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Ecological and Epidemiological Drivers of ESBL-Producing *E. coli* in  
Peridomestic Rats from Hong Kong

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2026

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Peridomestic Rats from Hong Kong

Catarina Alexandra da Costa Fernandes

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# Determinantes Ecológicos e Epidemiológicos da Presença de *E. coli* Produtora de ESBL em Ratos Peridomésticos de Hong Kong

## Resumo

O aumento de *Escherichia coli* produtora de beta-lactamases de espectro alargado (ESBL-EC) é considerado um ponto de viragem na história da resistência aos antimicrobianos (RAM). Atualmente, a RAM é reconhecida como um dos maiores desafios da saúde pública, acelerada pelo uso inapropriado de antimicrobianos e pela crescente interligação entre humanos, animais e ambiente. Contudo, o papel da vida selvagem na ecologia e disseminação da RAM continua pouco compreendido. Os ratos peridomésticos que prosperam em estreita proximidade com humanos e animais de produção podem servir como sentinelas valiosas da evolução da RAM.

Este estudo investigou a distribuição e os fatores associados ao transporte de ESBL-EC por espécies de *Rattus* capturadas em diferentes contextos ecológicos em Hong Kong. Foram capturados 221 ratos em áreas urbanas, explorações avícolas, explorações suinícolas e centros hípicas. Após o rastreio fenotípico para deteção de ESBL-EC, foi realizada uma análise de regressão logística para identificar fatores de risco associados à sua presença. A presença de ESBL-EC foi detetada em 58,8% das amostras. A espécie *Rattus tanezumi* apresentou uma probabilidade significativamente inferior de ser portadora de ESBL-EC em comparação com *R. norvegicus* (OR = 0,13; IC<sub>95%</sub>: 0,06 – 0,27;  $p < 0,001$ ). O contexto ambiental também revelou ter influência, observando-se níveis elevados em áreas urbanas e uma tendência acentuada para maior probabilidade de os ratos provenientes de explorações avícolas serem portadores, comparativamente aos da cidade (OR = 2,59; IC<sub>95%</sub>: 1,01 – 7,22;  $p = 0,055$ ). O comprimento corporal, utilizado como *proxy* da idade, foi associado de forma positiva à probabilidade da espécie *R. norvegicus* ser portadora de ESBL-EC (OR = 1,14; IC<sub>95%</sub>: 1,04 – 1,26;  $p = 0,006$ ), ao contrário do *R. tanezumi*, sugerindo padrões de exposição distintos entre espécies.

Estes resultados contribuem para uma melhor compreensão de como a ecologia do hospedeiro molda o envolvimento da vida selvagem na disseminação da RAM. Compreender os determinantes ecológicos da RAM nas espécies selvagens é essencial para o desenvolvimento de abordagens sustentáveis e eficazes na perspetiva de Uma Saúde, destinadas a mitigar a propagação da resistência.

Palavras-chave: resistência aos antimicrobianos; *Escherichia coli*; beta-lactamases; saúde pública; *Rattus*.

# Ecological and Epidemiological Drivers of ESBL-Producing *E. coli* in Peridomestic Rats from Hong Kong

## Abstract

The rise of extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL-EC) has been considered a turning point in antimicrobial resistance (AMR) history. AMR is now widely perceived as one of the most pressing challenges in public health, accelerated by the misuse of antimicrobials and the growing interconnection between humans, animals, and the environment. However, the role of wildlife in the ecology and dissemination of AMR remains poorly understood. Peridomestic rats, which thrive in close proximity to humans and livestock, may serve as valuable sentinels for AMR evolution.

This study investigated the distribution and predictors of ESBL-EC carriage in *Rattus* species captured across different ecological settings in Hong Kong. 221 rats were collected from city areas, chicken farms, pig farms, and horse-riding schools. Phenotypic screening for ESBL-EC was performed, and a logistic regression analysis was developed to identify risk factors associated with carriage. ESBL-EC was detected in 58.8% of samples. *Rattus tanezumi* showed significantly lower odds of ESBL-EC carriage compared to *R. norvegicus* (OR = 0.13; CI<sub>95%</sub>: 0.06 – 0.27;  $p < 0.001$ ). The environmental context further influenced carriage, with city areas showing high carriage levels and rats from chicken farms displaying a strong trend toward higher odds compared to those from city areas (OR = 2.59; CI<sub>95%</sub>: 1.01 – 7.22;  $p = 0.055$ ). Body length, used as a proxy for age, was positively associated with carriage in *R. norvegicus* (OR = 1.14; CI<sub>95%</sub>: 1.04 – 1.26;  $p = 0.006$ ), but not in *R. tanezumi*, suggesting distinct exposure patterns between species.

Together, these findings contribute to a broader understanding of how host ecology shapes wildlife involvement in AMR dissemination. Understanding ecological drivers of AMR in wildlife species is essential for the development of effective and sustainable One Health approaches aimed at mitigating resistance spread.

Key-words: antimicrobial resistance; *Escherichia coli*; beta-lactamases; public health; *Rattus*.

# Determinantes Ecológicos e Epidemiológicos da Presença de *E. coli* Produtora de ESBL em Ratos Peridomésticos de Hong Kong

## Resumo Alargado

A resistência aos antimicrobianos (RAM) constitui atualmente um dos maiores desafios para a saúde pública global, afetando simultaneamente os setores humano, animal e ambiental. A emergência e disseminação de bactérias multirresistentes, em particular *Escherichia coli* produtora de beta-lactamases de espectro alargado (ESBL-EC), representam uma ameaça devido ao impacto clínico e económico associado. Embora a resistência seja um fenómeno que ocorre naturalmente, o seu ritmo de evolução tem sido acelerado pela ação humana, incluindo o uso inapropriado de antibióticos, práticas de saneamento insuficientes, falhas no tratamento de resíduos e elevada densidade populacional. Este contexto favorece a circulação de genes de resistência, tornando essencial uma abordagem integrada de Uma Saúde. Apesar disso, o papel da vida selvagem, particularmente de espécies peridomésticas, na ecologia e disseminação da RAM permanece subinvestigado.

As espécies de ratos peridomésticos, como *Rattus norvegicus* e *Rattus tanezumi*, vivem em proximidade constante com a população humana e com sistemas de produção animal, sendo expostos a uma grande variedade de fontes ambientais contaminadas, desde resíduos urbanos e efluentes até subprodutos agrícolas. Estas espécies oportunistas ingerem alimentos descartados, resíduos domésticos e materiais presentes nas explorações. Consequentemente, têm sido propostas como sentinelas da contaminação ambiental por genes de resistência a antimicrobianos e como possíveis facilitadores da circulação de bactérias resistentes. Em regiões fortemente urbanizadas, como Hong Kong, a presença constante de ratos na interface Homem-animal-ambiente torna estes animais relevantes para compreender a ecologia da resistência antimicrobiana.

Este estudo investigou a distribuição e os fatores associados ao transporte de ESBL-EC por espécies de *Rattus* capturadas em diferentes contextos ecológicos de Hong Kong. Derivada de um projeto mais amplo dedicado à caracterização molecular de isolados resistentes em roedores peridomésticos, o presente estudo adota uma perspetiva epidemiológica, com o objetivo de identificar características que influenciam a probabilidade de um rato ser portador de ESBL-EC.

Entre outubro de 2020 e agosto de 2021, foram capturados 221 ratos em 16 locais de Hong Kong. Os locais incluíram áreas urbanas, explorações avícolas, explorações suínolas e centros hípicas, selecionados de modo a abrangerem diferentes contextos ambientais. Após a captura, foram registados parâmetros morfológicos e demográficos, e colhidas amostras cecais para deteção fenotípica de ESBL-EC. A identificação bacteriana foi realizada através

do método MALDI-TOF, sendo a produção de ESBL confirmada por testes fenotípicos. Após análise de estatística descritiva e univariada, foi desenvolvido um modelo de regressão logística para identificar fatores associados à presença de ESBL-EC.

A presença de ESBL-EC foi confirmada em 58,8% das amostras. A espécie *R. norvegicus* apresentou uma probabilidade superior de ser portadora de ESBL-EC comparativamente a *R. tanezumii*. Os resultados do modelo mostraram que *R. tanezumii* tinha apenas 13% da probabilidade de ser portadora, comparativamente com a probabilidade observada para *R. norvegicus* (OR = 0,13; IC<sub>95%</sub>: 0,06 – 0,27; p < 0,001), mesmo após o controlo de outros fatores, demonstrando que a espécie constitui um dos determinantes mais fortes. Este resultado é coerente com as diferenças ecológicas entre as duas espécies: *R. norvegicus* é geralmente mais associado a ambientes de elevada antropização, com maior contacto com esgotos, resíduos orgânicos, ambientes húmidos e áreas densamente povoadas, condições propícias à circulação de *Enterobacteriaceae* resistentes.

O contexto ecológico e ambiental revelou-se igualmente relevante. As áreas urbanas apresentaram uma maior proporção de ratos portadores, refletindo a pressão ambiental associada à densidade populacional e proximidade a infraestruturas humanas. Além disso, observou-se uma tendência acentuada para uma maior probabilidade de ratos provenientes de explorações avícolas serem portadores de ESBL-EC, comparativamente aos capturados em áreas urbanas (OR = 2,59; IC<sub>95%</sub>: 1,01 – 7,22; p = 0,055). Este resultado sugere que os sistemas avícolas podem constituir fontes importantes de pressão seletiva e exposição a ESBL-EC, resultado compatível com estudos prévios da região que reportam elevada presença de ESBL-EC em aves de produção e nos seus efluentes. As explorações suínícolas, por sua vez, não demonstraram impacto estatisticamente significativo no modelo final.

O comprimento corporal dos animais, utilizado como *proxy* da idade, mostrou ser um preditor relevante, mas dependente da espécie. Em *R. norvegicus*, observou-se uma associação positiva entre tamanho e probabilidade de ser portador (OR = 1,14; IC<sub>95%</sub>: 1,04 – 1,26; p = 0,006), sugerindo exposição cumulativa ao longo do tempo e coerente com padrões de comportamento e ecologia da espécie. Já em *R. tanezumii*, não se verificou uma associação significativa, provavelmente devido a diferenças no uso do habitat, deslocação e comportamentos alimentares e sociais.

O modelo de regressão logística final, que incluiu espécie de rato, local de captura, comprimento corporal centrado por espécie e interação de espécie e tamanho, apresentou boa capacidade discriminativa (AUC = 0,80) e classificou corretamente 161 de 211 indivíduos. Não foram observados problemas de multicolinearidade entre os preditores, indicando estabilidade dos coeficientes. O desempenho do modelo sugere que características

ecológicas das espécies de ratos, associadas ao contexto ambiental, explicam uma parte substancial da variação na probabilidade de os ratos serem portadores de ESBL-EC.

Adicionalmente, a análise descritiva dos dados genotípicos revelou a presença de genes de resistência amplamente descritos globalmente, incluindo as variantes CTX-M-14, CTX-M-55, TEM-1 e CTX-M-15. Embora a caracterização genética detalhada não tenha sido o foco do estudo, estes padrões sugerem que os ratos são portadores de populações bacterianas compatíveis com as circulantes nos setores humano, animal e ambiental, o que sustenta o seu papel como bons indicadores da circulação de genes de resistência clinicamente relevantes.

Os resultados deste estudo têm implicações importantes na compreensão da ecologia da RAM e no desenvolvimento de estratégias de vigilância. Em primeiro lugar, sugerem que a colonização por ESBL-EC não é homogénea entre espécies de roedores, sendo fortemente influenciada pela ecologia específica do hospedeiro. *Rattus norvegicus*, mais associado a infraestruturas urbanas e a ambientes com elevado impacto humano, apresentou maior probabilidade de ser portador de ESBL-EC, sugerindo que a espécie poderá desempenhar um papel mais relevante na disseminação ambiental deste tipo de resistência. Em segundo lugar, os resultados evidenciam que ambientes urbanos e sistemas avícolas representam contextos de maior risco, sublinhando a importância de considerar gradientes de antropização e práticas de produção animal na avaliação de risco. Modelos de vigilância que incluam espécies sentinela podem fornecer informação valiosa sobre tendências espaciais e temporais da RAM.

