IPS Journal of Applied Microbiology and Biotechnology
ISSN: 3115-5685
IPS J Appl Microbiol Biotech, 6(1): 311-316 (2026)
DOI: https://doi.org/10.54117/ijamb.v6i1.115

Potential Spread of Antimicrobial Resistant Gram-negative Bacteria by Houseflies (*Musca domestica*) in Urban Areas, Dar es Salaam, Tanzania

Farahani H. Nikuli and Kennedy D. Mwambete*

Department of Pharmaceutical Microbiology, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania

*Corresponding author email: kennedy.mwambete@muhas.ac.tz; Tel.: +255(0)757800840

Abstract (150-300 words)

Background: Owing to their abundance and proximity to human surroundings, houseflies (Musca domestica) are a major annoyance and serve as carriers of infectious disease-causing microorganisms to both humans and animals. They can spread many infections through ingestion of contaminated food, drinks, water, and other items as they dwell and feed on waste and decomposing matter. Our study aimed to determine the rate of antibiotic-resistant bacteria colonizing houseflies against commonly used antibiotics.

Materials and methods: Houseflies were collected from the randomly selected fast-food and beverage vending sites using sterilized trapping nets and deposited in capped containers and aseptically transported to the Pharmaceutical Laboratory within 2 hours of collection for analysis. Then each housefly was transferred into a universal bottle containing sterile normal saline and thoroughly shaken for a few seconds. An aliquot of the solution was sub-cultured on MacConkey agar and incubated at 28°C for 24 hours. The resultant discrete colonies were isolated and identified using the conventional methods, then subjected to antibiotic sensitivity tests against 6 selected antibiotics. The multiple antibiotic resistance index (MARI) was determined to ascertain the antibiotic resistance level.

Results: The study indicates that houseflies carried a significant number of antibiotic-resistant bacteria that pose threats to public health. *Escherichia coli*, *P. aeruginosa*, *P. vulgaris* and *K. oxytoca* are four Gram-negative bacterial species that colonized the collected houseflies. Over half (54%) of the bacteria were resistant to the antibiotics. The majority (93%) of the bacteria exhibited MARI above 0.2, suggesting that they were previously exposed to antibiotics.

Conclusion: The antibiotic resistance among the isolated bacteria was notably high. Most of the bacteria exhibited a MARI higher than 0.2, indicating prior exposure to the antibiotics. Isolation of the enteric bacteria points to unsanitary surroundings or inadequate hygiene among vendors.

Keywords: *Antibiotic resistance, bacteria colonizing houseflies, Pseudomonas aeruginosa, Escherichia coli, Klebsiella oxytoca, Proteus vulgaris.*

Article History
Received: 18 Jan 2026
Accepted: 06 Feb 2026
Published: 10 Feb 2026
Scan QR code to view◆

How to cite this paper: Nikuli, F. H., & Mwambete, K. D. (2026). Potential Spread of Antimicrobial Resistant Gram-negative Bacteria by Houseflies (Musca domestica) in Urban Areas, Dar es Salaam, Tanzania. *IPS Journal of Applied Microbiology and Biotechnology*, *6*(1), 311–316. https://doi.org/10.54117/ijamb.v6i1.115

Introduction

Insects in the order *Diptera*, such as flies, have one pair of wings for flight and a residual second pair of wings, known as knobs, which are used for balance. They frequently feed on decaying stuff, such as garbage and animal and human feces, where bacteria readily become attached to the sticky leg pads, hairs, electrostatically charged exoskeletons, and sponging mouthparts of flies (Sarwar, 2020). Thus, as the bacteria proliferate in the mouth, regurgitation sites, and digestive system, houseflies can "bio-enhance" their spread (Amendt *et al*., 2021). Adult flies can travel up to 20 kilometers in one lifetime, facilitating transmission of different microorganisms from dump and unsanitary environments to other places, such as healthcare facilities, nursery units, drainage systems, households or food/beverage vending sites (Nayduch *et al*., 2023; Gwenzi *et al*., 2021).

Houseflies are more likely to transmit foodborne diseases, particularly those contaminated by antibiotic-resistant bacteria, due to their habitats and life cycle (Gwenzi *et al*., 2021; Onwugamba *et al*., 2018). This method of transmission is influenced by a number of factors, including the environment, fly density, food chain, health services, and the closeness of animal farms and humans (Ajibola, 2022). Foodborne illnesses pose a serious risk to our communities and have both real and potential financial repercussions. These foodborne illnesses have a significant negative impact on public health by causing gastrointestinal and other clinical symptoms in consumers (DuPoint, 2007; Awad *et al*., 2024; White *et al*., 2004). The main way that antimicrobial-resistant foodborne bacteria were

---

contracted is by consuming contaminated animal-derived food or water (Rossi *et al*., 2023; Gourama, 2020; White *et al*., 2004).

