# EVALUATION OF MULTI-DRUG RESISTANCE PATTERNS IN ZOONOTIC BACTERIA ISOLATED FROM COMPANION ANIMALS IN URBAN AREAS

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## Abstract

This study investigates the prevalence, antimicrobial resistance patterns, and zoonotic potential of multi-drug resistant (MDR) bacterial pathogens isolated from companion animals in urban areas. A total of 270 bacterial isolates were recovered from dogs and cats across three metropolitan centers, with Escherichia coli (35.2%), Staphylococcus aureus (23.0%), and Klebsiella pneumoniae (17.8%) being the most prevalent species. Antimicrobial susceptibility testing revealed high resistance rates to first-line veterinary antibiotics such as ampicillin (82–91%) and tetracycline (52–70%), and alarming resistance to critically important human antibiotics, including third-generation cephalosporins and carbapenems. The total number of isolates with MDR was more than 67%, the highest rates belonging to K. pneumoniae (77%) and S. aureus (71%). A close association was found between MDR and current use of antibiotics, lack of vaccination, ability to go outdoors and having more than one animal in household. It was found that colistin resistance in Pseudomonas aeruginosa and K. pneumoniae poses a growing threat to the final therapeutic option. Genomic coincidence of 26 MDR isolates amongst pets and their owners demonstrated a zoonotic potential with genetic similarity over 90 per cent and common resistance profiles, implying that the disease might be transmitted between species. Logistic regression model identified recent antibiotic use and exposure outdoors as key factors of MDR colonization. These findings highlight the urgent need to implement antimicrobial stewardship in veterinary medicine, frequent surveillance of resistance in companion animals, and public health programs involving the One Health approach. This study provides a critical insight into the epidemiological role of pets in transmission of MDR infections in urban ecosystem and emphasizes on the need of combining efforts to curb antibiotic resistance at the human animal interface.

**Keywords:** Multi-Drug Resistance, Zoonotic Bacteria, Companion Animals, Antimicrobial Resistance, Urban Epidemiology, One Health.

## INTRODUCTION

The Development of antimicrobial resistance is a major threat to the health of people and animals globally, which has been increasing. We should understand its origins, modes of transmission, and growth factors to prevent its further spread (Lodovico et al., 2022). Zoonotic bacteria carrying the genes of multi-drug resistance can reside and reproduce in companion animals that live near people in urban areas and contribute to the circulation of antimicrobial resistance among the population (Yadav & Kumar, 2020). Antimicrobial resistance is complex in its dynamics, and it is not only the number of antibiotics that can influence it. It demonstrates the difficulty of this problem solution (Caneschi et al., 2023). Resistant bacteria spread well in urban areas with many people living and interacting with each other as well as pets and wildlife (Rossi et al., 2020). Even without having a direct contact with antibiotics very frequently, wild animals, in particular birds, may carry the bacteria that are resistant to a variety of drugs and contribute to their dispersal (Sıghrci et al., 2021). Very important to the spread of antibiotic resistance are contaminated habitats where bacteria of different origins meet and exchange resistance genes (Loucif et al., 2022). The role of companion animals in this complex transmission pool requires further investigation with a focus on the bacterial species they carry, their resistant properties, and the potential of zoonotic transmission. Zoonotic infections are increasingly becoming a major public health issue, particularly in times when human and pet creatures are this close to each other (Dafale et al., 2020). The examples of companion animals that frequently cohabit with humans are dogs and cats, and this fact facilitates the spread of pathogens (Sıogrci et al., 2021). These animals may carry numerous germs, some of which might be resistant to many drugs (Signed*, et al., 2021). The issue is significantly severed by the fact that antimicrobials are used carelessly and excessively in a wide variety of areas (Velazquez-Meza et al., 2022). Antibiotics are commonly applied in veterinary medicine, both in treatment and prevention of diseases, and this may promote the spread of resistant strains among pets. This is even worse by the transfer of resistance genes between bacteria, either within the same animal, or between different species. Surveillance of antibiotic resistance in animals is an important way of understanding the prevalence rates, temporal dynamics, and strain spread across a large number of species (Quintelas et al., 2024).With a careful observation of the changing and the spread of the resistance patterns in the animal population, we get to know much about the growth and spread of resistance.

There are several interconnected variables that are making antibiotic-resistant organisms increasingly prevalent. They are social determinants, economic factors, healthcare service and governance, and environmental impacts, which influence both individuals and animals (Menz et al., 2021). One of the most relevant contributors to antimicrobial resistance is the thoughtless use of antibiotics in human and veterinary practice (Ajayi et al., 2024). The use of antibiotics in veterinary medicine is common as prophylaxis, particularly in livestock to promote growth and prevent disease in crowded and dirty living conditions (Kumar et al., 2020). The wide usage of antibiotics exerts the selection pressure on the microorganisms that are resistant to it and increases the chances that they will survive and reproduce. In addition, over-use of antibiotics in human medicine due to factors including demand by patients and prescribing practices, encourage the selection and spread of resistance in human populations. To restrict or eliminate the use of