Por fim, este trabalho reforça a necessidade de integrar a vida selvagem e, em particular, espécies peridomésticas, nas estratégias de monitorização e mitigação da RAM. Em Hong Kong, apesar dos progressos registados nos planos estratégicos nacionais para controlo da RAM, os programas de vigilância ainda não contemplam a vida selvagem como componente formal. Os resultados apresentados mostram que os ratos peridomésticos não só refletem a pressão antimicrobiana ambiental existente como também podem atuar como potenciais vetores de disseminação de populações bacterianas resistentes entre ambientes urbanos e agrícolas. A inclusão destes animais em programas de monitorização pode contribuir para uma deteção precoce de resistências emergentes e para uma estratégia verdadeiramente integrada no contexto de Uma Saúde.

Em síntese, este estudo demonstrou que a ecologia do hospedeiro e o contexto ambiental podem desempenhar um papel determinante no transporte de ESBL-EC em ratos peridomésticos. Estes resultados representam um passo importante na compreensão da

forma como a RAM circula entre ecossistemas complexos e destaca a importância de abordagens integradas que considerem múltiplos reservatórios.

Palavras-chave: resistência aos antimicrobianos; *Escherichia coli*; beta-lactamases; saúde pública; *Rattus*.

## Table of Contents

Acknowledgments.....	iii
Resumo .....	v
Abstract.....	vi
Resumo Alargado .....	vii
Figure Index.....	xii
Table Index .....	xiii
List of abbreviations .....	xiv
1. Introduction .....	1
2. Internship Report.....	1
3. Literature review.....	3
3.1. Antimicrobial resistance as a global challenge.....	3
3.2. Extended-Spectrum Beta-Lactamase-producing <i>Escherichia coli</i> .....	5
3.3. Wildlife and livestock as drivers of AMR .....	7
3.4. Rodents as sentinels of AMR.....	8
3.5. The Hong Kong context .....	9
3.6. Research gaps .....	11
4. Materials and Methods .....	12
4.1. Objectives.....	12
4.2. Sampling .....	12
4.3. Study population.....	14
4.4. Dataset and data cleaning .....	14
4.5. Statistical analysis .....	15
5. Results.....	19
5.1. Carriage by rat species.....	19
5.2. Carriage by location type and region .....	19
5.3. Carriage by developmental stage, sex, and presence of cutaneous lesions ...	21
5.4. Carriage by body length.....	21
5.5. Risk factor model.....	22
5.5.1. Univariable analysis.....	22
5.5.2. Multivariable analysis.....	23
5.6. Genotypic findings .....	25
6. Discussion.....	27
7. Conclusion .....	34
8. Bibliography .....	35

## Figure Index

Figure 1. Geographic distribution of capture sites in Hong Kong.. ..	13
Figure 2. Correlation matrix between continuous variables.....	17
Figure 3. ESBL-EC carriage proportion by rat species.....	19
Figure 4. ESBL-EC carriage proportion by location type and region, separated by rat species. .....	20
Figure 5. ESBL-EC carriage proportion by developmental stage, sex, and presence of cutaneous lesions, separated by rat species. ....	21
Figure 6. Distribution of body length by ESBL-EC carriage status, separated by rat species. .....	22

## Table Index

Table 1. Variables included in the final working dataset.....	15
Table 2. Variables that met the inclusion criteria in univariable logistic regression.....	23
Table 3. Summary of the final logistic regression model predicting ESBL-EC carriage in peridomestic rats.....	24
Table 4. Classification table of predicted versus observed ESBL-EC carriage status .....	25
Table 5. Variance Inflation Factor values for predictors in the final model.....	25
Table 6. Distribution of ESBL genes among isolates according to rat species and sampling location.....	26
Table 7. ESBL gene combinations identified among isolates carrying more than one ESBL resistance gene .....	26

## **List of abbreviations**

AIC – Akaike’s Information Criterion

AMR – Antimicrobial resistance

ARGs – Antibiotic resistance genes

AUC – Area under the curve

DNA – Deoxyribonucleic acid

ESBL – Extended-spectrum beta-lactamase

ESBL-EC – Extended-spectrum beta-lactamase-producing *Escherichia coli*

GPS – Global positioning system

MALDITOF – Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

RN – *Rattus norvegicus*

ROC – Receiver operating characteristic

RT – *Rattus tanezumi*

VIF – Variance inflation factor

VPHI – Veterinary Public Health Institute

WHO – World Health Organization

## **1. Introduction**

This work was developed as part of my Integrated Master's in Veterinary Medicine. The first part describes the curricular internship, explaining the context, activities, and skills acquired. It then focuses on antimicrobial resistance, with special attention to the potential role of peridomestic rats as both sentinels and reservoirs of extended-spectrum beta-lactamase-producing *Escherichia coli*, in Hong Kong. The following sections detail the materials and methods applied in the study and the main results obtained, concluding with a discussion of the findings and their interpretation in relation to existing literature, along with final considerations and perspectives for future research directions.

## **2. Internship Report**

Between September 2024 and February 2025, a preparatory traineeship was completed under the supervision of Professor Telmo Nunes at the Faculty of Veterinary Medicine, University of Lisbon, totaling 380 hours. During this period, core competencies essential for the subsequent internship and project were developed. The traineeship focused on learning the R programming language, reviewing fundamental epidemiological concepts, and consolidating knowledge of basic statistical analysis. The R training included data preprocessing, exploratory data analysis, and the creation of visual outputs such as graphs and maps. These competencies were developed through practical tutorials and problem-based exercises. Additionally, introductory tutorials on Microsoft Power BI were completed to further enhance data visualization and interpretation skills.

Following the traineeship coursework, a curricular internship took place on-site at the Veterinary Public Health Institute (VPHI) of the University of Bern, under the mentorship of Professor Dr. Ioannis Magouras and Dr. Luís Pedro Carmo. The internship comprised 500 hours, from March to May 2025, and provided the opportunity to work within a multidisciplinary and international team involved in diverse research areas. This experience exposed me to various research workflows and problem-solving methodologies in the field of epidemiology and veterinary public health.

The initial weeks were dedicated to defining the research questions of the dissertation project. During this time, relevant literature on multiple topics was reviewed, and the background of the broader project from which the study's data originated was examined. The following weeks were focused on data cleaning, descriptive analysis, and statistical analysis in R. These facilitated the application and consolidation of previously developed skills, while allowing for the acquisition of new competencies through practical experience.

In addition to project-related tasks, regular participation in biweekly team meetings provided updates on ongoing projects and institutional activities. Monthly journal club sessions, led by PhD students and residents from the European College of Veterinary Public Health, involved critical discussions of recent scientific publications. These gatherings were particularly enriching, offering the opportunity to engage with current issues in veterinary public health and One Health, while developing scientific communication and reasoning skills. Participation in scientific coffee breaks and educational webinars organized by the VPHI further contributed to continuous learning in a wide range of research areas and encouraged interdisciplinary approaches.

In April, I was provided with the opportunity to attend a statistics course using the NCSS software, organized by members of the VPHI team. This training served as a valuable complement to the previous preparation, particularly by reinforcing the understanding of how to select appropriate statistical tests and interpret results within applied epidemiological contexts.

The internship provided a structured and supportive learning environment, allowing the application of the skills developed over the preceding months, while gaining practical experience in a research setting. The unique opportunity to interact with professionals at different academic levels and backgrounds helped me expand my perspective on the various dimensions of veterinary public health research and provided me with invaluable advice and insights. This experience deepened my understanding of the interdisciplinary nature of epidemiology and underscored the importance of collaboration in addressing global health challenges.

The work developed during this period is currently being prepared as a manuscript for submission to a peer-reviewed scientific journal, representing an important step toward contributing to the scientific community.

### **3. Literature review**

#### **3.1. Antimicrobial resistance as a global challenge**

Antimicrobial resistance (AMR) is now widely perceived as one of the most pressing challenges in healthcare, causing 1.14 million deaths in 2021 and being associated with 4.71 million deaths (Naghavi et al. 2024). Antibiotics, antifungals, antivirals, disinfectants, and food preservatives are products used to control or eliminate microorganisms. Antibiotics fundamentally changed medicine when they were first discovered, allowing for effective combat against bacterial infections. But, over time, bacteria have developed strategies to evade these substances (Salam et al. 2023). The phenomenon of AMR arises when microorganisms like bacteria, fungi, viruses, and parasites stop responding to medicines that were once effective against them (EClinicalMedicine 2021). Despite being a naturally occurring process, it has been significantly accelerated by human action. Practices such as overuse of antimicrobials in human and veterinary medicine, poor sanitation measures, and environmental pollution have created ideal circumstances for resistant bacteria to emerge and spread (Peterson and Kaur 2018). Bacteria gain resistance through genetic mutations or horizontal gene transfer, which involves the acquisition of genes from other microorganisms, often via plasmids or bacteriophages. The most common resistant mechanisms consist of drug-degrading enzymes, efflux pumps, altered antimicrobial targets, or changes in cell membrane permeability (Ferri et al. 2017).

To tackle this problem, a wide range of contributing factors must be considered. Lack of education remains a fundamental barrier, for only with global awareness can this issue be truthfully faced by everyone and every sector. Other drivers include permissive legislation, inadequate infection control, limited access to quality medical care, and the continued overuse of antimicrobials (Sharma et al. 2024). Antibiotic misuse can, in a way, be explained by the selection of antimicrobial treatments when their application is not necessary, diagnostic uncertainty, financial incentives, or the circulation of low-quality medicine with sub-therapeutic doses (WHO 2015).

Antimicrobial stewardship varies across the world and has changed over time, with the European Union banning their use as growth promoters and restricting prophylactic and metaphylactic use, as set out in Regulation (EU) 2019/6 (European Parliament and Council of the European Union 2019). In other regions, however, such practices remain common, and large amounts of antibiotics are used in livestock production, with agriculture and aquaculture representing particularly relevant sources of selection pressure (Abushaheen et al. 2020). This intensive use enables resistance genes to spread among both commensal and pathogenic bacteria, contaminating the surrounding environment and moving through soil, crops, animals, and ultimately entering the food chain (Ferri et al. 2017). Aquaculture mirrors this trend, with

antibiotics being frequently used in sub-therapeutic doses. The aquatic environment becomes a reservoir where resistant bacteria and genes can be refined before entering terrestrial habitats (Hossain et al. 2022). Residues from farms, hospitals, the pharmaceutical industry, and households reach water, sediment, and soil, creating gradients of antimicrobial concentrations that continue to favor resistant strains (Aslam et al. 2024).

These drivers end up translating into direct consequences for healthcare systems. Resistant infections complicate treatments, increase the risk of death, and endanger medical activities that depend on antimicrobials, from chemotherapy and organ transplants to routine dental procedures (Salam et al. 2023), forcing clinicians to rely on more expensive last-resort drugs (WHO 2015). AMR has major economic consequences, such as reduced human and animal productivity, worsened animal welfare, and longer costly hospital stays. At a global level, AMR also exacerbates inequality between populations, with low-income countries facing higher rates of infectious diseases and lacking adequate diagnostic tools, surveillance, or infection control measures, leaving them particularly vulnerable (Aslam et al. 2024).

Bacteria are able to render themselves resistant much faster than new antimicrobials are being developed. Pharmaceutical companies have opted to invest in more profitable medicines, given that once a new antimicrobial is introduced, it quickly becomes ineffective, which leads to financial losses (Ferri et al. 2017). This makes the introduction of new antibiotics rather difficult, and the beta-lactam class is a great example (Bonomo 2017). A further obstacle is the scarcity of updated and relevant data, challenging the ability to quantify antimicrobial use across sectors and assess the true burden of resistant infections (Abushaheen et al. 2020). Although progress has been made regarding AMR and antimicrobial use surveillance systems, available data are concentrated in European and other high-income countries. Reporting systems and data quality vary widely across the globe, with substantial data gaps in many large parts of the world, especially low- and middle-income regions (Sanders et al. 2020; WHO 2025).

In 2015, the World Health Organization (WHO) released the global action plan on antimicrobial resistance with guidelines on how to address this threat, by slowing it down and eventually reversing it. The aim was to preserve effective treatment options with safe and good quality products available for everyone. However, they also point out that it requires not only strong international political will and cooperation, but also technical and financial investment to create robust national action plans (WHO 2015).

Projections are alarming, estimating 169 million cumulative deaths associated with AMR from 2025 to 2050, with Asia, Oceania, and sub-Saharan Africa expected to face a higher

burden (Naghavi et al. 2024). Given these global trends, there is a pressing need to investigate how resistance emerges and circulates within local and global reservoirs.