It is also widely acknowledged that the use of antimicrobials, whether for growth promotion, prophylaxis, or treatment, can select for resistant bacterial pathogens and that these pathogens can spread through the food chain, despite the fact that opinions on the health burden imposed by resistance in foodborne bacterial pathogens vary greatly (Veloo *et al*., 2022; Sinnott-Stutzman & Sykes, 2021; Gourama, 2004). Previous research has shown that houseflies collected from hospitals, slaughterhouses, and landfills may spread antibiotic-resistant illnesses (Shahanaz *et al*., 2025; Nayduch *et al*., 2023; Amendt *et al*., 2021). Furthermore, research indicates that due to their shared environments, bacteria from humans and animals appear to share genotypes with those carried by houseflies (Liu *et al*., 2022; Akter *et al*., 2020).

However, little is known about the potential for houseflies to spread antibiotic-resistant microorganisms such as bacteria that could potentially infect people in cities like Dar es Salaam. Therefore, we intended to determine the antibiotic susceptibility profiles of the Gram-negative bacteria that colonize houseflies in different Dar es Salaam City localities because of their inherent antibiotic resistance of Gram-negative bacteria and the health risks they provide to urban residents in several middle- and low-income countries, including Tanzania (Joachim *et al*., 2023; Zhou *et al*., 2023; Maleki & Gankhaki, 2022; White *et al*., 2004).

Materials and Methods

Study Area and Design

This was a cross-sectional and laboratory-based study conducted over a three-month period between February and April 2023. It involved the collection of houseflies from different localities of four administrative areas of Dar es Salaam City, namely Ilala, Kinondoni, Temeke, and Kigamboni. The city is Tanzania's most populous commercial hub, boasting over 9 million inhabitants according to the 2024 census, and covers an area of 1,590 square kilometers.

Sample Collection, Handling and Processing

Representative samples from randomly chosen fast-food or beverage vending locations were selected using the random stratified sampling technique. Fifteen (15) samples of houseflies were collected from different areas in each of the four districts. From 1 to 60, the houseflies were assigned numbers. A pre-sterilized net was used to trap houseflies, which were then kept inside a trapping net and individually placed into a sterile container. The samples of houseflies were collected and aseptically transported to the Pharmaceutical Microbiology Laboratory for analysis within two hours. In accordance with previous descriptions (Mendonça *et al*., 2024), each housefly was identified before being placed in a tube with 4mL of sterile normal saline. After 30 seconds of vigorous vortexing each tube, 0.5 mL of the solution was sub-cultured onto MacConkey agar and incubated for 24 to 48 hours at 28°C. The resultant colonies were isolated and identified using both colony morphological characteristics and biochemical tests (Procop *et al*., 2020).

Antibiotic Susceptibility Profiling

The identified bacteria were subjected to the antibiotic susceptibility tests (AST) against six commonly used and clinically relevant antibiotics, namely sulfamethoxazole and trimethoprim (SXT25), amoxicillin (AML30), ampicillin and cloxacillin (AMC30), erythromycin (E15), Augmentin (ACL30) and ciprofloxacin (CIP5) (Remedica, Limassol, Cyprus). The AST was performed using the Kirby-Bauer disc diffusion method on Mueller Hinton agar (Carl Roth, Germany). The diameters of inhibition zone (IZ) for each bacterial isolate were determined and categorized as resistant, intermediate or sensitive as per the Clinical Laboratory and Standards Institute (CLSI) guidelines. For statistical purposes and to ensure consistency of results, each of the test procedures was performed in duplicate. Consequently, all numerical values for the results are expressed as means.

Determination of MARI of the Isolated Bacteria

MARI is calculated as the ratio of the number of antibiotics to which an organism is resistant to the total number of antibiotics to which the organism is exposed. This metric provides valuable insight into the resistance patterns of bacteria. Bacteria having MARI ≥ 0.2 implies that they originate from a high-risk source of contamination where several antibiotics are irrationally used (Mir *et al*., 2022).

