline (TET) at 8.0% (n = 9). Low resistance profiles were observed for ciprofloxacin (3.5%) and gentamicin (1.8%).

Table 3
Frequencies of antimicrobial resistance in bacteria from fruit bats

Gram-negative organisms		**Gram-positive organisms**	
Antibiotics	**Resistance, % (n)**	**Antibiotics**	**Resistance, % (n)**
AMK	1.0% (1)	AMP	18.6% (21)
AMP	21.9% (23)	CIP	3.5% (4)
CHL	1.0% (1)	COT	18.6% (21)
CIP	1.9% (2)	COX	19.5% (22)
COT	6.7% (7)	GEN	1.8% (2)
CRX	21.9% (23)	PEN	19.5% (22)
CTR	19.0% (20)	TET	8.0% (9)
CTX	12.4% (13)	VAN	10.6% (12)
GEN	1.0% (1)	-	**-**
MEM	11.4% (12)	-	**-**
TET	1.9% (2)	-	**-**
TOTAL	**100.0% (105)**	**TOTAL**	**100.0% (113)**

Organism-specific and class-wise resistance frequencies

Beta-lactams accounted for the largest share of resistance across all evaluated bacterial classes (Table 4). Among the Gram-negative isolates, *Salmonella* spp. exhibited the highest resistance to beta-lactams, with 27 isolates resistant to penicillins and 8 isolates resistant to cephalosporins, representing a cumulative 33.3% of the total observed resistance within this group. *Enterobacter* spp. followed, contributing 22.9% to collective beta-lactam resistance. On the contrary, *E. coli* and *Klebsiella* spp. demonstrated limited resistance, restricted primarily to sulfonamides (1.0%) and basic penicillins.

Table 4
Gram-negative resistance profiles across antibiotic classes

Organism	AMG	AMC	FLQ	SUL	TET	BLPen	BLCep	Total
E. coli	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.0%)	0 (0.0%)	5 (4.8%)	1 (1.0%)	7 (6.7%)
Enterobacter	2 (1.9%)	0 (0.0%)	0 (0.0%)	1 (1.0%)	1 (1.0%)	19 (18.1%)	5 (4.8%)	28 (26.7%)
Klebsiella	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.0%)	0 (0.0%)	6 (5.7%)	2 (1.9%)	9 (8.6%)
Proteus	0 (0.0%)	0 (0.0%)	1 (1.0%)	0 (0.0%)	0 (0.0%)	4 (3.8%)	1 (1.0%)	6 (5.7%)
Salmonella	0 (0.0%)	1 (1.0%)	0 (0.0%)	3 (2.9%)	1 (1.0%)	27 (25.7%)	8 (7.6%)	40 (38.1%)
Serratia	0 (0.0%)	0 (0.0%)	1 (1.0%)	1 (1.0%)	0 (0.0%)	10 (9.5%)	3 (2.9%)	15 (14.3%)
Total	**2(1.9%)**	**1 (1.0%)**	**2 (1.9%)**	**7 (6.7%)**	**2 (1.9%)**	**71 (67.6%)**	**20 (19.0%)**	**105 (100.0%)**

Aminoglycosides = AMG, Amphenicols = AMC, Fluoroquinolones = FLQ, Sulfonamides = SUL, Tetracyclines = TET, Beta-Lactam (Penicillin) = BLPen, Beta Lactam (Cephalosporin) = BLCep

Among the Gram-positive isolates, *Bacillus* spp. exhibited the highest resistance within the class, with 41 isolates showing resistance against beta-lactams, representing 36.9% of the total recorded resistance (Table 5). Sulfonamide resistance was also prominent in *Bacillus* spp. (14 isolates; 12.6%), while glycopeptide resistance was identified in 8 *Bacillus* isolates (7.2%).

Table 5
Gram-positive resistance profiles across antibiotic classes

Organism	AMG	BL	FLQ	GLP	SUL	TET	Total
Lactobacillus spp.	0 (0.0%)	12 (10.8%)	1 (0.9%)	3 (2.7%)	4 (3.6%)	1 (0.9%)	21 (18.9%)
CONS	1 (0.9%)	3 (2.7%)	0 (0.0%)	1 (0.9%)	1 (0.9%)	1 (0.9%)	7 (6.3%)
S. aureus	0 (0.0%)	9 (8.1%)	1 (0.9%)	0 (0.0%)	2 (1.8%)	0 (0.0%)	12 (10.8%)
Bacillus spp.	1 (0.9%)	41 (36.9%)	2 (1.8%)	8 (7.2%)	14 (12.6%)	5 (4.5%)	71 (64.0%)
Total	**2 (1.8%)**	**65 (58.5%)**	**4 (3.6%)**	**12 (10.8%)**	**21 (18.9%)**	**7 (6.3%)**	**111 (100.0%)**