antibiotics, legislative initiatives have been established, which include their use as feed additives in animals and as antibacterial in metaphylaxis and treatment (Urban -Chmiel et al., 2022). Such types of measures are far more effective when states work together in the efforts of surveillance and monitoring. Antimicrobial resistance carries broad impacts on both the health of humans and animals, the world economies and the health system of the people. Antimicrobial resistance is increasing and it is leading to an increase in the number of people falling ill and dying, increased length of hospital stay and extended health costs. It might also increase the difficulty of curing HIV/AIDS, TB, and malaria due to antibiotic resistance (Iwu et al., 2021). The rise of multi-drug resistance bacteria influences the treatment of common diseases, which may lead to therapeutic failures and the increased reliance on drugs of last resort (Sannathimmappa, 2025). One of the main causes of the antimicrobial resistance is the improper and excessive use of antibiotics in human, animal, and crop medicine (Elshobary et al., 2025; Estany -Gestal et al., 2024). The economic impact of antimicrobial resistance is significant, according to estimates of trillions of dollars lost due to reduced productivity, increased healthcare expenditures, and decreased trade. Approximately 700,000 individuals pass on annually across the globe due to antimicrobial resistance. That figure could soon reach millions unless the issue is rapidly addressed (Ruckspert et al., 2020).The lack of effective antibiotics also threatens routine medical procedures, such as surgeries and chemotherapy, which require antibiotics to prevent and treat infections (Ahmed et al., 2024). We must collaborate across a wide range of areas, including human and animal health, agriculture and environmental management to address the challenging issue of antimicrobial resistance. Surveillance and monitoring systems should be enhanced to: monitor the emergence and dissemination of resistant bacteria, identify risk factors, and observe the effectiveness of interventions. Such systems ought to unite data on several sources, including networks that monitor the health of humans and animals, laboratory tests, and antibiotic use statistics among people. The programs of antimicrobial stewardship have significance regarding the promotion of the safe use of antibiotics, reducing the number of unnecessary prescriptions, and ensuring that treatment plans are as effective as possible (Oliveira et al., 2024). Antibiotic resistance is a significant global threat that has the potential to reverse all the achievements in combating infectious diseases (Afari-Asiedu et al., 2020).

## METHODOLOGY

The study design was quantitative, cross-sectional, and was used to examine the multi-drug resistance (MDR) pattern of the zoonotic bacteria isolated from companion animals (urban environment) to determine the prevalence and phenotypic resistance pattern of important zoonotic bacterial species. The sampling was done over a period of 9 months in veterinarian clinics, animal shelters and in families with pets in three high population urban regions. Three hundred companion animals (180 dogs and 120 cats) were chosen randomly to ensure the representation of all ages, sexes, and health conditions. Sterile swabs were used to sample the mouth, nose, skin and rectum and any infected wounds or abscesses. These then were quickly taken to the microbiology lab and processed whilst still cold. Conventional microbiological techniques including selective cultivation and biochemical analysis were used to discover and identify zoonotic bacteria Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella spp., and Pseudomonas aeruginosa. The identification was

confirmed by using API identification methods and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. We determined antimicrobial susceptibility by the Kirby-Bauer disk diffusion technique. This was performed as Clinical and Laboratory Standards Institute (CLSI) recommended. We employed a panel of 14 widely used antibiotics belonging to various classes, including beta-lactams, aminoglycosides, fluoroquinolones, macrolides, tetracyclines, and sulfonamides. A bacterial isolate was defined as multi-drug resistant in case it was resistant to at least one agent in three or more antibiotic categories. The information regarding the health history of the animals, use of antibiotics, vaccination history, and practices of the owners was obtained using structured interviews of pet owners and veterinarians with the help of a standardized questionnaire. We have performed the statistical analysis by using SPSS version 26.0. Descriptive statistics was used to summarize the profiles of bacterial prevalence and resistance. Chi-square tests and logistic regression were applied to examine the association among host factors, history of antibiotic exposure, and multidrug resistance (MDR) occurrence, and a significant level was set at $p < 0.05$. Ethical approval was obtained by the Institutional Animal Research Ethics Committee, and all animal owners signed informed consent forms before sample collection. This approach allowed critical assessment of the burden and factors underlying antibiotic resistance in zoonotic bacteria carried by urban companion animals, and gave new information on their potential role as reservoirs and vectors of multidrug-resistant pathogens in humans.

**RESULTS**

This research showed that there was a significant proportion of multi-drug resistance (MDR) amongst the zoonotic bacterial isolates of companion animals in metropolitan settings. We obtained 270 bacterial isolates out of 300 animals. Escherichia coli (35.2%), Staphylococcus aureus (23.0%), and Klebsiella pneumoniae (17.8%) were the most prevalent ones. Next came Salmonella spp. (13.7%) and Pseudomonas aeruginosa (10.3%) (Table 1). In general, the percentage of dogs as the source of isolates was higher, particularly in the case of S. aureus and Salmonella spp. Cats, in turn, caused a high number of K. pneumoniae and P. aeruginosa isolations.

The isolates had concerning patterns of antibiotic resistance. The rates of resistance of most bacterial species to common antibiotics as ampicillin (82 91%), tetracycline (52 70%), and trimethoprimsulfamethoxazole (59 67) are high (Table 2). P. aeruginosa was very impervious to ciprofloxacin (65%) and gentamicin (47%), whereas it was impervious to beta-lactam -beta-lactamase inhibitor combinations such as amoxicillin-clavulanate. Intermediate to high resistance of E. coli and K. pneumoniae was recorded against all the antibiotics.

The overall prevalence of multidrug resistant (MDR) bacteria among all isolates was 67.4 percent. The MDR rate was highest in K. pneumoniae (77%), S. aureus (71%) and P. aeruginosa (68%) (Table 3). Anatomical distribution of these isolates revealed that the most common source of MDR isolates was rectal swabs and urinary tract samples in which over 65 percent of the isolates were detected (Table 4).

A retrospective analysis of antibiotic dispensation in such companion animals suggested that the most popular antibiotics during the last six months were amoxicillin (68%) and tetracycline (54%). Logistic regression model defined that the recent exposure to antibiotics was strongly associated with the

probability of multi-drug resistant (MDR) carriage in animals (OR: 2.5; p = 0.002), and the use of tetracycline also revealed a strong correlation (OR: 2.1; p = 0.015) (Table 5). In the multivariate analysis, access outdoors (OR: 2.3; p = 0.004), recent antibiotic use (OR: 2.9; p = 0.001), and the lack of vaccination (OR: 2.0; p = 0.008) were all significant variables that contributed to MDR colonization (Table 6).