Statistical Analysis

IBM SPSS statistics software version 26 (Chicago, USA) was used for data analysis. According to each specific objective, tables and figures were used to summarize both independent variables (bacterial species, identified susceptibility, and antibiotics) and the dependent variables (IZ and MARI). ANOVA and regression analysis were used to examine the association and correlation among the variables under investigation; a difference was considered significant at $p < 0.05$.

Results

Study Areas and Sampling

Of the 60 houseflies collected, 55 yielded bacterial growth, of which 50 (91.7%) carried Gram-negative bacteria (**Table 1**). (corrected). There were comparatively more bacteria-loaded houseflies in the surveyed localities of Ilala district than in other districts. The most frequently isolated bacteria were *P. aeruginosa* and *E. coli* by 36.5% (n =20) each, followed by *P. vulgaris* (21.7%) as indicated in **Table 1**.

Table 1: Frequency of isolation of housefly-loaded bacteria per localities.

	Isolated Bacteria				
Locality	*P. aeruginosa*	*P. vulgaris*	*E. coli*	*K. oxytoca*	*Total*
Temeke	4(7.3)	1(1.8)	6(11.0)	1(1.8)	12(21.8)
Kigamboni	4(7.3)	5(9.1)	1(1.8)	2(3.6)	12(21.8)
Ilala	8(14.5)	1(1.8)	7(12.7)	0(0.0)	16(29.1)
Kinondoni	4(7.3)	5(9.0)	6(11.0)	0(0.0)	15(27.3)
Total	20(36.5)	12(21.7)	20(36.5)	3(5.4)	55 (100.0)

Chi-square test revealed an association between sampling locations and the frequency of bacterial isolation from houseflies (X^2 = 165.513; p<0.001; 2-sided). Multiple bacteria were present in ten (18.2%) houseflies in variable numbers/proportions as indicated in **Table 2**. Isolates of *E. coli* were obtained from all surveyed areas/sites. While *K. oxytoca* isolates were only found in Temeke district and Kigamboni (**Table 1**).

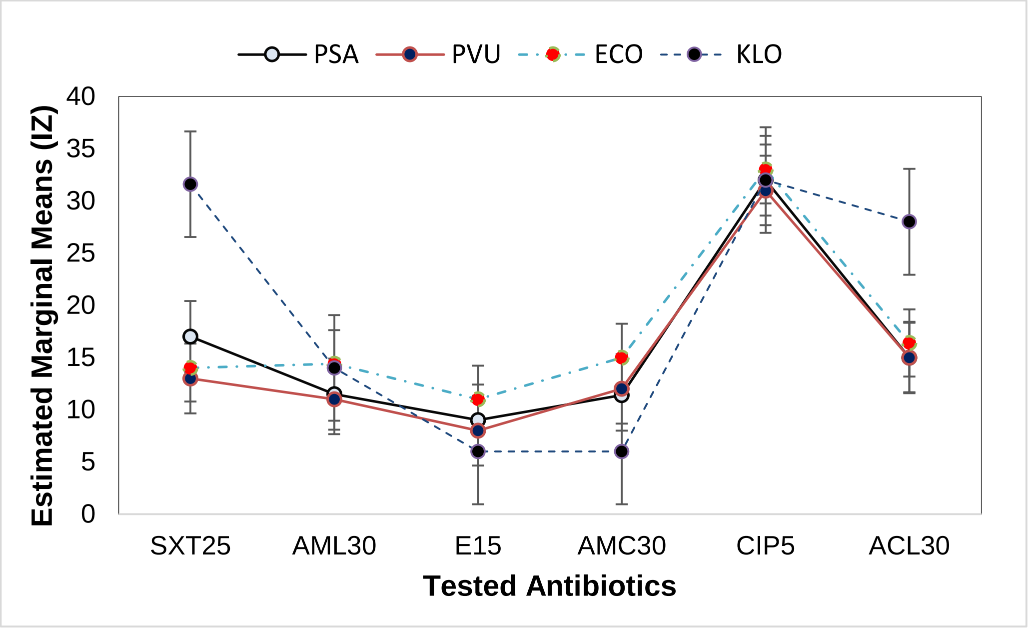
Table 2: Ten houseflies carrying multiple antibiotic-resistant bacteria

