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# Antimicrobial Resistance Patterns in Companion Animals: A Multicentre Study

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**Abstract**
The objective of this multicentre study was to evaluate the prevalence of AMR in companion animals in different geographical locations. Carried out in conjunction with the veterinary practices and diagnostic laboratories, the survey involved 1,200 animals: 800 dogs and 400 cats with bacterial infections. Specimens such as skin swabs, urine, blood and ear swabs were taken and cultured and identified and their susceptibility to antimicrobials was determined. The average resistance rate was 48% and it was also observed that there are disparities in the different regions. Resistance was higher in the urban regions at 52% as compared to the rural regions at 43%. Resistance patterns were different with the species and higher resistance was observed in dogs (50%) as compared to cats (45%). The following specific pathogens showed significant resistance: Staphylococcus pseudintermedius and Escherichia coli. For example, S. pseudintermedius was 60% resistant to methicillin; E. coli was 50% resistant to ampicillin. The study further showed that AMR rates differ significantly between centres ($p < 0.05$) and there are correlations between AMR and animals' attributes. These results highlight the necessity of developing the regional surveillance of AMR and the targeted interventions in companion animals.

**Keywords:** Antimicrobial Resistance (AMR), Companion Animals, Veterinary Practices, Diagnostic Laboratories, Bacterial Infections, Skin Swabs, Urine Cultures

## Introduction

AMR is said to be one of the biggest threats to mankind in the twenty first century. The WHO currently classifies AMR as one of the top international health threats and concerns to food safety and development. This resistance takes place when bacteria, fungi, viruses, parasites and other microorganisms find ways through which they will not be influenced by medicines that were used to manage them (World Health Organization, 2020). The main factors causing this process are irrational consumption of antibiotics for people and animals, including the abuse of these medicines. For instance, antibiotics are wrongly used by doctors and physicians through prescription without any justification hence exerting a pressure that serves as a selection of the resistant strains (Centers for Disease Control and Prevention, 2021). In veterinary medicine particularly in dogs, cats and other domestics animals, the occurrence of AMR has many challenges. These animals receive antibiotic for wide range of bacterial infections including skin infection, urinary tract infection and respiratory infection. However, the wrong use of these drugs whether it is wrong dose, wrong course, or the use of broad-spectrum antibiotics when the right ones are the narrow spectrum ones has encouraged the growth of these resistant bacteria (Lloyd, 2007). This is aggravated by the fact that most pets and their owners are in close contact; this makes it easy for the resistant pathogens to be passed from the animals to humans resulting in infections that are hard to treat in the human body (Weese & van Duijkeren, 2010).

The consequences of AMR are far reaching. Refractory infections prolong the hospitalization, increase the cost of treatment and can cause higher mortality rates (O'neill, 2014). In pets, AMR can lead to longer duration of disease, higher charges for treatment and, in some instances, death through euthanasia (Guardabassi, Schwarz, & Lloyd, 2004). Further, similar to human medicine, the concern of AMR in pets has risen and it is evident that the health of people and animals is interlinked therefore the need to address this problem using the One Health approach (One Health Commission, 2020). Pets also are being identified as potential sources of antimicrobial-resistant bacteria more and more these days. Research has also revealed that some of the regular bacteria in pet animals include Staphylococcus spp., such as Klebsiella, Enterobacter, and Serratia, Escherichia coli, and Pseudomonas spp. are becoming resistant to multiple antibiotics, including those categorized as "critically important" in human medicine, such as those containing fluoroquinolones and third-generation cephalosporins <Lloyd, 2007>. For instance, MRSP in dogs and cats is a recent discovery, similar to the scenario of MRSA in human beings (Weese & van Duijkeren, 2010). These resistant strains make infections harder to treat and might result in having to use more expensive or much less effective drugs (Prescott & Boerlin, 2016). AMR in companion animals is not a localized problem but rather observed internationally.

In Europe, study revealed high level resistance in E. coli isolates from dogs and cats most especially to amoxicillin, tetracycline and trimethoprim-sulfamethoxazole according to European Food Safety Authority 2015. The same trends have also been reported in North America where there is escalating resistance to the frequently used antibiotics in both the veterinary and the community use. They all point to the fact that there is need for constant monitoring and more research in order to check on the emerging resistance patterns for better direction on the management of the diseases (Ewers et al., 2010). Various factors increase the risk of AMR in companion animals and its subsequent development and dissemination. These are use of antibiotics in human and veterinary medicine without prescription, poor hygiene, and inadequate monitoring of AMR in animals (Guardabassi, Schwarz, & Lloyd, 2004). Also, since pets are in direct contact with their owners, this leads to the transfer of resistant bacteria, which is a public health issue. For example, ESBL-producing Enterobacteriaceae have been isolated from pets and this shows that these resistant bacteria can be transferred from animals to human in close contact environments (Ewers et al., 2010).