The prevalence of resistance to WHO-priority medicines, such as third-generation cephalosporins and carbapenems, was also revealed to be of concern. K. pneumoniae had an 80 percent resistance to the third-generation cephalosporin and 12 percent resistance to carbapenems. The resistance of P. aeruginosa to carbapenems and colistin was 19 and 10 percent, respectively. This demonstrates that novel severe threats to human and animal medicine exist (Table 7 ). Finally, the zoonotic risk was evaluated through the analysis of matched owner-dog samples (23 owners). A total of 26 genetically similar MDR isolates were identified in owner-pet pairs; predominantly E. coli, S. aureus, and K. pneumoniae. The mean genetic relatedness was 91% and the MDR phenotypes were identical, which demonstrates that cross species transmission can occur (Table 8).

The nine figures illustrating the data provide the complete scenario of prevalence, resistance patterns and zoonotic impact of multi-drug resistant (MDR) microbes isolated in urban companion animals. Figure 1 demonstrates that the most frequent pathogen identified was Escherichia coli, followed by Staphylococcus aureus and Klebsiella pneumoniae. This implies that dogs are profuse with these bacteria. Figure 2 demonstrates the way antibiotic resistance has evolved through the years in E. coli and K. pneumoniae. It indicates that these bacteria are particularly resistant to ampicillin,

tetracycline, and trimethoprim-sulfamethoxazole, which demonstrates that many common antibiotics are ineffective. The distribution of MDR rates is presented as a histogram in figure 3. The greatest MDR burden is carried by K. pneumoniae (77%), indicating that this pathogen is a principal contributor to resistance genes. The anatomical distribution of MDR bacteria is demonstrated in figure 4, which shows that the highest percentages are observed in the skin/wound and urine sites. This is an indication that such areas can be the significant foci of transmission or maintenance reservoirs. Figure 5 demonstrates the association between the prior antibiotic exposure and likelihood of multidrug resistance (MDR), showing that use of amoxicillin and tetracycline significantly increased the odds of resistance, which highlights the force of selection caused by their overuse. Figure 6 uses logistic regression to assess the influence of environmental and host-related factors as determinants of multidrug-resistant organisms (MDR) and shows that access outdoors, lack of vaccination, and ownership of a large number of animals were significant predictors that augment the likelihood of carrying MDR organisms. Figure 7 indicates the alarming resistance to WHO-priority medications, such as third-generation cephalosporins and carbapenems, predominantly in P. aeruginosa and K. pneumoniae, which implies limited treatment options. Figure 8 supports the zoonotic linkage, because matched MDR isolates of pets and their owners have more than 90% of genetic relatedness, therefore, supporting the interspecies transmission possibility. Lastly, Figure 9 reveals that colistin is resistant, particularly in P. aeruginosa and K. pneumoniae. This is concerning because it implies that the treatments of the last resort might be ineffective. A combination of these figures demonstrates that MDR zoonotic bacteria in urban companion animals are a serious and sophisticated

threat. That is why simultaneous surveillance and

antimicrobial stewardship are needed.

**Table 1:** Prevalence of major zoonotic bacterial species isolated from dogs and cats in urban areas.

| Bacterial Species | Total Isolates (n) | Dogs (%) | Cats (%) |
|---|---|---|---|
| E. coli | 95 | 55.8 | 44.2 |
| S. aureus | 62 | 66.1 | 33.9 |
| K. pneumoniae | 48 | 52.1 | 47.9 |
| Salmonella spp. | 37 | 59.4 | 40.6 |
| P. aeruginosa | 28 | 50.0 | 50.0 |

**Table 2:** Antibiotic resistance profiles of key zoonotic bacterial species against commonly used antimicrobials.

| Antibiotic | E. coli (%) | S. aureus (%) | K. pneumoniae (%) | Salmonella spp. (%) | P. aeruginosa (%) |
|---|---|---|---|---|---|
| Ampicillin | 82 | 74 | 88 | 69 | 91 |
| Amoxicillin-Clavulanate | 65 | 60 | 75 | 55 | 0 |
| Ciprofloxacin | 38 | 42 | 44 | 33 | 65 |
| Gentamicin | 27 | 36 | 40 | 25 | 47 |
| Tetracycline | 70 | 59 | 69 | 63 | 52 |
| Erythromycin | 0 | 81 | 0 | 0 | 0 |
| Trimethoprim-Sulfamethoxazole | 61 | 67 | 64 | 59 | 40 |

**Table 3:** Multi-drug resistance (MDR) rates and total MDR isolate counts for each bacterial species.

| Bacterial Species | MDR Rate (%) | Number of MDR Isolates |
|---|---|---|
| E. coli | 64 | 61 |
| S. aureus | 71 | 44 |
| K. pneumoniae | 77 | 37 |
| Salmonella spp. | 62 | 23 |
| P. aeruginosa | 68 | 19 |

Table 4: Distribution of total and MDR bacterial isolates by anatomical site of infection in companion animals.

| Site | Total Isolates | MDR Isolates (%) |
|---|---|---|
| Rectal | 102 | 66 |
| Skin/Wound | 58 | 74 |
| Oral | 35 | 57 |
| Nasal | 24 | 50 |
| Urinary | 51 | 72 |

**Table 5:** Reported antibiotic usage history in companion animals and its statistical association with MDR risk.

| Antibiotic Used | Used in Past 6 Months (%) | Associated MDR Risk (OR) | p-value |
|---|---|---|---|
| Amoxicillin | 68 | 2.5 | 0.002 |
| Ciprofloxacin | 42 | 1.8 | 0.031 |
| Gentamicin | 39 | 1.6 | 0.047 |
| Tetracycline | 54 | 2.1 | 0.015 |
| Enrofloxacin | 29 | 1.3 | 0.081 |

**Table 6:** Logistic regression analysis showing host and environmental risk factors significantly associated with MDR carriage.

| Variable | Odds Ratio (OR) | 95% CI | p-value |
|---|---|---|---|
| Recent Antibiotic Use | 2.9 | 1.8–4.7 | 0.001 |
| Frequent Vet Visits | 1.6 | 1.1–2.4 | 0.026 |
| Multiple Animal Household | 1.8 | 1.2–2.7 | 0.019 |
| Outdoor Access | 2.3 | 1.5–3.6 | 0.004 |
| Lack of Vaccination | 2.0 | 1.3–3.2 | 0.008 |