Sample	Bacteria isolated (antibiotic to which resisted)
1	*K. oxytoca* (E15); *E. coli* (All)
2	*P. vulgaris* (All); *P. aeruginosa* (AML30; E15, AMC30)
9	*E. coli* (SXT25, AML30, AMC30, ACL30); *P. aeruginosa* (AML30, E15, AMC30)
25	*P. aeruginosa* (SXT25), *E. coli* (E15, SXT25)
32	*P. aeruginosa* (E15, AML30, AMC30, ACL30); *P. aeruginosa* (AML30, AMC30)
33	*P. aeruginosa* (E15, ACL30); *E. coli* (E15, AML30, AMC30, ACL30);
35	*E. coli* (E15); *E. coli* (SXT25, E15; AML30)
39	*E. coli* (SXT25, E15; AML30, ACL30); *P. vulgaris* (E15, AMC30)
40	*E. coli* (SXT25, E15*); P. aeruginosa*(E15)
46	*P. vulgaris* (SXT25, AML30, E15); *P. aeruginosa* (AML30, E15; ACL30)

Keys: SXT25- sulfamethoxazole and trimethoprim; AML30- amoxicillin; AMC30- ampicillin and cloxacillin; E15-erythromycin; ACL30-Augmentin; and CIP5-ciprofloxacin.

Antibiotic Sensitivity Test Results

Thirty (54.5%) of the houseflies carried bacteria resistant to antibiotics, and of those, 10(33.3%) of them carried several antibiotic-resistant bacteria. Multiple bacterial carriage in houseflies was associated with antibiotic resistance (X^2= 87.05; df = 54; p< 0.001). The susceptibility of the isolated bacteria to the six widely used antibiotics varied noticeably. Statistical analysis using the population (estimated marginal) means for unequal sample sizes revealed clear differences in the susceptibility of the bacteria against the tested antibiotics, as indicated in **Figure 1**.



Keys: *PSE-P. aeruginosa; PRV-P. vulgaris; ECO-E. coli; KLO-K. oxytoca*

Figure 1: Sample size- based analysis of the antibiotic susceptibility profiles of the isolated bacteria

The AST profiles of the isolated bacterial species against the six chosen antibiotics are classified as sensitive, intermediate, and resistant. These profiles. A total of 30(54.5%) of the tested bacteria were resistant, while only about 30% (17) were susceptible to at least one of the antibiotics (**Table 3**). Nevertheless, CIP5 is the only antibiotic to which none of the isolates were resistant (**Table 4**).

Table 1: Generalized antibiotic sensitivity results of the isolated bacteria

AST remarks	Isolated Bacteria				Total
	P. aeruginosa	***P. vulgaris***	***E. coli***	***K. oxytoca***	
Sensitive	7(12.7)	3(5.5)	6(10.9)	1 (1.8)	17(31.0)
Intermediate	3(5.5)	2(3.6)	3(3.6)	0(0.0)	8(14.5)
Resistant	10(18.2)	7(12.7)	11(21.9)	2(3.6)	30(54.5)
Total	20(36.4)	12(21.8)	20(36.4)	3(5.5)	55(100.0)

Over 75% of the isolated bacteria exhibited resistance to AML30, E15, and AMC30. The isolates of *E. coli* showed different antibiotic resistance rates (50–70%) as shown in **Table 4**. Of the isolates of *P. vulgaris*, only one (8.3%) exhibited intermediate resistance to CIP5 (data not included).

Table 2: The rate of antibiotic resistance exerted by the bacteria colonizing the houseflies

Isolated Bacteria	Tested Antibiotics				
	SXT25	AML30	E15	AMC30	ACL30
P. aeruginosa (n =20)	10(50.0)	15(75.0)	18(90.0)	11(66.0)	11(66.0)
P. vulgaris (n =12)	7(58.3)	8(66.7)	11(91.7)	9(75.0)	5(41.7)
E. coli (n = 20)	11(66.0)	12(60.0)	14(70.0)	10(50.0)	10(50.0)
K. oxytoca (n =3)	0(0.0)	0(0.0)	1(33.0)	1(33.0)	0(0.0)
Resistance rate per antibiotic	28(68.3)	35(85.4)	34(82.9)	31(75.6)	26(63.4)

MARI of the bacteria carried by the houseflies

As can be shown in Table 5, 47 (85.4%) of the tested bacteria had MARI over the cutoff (≥0.2), indicating that they originated from a high-risk source of contamination by antibiotic-resistant bacteria.