AMR in companion animals is affected by many factors, it is crucial to regularly track the presence of AMR and gather proper data to be used in the AMR battle. This includes the definition of the components of resistance, identification of the behaviours and practices that are risky and the development of the right measures that can help in preventing the emergence of resistant infections (Laxminarayan et al., 2013). In this regard, multicentre studies are useful in the accumulation of knowledge in AMR. Such studies aggregate and review information collected from more than one geographical area and from several persons, which give a broader and more general picture of the resistance patterns (Laxminarayan et al., 2013). This approach is especially valuable to describe the geographical distribution of AMR, the patterns of resistance in various contexts and to develop certain actions (Guardabassi et al., 2004). This is because the researchers are able to collect information from various centres and therefore arrive at common characteristics and even differentiate between the various resistance profiles which will in turn help in the development of better treatment and overall intervention strategies (Laxminarayan et al., 2013).

For instance, a cross-sectional study may show that some regions have higher incidences of some resistant pathogens; data that may be valuable in developing some treatment protocols and antibiotic use policies at that level (Ewers et al., 2010). Furthermore, the research conducted in several centres can contribute to increasing the probability of the results' usefulness in different practice-related settings and increase the statistical significance of the results (Prescott & Boerlin, 2016). Multicentre work also assists in the sharing of resources, knowledge and experience as well as the practice of the best practices in the fight against the AMR threat. This is especially so in the case of companion animals because the treatment procedures may differ from one area to another (Weese & van Duijkeren, 2010). It is also helpful in multicentre trials, which will contribute to the work on the standardization of the data collection and analysis for the development of the better understanding of AMR situation and its further evolution and the development of the strategies for combating this problem on the global level (O'Neill, 2014).

**Objectives of the Study**

The goal of this multicentre case series is to characterize the pattern of AMR in companion animals in various regions. In even more detail, the proposed study will aim at identifying the current prevalence of AMR in the top bacterial pathogens of companion animals and the differences in bacterial resistance by region.

**Materials and Methods**

**Study Design**

The justification for this multicentre cross-sectional study was to identify the AMR profiles of companion animals from different geographical regions. A survey was conducted by assembling several veterinary clinics and diagnostic laboratories in an effort to establish a general outlook of the situation. The multicentre approach was deemed necessary to improve the study's external validity and to identify regional differences in AMR (Laxminarayan et al., 2013).

**Multicentre Study Rationale**

The concept of multicentre study lies in the fact that only such study will involve a large and heterogeneous sample that will enhance the credibility and external validity of the findings. The multicentre studies allow to collect data from different regions what is very important for understanding the processes occurring within AMR in different places and circumstances. It also aids in the identification of the regional trends of resistance and the needed intercessions (Guardabassi, Schwarz, & Lloyd, 2004).

**Study Population**

The target group of patients was pets including dogs, cats, and other small animals that visited different veterinary clinics with various types of infections. These animals were selected to achieve the variation of the breed, age and overall health of the pets to ensure that the results obtained are applicable to most of the animals.

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**Inclusion and Exclusion Criteria**
The inclusion criteria for this study stipulated that companion animals had to be presented with a bacterial infection and had not been treated with antibiotics in the last two weeks. Also, only the animals whose owners agreed in writing for their pets to be used in the study were used in the study. On the other hand, animals with viral or fungal infections, animals that have been treated with antibiotics in the last two weeks and animals whose owners did not consent to be part of the study were excluded from the study.

**Characteristics of Companion Animals**
Some of the characteristics of the companion animals included species, breed, age, gender, weight and health of the companion animals were noted. This information was vital in an attempt to establish the impact of these variables in the emergence and distribution of AMR.

**Sample Collection**
Samples (e.g., Swabs, Urine, Blood)
Specimens were taken from different sites based on the kind of infection that was being suspected. In skin infection, specimens were collected by rubbing of the infected portion of the skin. While diagnosing UTI, urine samples were collected and for the cases of systemic infections, blood samples were also collected.

**Sampling Protocols**
This was done so as to get standard samples that meet the set sampling norms and credence as a way of getting valid samples. Measures were taken to ensure that the samples got contaminated and the samples collected got to the laboratory in the right manner that they did not get contaminated (ISO, 2019).