### **3.2. Extended-Spectrum Beta-Lactamase-producing *Escherichia coli***

*Escherichia coli* is an extremely adaptable microorganism that inhabits the gut of animals and humans, usually coexisting with its hosts in a balanced way. However, despite its commensal role, it can also be responsible for a plethora of infections (Ribeiro et al. 2024) and represents a concern for food safety and security (Husna et al. 2023). It is frequently implicated in bloodstream infections and urinary tract infections (UTIs) worldwide (Vittecoq et al. 2016), but also in sepsis, gastroenteritis, and meningitis (Aljohni et al. 2025). *E. coli* is also the most common bacterial host for extended-spectrum beta-lactamases (ESBL) genes. Early detection of extended-spectrum beta-lactamase-producing *E. coli* (ESBL-EC) is essential, since such infections often lead to longer hospital stays, expensive treatment, and higher morbidity due to reduced antibiotic efficacy (Husna et al. 2023). ESBL-producing *Enterobacterales* overall are linked to considerable mortality and health burdens in both humans and animals (Benavides et al. 2024).

A meta-analysis on the global prevalence of ESBLs in coexisting *E. coli* and *Klebsiella pneumoniae*, covering 1990-2022, reported pooled prevalence values of 33% in humans, 33.5% in animals, and nearly 57% in the environment (Ramatla et al. 2023). In Egypt, almost 60% of *E. coli* isolates from UTIs were ESBL-producers, and 62% of those showed multidrug resistance (Hassuna et al. 2020). Risk factors for ESBL-EC colonization include antibiotic misuse and high antimicrobial pressure (Gudiol et al. 2009).

The central mechanism behind this challenge is resistance to beta-lactams, which are one of the most prescribed antibiotic classes (Bush and Bradford 2016). Their bactericidal action occurs through inhibition of penicillin-binding proteins, consequently stopping bacterial cell wall synthesis. To counteract this action, Gram-negative bacteria have developed several mechanisms and the production of beta-lactamases, enzymes that hydrolyze beta-lactams, remains one of the most relevant (Bonomo 2017). Within this group, ESBLs confer resistance to third-generation cephalosporins such as cefotaxime, ceftazidime, and ceftriaxone, along with monobactams, but not carbapenems and cephamycins (Pitout and Laupland 2008).

Two classification systems are commonly used for beta-lactamases, reflecting their diversity and range of resistance. The Ambler system categorizes them into four classes, from A to D, according to molecular structure, with most ESBLs belonging to class A. On the other hand, the Bush-Jacoby-Medeiros considers groups 1 to 3 depending on the degradation of beta-lactam substrates and the effects of inhibitors, with most ESBLs integrating group 2 (Aljohni et al. 2025). The TEM, SHV, and CTX-M families are the most clinically relevant

(Bonomo 2017) and are especially important because they have driven the evolution of resistance to extended-spectrum cephalosporins and other beta-lactams (Aljohani et al. 2025). Since the 2000s, *E. coli* isolates carrying CTX-M genes have emerged as the dominant ESBL-producers threatening public health at a global level, with CTX-M-15 being the most frequently detected, followed by CTX-M-14, particularly common in South-East Asia (Peirano and Pitout 2019). The biochemical diversity of these enzymes reflects the adaptive potential of bacteria under sustained antibiotic pressures.

Horizontal gene transfer allowed for the rapid dissemination of ESBL-encoding genes. Conjugation, the transfer of genetic material during direct cell contact, played a central role in disseminating *bla* genes such as *bla*<sub>CTX-M</sub>, *bla*<sub>TEM</sub>, and *bla*<sub>SHV</sub>. Mutations within these genes under high selection pressures have further expanded their resistance profiles (Aljohani et al. 2025), while mobile genetic elements (e.g., insertion sequences, transposons, integrons, and plasmids) facilitated both intracellular and intercellular mobility (Peirano and Pitout 2019). The widespread detection of these genes in different settings shows how molecular evolution can translate into epidemiological threats.

The rise of ESBL-EC has been considered a turning point in AMR history. Carbapenems used to be efficient against them, but their overuse has led to the emergence of carbapenem-resistant pathogens, and the same is happening with colistin, narrowing treatment options (WHO 2021). Reflecting this urgency, third-generation cephalosporin-resistant *Enterobacteriaceae* remain at the top of the WHO Priority Pathogens List, last updated in 2024 (WHO 2024). ESBL and carbapenem-resistant *E. coli* are implicated in a wide spectrum of healthcare-associated infections, especially in vulnerable patients, but are also increasingly reported in community-acquired infections (Aljohani et al. 2025).

The molecular epidemiology of ESBLs is becoming increasingly complex, with blurred boundaries (Peirano and Pitout 2019). In South Asia, the heavy use of cephalosporins in livestock may have triggered the expansion of ESBL-producers into the food chain (Husna et al. 2023). Aquaculture, livestock farming, and industrial discharges further contribute to their spread in the environment (Ribeiro et al. 2024). Evidence from southern Malawi shows worrying contamination levels with ESBL-producing *Enterobacteriaceae* in rivers, dams, and households, influenced by urbanization and seasonality, and raising concerns about environmental transmission at the community level (Cocker et al. 2023).

The level of dispersal of this pathogen reflects the complexity of transmission pathways between the human, animal, and environmental sectors, where reservoirs overlap and reinforce each other (Ribeiro et al. 2024). While ESBL-EC has been extensively studied in

clinical and livestock contexts, such efforts have not been applied to understanding its occurrence in wildlife species.

### **3.3. Wildlife and livestock as drivers of AMR**

Given the significant impact AMR causes in various sectors, such as economy and health, it is logical that further research should be focused on studying how each domain influences and takes part in the dissemination of AMR between bacterial populations (Vittecoq et al. 2016). Zoonotic pathogens are responsible for most of the new emerging diseases (Sabour et al. 2022), and human population growth continues to alter natural habitats, creating new contact points between wildlife, humans, and domestic animals. This increases the opportunities for resistance to circulate across species boundaries (Doyle et al. 2025). Identifying the original source of resistance determinants is often difficult, given that resistant bacteria can move across ecosystems and over large distances (Magouras et al. 2017).

Wildlife sits at the intersection of the animal and environmental domains, acting both as a recipient and a spreader of resistance. *E. coli* is commonly detected in wild species, but the true role of wildlife in the circulation of ESBL-EC and other resistant bacteria remains understudied, with many knowledge gaps to fill (Vezeau and Kahn 2024b). Clinically relevant resistant bacteria and genes are frequently detected in wildlife, particularly in urban environments, and are generally interpreted as good indicators of anthropogenic contamination with resistant bacteria. (Laborda et al. 2022). Biology, ecology, and geography can all influence colonization by resistant bacteria in a way not fully understood yet. The biggest certainty, however, is the rising number of diverse AMR bacteria in wildlife (Dolejska and Literak 2019).

Peridomestic species, characterized by being wildlife species that live in close proximity to human settlements and waste sources, are particularly important in this context. They present foraging and scavenging behavior, feeding on waste, leftovers, landfills, wastewater, and agricultural soil, consequently increasing their exposure to contaminated items (Doyle et al. 2025). *Enterobacteriaceae* such as *E. coli* are spread mainly through fecal shedding, meaning both hosts and environments they inhabit represent potential reservoirs with multiple overlapping transmission pathways (Magouras et al. 2017).

The environment itself can serve as a source and reservoir for both novel and existing antibiotic resistance genes (ARGs), but its role in wildlife-mediated dissemination is poorly understood. Wild species can act as sentinels of this contamination, reflecting the impact of human activities and potentially helping to evaluate the success of mitigation strategies (Doyle et al. 2025). Livestock farms are another source of resistance. Antibiotics are used in abundance in animal production, and conventional waste treatment systems are unable to fully

eliminate antimicrobial residues and ARGs, leaving them to be released into soil and water(He et al. 2020). Omnivorous wildlife species that forage in or near farms are particularly likely to be exposed, placing them in the middle of human-domestic animal-wildlife transmission cycles (Vittecoq et al. 2016).

### **3.4. Rodents as sentinels of AMR**

Within peridomestic species, rats have deserved increasing attention over the years. Not only because they are carriers of several zoonotic pathogens like *Yersinia* and *Leptospira*, but also due to their ubiquitous presence across sylvatic, agricultural, and especially urbanized settings, being consistently exposed to human action (Sabour et al. 2022). Their global distribution, along with their opportunistic feeding behavior, makes them a powerful indicator of resistant bacteria in the environment (Schaufler et al. 2018), placing them at the frontline of environmental exposure and bacterial exchange. Animals inhabiting settings heavily impacted by human action tends to show the highest diversity of ARGs, including resistance mechanisms of major clinical concern (Vittecoq et al. 2016). A study in India highlighted their part as sentinels of environmental AMR spillover, where 89% of rodents sampled carried more than one zoonotic bacteria, and over half harbored multiple ARGs, posing a risk to humans, livestock, and pets (Devanathan et al. 2024).

Globally, there are numerous rodent species (Witmer 2022), but members of the *Rattus* genus stand out as some of the most invasive pests in urban and peri-urban settings (Shukla and Wilmers 2024). They damage stored food, crops, and infrastructure (Aplin et al. 2003), and they are likely facilitators of resistance development in their own microbiota by directly ingesting residues present in household, hospital, industrial, and agricultural waste (Uea-Anuwong et al. 2023). *Rattus norvegicus* is thought to have originated in Asia (Modlinska and Pisula 2020) but has spread worldwide and became the classic urban pest, also known as the Norway rat or brown rat. *Rattus tanezumi* is known as the Asian “house rat”, part of the *Rattus rattus* complex, being closely related to *R. rattus*, the European “black rat” (Aplin et al. 2003). Together, these species are ubiquitous carriers of zoonotic pathogens (Himsworth et al. 2014).

*E. coli* is one of the most frequently detected bacteria in peridomestic rats, carried in their intestinal tract and shed in feces. Considering ESBL studies in rats, the most commonly found is *bla*<sub>CTX-M</sub> (61%), followed by *bla*<sub>TEM</sub> (26%) and *bla*<sub>SHV</sub> (13%) (Uea-Anuwong et al. 2023). Many isolates from rat harbor plasmid-mediated resistance genes, enabling horizontal gene transfer within the gut, highlighting their role as facilitators of AMR development and selection (Uea-Anuwong et al. 2023). In this sense, wild rats are likely active participants in AMR spread to other wildlife, food products, and environmental settings (Landolsi et al. 2023). The ability to disseminate *E. coli* strains makes rats a key hotspot for further evolution of pathogenic

bacteria and resistance genes (Himsworth et al. 2015), disseminating these through feces, urine, and direct contact with humans, livestock, and domestic animals (Doyle et al. 2025).

Rodent-borne bacterial infections are, in fact, a huge public health risk, reinforcing the importance of research into their role in pathogen transmission for the establishment of control and hygiene strategies (Sabour et al. 2022). However, wildlife species are mentioned in the National Action Plans of only six European countries and one Latin American country, with only two actually applying specific measures (Benavides et al. 2024).

### **3.5. The Hong Kong context**

In 2015, Cheng et al. alerted that “antimicrobial resistant bacteria are either highly prevalent or increasing rapidly in Hong Kong and China”. According to WHO, the Western Pacific Region is predicted to face severe health and economic consequences due to AMR from 2020 to 2030 (WHO 2023).

Hong Kong’s situation is shaped by its unique context. It is one of the most densely populated and urbanized regions in the world (Lang et al. 2019), where pressure on both environmental and agricultural domains can quickly affect the human population (Cheng et al. 2025). In July 2017, the High Level Steering Committee on Antimicrobial Resistance introduced the Hong Kong Strategy and Action Plan on Antimicrobial Resistance (2017 – 2022), aiming to control the local impact of AMR. A mid-term evaluation in 2020 acknowledged progress but also persistent challenges, especially after the highly consequential COVID-19 pandemic (The Government of the Hong Kong Special Administrative Region 2022). Interviews with people from distinct sectors revealed weak collaboration between sectors, continued misuse and over-prescription of antimicrobials, and insufficient resources (Song et al. 2022).

Food safety and animal production play a preponderant part as well since Hong Kong relies heavily on imported food and is thus exposed to resistant bacteria circulating in international livestock systems with varying antimicrobial stewardship standards. Locally, third-generation cephalosporin-resistant *Enterobacteriaceae* are endemic. Between 2010 and 2023, ESBL-EC was frequently detected in the swine (51.5%) and poultry (86.3%) segments, while ESBL-producing *Enterobacteriaceae* were also found in common food products such as ready-to-eat vegetables, sashimi, and braised dishes (Cheng et al. 2025). An earlier study reported an overall ESBL-EC prevalence of 28.5% across animal populations, with food-producing animals showing higher rates than pets and rodents (Ho et al. 2011).