Table 3: MARI of the isolated bacteria colonizing houseflies

	Multiple antibiotic resistance indices				
Bacteria	0.17	0.33	0.50	0.67	0.83
P. aeruginosa	3(5.5)	3(5.5)	7(12.7)	4(7.2)	3(5.5)
P. vulgaris	1(1.8)	2(3.6)	1(1.8)	5(9.0)	3(5.5)
E. coli	3(5.5)	3(5.5)	4(7.2)	7(12.7)	3(5.5)
K. oxytoca	1(1.8)	2(3.6)	0(0.0)	0(0.0)	0(0.0)
Total	8(13.2)	10(17.0)	12(22.6)	16(30.2)	9(17.0)

A negative Pearson's correlation was revealed between ZI and MARI (R = -0.317; p < 0.001; 2-sided). When the types of antibiotics and tested bacteria were controlled for, regression analysis showed that a 0.993 unit decrease in ZI resulted into a 0.382 (p<0.001) increase in MARI.

Discussion

Microorganisms, including bacteria, colonize both living matter such as wounds and insects, and can temporarily colonize non-viable things such as papers, foodstuffs and clothes, provided that their minimum nutritional requirements are met (Cockell, 2021). The persistent association of houseflies with humans, animals, food, refuse, and excreta makes them potential mechanical or biological vectors for the dissemination of pathogenic and multidrug-resistant bacteria (Yin *et al*. 2022; Amendt *et al*., 2021). In most low- and middle-income countries, major cities like Dar es Salaam, their inhabitants reside in congested areas without proper waste disposal infrastructures, which leads to frequent outbreaks of foodborne and other related infectious diseases (Yin *et al*. 2022). Because of living in proximity to humans (synanthropic characteristic) and the tendency to feed on animal and human wastes and decaying matter (coprophagic nature), houseflies play a major role in spreading foodborne infectious diseases (Rossi *et al*., 2023; Yin *et al*. 2022; Amendt *et al*., 2021).

Three bacterial species were isolated from the houseflies, namely *E. coli*, *P. aeruginosa*, and *P. vulgaris*, which exerted resistance rates ranging from 50% to 100% against four of the 6 tested antibiotics, suggesting that they could be excellent vectors for dissemination of the antibiotic-resistant bacteria and thus spreading AMR in the community (Monyama *et al*., 2023; Akter *et al*., 2020). Pseudomonas aeruginosa is regularly found in soil and water, but it is an opportunistic pathogen that causes various infections in humans (Paprocka *et al*., 2022). While *K. oxytoca* is part of the normal gut flora and can also cause several infections, including urinary tract infections (UTIs) (Abass *et al*., 2020). Both *P. aeruginosa* and *K. oxytoca* are also major causes of healthcare-associated infections attributable to significant morbidity in humans, which are difficult to manage with first-line antibiotics (Joachim *et al*., 2023; Yin *et al*., 2022). *Proteus vulgaris* are well-known for their opportunistic pathogenicity and also cause UTIs, respiratory tract infections, and pyogenic lesions (Wu *et al*., 2024); though they reside in the soil, water, and fecal matter (Baruah, 2024). *Escherichia coli* is part of the normal gut flora but can also cause infections in humans such as UTIs and diarrheal diseases, and hemolytic uremic syndrome, which are contracted through eating contaminated food (Baruah, 2024; Nowicki *et al*., 2021). Except for *P. aeruginosa*, the rest of the isolated bacteria are normal flora of the human gut (enteric bacteria) (Wu *et al*., 2022). Thus, their isolation from the houseflies indicates that they originated from fecal or decaying matter (Baruah, 2024). Recovery of these bacteria from houseflies found nearby fast food, beverages and grocery vending areas is of great public concern and echoes unsanitary or unhygienic conditions (Rossi *et al*., 2023; Nowicki *et al*., 2023). Usually, except in severe cases, many food-related infections are asymptomatic, and therefore they are hardly reported or documented (Maleki & Gankhaki, 2022), and they may at the beginning be harmless. However, they may proliferate in food and cause serious foodborne infections (Adley & Ryan, 2025; Abebe *et al*., 2020). Nevertheless, most of the isolated bacteria are opportunistic pathogens. They may be dangerous for immunocompromised individuals if they ingest too large amounts of such bacteria-contaminated foodstuffs (Rossi *et al*., 2023).

Our study observed 8.3% intermediate resistance to CIP5 (ciprofloxacin) in *Proteus* spp., contrary to some previous researchers who reported susceptibility to the antibiotic (Araújo *et al*., 2023). Despite being resistant to several widely used antimicrobial agents, *Klebsiella oxytoca* is becoming a significant cause of hospital-acquired infections in humans (Abebe, 2020; Abbas *et al*., 2020). In this study, it only exhibited resistance against AMC30 and E15O. Our observation differs from a previous study that reported varying degrees of resistance (40–80%) of *K. oxytoca* against CIP, suggesting prior exposure to the antibiotic to be the cause (Araújo *et al*., 2023). The observed differences in resistance may be due to types of antibiotics to which we were previously exposed and/or sources of the bacteria.