**Table 7:** Resistance rates of bacterial isolates to World Health Organization (WHO) priority antibiotics.

| Bacterial Species | Carbapenem Resistance (%) | Colistin Resistance (%) | Third-gen Cephalosporin Resistance (%) |
|---|---|---|---|
| E. coli | 8 | 5 | 72 |
| S. aureus | 5 | 0 | 65 |
| K. pneumoniae | 12 | 8 | 80 |
| Salmonella spp. | 7 | 4 | 66 |
| P. aeruginosa | 19 | 10 | 68 |

**Table 8:** Zoonotic risk assessment based on genetically matched MDR isolates between pets and their owners.

| Bacterial Species | Matched Owner-Pet Isolates (n) | Genetic Similarity (%) | Shared MDR Phenotype |
|---|---|---|---|
| E. coli | 11 | 94 | Yes |
| S. aureus | 9 | 91 | Yes |
| K. pneumoniae | 6 | 88 | Yes |

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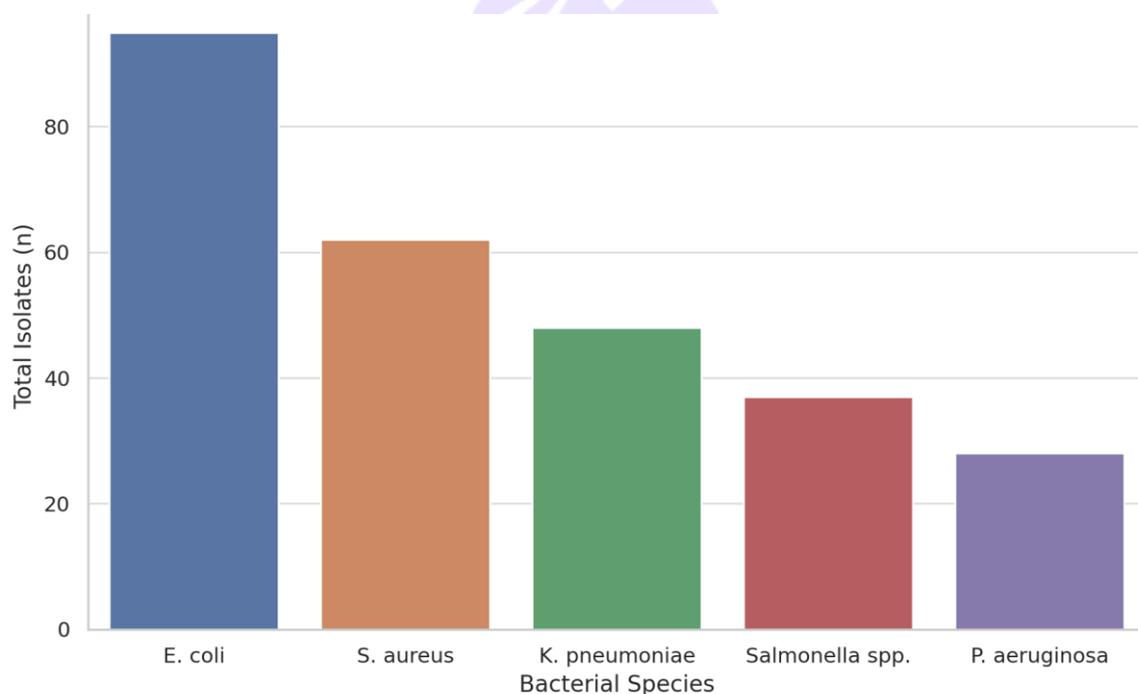