China has consistently remained the largest consumer of antimicrobials in livestock production, accounting for approximately 45% of global use in 2017 and projected to remain dominant through 2030 (Tiseo et al. 2020; Mulchandani et al. 2023). Food culture in Asia also plays a role, relying mainly on wet markets where unsafe and unhygienic practices prevail. The

proximity of people, food products, animals, and even pests gives way to the dissemination of resistant pathogens. In one study, almost 89% of chicken meat samples from 29 markets were contaminated with ESBL-EC, *bla*<sub>CTX-M-1</sub> (80%) and *bla*<sub>TEM</sub> (63.7%) being the most frequent genes (Hasib et al. 2024).

As for the community in Hong Kong, stool samples collected in 2017 from healthy individuals revealed a 52.8% prevalence of ESBL-producing *Enterobacteriaceae*, with *E. coli* as the predominant species, mostly harboring *bla*<sub>CTX-M</sub> genes, as well as *bla*<sub>TEM</sub>. Carriage was associated with recent antibiotic consumption, and the authors emphasized the multifaceted routes of dissemination involving human, animal and environmental reservoirs (Kwok et al. 2020).

Another critical issue is that Hong Kong particularly struggles with sustainable waste management. Over the past 30 years, the city has seen a 68% increase in waste disposal, with landfills nearing saturation (Cheng et al. 2022). Domestic waste, mainly food waste, accounts for the majority. Limited public awareness of waste handling and recycling remains a big challenge of local solid waste management (Li et al. 2023). This is particularly concerning, given that overloaded and poorly managed waste systems can create reservoirs for resistant organisms, contributing to the AMR problem.

With the goal to renew and improve the adopted measures, a new Hong Kong Strategy and Action Plan on Antimicrobial Resistance (2023 – 2027) was launched, focusing on six key areas, including the optimization of antimicrobial use in humans and animals. ESBL-EC is included as an indicator, with a target to gradually reduce its prevalence in livestock by 2027 (The Government of the Hong Kong Special Administrative Region 2022). However, important gaps remain. Wildlife species, including peridomestic rats, are not currently integrated into AMR surveillance or resistance monitoring frameworks. In Southeast Asia, two common peridomestic rats, *R. norvegicus* and *R. tanezumi*, are known carriers of several zoonotic pathogens (Li et al. 2023), highlighting the need for their inclusion in future surveillance efforts. In fact, the first rat-borne Hepatitis E human case was recently reported in Hong Kong, a reminder of the fundamental role that these species have in the evolution and transmission of zoonotic agents (Sridhar et al. 2021). Detailed strategies for waste management are also absent, even though poor disposal practices represent both environmental and public health risks, attracting synanthropic animals like rodents.

Ultimately, Hong Kong's attempt to adopt a One Health approach to AMR has encountered persistent barriers due to the complexity of the issue, cultural beliefs, and resource limitations (Cheng et al. 2025).

### **3.6. Research gaps**

Antimicrobial resistance might be widely recognized as a One Health challenge, but our understanding of its ecology is still fragmentary. We know relatively little about how resistant bacteria compare across different settings, and we lack clarity on which ecological traits make some species, such as peridomestic rats, more likely to carry and spread resistance.

Wildlife is dangerously overlooked and absent from surveillance programs, even though they may act not only as reservoirs but also as sentinels that mirror the level of environmental and community contamination. What drives resistance carriage in wild species is still poorly understood, leaving an important blind spot in AMR epidemiology, especially when it comes to ESBLs, which continue to rise.

Without more consistent approaches, it becomes difficult to produce data that are truly comparable and useful for risk assessment or to design effective surveillance systems that include wildlife.

## **4. Materials and Methods**

### **4.1. Objectives**

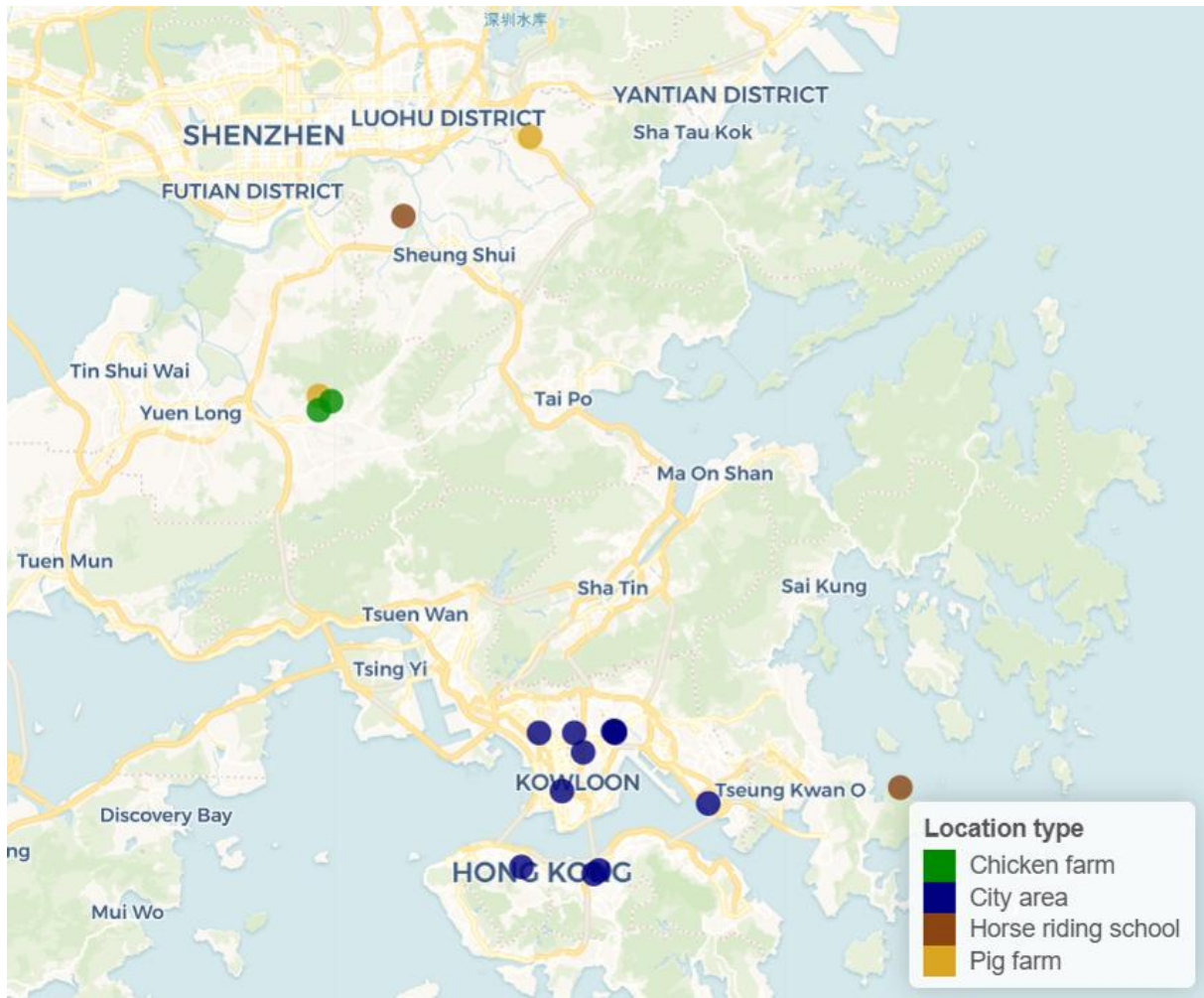
This study was part of a broader project conducted in Hong Kong that investigated antimicrobial resistance and zoonotic diseases in *Rattus* species across various ecological areas. The original study primarily focused on the molecular characterization of Extended-Spectrum Beta-Lactamase-producing *Escherichia coli*. The present study explores the epidemiological aspects of ESBL-EC carriage, aiming to identify risk factors associated with its occurrence in peridomestic rat populations.

We hypothesized that peridomestic rat species would demonstrate potential as sentinels and reservoirs for antimicrobial resistance. In this context, the analysis sought not only to assess ESBL-EC carriage but also to evaluate the role of these species in reflecting environmental contamination and contributing to resistance transmission dynamics.

Fieldwork, including rat capture, sample collection, and microbiological and laboratory procedures, was carried out by the Hong Kong research team. All procedures involving animal capture, handling, and sampling were approved by the Animal Research Ethics Subcommittee of the City University of Hong Kong (Internal Ref: A-0380). The present work focused on the epidemiological and statistical analysis of the dataset, including data cleaning, variable selection, and modelling, which were conducted by the author. The dataset provided for this analysis comprised information on rat characteristics, capture locations, ESBL-EC carriage status, and detected resistance genes.

### **4.2. Sampling**

The study followed a cross-sectional observational design, analyzing data from rats captured across different settings. Rat sampling occurred between October 2020 and August 2021 across 16 locations in Hong Kong Island, Kowloon, and the New Territories. Sampling sites were selected based on ecological diversity and prior knowledge of rat activity. The study involved both urban and suburban environments, including ten city areas, two poultry farms, two swine farms, and two horse-riding schools. The global positioning system (GPS) coordinates of each cage were recorded along with the corresponding unique numerical identifier. Figure 1 shows capture sites, showing approximate cluster locations.



**Figure 1. Geographic distribution of capture sites in Hong Kong.**

Map showing the main sampling locations where peridomestic rats (*R. norvegicus* and *R. tanezumii*) were captured. Each point represents an aggregated capture site.

Following capture, euthanasia and *post-mortem* examinations were performed, during which cecum swabs were aseptically collected for microbiological analysis.

Each rat was pinned laterally on the dissection board without stretching: one pin at the tip of the nose, another beside the anus, and a third pin at the tip of the tail. Several parameters were noted in paper form: sex, developmental stage, body weight (g), body length (cm), tail length (cm), skull size (mm), pes length (cm), ear length (cm), presence of skin wounds and ectoparasites, date and location of capture. Skull length was measured using a Vernier caliper, while body length, tail, pes, and ear length were measured with a ruler.

Both phenotypic and genotypic methods were employed to characterize ESBL-EC isolates, following standardized protocols. Briefly, cecum swabs were enriched in selective broth and cultured on Brilliance ESBL agar. Colonies suggestive of ESBL-EC were selected for species identification using matrix-assisted laser desorption/ionization time-of-flight mass

spectrometry (MALDITOF), and ESBL production was verified by combination disk testing. DNA was then extracted for whole genome sequencing (Illumina MiniSeq Sequencer).

### **4.3. Study population**

A total of 221 rats were included in the original study population. Four different species were identified: *Rattus norvegicus* (n = 144), *Rattus tanezumi* (n = 67), *Rattus andamanensis* (n = 8), and *Nivitenter huang* (n = 2). These rats were captured in city areas (n = 104), poultry farms (n = 44), swine farms (n = 52), and horse-riding schools (n = 21). For the purpose of this analysis, only peridomestic species were considered and, therefore, *R. andamanensis* and *N. huang* were excluded.

Detailed protocols on sample collection, rat species identification, and microbiological processing were described by Uea-Anuwong et al. (2024).

### **4.4. Dataset and data cleaning**

Data was collected in Excel and contained information regarding rat population characteristics, the locations of capture, and phenotypic and genotypic data related to ESBL-EC.

As a first step, variable names were adjusted in Microsoft Office Excel 2019 to conform to RStudio's syntax requirements. Data cleaning, visualization, and statistical analysis were performed using R software (version 4.4.1) via RStudio. Descriptive tables were subsequently formatted in Microsoft Excel 2019 for presentation purposes.

Data cleaning involved transforming the raw dataset to enhance its structure for analysis. Key steps included converting categorical variables into factors with defined levels, addressing missing data, and renaming variables.

The final working dataset comprised a total of 23 variables: 7 quantitative and 16 qualitative. These included morphological traits, demographic descriptors, and information related to the surrounding human population, as shown in Table 1.