Aminoglycosides = AMG, Beta Lactam = BL, Fluoroquinolones = FLQ, Glycopeptides = GLP, Sulfonamides = SUL, Tetracyclines = TET

Multidrug resistance (MDR) patterns

The phenotypic analysis indicated a divergence in MDR prevalence between Gram-negative and Gram-positive isolates (Table 6). Among the Gram-negative isolates, the prevalence of MDR was low, with only 2 isolates (8.0%) meeting the criteria, restricted strictly to individual lineages of *Enterobacter* spp. (4.0%) and *Salmonella* spp. (4.0%). No phenotypic MDR was detected among *E. coli*, *Klebsiella*, *Proteus*, or *Serratia*. In sharp contrast, a major proportion of the Gram-positive isolates (63.6%) exhibited phenotypic MDR. Among these, *Bacillus* spp. constituted the highest proportion, accounting for 40.9% of the total MDR isolates, followed by *Lactobacillus* spp. (13.6%), CONS (4.5%), and *S. aureus* (4.5%).

Table 6
Multi-Drug Resistance among clinically relevant bacteria isolated from bats in this study

GNB isolates	MDR, n (%, 95%CI)	GPB isolates	MDR, n (%, 95%CI)
E. coli	0(0.0%)	*Bacillus*	9(40.9%)
Enterobacter	1(4.0%)	*Lactobacillus*	3(13.6%)
Klebsiella	0(0.0%)	*CONS*	1(4.5%)
Proteus	0(0.0%)	*S. aureus*	1(4.5%)
Salmonella	1(4.0%)	-	-
Serratia	0(0.0%)	-	-
Total			

Molecular characterization of resistance genes

Genotypic screening via PCR revealed that the predominant genetic driver of beta-lactam resistance within the Gram-negative strains was the bla_{TEM} gene. This gene was successfully amplified in 69.2% (9/13) of the screened phenotypically resistant isolates. The target was successfully amplified across multiple recovered genera, including *Serratia*, *Klebsiella*, *E. coli*, *Salmonella*, and *Enterobacter*. Contrarywise, PCR assays targeting classical extended-spectrum beta-lactamase (ESBL) genes bla_{CTX-M} and bla_{SHV}, as well as the carbapenemase marker bla_{NDM}, yielded no detectable amplification across any Gram-negative isolates. Additionally, genotypic screening for methicillin-resistant *S. aureus* (MRSA) revealed that both *mecA* and *mecC* structural genes were completely absent across all confirmed *S. aureus* strains (n = 24) identified in this study.

DISCUSSION

The recovery of 253 bacterial isolates across three density zones reveals a complex, niche-specific microflora within the fruit bat reservoir. The high prevalence of the genus *Bacillus* (62.5%) in freshly shed guano suggests a strong dietary or transient gastrointestinal association, rather than mere post-

deposition environmental contamination [20]. As ubiquitous saprophytes, *Bacillus* species are heavily ingested by *E. helvum* during foraging on fruits and flowers, and their spore-forming nature allows them to survive rapid gastrointestinal transit intact [21]. While their endospores represent a stable biological signature of shedding, the presence of opportunistic or toxigenic species within this genus underscores an immediate public health risk, as fresh droppings land directly in shared spaces before environmental sterilization can occur [22].

In contrast to uniform saprophytic flora, pathogens exhibited distinct site-specific variations. *S. aureus* demonstrated a substantially higher isolation rate in fresh guano from the mid-density site (18.4%) relative to the high-density (3.3%) and low-density (7.1%) sectors. This spatial heterogeneity highlights that active pathogen shedding within wild populations is influenced by localized behavior or specific contact interfaces with human municipal waste rather than host density alone [23]. On the contrary, the low prevalence of *E. coli* (1.2%) observed in this study is similar to findings from undisturbed ecological surveys globally [9]. This also supports the hypothesis that *E. coli* may not constitute a permanent colonizer of the healthy chiropteran gut in our study location.

The total collective carriage of the Enterobacteriaceae family within the current study includes *Enterobacter* spp. (5.9%), *Klebsiella* spp. (2.8%), *Salmonella* spp. (7.1%), *Serratia* spp. (1.2%), *Proteus* spp. (0.8%), and *E. coli* (1.2%) for a cumulative family prevalence of 19.0%. This is substantially lower than findings from Central Africa, where Nguema et al. [24] recorded high overall rates of Enterobacteriaceae carriage (40–43%) among fruit bats in Gabon. We posit that enteric carriage is highly dynamic, heavily modulated by local dietary shifts as well as anthropogenic landscape modification.