The high isolation rate of intestinal bacteria, particularly *E. coli*, in foodstuffs is an indication of fecal contamination (Nowicki *et al*., 2021). This is a consequence of the houseflies coming in contact with feces and other animal waste. Bacteria residing in the human gut when they are constantly exposed to antibiotics may develop resistance (Shahanaz *et al*., 2025; Liu *et al*., 2022). Results indicate that all isolates of *E. coli* and over 70% of isolates of *P. vulgaris* and *P. aeruginosa* were resistant to E15. The antibiotic (E15) has a different mode of action from the beta-lactams (AML30 and AMC30) or sulpha-derivatives (SXT25); it acts by inhibiting protein synthesis, retarding bacterial growth. Therefore, the E15 could be an alternative agent for bacteria resistant to the two types of antibiotics (Zhou *et al*., 2023; Impey *et al*., 2020).

Findings show a significant contribution of houseflies in disseminating the pathogenic and antibiotic-resistant bacteria. Slightly more than half of the isolated bacteria were resistant to tested antibiotics. The isolation of multiple bacteria from a single source/housefly suggests the possibility of exchange of antibiotic resistance traits among them (Liu *et al*., 2022). The majority of the isolates of *P. aeruginosa* and *E. coli* produced MARI above 0.2. Microorganisms exhibiting MARI greater than or equal to 0.2 confirm the presence of multi-drug-resistant traits originating from the environment where there is an abuse of antibiotics (Joachim *et al*.,2023; Maleki & Gankhaki, 2022).

Some of the limitations of our study include the small sample size, not employing enrichment media for detection of fastidious bacterial species found in low amounts. Because of high research cost, cutting-edge techniques could not be employed to investigate the resistance genes. Moreover, the short period of study, and seasonal variability were not considered as it may also affect the presence of bacteria in the environment. Lastly, the bacterial counts were not performed, as was not the scope of the study.

Conclusion

Escherichia coli, *P. aeruginosa*, *P. vulgaris*, and *K. oxytoca* are opportunistic Gram-negative bacteria that were recovered from houseflies. Slightly more than half of the isolated bacteria from the houseflies were resistant to the tested antibiotics. The majority of the bacteria had a MAR index greater than 0.2, suggesting that the bacteria were previously exposed to antibiotics. Additionally, enteric bacteria were identified, demonstrating the unclean conditions in the areas investigated or among the fast food and beverage vendors.

We recommend public health education among the city residents on sanitary conditions in food vending areas and personal hygiene for the vendors. Further studies should be conducted to quantify the bacterial contamination, expand the study areas and increase the sample size to increase the possibility of isolating more bacterial species that colonize the houseflies and thus could be implicated in spreading AMR.

Acknowledgments

The authors wish to thank the Ministry of Education (Tanzania) through MUHAS, and the Ministry of President's Office and Local Government Authorities for their administrative support and Mr. Ezekiel M. Marandu and Ms. Baswaswa Mbozi for their technical assistance.

Conflict of Interests

Authors declare no potential conflicts.

Funding

This research did not receive specific donor funding.

Ethical Approval

The study was approved by the School of Pharmacy Research Committee and the University IRB.

Authors Contributions

Mwambete: designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript.

Nikuli: collected and processed the samples, managed the literature searches, and revised the manuscript. Finally, both authors read and approved the final manuscript.

Availability of Data and Materials

Data can be accessed from the MUHAS repository-database as both soft-and hard-copies.

References

Abbas, A. F., Al-Saadi, A. G. M., & Alkhudhairy, M. K. (2020). Biofilm formation and virulence determinants of Klebsiella oxytoca clinical isolates from patients with colorectal cancer. *Journal of Gastrointestinal Cancer*, 51(3), 855-860.10.1007/s12029-019-00317-7

Abebe, G. M. (2020). Detection of biofilm formation and antibiotic resistance in Klebsiella oxytoca and Klebsiella pneumoniae from animal origin foods. *International Journal of Microbiology and Biotechnology*, 5(3), 120.10.11648/j.ijmb.20200503.17

Adley CC, Ryan MP. (2025). The nature and Extent of Foodborne Diseases. In Antimicrobial Food Packaging. (Eds). Academic Press, UK, pp.3-14.10.1016/B978. 10.1016/B978-0-323-90747-7.00002-8