**Table 1. Variables included in the final working dataset**

Variable name	Description	Values / Levels
Rat_no.	Sequential capture number assigned to each individual rat	e.g.: 1, 2, 3, 4
Rat_ID	Unique identifier for each rat captured	e.g.: YT0201, NC0101
Region	Main geographical region of the capture site	Hong Kong Island, Kowloon, New Territories
District	District correspondent to capture site	e.g.: Yau Tsim Mong, Yuen Long, Kowloon City
Location	Specific site where the rat was captured	e.g.: around street market, private place (grocery shop), seafood wholesale market
Location_type	Category of location	City area, Chicken farm, Horse riding school, Pig farm
Avg_inc	Level of average population income in the capture area	Low, Medium, High
Pop_dens	Level of human population density in the capture area	Low, Medium, High
Area_type	Category of area	Urban, Suburban
Date	Date of capture	-
body_wt_g	Rat body weight in grams	-
body_cm	Rat body length in centimeters	-
skull_mm	Rat skull width in millimeters	-
ear_cm	Rat ear length in centimeters	-
tail_cm	Rat tail length in centimeters	-
pes_cm	Rat hind foot length in centimeters	-
Sex	Rat biological sex	M, F
Dvplt	Rat developmental stage	Young, Adult
Species	Rat species	<i>Rattus norvegicus</i> , <i>Rattus tanezumi</i>
Skin_wound	Presence of visible cutaneous lesions	Yes/No
Ectoparasite	Presence of ectoparasites	Yes/No
Pregnancy	Pregnancy status of female rats	Yes/No
ESBL_genes	ESBL-EC carriage status	Yes/No

#### 4.5. Statistical analysis

Descriptive statistics were initially employed to explore the dataset and better understand data distribution, as well as detect relevant patterns. Bar plots were used to visualize categorical variables, while box plots and histograms were used to examine continuous variables.

Based on insights from the descriptive analysis, hypotheses were formulated regarding differences in ESBL-EC carriage proportion. Hypotheses tested included differences in ESBL-EC carriage between *R. norvegicus* and *R. tanezumii*, both overall and when stratified by region and location type. Comparisons were also conducted between poultry and swine farms to examine differences in ESBL-EC carriage across farm types. Within-species analysis was carried out to explore whether ESBL-EC carriage varied according to developmental stage, sex, or the presence of skin wounds.

Pearson's chi-square test was used to evaluate associations between categorical variables. When the expected cell counts in contingency tables were below five, Fisher's exact test was applied instead (Nowacki 2017).

In addition, the relationship between body length and ESBL-EC carriage was examined. As the distribution of body length data was non-normal, the Wilcoxon rank-sum test was used.

Finally, a logistic regression analysis was developed using a generalized linear model with a binomial distribution and a logit link function to investigate factors associated with ESBL-EC carriage in rats. Binary logistic regression was used to evaluate the relationship between one binary outcome and multiple independent variables. The regression estimates the probability of an event occurring based on the explanatory predictors (Park 2013). The final model was obtained following a "purposeful selection" strategy, in which variables were chosen based on both statistical significance and biological plausibility.

To assess potential multicollinearity among continuous variables, a correlation matrix was created (Figure 2). This step was essential, as multicollinearity can distort the estimated contribution of each coefficient and reduce model interpretability (Park 2013). All numeric variables represented size-related metrics and, as expected, exhibited strong intercorrelations. For further modeling, body length was selected as a proxy for rats' age (de Santiago et al. 2015), given its statistical and biological relevance. Ear size was also retained for consideration since it showed a weaker correlation with the other size metrics.

Variable	body_wt_g	skull_mm	ear_cm	body_cm	tail_cm	pes_cm
body_wt_g	1.00	0.87	0.63	0.89	0.76	0.74
skull_mm	0.87	1.00	0.61	0.85	0.71	0.74
ear_cm	0.63	0.61	1.00	0.62	0.66	0.48
body_cm	0.89	0.85	0.62	1.00	0.74	0.74
tail_cm	0.76	0.71	0.66	0.74	1.00	0.62
pes_cm	0.74	0.74	0.48	0.74	0.62	1.00

**Figure 2. Correlation matrix between continuous variables.**

Matrix showing Pearson correlation coefficients between continuous variables measured in captured rats. Strong positive correlations are indicated in darker shades.

Model development was based on methods described by Bursac et al. (2008) and Zhang (2016). A univariate logistic regression was performed for each potential predictor. Coefficients showing a p-value below 0.2 were selected for inclusion in the first multivariable model.

Following this screening, a multivariable model was constructed using all selected variables. Due to redundancy between location-related variables, population density and region were removed from the model to avoid collinearity and overlap with location type. This resulted in a reduced preliminary full model.

Variables with p-values above 0.15 and limited contribution to the model were selected for exclusion. This step resulted in a simplified model containing only rat species and location type as predictors for ESBL-EC carriage, the outcome.

To assess potential confounding, the coefficients from this reduced model were compared to those from the preliminary full model. A relative change exceeding 15% indicated the presence of a confounding effect, meaning that excluded variables influenced the estimates of the retained ones (Bursac et al. 2008). Variables identified as potential confounders were reintroduced into the model one at a time (body length, ear size, developmental stage, and presence of skin wounds) to evaluate their impact on the estimates. Confounding variables were retained in the model as described by Zhang (2016).

To account for differences in body length range between species, the variable was mean-centered within species. This species-specific centering involved subtracting the average body length of each species from the individual values, resulting in a new variable

(*body\_cm\_sc*). This adjustment allowed the effect of body length to be interpreted relative to the mean size of each species.

As a final step, potential interaction effects between the independent variables were explored. An interaction indicates that the effect of one predictor on the outcome depends on the level of another predictor (Zhang 2016). Based on biological plausibility, an interaction between rat species and body length was examined.

Throughout the model-building process, comparisons between models were performed using Akaike's Information Criterion (AIC) and likelihood ratio tests via analysis of variance (ANOVA), as recommended by Bevans (2023) and Zhang (2016). The level of significance used was 5%.

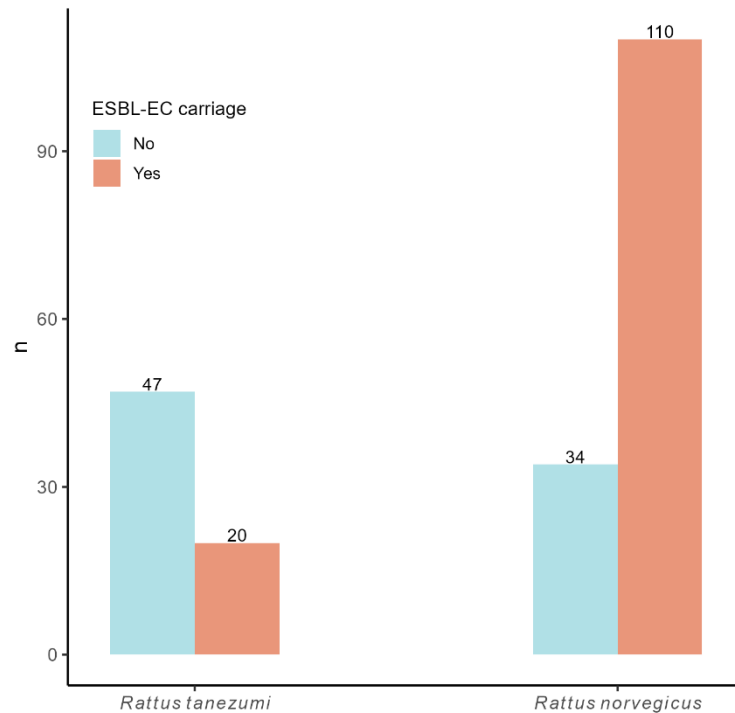
Overall model performance and reliability were evaluated using multiple diagnostic approaches. Model fit was assessed via a likelihood ratio test comparing the final model to the null model, which assumes a constant probability of ESBL-EC carriage across all rats. Predictive accuracy and discrimination (the ability to distinguish between rats that carried ESBL-EC and those that did not) were examined using a classification table and a Receiver Operating Characteristic (ROC) curve, with the Area Under the Curve (AUC) serving as a summary measure of model performance. Higher AUC values indicate stronger discriminative ability (Boateng et al. 2019). Multicollinearity occurs when predictor variables are highly correlated, distorting their estimated effects. Variance Inflation Factor (VIF) values were calculated for each predictor in the model, quantifying the extent to which the variance of a regression coefficient was increased due to correlation among predictors (Shrestha 2020).

The original dataset contained genotypic information. This aspect was not analyzed in detail to avoid overlap with the objectives of the primary project. However, a brief frequency analysis was performed to provide context on the distribution of ESBL genes.

## 5. Results

### 5.1. Carriage by rat species

Phenotypic screening identified 134 ESBL-EC isolates in 130 of 221 cecal samples (58.8%), with 110 samples from *R. norvegicus* (RN) and 20 from *R. tanezumi* (RT) (Figure 3). Four rats (2 RN and 1 RT from city areas, and 1 RN from a chicken farm) carried two distinct ESBL-EC isolates.

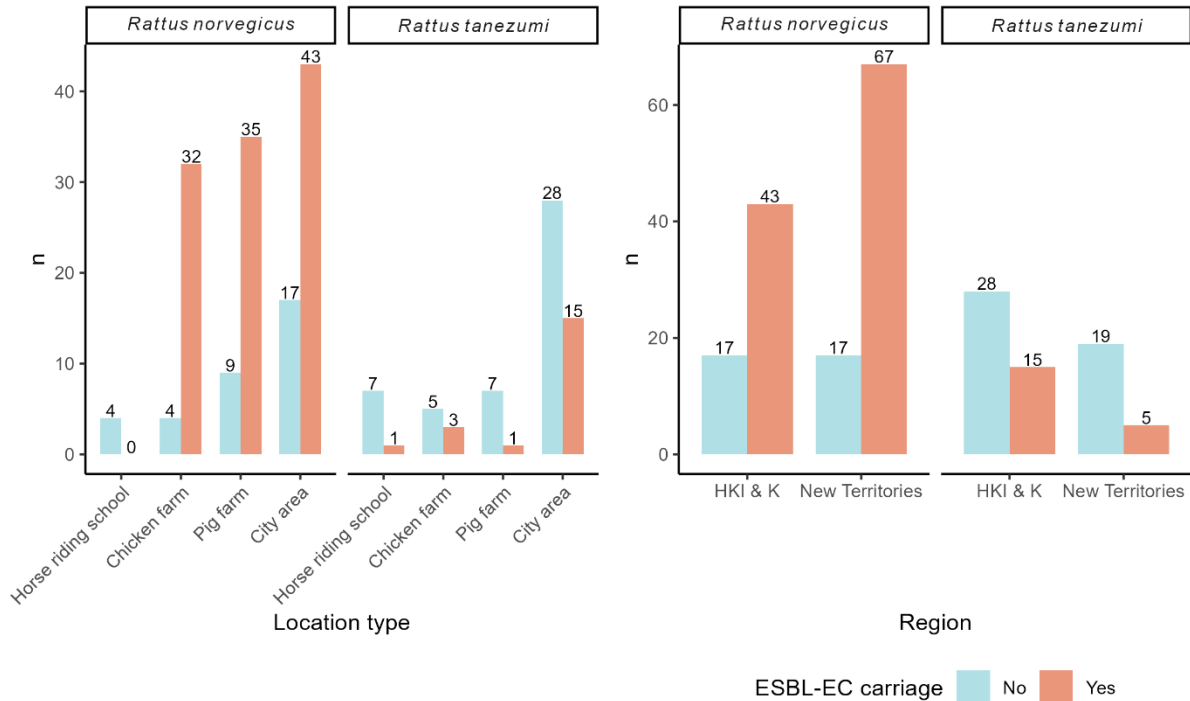


**Figure 3. ESBL-EC carriage proportion by rat species.**

A significantly higher proportion of *R. norvegicus* carried ESBL-EC compared to *R. tanezumi* (76.4% vs. 29.9%, respectively;  $\chi^2 = 39.93$ ,  $p < 0.001$ ). The association between rat species and ESBL-EC carriage was statistically significant.

### 5.2. Carriage by location type and region

Among the positive cases, 58 originated from city areas (RN = 43; RT = 15), 36 from pig farms (RN = 35; RT = 1), 35 from chicken farms (RN = 32; RT = 3), and one from a horse-riding school (RT). When grouped by geographic region, 72 positive samples were from the New Territories (RN = 67; RT = 5), while 58 were from Hong Kong Island and Kowloon (RN = 43; RT = 15) (Figure 4).



**Figure 4. ESBL-EC carriage proportion by location type and region, separated by rat species.**

When stratified by rat species, significant differences in ESBL-EC carriage were observed between *R. norvegicus* and *R. tanezumi* in several ecological settings.

In city areas, 72% of *R. norvegicus* carried ESBL-EC compared to 35% of *R. tanezumi* ( $\chi^2 = 12.32$ ,  $p < 0.001$ ). Similar differences were observed within farm environments where Fisher's exact test identified a significantly higher carriage proportion in *R. norvegicus* on both chicken farms (89% vs. 38%;  $p = 0.005$ ) and pig farms (80% vs. 13%;  $p < 0.001$ ).