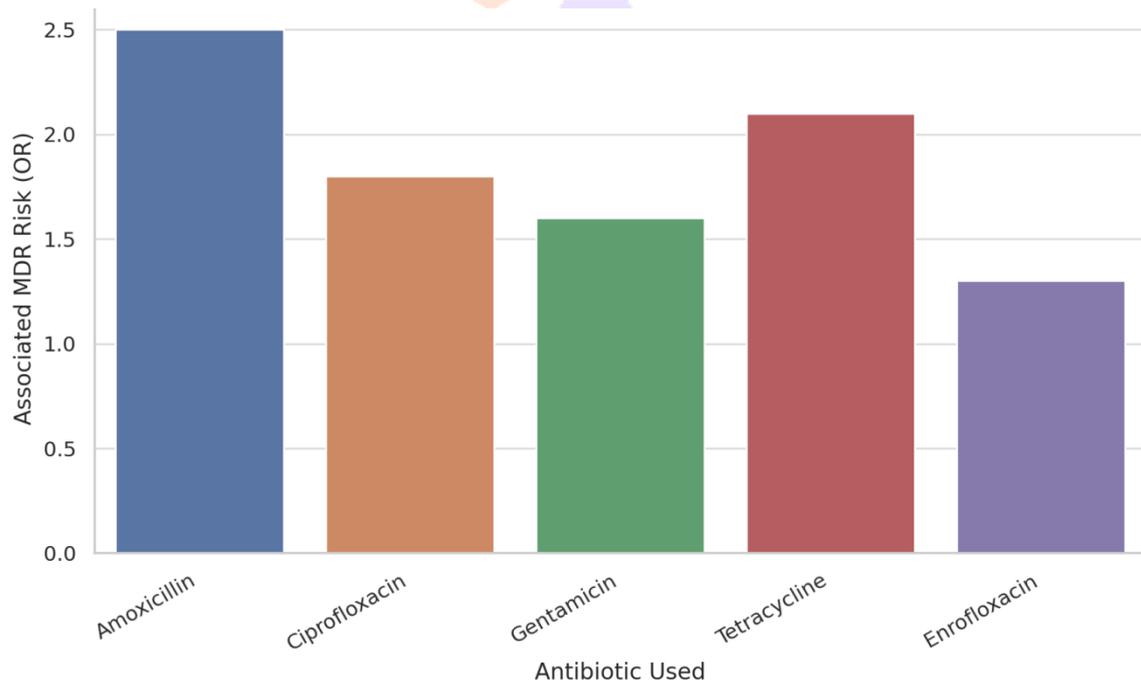**Figure 1:** Prevalence of bacterial isolates from urban companion animals.
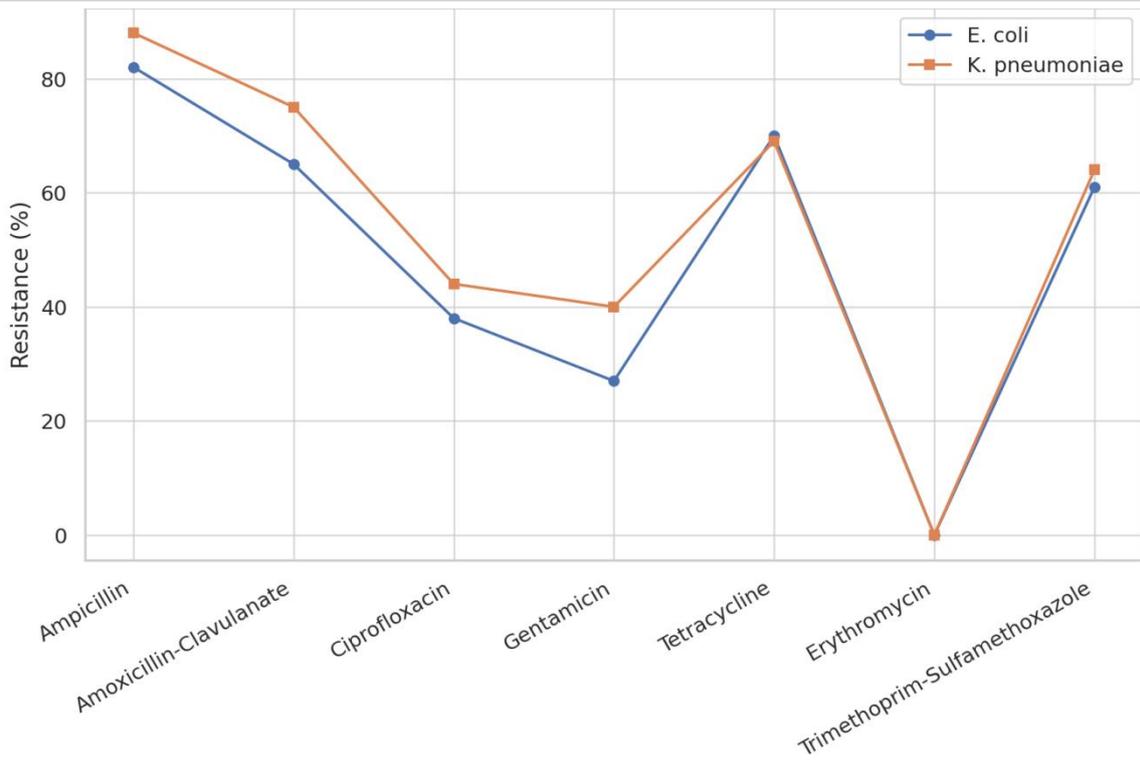
**Figure 2:** Resistance profiles of E. coli and K. pneumoniae against common antibiotics.
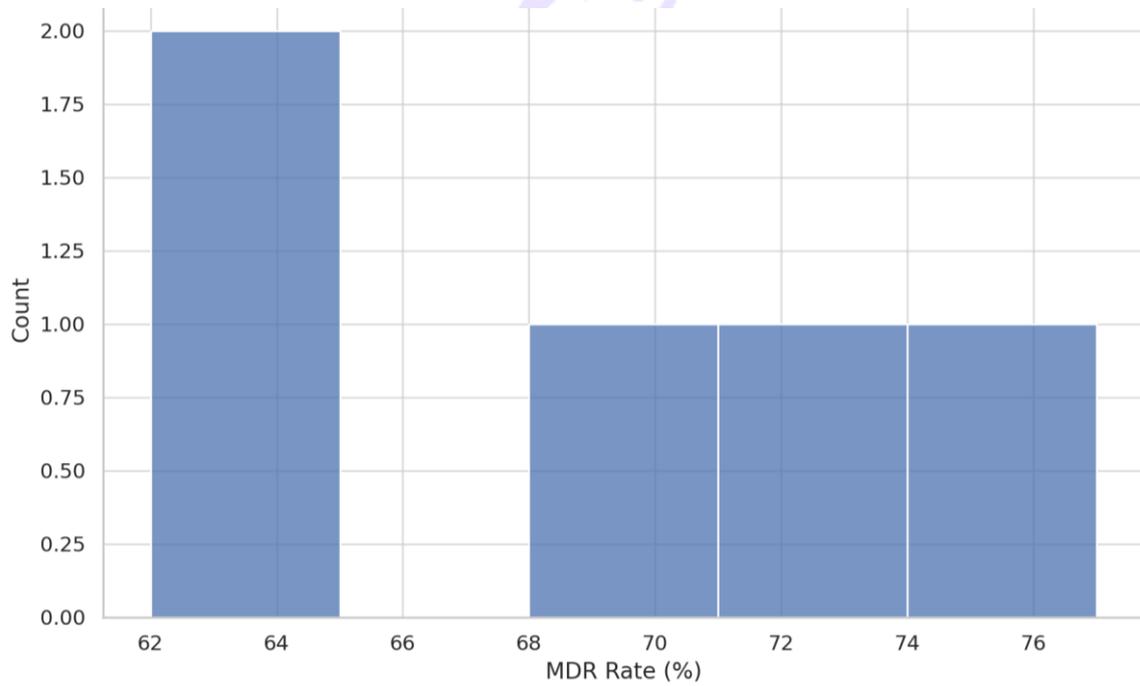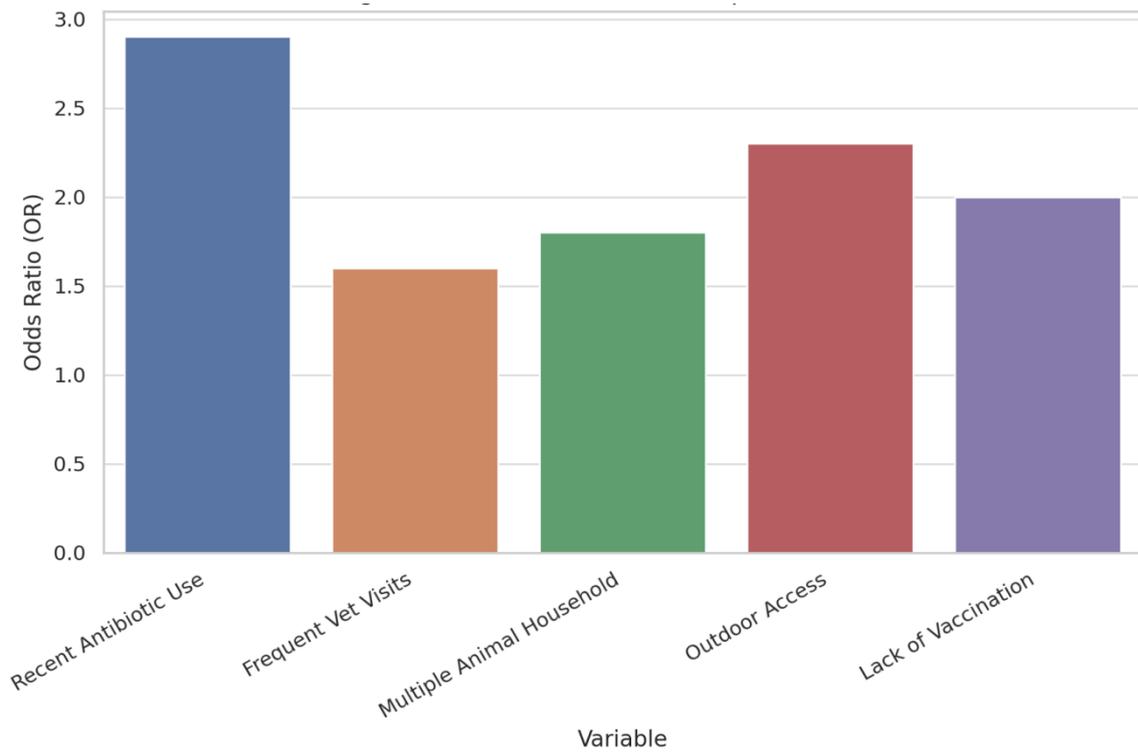