Ajibola, O. E. (2022). Housefly: Common zoonotic diseases transmitted and control. 6:1-10. 10.22034/jzd.2022.14378

Akter, S., Sabuj, A. A. M., Haque, Z. F., Kafi, M. A., Rahman, M. T., & Saha, S. (2020). Detection of antibiotic-resistant bacteria and their resistance genes from houseflies. *Veterinary world*, 13(2), 266.10.14202/vetworld.2020.266-274

Amendt, J., Bugelli, V., & Bernhardt, V. (2021). Time flies-age grading of adult flies for the estimation of the post-mortem interval. *Diagnostics, 11(2)*, 152.10.3390/diagnostics11020152

Araújo, D., Castro, J., Matos, F., Oliveira, R., Ramos, C., Almeida, C., & Silva, S. (2023). Exploring the prevalence and antibiotic resistance profile of Klebsiella pneumoniae and Klebsiella oxytoca isolated from clinically ill companion animals from

North of Portugal. *Research in Veterinary Science*, *159*, 183-188.10.1016/j.rvsc.2023.04.009

Awad, D. A., Masoud, H. A., & Hamad, A. (2024). Climate changes and food-borne pathogens: the impact on human health and mitigation strategy. Climatic Change, 177(6), 92. https://doi.org/10.1007/s10584-024-03748-9

Baruah, S. M. (2024). The Versatile and Pathogenic Faces of Escherichia coli. *Assam Journal of Internal Medicine*, *14*(1), 1-2.10.4103/ajoim.ajoim_15_24

Cockell, C. S. (2021). Are microorganisms everywhere they can be? *Environmental Microbiology*, *23*(11),6355-6363. 10.1111/1462-2920.15825

DuPont, H. L. (2007). The growing threat of foodborne bacterial enteropathogens of animal origin. Clinical infectious diseases, 45(10), 1353-1361. https://doi.org/10.1086/522662

Gourama, H. (2020). Foodborne pathogens. In Food safety engineering (pp. 25-49). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-030-42660-6_2

Gwenzi, W., Chaukura, N., Muisa-Zikali, N., Teta, C., Musvuugwa, T., Rzymski, P., & Abia, A. L. K. (2021). Insects, rodents, and pets as reservoirs, vectors, and sentinels of antimicrobial resistance. *Antibiotics*, *10*(1), 68.10.3390/antibiotics10010068

Impey, R. E., Hawkins, D. A., Sutton, J. M., & Soares da Costa, T. P. (2020). Overcoming intrinsic and acquired resistance mechanisms associated with the cell wall of gram-negative bacteria. *Antibiotics*, *9*(9), 623. 10.3390/antibiotics9090623

Joachim, A., Manyahi, J., Issa, H., Lwoga, J., Msafiri, F., & Majigo, M. (2023). Predominance of Multidrug-Resistant Gram-Negative Bacteria on Contaminated Surfaces at a Tertiary Hospital in Tanzania: A Call to Strengthening Environmental Infection Prevention and Control Measures: J. Manyahi *et al*. *Current Microbiology*, *80*(5), 148.10.1007/s00284-023-03254-8

Liu, G., Thomsen, L. E., & Olsen, J. E. (2022). Antimicrobial-induced horizontal transfer of antimicrobial resistance genes in bacteria: a mini-review. *Journal of Antimicrobial Chemotherapy*, *77*(3), 556-567. DOI: 10.1093/jac/dkab450

Maleki, S., & Gankhaki, A. (2022). Evaluation of Influencing Factors on Integrated Solid Waste Management in Cities: A Case Study on the Coastal Cities of Bushehr Province. *Urban Structure and Function Studies*, *9*(31), 229-252. 10.22080/USFS.2022.3630

Mendonça, P. M., Cortinhas, L. B., Garção-Neto, C. H., & Queiroz, M. M. D. C. (2024). Brief Review of Morphological Characters in the Identification of Muscomorpha (Diptera) of Sanitary and Forensic Importance. *Diversity*, *16*(10), 599.10.3390/d16100599

Mir, R., Salari, S., Najimi, M., & Rashki, A. (2022). Determination of frequency, multiple antibiotic resistance index and resistotype of Salmonella spp. in chicken meat collected from southeast of Iran. *Veterinary medicine and science*, *8*(1), 229-236.Cockell CS. Are microorganisms everywhere they can be? Environ Microbiol. 2021,23:6355-63. 10.1111/1462-2920.15825.