However, when comparing ESBL-EC carriage within each rat species across farm types, no significant differences were detected. In *R. norvegicus*, carriage proportions were not significantly different between pig and chicken farms ( $\chi^2 = 0.676$ ,  $p = 0.411$ ). Similarly, no significant difference was found for *R. tanezumi* with a Fisher's exact test ( $p = 0.569$ ).

Comparison between regions revealed similar significant differences between the two rat species. In urban areas (Hong Kong Island and Kowloon), 72% of *R. norvegicus* were positive compared to 40% of *R. tanezumi* ( $\chi^2 = 12.32$ ,  $p < 0.001$ ). In the New Territories, 80% of *R. norvegicus* were positive compared to 21% of *R. tanezumi* individuals ( $\chi^2 = 26.58$ ,  $p < 0.001$ ).

### 5.3. Carriage by developmental stage, sex, and presence of cutaneous lesions

Among adult rats, 68% (85/125) were positive for ESBL-EC, with 77 cases in RN and 8 in RT. As for the young category, 52% (45/86) carried ESBL-EC (RN = 33; RT = 12) (Figure 5). No statistically significant differences were observed between ESBL-EC carriage and developmental stage in either rat species (*R. norvegicus*,  $p = 0.187$ ; *R. tanezumi*,  $p = 0.876$ ).

Regarding sex, 65% (69/106) of female rats were ESBL-EC positive (RN = 57; RT = 12), compared to 58% (61/105) of male rats (RN = 53; RT = 8) (Figure 5). There were no significant differences based on sex (*R. norvegicus*,  $p = 0.412$ ; *R. tanezumi*,  $p = 0.089$ ).

As for the presence of skin wounds, 70% (31/44) of rats with visible wounds carried ESBL-EC (RN = 28; RT = 3), while 59% (99/167) of rats without skin wounds were ESBL-EC positive (Figure 5). The presence of skin wounds also showed no significant relationship with ESBL-EC carriage in *R. norvegicus* ( $p = 0.068$ ) or *R. tanezumi* ( $p = 0.739$ ).

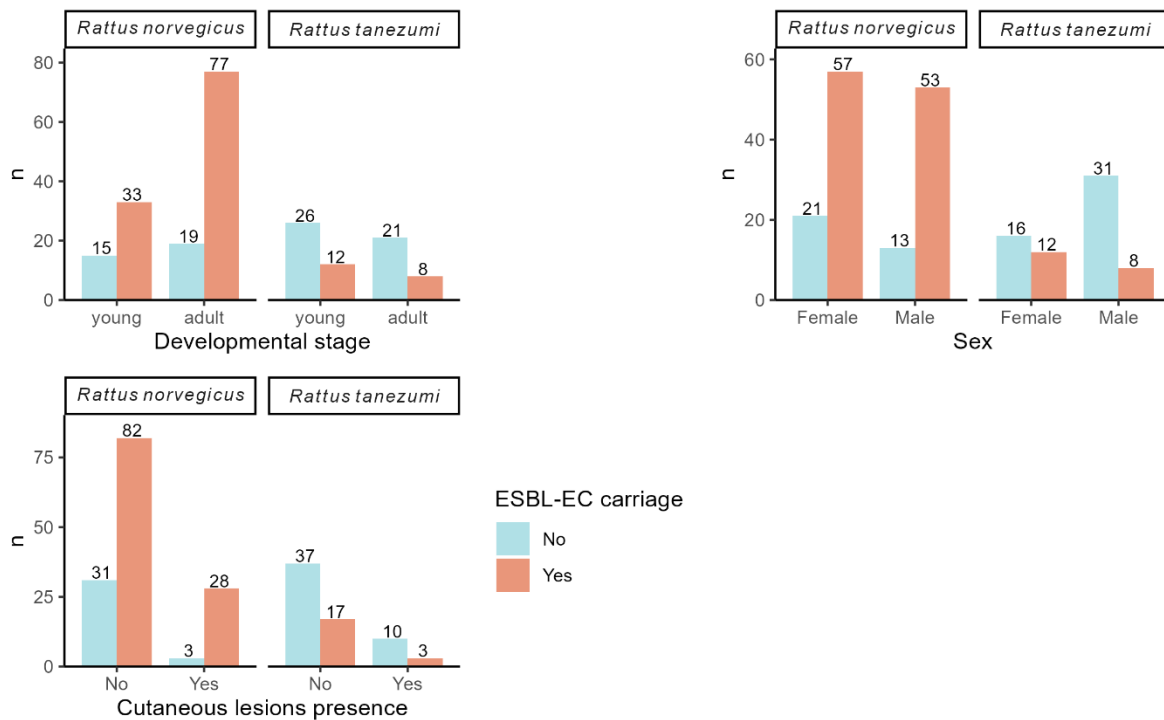
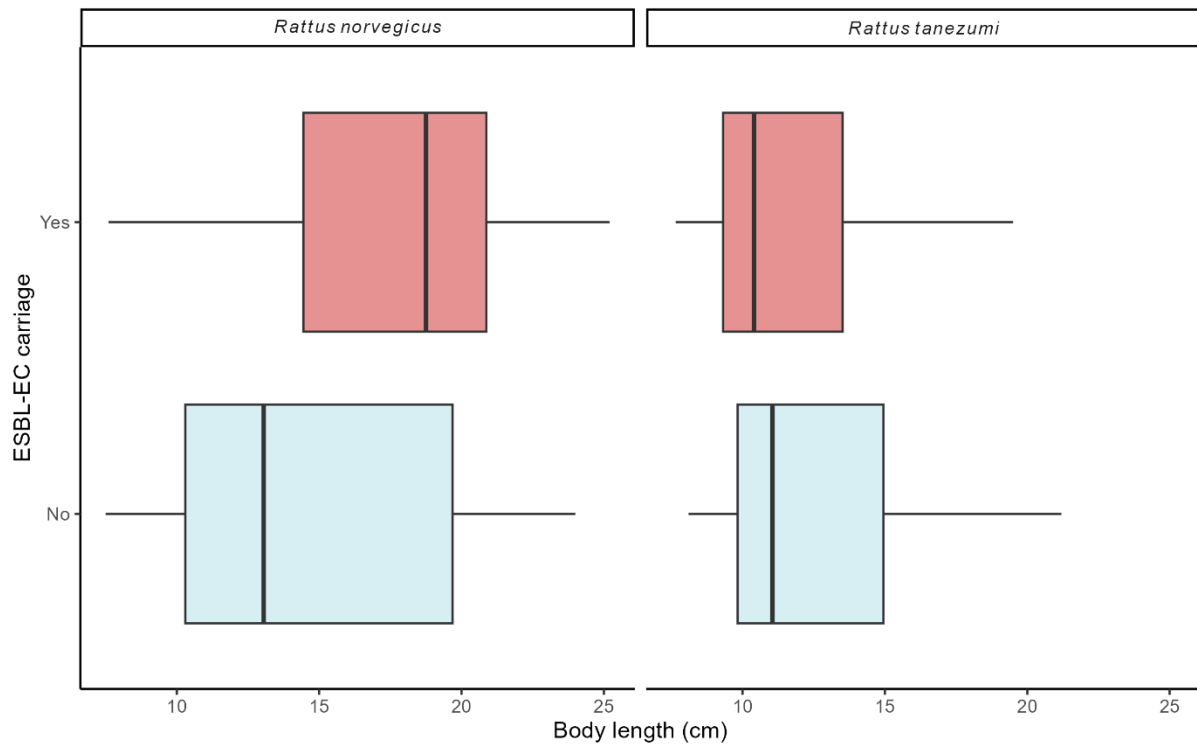


Figure 5. ESBL-EC carriage proportion by developmental stage, sex, and presence of cutaneous lesions, separated by rat species.

### 5.4. Carriage by body length

Body length in *R. norvegicus* ranged from 7.50 cm to 25.20 cm, with a mean value of 17.10 cm and a median of 18.60 cm. ESBL-EC carriers had higher average and median body length values (17.76 cm; 18.75 cm) compared to non-carriers (14.97 cm; 13.05 cm) (Figure 6).

In *R. tanezumi*, body length ranged from 7.65 cm to 21.20 cm, with a mean of 12.13 cm and a median of 10.50 cm. ESBL-EC carriers had lower average and median body length values (11.58 cm; 10.40 cm) than non-carriers (12.36 cm; 10.80 cm) (Figure 6).



**Figure 6. Distribution of body length by ESBL-EC carriage status, separated by rat species.**

Body length distribution was assessed using the Shapiro-Wilk normality test. Data were not normally distributed within each species (RN:  $p < 0.001$ ; RT:  $p < 0.001$ ). Consequently, the Wilcoxon rank-sum test was used to compare body length by ESBL-EC carriage status. Among *R. norvegicus*, a statistically significant difference was found, with carriers showing greater body lengths than non-carriers ( $p = 0.007$ ), although the effect size was small ( $r = 0.225$ ).

For *R. tanezumi*, no significant difference in body length was observed between ESBL-EC positive and negative individuals ( $p = 0.343$ ), and the effect size was also small ( $r = 0.118$ ).

## 5.5. Risk factor model

### 5.5.1. Univariable analysis

In the univariable analysis, several variables showed a significant association with ESBL-EC carriage at the 5% level of significance (Table 2).

**Table 2. Variables that met the inclusion criteria in univariable logistic regression**

Variable	Level	$\beta$	S.E.	p-value
Body weight (g)		0.006	0.001	< 0.001
Body length (cm)		0.156	0.033	< 0.001
Ear length (cm)		1.023	0.562	0.069
Species	<i>R. tanezumi</i>	-2.028	0.331	< 0.001
	Horse riding			
Location type	school	-2.652	1.063	0.013
	Chicken farm	1.104	0.423	0.009
	Pig farm	0.557	0.360	0.122
Dvplt	Adult	0.661	0.289	0.022
Region	HKI & K	-0.439	0.285	0.123
Area type	Urban	-0.439	0.285	0.123
Pop. Dens.	High	-0.856	0.388	0.027
	Medium	-0.198	0.326	0.542*
Skin wound	Yes	0.493	0.366	0.178

Variables that met the inclusion criteria ( $p < 0.2$ ) for the preliminary multivariable model. \*Included due to the statistical significance observed in the other levels.  $\beta$ : regression coefficient; S.E.: standard error.

Despite having a weaker impact and model fit than body length values, body weight showed a significant positive association with each gram increase corresponding to a 0.01 increase in the log-odds of carriage ( $p < 0.001$ ). Each centimeter increase in body length represented a 0.16 increase in the log-odds ( $p < 0.001$ ). Rat species also had a strong effect, with *R. tanezumi* showing a 2.03 decrease in the log-odds of ESBL-EC carriage ( $p < 0.001$ ) compared to *R. norvegicus*. Regarding developmental stage, adult rats showed a 0.66 increase in the log-odds of carriage compared to younger rats ( $p = 0.022$ ).

As for location type, horse riding schools had a 2.65 decrease in the log-odds of ESBL-EC carriage compared to those from city areas ( $p = 0.013$ ), while those from chicken farms had a 1.10 increase ( $p = 0.009$ ). Pig farms did not present a statistically significant increase in carriage odds compared to city areas.

Other tested predictors, including ear size, region, area type, population density, presence of skin wounds, sex, presence of ectoparasites, and pregnancy, were not significantly associated with ESBL-EC carriage at the 5% level. Location and district were not analyzed due to sparse data in each unique category.

### 5.5.2. Multivariable analysis

After the exclusion of variables from the preliminary full model, rat species was found to have a confounding effect on body length. As a result, both variables were retained in the final model.

The interaction term between species-centered body length and rat species was statistically significant. A likelihood ratio test comparing models with and without the interaction term showed a statistically significant improvement in model fit when the interaction was included ( $\chi^2 = 6.27$ ,  $p = 0.012$ ).

The final model was therefore defined to include rat species, species-centered body length, location type, and the interaction term, as shown in Table 3.

**Table 3. Summary of the final logistic regression model predicting ESBL-EC carriage in peridomestic rats**

Predictor	Category	$\beta$	S.E.	p-value	OR	CI 95%
Intercept		1.203	0.294	< 0.001	3.33	1.91 - 6.11
Species	<i>R. tanezumi</i>	-0.203	0.370	< 0.001	0.13	0.06 - 0.27
Body length (species-centered)		0.133	0.049	0.001	1.14	1.04 - 1.26
Species x Body length		-0.246	0.102	0.012	0.78	0.63 - 0.95
Location type	Horse riding school	-2.148	1.090	0.049	0.12	0.01 - 0.68
	Chicken farm	0.951	0.496	0.055	2.59	1.01 - 7.22
	Pig farm	-0.221	0.443	0.618	0.80	0.33 - 1.91

$\beta$ : regression coefficient; S.E.: standard error; OR: odds ratio; CI: confidence interval.