**Figure 3:** Histogram showing MDR rate distribution by bacterial species.
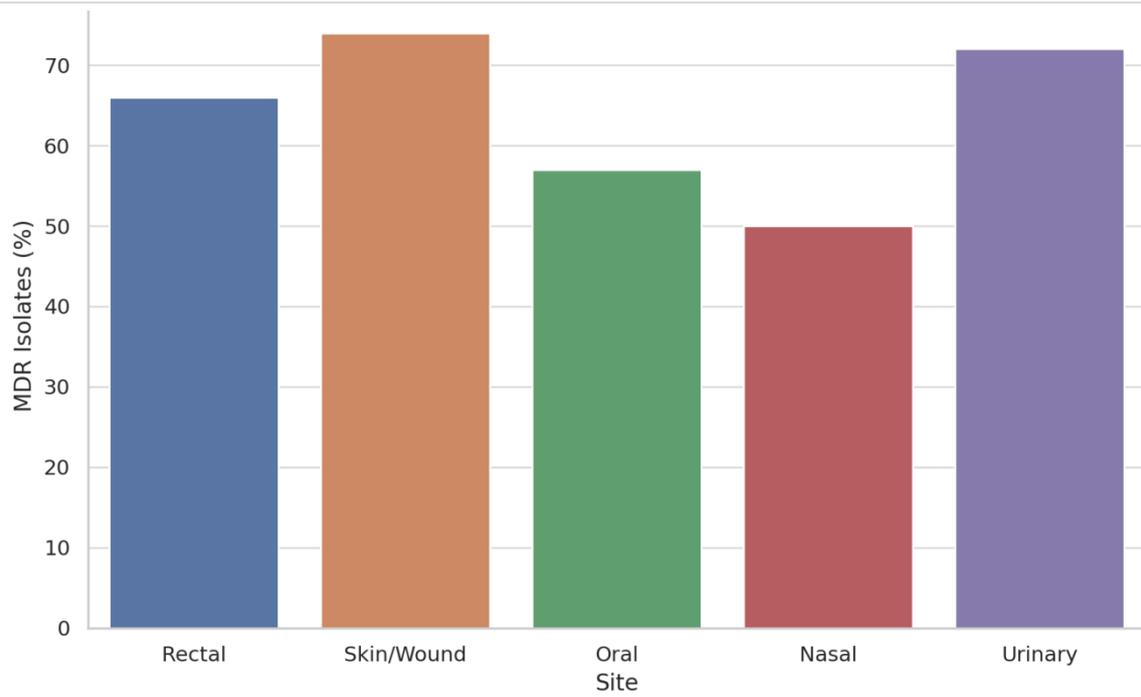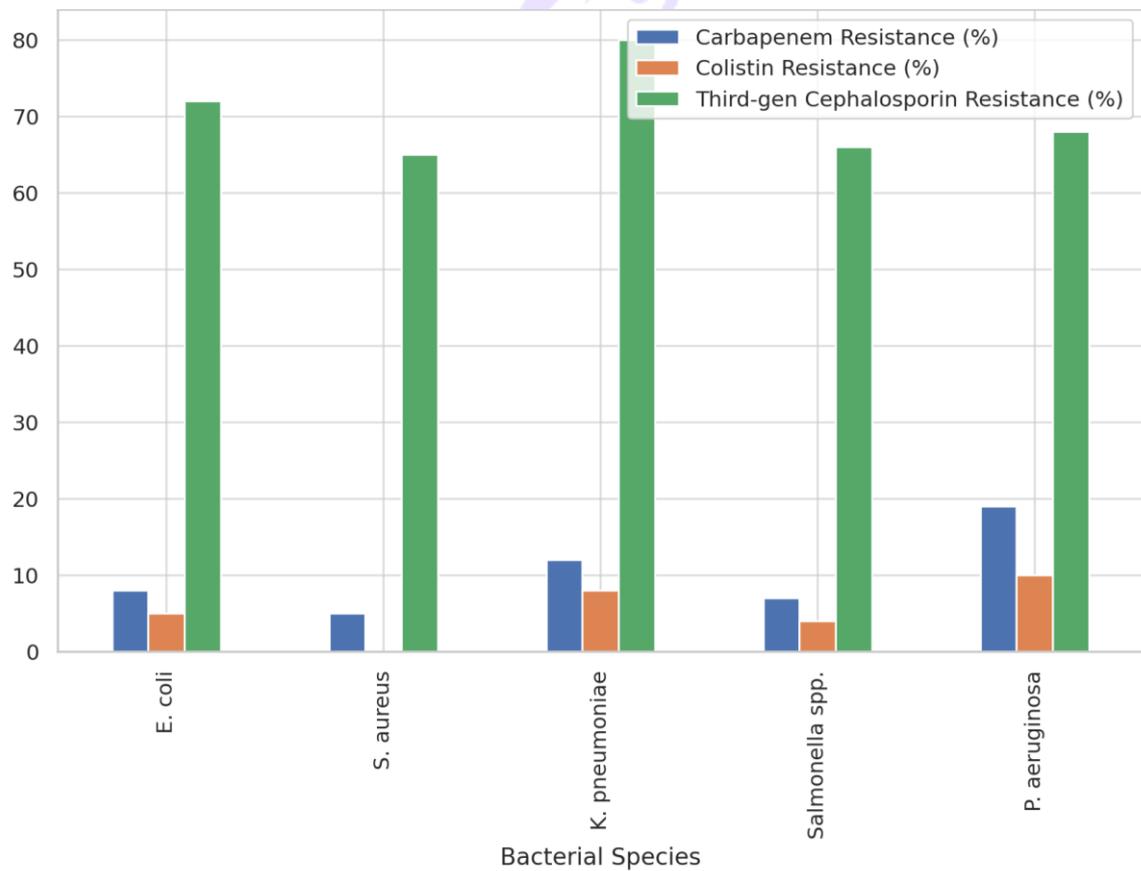
**Figure 4:** MDR occurrence across different anatomical sites of infection.
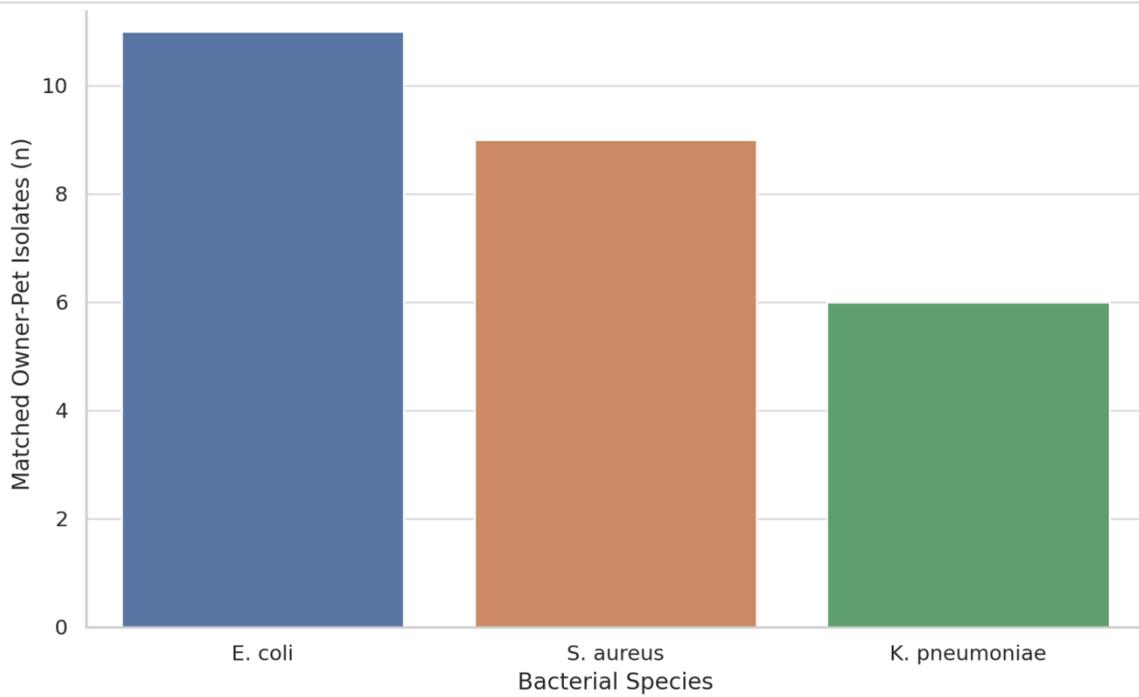