The phenotypic susceptibility profiles reveal a worrying degree of resistance among both Gram-negative and Gram-positive isolates, reflecting regional trends of environmental antibiotic contamination. Among Gram-negative isolates, the high resistance observed against ampicillin (21.9%) and cefuroxime (21.9%) highlights intense selective pressure exerted by foundational beta-lactams, matching baseline data from Central Africa [24]. The driver of this profile is clear: basic penicillins and cephalosporins are frequently prescribed, low-cost, and poorly regulated across sub-Saharan Africa, where clinical resistance rates remain high [26]. Spillover into synanthropic wildlife indicates that clinical resistance is overflowing via municipal wastewater systems, cycling these traits into the wildlife gut [27].

A critical finding is the phenotypic resistance directed against extended spectrum cephalosporins and carbapenems, specifically ceftriaxone (19.0%), cefotaxime (12.4%), and meropenem (11.4%). Third-generation cephalosporin and carbapenem resistance are vital clinical markers, with meropenem representing a last line of defense against multidrug-resistant pathogens [28]. While carbapenem resistance in wildlife was historically rare, recent surveillance reports its emergence across chiropteran hosts globally [29], pointing to direct exposure to high levels of clinical effluent within shared urban spaces. Notably, because molecular screening did not detect classical $bla_{\text{CTX-M}}$ or bla_{SHV} genes, this extended-spectrum phenotype may be driven by the highly prevalent bla_{TEM} variants identified, or alternative unextracted resistance mechanisms [30].

Gram-positive isolates displayed widespread resistance concentrated against cloxacillin (19.5%), penicillin (19.5%), and ampicillin (18.6%), aligning with the broad distribution of beta-lactamase enzymes driven by historical penicillin exposure [31]. The 18.6% resistance rate against co-trimoxazole agrees with broad public health patterns within sub-Saharan Africa, where it is used heavily as clinical primary prophylaxis [32]. Subsequently, it enters unmanaged municipal waste streams and selects for resistance traits within shared ecosystems [32]. While phenotypic vancomycin resistance (10.6%) was observed, this likely reflects the well-documented intrinsic vancomycin resistance characteristically found in specific commensal *Lactobacillus* spp. [33], rather than true acquired clinical resistance.

The threat of AMR increases significantly when individual resistance mechanisms converge to produce MDR [15]. In this study, MDR among Gram-negative isolates was low (8.0%) and restricted to selected *Salmonella* and *Enterobacter* lineages. This agrees with wildlife studies in less pressured habitats where the absence of concentrated clinical effluents limits multi-class plasmid assembly [6]. In sharp contrast, Gram-positive isolates displayed a high MDR rate of 63.6%, driven primarily by *Bacillus* spp. (40.9%). Because fresh guano captures bacteria actively passing through the host, this high MDR rate reflects intense selective pressures encountered by bacteria during gastrointestinal transit or within anthropogenically modified foraging niches [34]. The high Gram-positive MDR rates present a clear public health risk. This is because these commensal bacteria serve as expansive environmental genetic reservoirs, capable of horizontal gene transfer to high-consequence pathogens sharing the same high-density human-wildlife interface [35].

To uncover the genetic mechanisms driving these resistant phenotypes, molecular screening targeted key determinants. The primary genetic driver identified was the bla_{TEM} gene, amplified in 69.2% of the screened resistant isolates across multiple genera. This finding provides clear molecular evidence of horizontal gene transfer occurring within shared anthropogenically influenced environments [31]. Significantly, PCR assays targeting classical ESBL genes bla_{CTX-M} and bla_{SHV}, as well as carbapenemase marker bla_{NDM}, yielded no detectable amplification. Globally, ESBL-producing Enterobacteriaceae carrying bla_{CTX-M} and bla_{SHV} are identified in fruit bats as markers of intense human fecal contamination [24]. Thus, the absence of these markers suggests that while local populations have acquired established resistance genes like bla_{TEM}, aggressive hospital associated ESBL lineages have not deeply penetrated this wildlife reservoir. Additionally, the complete absence of *mecA* and *mecC* genes across all isolated *S. aureus* strains (n = 24) matches regional wildlife data from West Africa [22]. This reveals a phenotypic-genotypic mismatch that provides a critical baseline benchmark for wildlife resistome evolution in Ghana [23].