The intercept represents the baseline case: a *Rattus norvegicus* individual with average body length captured in a city area, with an OR of 3.33 (CI<sub>95%</sub>: 1.91 – 6.11;  $p < 0.001$ ). Compared to *R. norvegicus*, *R. tanezumi* showed lower odds of ESBL-EC carriage (OR = 0.13; CI<sub>95%</sub>: 0.06 – 0.27;  $p < 0.001$ ), holding all other variables constant.

Species-centered body length was positively associated with ESBL-EC carriage in *R. norvegicus*, indicating increased odds with greater body size (OR = 1.14; CI<sub>95%</sub>: 1.04 – 1.26;  $p = 0.006$ ). A statistically significant interaction between rat species and species-centered body length was observed (OR = 0.78; CI<sub>95%</sub>: 0.63 – 0.95;  $p = 0.016$ ).

Regarding location type, rats captured at horse-riding schools had lower odds of ESBL-EC carriage compared to those from city areas (OR = 0.12; CI<sub>95%</sub>: 0.01 – 0.68;  $p = 0.049$ ). Despite the non-significant results, rats from chicken farms showed higher odds of ESBL-EC carriage (OR = 2.59; CI<sub>95%</sub>: 1.01 – 7.22;  $p = 0.055$ ) and pig farms showed slightly lower odds (OR = 0.80; CI<sub>95%</sub>: 0.33 – 1.91;  $p = 0.618$ ).

The likelihood ratio test comparing the final model to the null model confirmed that the final model provided a significantly better fit ( $\chi^2 = 65.01$ ,  $p < 0.001$ ).

A classification table (Table 4) was generated, comparing predicted outcomes (at a 0.5 probability threshold) with observed classifications. The model correctly classified 161 out of 211 rats, corresponding to an overall accuracy of 76.3%. This output showed good sensitivity (83.8%), reflecting its ability to correctly identify ESBL-EC carriers, and reasonable specificity

(64.2%), indicating correct identification of non-carriers. These results suggest that the model has effective predictive performance.

**Table 4. Classification table of predicted versus observed ESBL-EC carriage status**

Predicted	Observed		Total
	0	1	
0	52	21	73
1	29	109	138
Total	81	130	211

The resulting AUC value from the ROC curve was 0.80, indicating good discriminative ability. This suggested that the model correctly differentiated between carriers and non-carriers in approximately 80% of cases. The AUC values range from 0.5 (no discrimination) to 1.0 (perfect discrimination), with values above 0.8 generally considered strong (Boateng et al. 2019).

VIF values above 5 suggest moderate multicollinearity, and values above 10 indicate severe multicollinearity (Shrestha 2020). All predictors in the final model had VIF values, adjusted for degrees of freedom, well below the thresholds (Table 5). These results confirmed that multicollinearity was not a concern and that the model's estimates were stable and reliable.

**Table 5. Variance Inflation Factor values for predictors in the final model**

Predictor	Adjusted VIF
Species	1.06
Body length (species-centered)	1.21
Species x Body length	1.19
Location type	1.05

## 5.6. Genotypic findings

A brief frequency analysis of genotypic data revealed a diverse distribution of ESBL resistance genes among the isolates (Table 6). The CTX-M variants were predominant, with *bla*<sub>CTX-M-14</sub> being the most frequently detected gene among ESBL-EC isolates (39.6%), followed by *bla*<sub>CTX-M-55</sub> (23.1%) and *bla*<sub>CTX-M-15</sub> (14.2%). Co-occurrence of ESBL genes was frequently observed (Table 7). The original *bla*<sub>TEM-1</sub> variant was also detected in 31.3% of ESBL-EC isolates.

**Table 6. Distribution of ESBL genes among isolates according to rat species and sampling location**

Gene	Total n (%)	RN	RT	City area	Chicken farm	Pig farm	Horse riding school
<i>bla</i> <sub>CTX-M-14</sub>	53 (39.6%)	47	6	17	10	26	0
<i>bla</i> <sub>CTX-M-55</sub>	31 (23.1%)	25	6	21	10	0	0
<i>bla</i> <sub>CTX-M-15</sub>	19 (14.2%)	14	5	8	2	8	1
<i>bla</i> <sub>OXA-10</sub>	19 (14.2%)	17	2	7	11	1	0
<i>bla</i> <sub>CTX-M-65</sub>	17 (12.7%)	16	1	3	12	2	0
<i>bla</i> <sub>CTX-M-27</sub>	7 (5.2%)	4	3	6	1	0	0
<i>bla</i> <sub>CTX-M-3</sub>	3 (2.2%)	3	0	3	0	0	0
<i>bla</i> <sub>CTX-M-130</sub>	2 (1.5%)	2	0	2	0	0	0
<i>bla</i> <sub>CTX-M-24</sub>	1 (0.7%)	1	0	1	0	0	0
<i>bla</i> <sub>CTX-M-267</sub>	1 (0.7%)	1	0	1	0	0	0
<i>bla</i> <sub>CTX-M-64</sub>	1 (0.7%)	1	0	0	1	0	0

RN: *Rattus norvegicus*; RT: *Rattus tanezumi*. Absolute counts (n) and percentages are presented based on the total number of ESBL-EC isolates (n = 134).

**Table 7. ESBL gene combinations identified among isolates carrying more than one ESBL resistance gene**

ESBL Gene Combination	Total n	Rat species	Location type
<i>bla</i> <sub>CTX-M-65</sub> + <i>bla</i> <sub>OXA-10</sub>	13	RN; RT	Chicken farm; City area; Pig farm
<i>bla</i> <sub>CTX-M-55</sub> + <i>bla</i> <sub>OXA-10</sub>	4	RN; RT	City area
<i>bla</i> <sub>CTX-M-14</sub> + <i>bla</i> <sub>CTX-M-55</sub>	1	RN	City area
<i>bla</i> <sub>CTX-M-14</sub> + <i>bla</i> <sub>OXA-10</sub>	1	RN	Chicken farm
<i>bla</i> <sub>CTX-M-27</sub> + <i>bla</i> <sub>OXA-10</sub>	1	RN	City area

RN: *Rattus norvegicus*; RT: *Rattus tanezumi*.

## 6. Discussion

This study investigated the overall distribution and predictors of extended-spectrum beta-lactamase-producing *Escherichia coli* carriage in wild peridomestic rats (*R. norvegicus* and *R. tanezumi*) trapped in urban and agricultural settings in Hong Kong. The overall ESBL-EC carriage proportion was 58.8%, with significant variation between rat species and locations. These results are higher than those reported in previous studies on rodents from Tunis (Landolsi et al. 2023), Gabon (Onanga et al. 2020), Vancouver (Himsworth et al. 2015), and Berlin (Guenther et al. 2013). A previous investigation conducted in Hong Kong found a 7% ESBL-EC prevalence among wild rats (Ho et al. 2015), although differences in sampled rat species, biological material, and methodology limit direct comparison.

*R. norvegicus* showed significantly higher odds of ESBL-EC carriage compared to *R. tanezumi*, and environmental settings further influenced carriage, with city areas and chicken farms associated with increased odds. Body length, used as a proxy for age, was positively associated with carriage in *R. norvegicus* but not in *R. tanezumi*, suggesting species-specific dynamics of antimicrobial resistance exposure. As for genotypic data, CTX-M-14 was the most commonly detected ESBL subtype, followed by TEM-1, CTX-M-55, and CTX-M-15.

A particularly interesting observation was the difference in ESBL-EC carriage between the two most prevalent *Rattus* species included in the analysis. *Rattus norvegicus* exhibited a significantly higher carriage proportion (76.4%) compared to *R. tanezumi* (29.9%). This difference remained statistically significant after adjusting for body length and location type in a multivariable logistic regression model. Rat species was the most influential predictor of ESBL-EC carriage, with *R. tanezumi* showing approximately 87% lower odds of carriage relative to *R. norvegicus*. These findings are consistent with those of Ho et al. (2015), who observed higher ESBL-EC carriage rates in *R. norvegicus* compared to other rat species. The study also found no association between sex or maturity and the odds of carriage, reinforcing that rat species is a key determinant of ESBL-EC carriage within the peridomestic species analysed. Similarly, Himsworth et al. (2015) reported lower odds of *E. coli* carriage in black rats than in brown rats. The disparity in ESBL-EC carriage observed likely reflects fundamental differences in the behavioral ecology and habitat preferences of the two species. Even when species share the same environment, their ecological traits, such as how they use habitat resources and how they move within and between habitats, can influence their exposure to contaminants and resistant bacteria (Vittecoq et al. 2016). *Rattus norvegicus* (brown rat) is closely associated with human dwellings and perceived as an urban pest (Modlinska and Pisula 2020). They can adapt to different environments and food availability, and commonly inhabit sewage systems, garbage sites, poorly maintained infrastructure, and areas with abundant human refuse (Sacchi et al. 2008). Studies have shown that brown rats adjust easily,

showing a less pronounced neophobic response, a typical rodent reaction to novel objects and food (Modlinska and Stryjek 2016; Koizumi et al. 2021). Other previous studies support the interpretation that omnivorous and ferocious feeding habits increase *R. norvegicus*' contact with contaminated materials, including human food waste and wastewater, facilitating AMR acquisition (Guenther et al. 2013; Ho et al. 2015). These characteristics make it a particularly relevant species for understanding the environmental distribution of AMR and for monitoring zoonotic risks at the human-animal-environment interface. In contrast, *R. tanezumi* exhibits more arboreal tendencies. It is widely regarded as an agricultural pest in Asia and the Pacific region (Aplin et al. 2003). While it can adapt to urban areas, as an opportunistic omnivore, its diet more commonly consists of plant parts, seeds, fruit, and insects (Htwe and Singleton 2014; Stuart et al. 2015). *Rattus tanezumi* is also a better climber than *R. norvegicus*, which typically is a terrestrial and scavenger rodent (Aplin et al. 2003; Kosoy et al. 2015). Interestingly, *R. tanezumi* has also demonstrated genetic adaptations to hypoxic environments, allowing it to expand to different ranges (Cao et al. 2024). These distinct biological, ecological, and behavioural characteristics shape the exposure risk of these rat species to resistant and zoonotic pathogens. It suggests that *R. norvegicus* and *R. tanezumi* may differ in their potential contribution to the dissemination of ESBL-EC, reflecting their distinct roles as carriers of AMR.

Accordingly, our findings emphasize the need for incorporating species-level ecological information into AMR surveillance efforts. Wildlife species that interact more closely with anthropogenic waste may contribute significantly to environmental AMR dissemination and also serve as an epidemiological bridge for clinically significant AMR transmission at the human-animal-environmental interface.

Body length was found to have a statistically significant association with ESBL-EC carriage in *R. norvegicus*, with each standard deviation increase corresponding to a 14% rise in the odds. This was consistent with the Wilcoxon rank-sum test, which showed that ESBL-EC carriers were significantly longer than non-carriers ( $p = 0.007$ ). Nevertheless, the effect size was small ( $r = 0.225$ ), indicating that, while the association is statistically significant, its practical biological relevance may be limited. This pattern could possibly reflect cumulative environmental exposure, as larger individuals are likely older and had more contact with contaminated sources. Greater energetic demands and potentially broader foraging ranges could also be an explanation. In contrast, no statistically significant association was observed between body length and ESBL-EC carriage in *R. tanezumi*. The trend appeared to be absent or even reversed, but it was not significant ( $p = 0.343$ ;  $r = 0.118$ ). The interaction between rat species and body length was still statistically significant in the final model, suggesting the association between body length and carriage is not consistent across species, possibly due to ecological variations.

Location type influenced ESBL-EC carriage in rats as well. Himsworth et al. (2015) also observed a significant association between place of capture and resistant *E. coli* carriage by wild rats. City areas showed a high proportion of ESBL-EC in both rat species. These are habitats marked by intense anthropogenic drivers. Urban environments are characterized by high human population density, sewage systems loaded with antibiotic residues, hospital and industrial effluents, and abundant human refuse, therefore representing hotspots for AMR occurrence (Dolejska and Literak 2019).