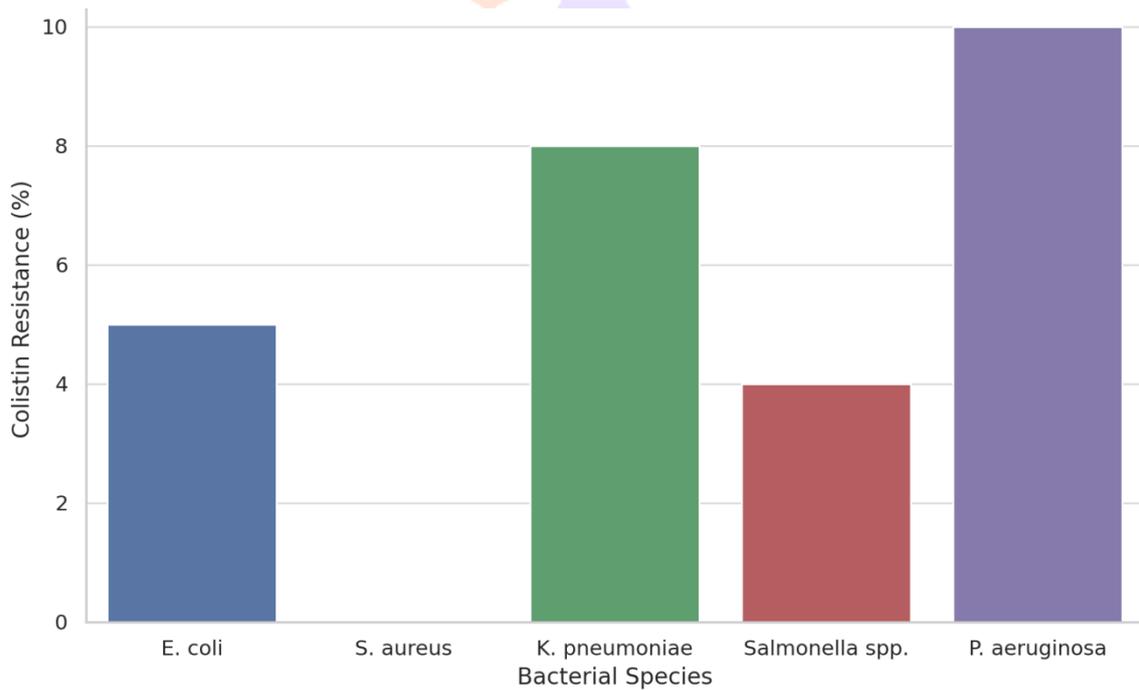**Figure 5:** Odds ratios of MDR linked to prior antibiotic use.