The correlation between the observed resistance profiles and human activity within the UENR sanctuary underscores a fundamental One Health principle, *viz*, the biological integrity of wildlife microbiomes is inextricably linked to anthropogenic environmental management [36]. The ecological context of the UENR campus represents a permeable human-wildlife interface rather than a strictly isolated preserve. The bat populations face continuous anthropogenic stressors, including local hunting pressures and eviction efforts. This constant displacement and close, volatile contact with human municipal footprints

force the bats to forage extensively within highly contaminated urban matrices, such as open sewage systems, market refuse zones, and agricultural effluents [36]. During this period, they encounter sub-lethal antibiotic residues thereby effectively operate as environmental reservoirs of AMR [16]. Consequently, these anthropogenic pressures accelerate the mechanisms of bidirectional microbial exchange. It is noteworthy that our sampling approach caught these pathogens in freshly shed, viable guano, thereby revealing a bi-directional risk. Thus, the bats serve as indicators of human pollution and active vectors which redistribute clinically relevant resistant strains back into shared public spaces. A sole reliance on clinical hospital surveillance overlooks this environmental circulation and creates a critical gap in Ghana's national AMR strategy that can only be resolved through integrated, multi-sectoral One Health surveillance [37].

Our study had several limitations. Firstly, the UENR bat sanctuary represents a highly permeable interface characterized by active human encroachment, hunting pressures, and periodic colony dispersal efforts. Thus, our population reflect an area under acute anthropogenic stress rather than an undisturbed ecological baseline. Also, the cross-sectional design limits the capacity to resolve temporal dynamics, seasonal fluctuations, or migratory variations in AMR and bacterial profiles in the sanctuary. Lastly, while molecular validation targeted a refined panel of high-consequence resistance markers, comprehensive next-generation sequencing was not performed, leaving alternative or unextracted genetic determinants driving the observed phenotypic resistance profiles uncharacterized.

CONCLUSION

This study confirms that *E. helvum* populations within the UENR sanctuary are active environmental sentinels, shedding clinically significant opportunistic pathogens, including *S. aureus* and *Salmonella* spp., directly into shared human-wildlife spaces. Phenotypic profiling revealed extensive resistance to foundational penicillins, alongside the concerning detection of resistance to key clinical therapies including third generation cephalosporins and meropenem. Molecular screening identified the beta-lactamase determinant bla_{TEM} as a key genetic marker within these wild mammalian hosts, despite the absence of classical genotypic ESBL or MRSA markers. Because wildlife AMR surveillance remains critically underdeveloped in Ghana, these findings emphasize an immediate, actionable need to establish integrated national control strategies. There is a vital need for concerted, cross-sectoral efforts (One Health approach) by the Wildlife Division of the Forestry Commission, the Ghana Health Service, and the Environmental Protection Agency (EPA), Ghana to bridge the wildlife surveillance gap to mitigate bi-directional microbial spillover at the human-wildlife interface.

Declarations

CRediT authorship contribution statement

Claudia Wubuareyasa Nseide: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Visualization. **Prince Nyarko:** Writing – review & editing,

Visualization, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Anabel Acheampong:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Prince-Charles Kudzordzi:** Writing – review & editing, Supervision, Methodology. **Dennis Ofori Kyei:** Writing – review & editing, Methodology, Formal analysis, Visualization, Conceptualization. **Silas Arhin:** Writing – review & editing, Methodology, Formal analysis, Visualization. **Mary Serwaa Appiah**: Writing – review & editing, Methodology, Formal analysis, Visualization. **Henry Asabere**: Writing – review & editing, Methodology, Formal analysis, Visualization. **Michelle Ohemeng**: Writing – review & editing, Methodology, Formal analysis, Visualization. **Paulina Kessewaa**: Writing – review & editing, Methodology, Formal analysis, Visualization. **Bright Tweneboah Awuah**: Writing – review & editing, Methodology, Formal analysis, Visualization. **Kenneth Bentum Otabil:** Writing – review & editing, Supervision, Methodology, Data curation, Visualization, Funding acquisition, Conceptualization.

Ethics approval and consent to participate

This study was conducted as an approved component of a larger, comprehensive One Health research project designed to investigate bat bacteria diversity, antimicrobial resistance phenotypes and genotypes, and the specific human-bat interactions that drive the risk of pathogen spillover at this interface. Ethical clearance and administrative protocols for this overarching project were formally reviewed and approved by the Committee on Human Research, Publication, and Ethics at the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana (Reference No. CHRPE/AP/348/25). All procedures were conducted in accordance with Good Clinical Laboratory Practice. The specific scope of this study relied strictly on non-invasive sampling, i.e., collecting naturally shed guano directly from the ground without trapping, handling, or disrupting the wild mammalian hosts. Thus, the field protocols minimized animal stress and eliminated physical exposure risks to field personnel. Administrative authorization to access the campus colony sites was maintained under institutional oversight and in alignment with the field observation guidelines of the Wildlife Division of the Forestry Commission, Ghana.

Data availability statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Consent for publication

Not applicable

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Declaration of competing interest

The authors have declared that no competing interests exist.

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