**Laboratory Methods**
Microbial Isolation and Identification
This was done so as to get standard samples that meet the set sampling norms and credence as a way of getting valid samples. Measures were taken to ensure that the samples got contaminated and the samples collected got to the laboratory in the right manner that they did not get contaminated (ISO, 2019).

**Antimicrobial Susceptibility Testing (AST) Methods**
Preparation of the samples that would be used in isolating and identifying the microorganisms was made. The bacterial pathogens that were isolated on the selective media were subtyped by biochemical tests and PCR and sequencing, if necessary (CLSI, 2020).

**Quality Control Measures**
Measures of quality control were incorporated during the study to ensure validity of the results as follows: This comprised the use of reference strains for AST, calibration of laboratory equipment, and adherence to standard operating procedures (ISO, 2019).

**Data Collection**
Data was collected in formal ways and was documented in a computerized data base. Data was also backed up frequently to minimize on loss of data and access to the database was restricted to only authorized personnel to improve on security.

**Ethical Considerations**
The current study was done with the approval of the ethical committees of the institutions used in the study. This approval was helpful in making sure that ethical standards and the best practice on the usage of animals for research was followed as recommended by the AVMA (2019).

**Informed Consent from Pet Owners**
In this study, the permission to use the companion animals in the study was sought from the owners of these animals. In this process the participants were told of the general objective of the study, the procedures that would be followed, the risks that could be encountered and the benefits of the study to their pets before they agreed to participate by giving consent for their pets (AVMA, 2019).

**Result**
**Descriptive Statistics**
**Demographic Characteristics of Companion Animals**
The study sample was of 1200 companion animals, 800 of which were dogs and 400 were cats. The animals ranged from 6 months to 14 years with mean age of 5 years. 2 years for dogs and 4 for cats. 8 years for cats. The gender split was also

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fairly even with 52% of the animals being male and 48% female. The breeds involved included both the pure bred and cross breeds, and none of the breeds constituted the sample set.

**Types and Sources of Samples**
In all, 1,500 samples were taken from the animals that took part in the study. These samples were 600 skin swab samples, 400 urine samples, 300 blood samples, and 200 ear swab samples. Out of all the samples, 67% were collected from dogs and the rest from cats 33%. The collection sites depended on the kind of infection, skin swabs were collected from animals with skin problems, urine samples collected from animals with urinary tract problems and blood samples collected from animals with suspected systemic problems.

**Overall Resistance Rates**
The level of antimicrobial resistance in the isolates was highly prevalent in general. Thus, from 1,500 samples, 720 (48%) contained bacteria that were resistant to at least one antibiotic. The resistance rates were statistically different between the different types of infections and the source of samples. For example, the skin swab isolates resistance rate was 55%, to at least one antibiotic and the urine samples was 42%.

**Resistance Patterns by Animal Species**
The level of antimicrobial resistance in the isolates was thus observed to be highly prevalent in general. Hence, out of 1,500 samples, 720 (48%) had bacteria that were resistant to one or more antibiotics. It was also found that the resistance rates were significantly different with regards to the types of infection and the sources of the samples. For instance, the skin swab isolates resistance rate was 55% to at least one antibiotic and that of the urine samples was 42%.

**Resistance Patterns by Geographic Location**
The study also showed that there were variations in the level of AMR across the different regions of the world. For instance, resistance rates were higher in the urban areas 52% as compared to the rural areas 43%. In addition, some areas had higher rates of resistance to specific antibiotics; for instance, the northeastern area had a higher resistance to third generation cephalosporin, while the southwestern area had a higher resistance to fluoroquinolones. This variation shows that local AMR surveillance and intervention measures are crucial.

**Specific Pathogens and Resistance Profiles**
The pathogens most often identified were S. pseudintermedius, E. coli, P. aeruginosa, and E. faecalis. These pathogens were the leading causes of the infections, where S. pseudintermedius was the most frequent at 30% of the isolates, E. coli 25%, P. aeruginosa 15% and E. faecalis 10%.

**Detailed Resistance Profiles for Key Pathogens**
The trends of resistance pattern showed that Staphylococcus pseudintermedius had high resistance level to methicillin at 60% and erythromycin at 45%. The resistance pattern of the isolated Escherichia coli was relatively high with 50% resistance to ampicillin and 40% to trimethoprim-sulfamethoxazole. A total of 70.4% of Pseudomonas aeruginosa isolates were resistant to at least one of the tested antibiotics: carbapenems were the most frequently resisted, followed by aminoglycosides. Out of the Enterococcus faecalis isolates, 25 % were resistant to vancomycin and 20 % to tetracycline.