A recent study in Marrakesh reported alarming contamination levels with ESBL-EC in the municipal wastewater system, including treated effluents. From 72 samples, the authors recovered 364 resistant *Enterobacteriaceae* isolates, of which 134 were confirmed as ESBL-EC, with CTX-M-1 and CTX-M-15 being the most frequently detected subtypes (El Garraoui et al. 2025). Similarly, a study in Thailand demonstrated extensive environmental contamination levels by ESBL-EC across multiple settings, including wastewater systems from public hospitals, fresh markets, and landfill facilities. ESBL-EC was detected in up to 60% of market leachate samples, 80% of cockroaches, and remarkably, in the intestinal contents of all rats captured near these markets (Thamlikitkul et al. 2019). In Norway, where antimicrobial usage is tightly regulated, ESBL-EC was detected in wastewater and recreational water samples (Jørgensen et al. 2017). The most prevalent subtypes observed were CTX-M-15 and CTX-M-14 and detection was associated with high levels of *E. coli* contamination. The study suggests that the probability of detecting ESBL-EC increases with environmental contamination and possibly reflects colonization by commensal and pathogenic strains circulating in local human populations. Higher ESBL-EC carriage odds in rats inhabiting sewer tunnels were observed in Gabon (Onanga et al. 2020) and Berlin (Guenther et al. 2013). Collectively, these findings support the hypothesis that growing environmental contamination and proximity to human waste turn city rats into major reservoirs of resistant pathogens.

Horse-riding schools were associated with 88% lower odds of ESBL-EC carriage in rats compared to city areas. This suggests a reduced antimicrobial selection pressure in these less industrialized and less densely populated settings. The difference could also be influenced by more controlled waste management practices and lower quantities of antimicrobial usage, thereby reducing favorable circumstances for AMR development. However, the small number of rats sampled from these settings can lead to biased interpretations.

Chicken farms were associated with elevated odds of ESBL-EC carriage, with rats being 2.6 times more likely to be carriers, compared to those in city areas. A finding that, although not statistically significant (OR = 2.59; CI<sub>95%</sub>: 1.01 – 7.22; p = 0.055), is biologically plausible and may indicate a trend. Poultry farms are known hotspots for AMR bacteria due to

intensive antibiotic use, high-density animal populations, and inadequate waste management systems. A study in the Netherlands investigated ESBL-EC prevalence in poultry settings across multiple farms. Sampling included animals and various environmental sources (e.g., surface water, rinse water, soil, and feces from other farm animals), and a widespread ESBL-EC contamination was observed (Blaak et al. 2015). More recent evidence from poultry production systems reinforces this concern. A study conducted in Lithuania detected ESBL-EC in up to 57.5% of broiler samples and 25% of environmental samples within the first days of production, highlighting rapid colonization and environmental contamination (Kasparaviciene et al. 2025). In Selangor, a 37.2% prevalence of ESBL-EC was reported on a poultry farm, including isolates from healthy chickens and environmental samples. Animal feed and drinking water were also identified as potential sources of exposure (Aliyu et al. 2024). Rats inhabiting these environments forage and feed on contaminated materials, potentially favoring the spread of ESBL-EC and other resistant pathogens found in poultry farms. Interestingly, rats from pig farms showed slightly lower odds of ESBL-EC carriage compared to city rats (OR = 0.80; CI<sub>95%</sub>: 0.33 – 1.91; p = 0.618). However, a study on freshwater fish and pig food products sold at wet markets in Hong Kong revealed high levels of contamination by ESBL-producing *Enterobacteriaceae*, particularly in pig organs. Among these, 39.5% of the sampled pig organs were positive for ESBL-EC, with the CTX-M and TEM groups being the most detected in a similar pattern to those found in this study. Within the CTX-M group, *bla*<sub>CTX-M-55</sub> and *bla*<sub>CTX-M-14</sub> were among the most prevalent variants. (Sapugahawatte et al. 2020). Another study examined ESBL-EC prevalence in different livestock settings across China, showing 78.6% and 75.4% in the poultry and swine sectors, respectively. Genotypic findings revealed differences dependent on the animal species, with poultry isolates showing higher carriage rates of almost all genes (Liu et al. 2022). These non-significant results regarding rats captured in livestock farms may be partly due to the smaller sample size compared to those from city areas, but are still relevant to indicate potential trends.

A recent study in Hong Kong analyzed the gut microbiome and resistome of wild rats from various ecological settings. It found that rat species and capture location significantly shaped microbiome structure, with *R. norvegicus* and city rats exhibiting higher microbial diversity. Regarding the resistome, the authors identified high-risk antimicrobial resistance genes (ARG) and associated host bacteria, including *E. coli*. Rat species and habitat also influenced the abundance of ARGs located on plasmids or mobile genetic elements, with *R. norvegicus* and rats from livestock farms showing significantly higher levels. These results highlighted not only the panoply of resistant pathogens accessible to rats but also their potential involvement in AMR dissemination, particularly within agricultural environments (Wen et al. 2025). Animal production systems provide the necessary conditions for microorganisms

to develop and amplify resistance genes, which can be spread through contaminated products, environmental leakage, or farmers (Robinson et al. 2016). Rats are important disseminators and require special attention from all stakeholders, including farmers (Dominguez et al. 2023).

This study's findings also revealed consistent differences between rat species across all locations. Regardless of ecological settings, *R. norvegicus* maintained a significantly higher ESBL-EC carriage proportion than *R. tanezumi*. This supports the idea that species-specific behavior and microhabitat use mediate AMR exposure more strongly than environmental settings alone, underscoring the need for wildlife surveillance strategies tailored to species (Vezeau and Kahn 2024a). It also supports the view that wild rats can serve as indicators of local AMR pressure and highlights the importance of environmental context in shaping resistance dynamics (Ramey 2021). Interestingly, four rats in our study carried two distinct ESBL-EC strains, three of which were *R. norvegicus* captured in city areas and on a chicken farm. This reinforces the model's identification of *R. norvegicus* and highly anthropogenic environments as risk factors for ESBL-EC carriage. It may suggest that brown rats inhabiting urban and agricultural settings are repeatedly exposed to multiple contamination sources, reflecting both the intensive pressure and the close contact of this species with human-associated waste and microbial reservoirs.

Although not deeply explored, genotypic data appeared to be consistent with ESBL-EC molecular patterns reported in previous studies from Hong Kong, mainland China, and globally (Peirano and Pitout 2019; Kwok et al. 2020; Sapugahawatte et al. 2020; Hasib et al. 2024). The predominance of CTX-M variants, particularly CTX-M-14, CTX-M-55, and CTX-M-15, mirrors trends seen in human, livestock, and environmental isolates. Similar ESBL gene profiles reinforce the view that wild rats act as effective sentinels and are shared reservoirs for both environmental and community-level contamination.

Several limitations should be considered when interpreting the results of this study. Although the overall sample size was substantial, some categories such as horse-riding schools and *R. tanezumi* on farms were represented by fewer individuals, limiting statistical power for certain comparisons. The cross-sectional nature of the data further restricts causal inference. The associations identified between host species, environmental settings, and ESBL-EC carriage should therefore be interpreted as correlational and not causal. The study was conducted in selected locations across Hong Kong, which may not fully capture the ecological variability of peridomestic rat populations in other regions. Similarly, the analysis focused on *R. norvegicus* and *R. tanezumi*, two of the most common peridomestic species in Hong Kong, meaning results may not apply to other rodent or wildlife species. Regarding body length, it was used as a proxy for age to reflect potential cumulative exposure to environmental

contamination. While it is biologically possible, body length may also be influenced by other factors like nutrition and habitat, which could affect its precision as an age indicator. The variable was mean-centered within species before modelling to improve comparability between species, a common statistical procedure. However, the model assumes that relationships are approximately linear and that observations are independent, thus minor deviations from these assumptions cannot be entirely excluded. As for genotypic data, it was only briefly explored to avoid overlap with the primary project, and further molecular analysis would provide deeper insight into gene diversity and transmission pathways. Finally, although several biologically plausible predictors were included, other unmeasured factors such as diet, seasonality or proximity to specific waste sources, may have influenced ESBL-EC carriage as well, and some residual confounding cannot be excluded.

Nevertheless, this study contributes to a growing body of literature emphasizing the ecological dimensions of AMR and a solid foundation for future research aimed at better understanding causal mechanisms and transmission dynamics. Surveillance and mitigation strategies need to be redirected to reflect the distinct risk levels that different wildlife species pose in acquiring and transmitting specific resistant bacteria. The observed differences between rat species, interaction with body length, and environmental variation in carriage all point to the complexity of host-pathogen-environment interactions. Each rat species has its own biological and ecological specificities that must be considered not only to refine rodent management, but to better understand how they influence pathogen carriage (Kosoy et al. 2015). In this context, *R. norvegicus* emerges as a particularly promising sentinel species for a clinically important pathogen, ESBL-EC. Its high carriage proportion, commensal lifestyle, and behavioral plasticity position it as a bridge host between environmental, agricultural, and human AMR sources. These findings also reinforce the One Health framework, which emphasizes the interconnectedness of the human, animal, and environmental sectors (Soma et al. 2024). Rats, particularly *R. norvegicus*, are uniquely positioned within this interface. As such, they may serve as both sentinels and vectors in the broader landscape of AMR transmission.

Mitigating the risk of AMR dissemination through wildlife requires a multifaceted approach that addresses selection pressures across sectors. It is urgent to recognize and interrupt the multiple transmission routes in order to slow the cycle and prevent the risk that resistant pathogens like ESBL-EC pose to public health. From a healthcare perspective, the spread of ESBL-EC beyond hospitals into environmental and wildlife reservoirs poses major challenges for infection control. These pathogens compromise first-line therapies, increase treatment costs, and elevate morbidity and mortality risks. Their presence in commensal species like rats suggests that resistant strains are becoming deeply entrenched in the urban

microbiome. Intervention strategies should begin at the source. Improved wastewater treatment is essential to effectively remove resistant bacteria and ARG before they reach natural habitats and urban areas (El Garraoui et al. 2025). Farm biosecurity measures, including proper sanitation, controlled access, and regulated antibiotic use, can further limit the spillover of resistance determinants (Soma et al. 2024) and should be prioritized over antimicrobial use for disease control. Targeted research is needed to elucidate the precise role of wildlife species, including wild rats, in the propagation of AMR. As well as to inform the development of standardized, cross-sectoral surveillance systems that monitor resistance at the human-animal-environment interface (Dolejska and Literak 2019). The lack of attention given to wildlife in surveillance systems and standardized measures to use them as sentinels for AMR evolution hinders an effective One Health approach (Doyle et al. 2025). Rodent management protocols must also be part of a comprehensive AMR control strategy, since proper waste disposal and food management practices have been shown to significantly reduce rodent presence in urban areas, thereby limiting opportunities for them to act as AMR vectors (Shukla and Wilmers 2024). However, sustainable long-term control requires innovation in population management strategies and the active involvement of local communities. Public awareness campaigns and education on the health implications of rodent infestations can foster community-led interventions and improve sanitation behaviors (Witmer 2022). Community sensibilization must also be focused on improving antimicrobial stewardship.

Ultimately, addressing AMR like ESBL-EC in wild rats is not only a matter of ecological insight, but also of coordinated, cross-disciplinary action. Nevertheless, considering the ecological characteristics of each species allows for the identification of suitable wildlife sentinels that can be monitored practically and cost-effectively at crucial One Health points along the AMR transmission pathway (Doyle et al. 2025).

## 7. Conclusion

This study takes a closer look at which factors, ecological and epidemiological, seem to shape carriage of extended-spectrum beta-lactamase-producing *E. coli* in peridomestic rats from Hong Kong. The initial hypothesis that these species might actually play a bigger role than we tend to assume as sentinels or even as reservoirs for antimicrobial resistant bacteria and determinants was supported by the obtained results. In particular, *R. norvegicus* showed high carriage across urban and agricultural settings, suggesting that these rodents may reflect environmental and community contamination levels.

Together, these findings contribute to a broader understanding of how host ecology shapes wildlife involvement in AMR dissemination and emphasize the importance of incorporating targeted measures in surveillance strategies. Integrating wildlife species ecology into monitoring protocols, such as National Action Plans, could sharpen the One Health approach and maybe even allow for earlier detection of emerging resistance trends.

Antimicrobial resistance continues to pose a major public health threat, amplified by the current wave of environmental degradation. Addressing it requires coordinated and multidisciplinary action as the opportunity to contain AMR narrows. Studies like this one highlight the urgency of understanding how resistance continues to develop and move across interconnected systems, an essential, comprehensive step toward designing effective and sustainable control strategies.

Ultimately, this study reflects the challenging yet fundamental nature of the One Health approach. It can only be truly optimized through coordinated efforts across sectors, supported by adequate resources and aligned objectives, to foster an integrated understanding that enables effective prevention rather than reactive responses.

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