Monyama, M. C., Taioe, O. M., Nkhebenyane, J. S., van Wyk, D., Ramatla, T., & Thekisoe, O. M. (2023). Bacterial communities associated with houseflies (Musca domestica L.) inhabiting hospices in South Africa. *Microorganisms*, *11*(6), 1440.10.3390/microorganisms11061440

Nayduch, D., Neupane, S., Pickens, V., Purvis, T., & Olds, C. (2023). House flies are underappreciated yet important reservoirs and vectors of microbial threats to animal and human health. *Microorganisms*, *11*(3), 583.10.3390/microorganisms11030583

Nowicki, S., Delaurent, Z. R., de Villiers, E. P., Githinji, G., & Charles, K. J. (2021). The utility of Escherichia coli as a contamination indicator for rural drinking water: Evidence from whole genome sequencing. *PLoS One*, *16*(1), e0245910.10.1371/journal.pone.0245910

Onwugamba, F. C., Fitzgerald, J. R., Rochon, K., Guardabassi, L., Alabi, A., Kühne, S., Grobusch, M.P. & Schaumburg, F. (2018). The role of ‘filth flies’ in the spread of antimicrobial resistance. *Travel medicine and infectious disease*, *22*, 8-17.10.1016/j.tmaid.2018.02.007

Paprocka, P., Durnaś, B., Mańkowska, A., Król, G., Wollny, T., & Bucki, R. (2022). Pseudomonas aeruginosa infections in cancer patients. *Pathogens*, *11*(6), 679.10.3390/pathogens11060679

Procop, G. W., Church, D. L., Hall, G. S., & Janda, W. M. (2020). *Koneman's color atlas and textbook of diagnostic microbiology*. Jones & Bartlett Learning.

Rossi, F., Santonicola, S., Amadoro, C., Marino, L., & Colavita, G. (2023). Recent records on bacterial opportunistic infections via the dietary route. *Microorganisms*, *12*(1), 69.10.3390/microorganisms12010069

Sarwar, M. (2020). Typical flies: Natural history, lifestyle and diversity of Diptera. *Life cycle and development of Diptera*, 1-50.10.5772/intechopen.91391

Shahanaz, E., Zwally, K. M., Powers, C., Lyons, B., Kaufman, P., Athrey, G., & Taylor, T. M. (2025). Flies as vectors of foodborne pathogens through food animal production: Factors affecting pathogen and antimicrobial resistance transmission. *Journal of Food Protection*, 100537.10.1016/j.jfp.2025.100537

Sinnott-Stutzman, V., & Sykes, J. E. (2021). Gram-negative bacterial infections. In Greene's Infectious Diseases of the Dog and Cat (pp. 643-654). WB Saunders. https://doi.org/10.1016/B978-0-323-50934-3.00053-7

Veloo, Y., Thahir, S. S., Rajendiran, S., Hock, L. K., Ahmad, N., Muthu, V., & Shaharudin, R. (2022). Multidrug-resistant gram-negative bacteria and extended-spectrum β-lactamase-producing Klebsiella pneumoniae from the poultry farm environment. *Microbiology Spectrum*, *10*(3), e02694-21. 10.1128/spectrum.02694-21

White, D. G., Zhao, S., Singh, R., & McDermott, P. F. (2004). Antimicrobial resistance among gram-negative foodborne bacterial pathogens associated with foods of animal origin. Foodbourne Pathogens & Disease, 1(3), 137-152. https://doi.org/10.1089/fpd.2004.1.137

Wu, J., Zhou, Q., Qi, H., Lan, W., Yang, S., Yang, S., Fan, Z., & Zhang, A. (2024). Antimicrobial resistance spectrum and virulence characterization of *Escherichia coli, Klebsiella pneumoniae* and *Proteus mirabilis* isolated from asymptomatic and diarrheal rhesus monkeys. *Microbiological Research*, *282*, 127633.10.1016/j.micres.2024.127633

Yin, J. H., Kelly, P. J., & Wang, C. (2022). Flies as vectors and potential sentinels for bacterial pathogens and antimicrobial resistance: A review. *Veterinary Sciences*, *9*(6), 300. 10.3390/vetsci9060300

Zhou, G., Wang, Q., Wang, Y., Wen, X., Peng, H., Peng, R., Shi, Q., Xie, X. & Li, L. (2023). Outer membrane porins contribute to antimicrobial resistance in gram-negative bacteria. *Microorganisms*, *11*(7), 1690.10.3390/microorganisms11071690

•*Thank you for publishing with us.*