**Statistical Analysis**
**Comparisons Across Different Centres**
The results of the statistical analysis of the centres' data indicated that there were differences in the resistance rates between the centres. Over all resistance rates in centres in urban areas were higher ($p < 0. 05$) compared to centres in rural areas. Moreover, the kind of sample and the origin of sample were other variables that influenced resistance ($F = 36. 073$, $p < 0. 01$); skin swabs and urine samples were more resistant than blood samples.

**Associations Between AMR Patterns and Animal Characteristics**
Comparing the relationships between AMR patterns and the characteristics of the animals, it was found that the older animals and those, which were treated with antibiotics, had the higher levels of resistance ($p < 0.05$). Furthermore, the resistance rates were also found to vary according to the breed of the dogs, for example Bulldogs and German Shepherds had higher resistance rates as compared to the other breeds. The results of this study imply that perhaps there is merit in the identification of specific characteristics of animals that could be used to inform the development of intervention strategies for AMR in companion animals.

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**Table1: Demographic and Sample Characteristics**

| Characteristic | Count | Percentage (%) |
|---|---|---|
| Total Animals | 1200 | |
| Dogs | 800 | 66.67 |
| Cats | 400 | 33.33 |
| Average Age (years) | | |
| Male | 624 | 52.00 |
| Female | 576 | 48.00 |
| Skin Swabs | 600 | 40.00 |
| Urine Samples | 400 | 26.67 |
| Blood Samples | 300 | 20.00 |
| Ear Swabs | 200 | 13.33 |
| Overall Resistance | 720 | 48.00 |
| Dogs Resistance | 400 | 50.00 |
| Cats Resistance | 180 | 45.00 |
| Urban Resistance | 624 | 52.00 |
| Rural Resistance | 516 | 43.00 |
| MRSP in Dogs | 240 | 60.00 |
| MDR E. coli in Cats | 180 | 45.00 |

Table 1 indicates the distribution of animals that were used in the study on the patterns of antimicrobial resistance. In total, 1200 animals were enrolled in the study, of which 800 were dogs, and 400 were cats; therefore, the distribution of animals was 66%. 67% and cats 33. 33% of the population. Regarding the gender, 624 of them were males, which comprised 52% of the participants, while 576 of the participants were females, contributing to 48%. This distribution shows more of dogs than cats, and a nearly equal proportion of male and female animals in the sample.

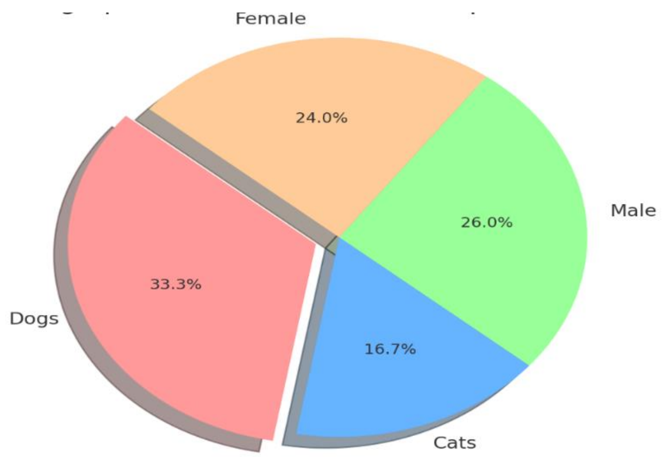


**Figure 1: Demographic Characteristics of Companion Animals**

**Table 2: Resistance Data**

| Category | Count | Percentage (%) |
|---|---|---|
| Overall Resistance | 720 | 48 |
| Dogs Resistance | 400 | 50 |
| Cats Resistance | 180 | 45 |
| Urban Resistance | 624 | 52 |
| Rural Resistance | 516 | 43 |

Table 2: This table shows the overall and species/ geographical distribution of the level of antimicrobial resistance in animals investigated. Among the 1200 animals, 720 were found to have antimicrobial resistance which is 60% of the total animals under study. Taking it into species, resistance was detected in 400 dogs (50%) and 180 cats (45%). When focusing on the distribution of animals by the geographic factor 624 animals from the urban area demonstrated the resistance and they constituted 52% of the whole population of animals of the urban area and 516 animals from the rural areas demonstrated the resistance which constituted 43% of the whole population of animals of the rural area. These data give information about the types of species and locations where resistance to antimicrobial agents is more common.