**Figure 6:** Key host and environmental predictors of MDR in logistic regression



**Figure 7:** Resistance to WHO-classified critical antibiotics.

**Figure 8:** Number of genetically matched MDR isolates shared between pets and owners.



**Figure 9:** Colistin resistance percentage by bacterial species

.**DISCUSSION**

It is not the only initiative since the WHO Global Action Plan on Antimicrobial Resistance endeavors to enhance surveillance and research, reduce infection rates, and optimize the usability of antimicrobials regarding people, animals, and the environment (Estany-Gestal et al., 2024). Despite over 80 years of using antibiotics, microorganisms have managed to evade their impacts (Wang et al., 2023). The misuse of antimicrobials is a sophisticated topic that should be well comprehended to develop efficient methods to

combat AMR (Kasimanickam et al., 2021). AMR is difficult to turn back completely; however, great progress can be made in mitigating its consequences. One should also monitor the situation with antimicrobial resistance in animals, in particular, pets, as they are in close contact with people and may transmit diseases to humans (Tang et al., 2023). The complexity of antimicrobial resistance requires a comprehensive strategy that couples monitoring and stewardship with balanced action in a number of areas. To understand the origins and routes of spread of infections, a "One Health" approach is necessary to develop proper strategies of mitigation (Bulteel et al., 2020; Endale et al., 2023). The professionals who can play a key role in the human -animal -environment triad are veterinarians, who should operate in collaboration with human medical professionals as part of a One Health approach (Caneschi et al., 2023). Our response to antimicrobial resistance should be a One Health strategy that unites health care professionals, hospitals, and the community to administer antibiotics responsibly in humans, animals, and the environment (Chandra et al., 2021). Without acting quickly, we may face a so-called post-antibiotic era, i.e., the situation when newly discovered antibiotics become fewer, some antibiotics become overused, and others underused, healthcare becomes more expensive, and more morbidity and mortality occur (Chandra et al., 2021). The role of the community is highly emphasized to combat antimicrobial resistance within a One Health approach in low-and-middle-income countries due to the contribution of social and systemic factors that result in antimicrobial use (Mitchell et al., 2021). The spread of resistant germs can be halted by educational campaigns, better sanitation and better hygiene.

**CONCLUSION**

The results of this study provide significant evidence that companion animals living in urban environment serve as significant reservoirs of multi-drug resistant (MDR) zoonotic bacteria, which poses a direct threat to the public health of the veterinary and human populations. The high level of MDR bacteria particularly in Escherichia coli, Staphylococcus aureus, and Klebsiella pneumoniae indicates the grave extent of antibiotic resistance in pets. The resistance to critically important antibiotics, including third-generation cephalosporins, carbapenems, and colistin, was observed at the levels that reflect reduced treatment options in both veterinary and human medical practices. Also, the identification of genetically related MDR isolates in pet-owner pairs supports the potential bidirectional zoonotic transmission, which gives relevance to a One Health approach in surveillance and control. The presence of risk factors suggested that a history of antibiotic exposure, outdoor access, lack of vaccination, and living in multi-animal households significantly increases the likelihood of MDR colonization, highlighting the multifactorial nature of resistance transmission. High rates of resistance to commonly used veterinary antibiotics, such as ampicillin and tetracycline, demonstrate the selective pressure caused by over-prescription and misuse and, therefore, urgent antimicrobial stewardship interventions. These findings also reveal the significance of improving the veterinary clinics with better diagnostic capacity, routine microbiological monitoring, and stricter regulations concerning the administration of antibiotics to pets. We should educate the people and the veterinarians through mass awareness and educational programs so that they can learn not to use antibiotics in a manner that does not make sense and to ensure proper care of their pets. This contribution demonstrates valuable epidemiological data to the emerging field of study about MDR bacteria in pets

and illustrates the significance of the existence of regulations that consider not only the health of individuals and animals but also that of the environment. Unless we act deliberately, the unchecked transmission of antimicrobial resistance in companion animals can hamper international progress in combating infectious disease and accelerate the transition to a post-antibiotic era. Policymakers must be on the front foot, collaborate and engage the community to prevent the rising issue of antimicrobial resistance in urban areas.

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