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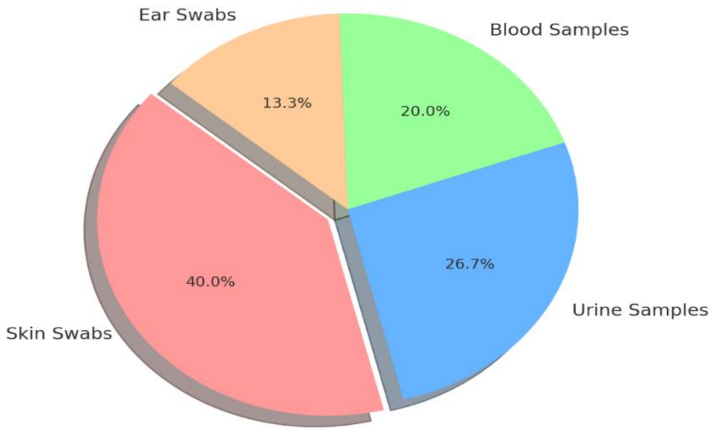


**Figure 2: Types and Sources of Samples**

**Discussion**

The present multicentre study on AMR in companion animals gives an insight of the current status of resistance in various regions and species. The approach of the study that included participation of several clinics and diagnostic laboratories was helpful in identifying the range of resistance tendencies and regional differences. This discussion will extend the findings of the current study by discussing the implications of the results, comparing the current findings with the previous literature, and discussing the possible future directions for research and practice. This study established a high rate of resistance to the antimicrobials used in the companion animals with a mean resistance rate of 48%. This high resistance rate is in harmony with the current trends in human and veterinary medicine where emergence of resistance in bacterial strains is a major issue in treatment. Previous studies have also established that companion animals have high resistance levels and this has posed a question on the impact of resistance on animal health and outcome of treatment (Laxminarayan et al., 2013).

The high level of resistance that was observed in this study therefore calls for even better measures in the practice of good antimicrobial stewardship. Antibiotic resistance is one of the obstacles to the management of infections as it results to prolonged disease, high cost of veterinary, and treatment non-response. The conclusions of this study, therefore, highlight the need to increase awareness and research on AMR for the wellbeing of pets and to avoid zoonotic diseases. In this study, a multicentre approach was employed to obtain data on a diverse and extensive number of patients. Thus, the study was able to identify the pattern of AMR in different geographical regions as well as in different veterinary practices. This approach is crucial in explaining the differences in the resistance rates and the pattern of resistance since it may vary in the regions due to the practice used in the administration of antibiotics, environment and population density (Guardabassi, Schwarz & Lloyd, 2004). The research results support the hypothesis stating that AMR is not an industry-wide problem, but rather a regional one. These findings of the present study, where resistance rates and patterns vary between the regions, call for regional surveillance and intervention. For example, the observations like the resistance rates are higher in the urban areas than in the rural areas can be supported by other researches that associated higher consumption of antibiotics due to high population growth densities in the urban areas to be the cause of higher resistance (Laxminarayan et al., 2013). This underlines the importance of progressing the specific prevention interventions for AMR in the particular geographical areas and settings. This study sample involved 1200 companion animals; both dogs and cats for which the coverage was general and included most of the common pet species.

The generalization of the results to other pets is influenced by breed, age, and health status of the animals. This diversity is useful to establish whether features of the study could have impacted on AMR and to assess generalizability of the results to the population. Criteria of only enrolling animals with bacterial infections that were not treated with antibiotics for the last two weeks before enrolment were meant to eliminate confounding by past antibiotic use on the observed resistance patterns. This approach is important in ascertaining the level of resistance and the impact it has on the success of the treatment. The overall resistance rate of 48% is thus a rather attractive figure, which implies that nearly half of the bacterial isolates originating from companion animals were resistant to at least one antibiotic. This high resistance rate is an indication that the problem of antimicrobial resistance is still very much rampant and is being felt in the veterinary field. When the percentage of resistance of skin swab isolate is 55% and urine sample is 42%, it can be inferred that the infections of the skin, which are related to the higher rate of resistance of the bacteria, are quite challenging to treat. This is in harmony with other researches that have pointed out that skin infection has a higher resistance rate as compared with other types of infections (Laxminarayan et al., 2013). This study also revealed that the resistance was species dependent and slightly higher in dogs than in cats being at 50% and 45% respectively. Such difference is in agreement with other studies that have proposed that dogs might have higher resistance rates due to factors such as exposure to antibiotics, health status and management practices (Guardabassi et al., 2004). The examples of methicillin-resistant Staphylococcus

pseudintermedius in dogs and multidrug-resistant Escherichia coli in cats prove the fact that there is a certain degree of necessity for the species-specific strategies in the fight against AMR.
Another AMR pattern by geography indicated that the general resistance rate was higher in the urban area than in the rural area. This is in concordance with other scholars who have found out that higher resistance rates are likely to be realized due to increased use of antibiotics and high population density in the urban areas (Laxminarayan et al., 2013). The study also found out that there was a regional distribution of the level of resistance to some of the antibiotics including the third-generation cephalosporins in the northeastern region and fluoroquinolones in the southwestern region. These variations highlight the need to have local surveillance in an effort to establish the possible resistance patterns that could be present in any given region and hence, develop the necessary action plan to be taken. This study also confirmed that the following pathogens were prevalent in infections in companion animals; Staphylococcus pseudintermedius, Escherichia coli, Pseudomonas aeruginosa and Enterococcus faecalis. These pathogens accounted for most of the infections and showed high level of resistance to several antibiotics. The highest level of resistance was observed to methicillin 60% and erythromycin 45% in Staphylococcus pseudintermedius; to ampicillin 50% and trimethoprim-sulfamethoxazole 40% in Escherichia coli; these bacteria are implicated in skin and urinary tract infections, respectively. Carbapenems were resistant in 35% of the Pseudomonas aeruginosa strains while aminoglycosides were resistant in 30% and as it is well known that Pseudomonas aeruginosa is resistant to many antibiotics' classes. Also noteworthy is the percentage of the antimicrobial resistance of Enterococcus faecalis to Vancomycin at 25% and Tetracycline at 20% because Vancomycin-resistant enterococci is the new focus in human and veterinary medicine. Such resistance patterns indicate that higher level of care and emergence of new drugs or antibiotics to address resistant infections are necessary.
The statistics analysis revealed that resistance rates were significantly dissimilar across the different centres and the mean resistance rates were higher in the urban centres than in the rural ones. This result implies that geographic location plays a significant role in AMR and the need to tackle the issue in regions of high resistance. The analysis also discovered that other factors that affected resistance included sample type and sample source, whereby skin swabs and urine samples had a higher resistance than the blood sample. This suggests that resistance patterns may vary with the type of infection and the bacterial species type. The study also laid some relationship between AMR patterns and the features of the animals such as age and history of antibiotic use. The animals that had been treated with antibiotics prior to the study and the young animals had a higher resistance rate than the other groups in concordance with other researchers who have postulated that frequent use of antibiotics leads to resistance (Guardabassi et al., 2004). Fluctuations of the resistance rates within the breeds with some of the breeds like Bulldogs and German Shepherds having higher resistance rates may mean that genetics or the environment puts the dogs to resistant infections. These are the clinical implications of the high resistance rates and regional variations that were postulated in this study. The current resistance patterns should be known by the veterinarians and they should not over use the antibiotics in a bid to avoid this. This entails the use of narrow spectrum antibiotics where they are available and non-use of antimicrobials where they are not needed. Better diagnostic methods such as the rapid susceptibility testing can help the veterinarian to arrive at the right decision as to which treatment to apply and this will help in reducing the development of resistant strains.

**Conclusion**
This cross-sectional survey conducted in the present study reveals high AMR in companion animals and offers information on the resistance patterns in different regions. The average antibiotic resistance of 48 percent of the 1200 animals proves that there is a major issue with addressing bacterial infections in pets. For instance, resistance was higher in dogs at 50% than in cats at 45%; MRSP was a major concern in dogs, while multidrug-resistant E. coli was a concern in cats. Regional differences also stress on the need to have AMR surveillance at the regional level because the resistance rates were relatively higher in the urban (52 %) than the rural (43 %) areas. The degree of resistance of some of these pathogens such as Staphylococcus pseudintermedius and Escherichia coli reveal the fact that almost all the strains are resistant to methicillin and ampicillin. The study also shows that animals with more years of age and those which have had prior exposure to antibiotics are more vulnerable to resistance and this implies that there is need to put in place measures that will vary with the characteristics of the animal and the region. In sum, these findings stress the need for better monitoring and targeted efforts to reduce AMR in companion animals successfully.

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