Cross-sectional study: Use of Antimicrobials in the Veterinary Clinics and Antibiotic Resistance

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Abstract
Antibiotics are used for the treatment and control of many types of microbial infections in a wide variety of animals and humans. Due to lack of prudent antibiotic use, antimicrobial resistance (AR) emerged as a global threat and pressing concern in public and animal health. AR is influenced by multifaceted interaction of human, agricultural production, and the environment. The objectives of this study were to perform cross-sectional data collection and analysis from veterinary clinics and antibiotic resistance patterns collected from diagnostic laboratory records. The pharmacy database from three animal hospitals was recorded for antibiotic use, prescribing practices, and personal opinions. AR data was collected from 88 recent clinical submissions and analyzed for the resistance/susceptibility of the isolates. The antibiotic classes commonly prescribed across the three veterinary clinics were cephalosporin, aminoglycoside, beta-lactam, quinolones, and tetracycline. Frequently prescribed antibiotic agents were used to treat dermatological infections, respiratory infections, ear infections, and urinary infections as 24%, 15%, 14%, and 12%, respectively. Based on the records from veterinary diagnostic laboratory, the submitted clinical samples were from seven animal species out of which 66% were from canine. Forty-three antimicrobial drugs were included in the resistance assay panel. From all of the clinical isolates (n=88), sixteen bacterial species were identified, predominantly Staphylococcus (29), E. coli (29), and Pseudomonas species (11). In order to promote judicious use of antimicrobial resistance and use, it is critical to have adequate knowledge and database from the current clinical practice settings which require a multidisciplinary perspective under One Health Initiative and implement feasible guidelines and antimicrobial stewardship.

Keywords
Antibiotic; Resistance; Veterinary clinic; Bacteria

Introduction
Antimicrobials were first used as therapeutics in veterinary medicine for the treatment of mastitis in dairy cows shortly after the use of antimicrobials has increased to such an extent in intensive agricultural production techniques as feed supplements [1,2]. In recent years, antimicrobial resistance in bacteria of animal origin, including food-producing animals, and companion animals, fish, and other aquatic animals as well as wild animals, has gained particular attention. Broader applications of antimicrobial agents have revolutionized medicine in many respects, but the application has been accompanied by a rapid appearance of resistant strains for many decades, resulting in a global health problem [3].

Overall, worldwide usage of antibiotics in both animal production and human medicine has increased in recent decades; agriculture accounts for the majority of drugs used, and the mass of antibiotics used for the production of terrestrial food animals is estimated to exceed the amount of drugs used in aquaculture [4]. Low and sub-therapeutic dose of antimicrobials plays a very important role for the improvement of feed efficiency, promotion of animal growth, and prevention and control of the diseases [5,6]. In veterinary medicine, food-producing animals are often regarded as posing the greatest risk for the transmission of antimicrobial-resistant organisms to humans via the food chain. However, circumstantial evidence indicated that transmission of antimicrobial resistant bacteria bi-directionally between humans and household animals occur; which has implications for the treatment options available for veterinary use as well as the health of companion animal patients, their owners and caretakers. Infection with resistant organisms can lead to longer and more severe infections, increased mortality and higher costs for treatment [3,7].

Each year in the United States, at least 2 million people acquire serious infections with bacteria that are resistant to one or more of the antibiotics designed to treat those infections. At least 23,000 people die each year as a direct result of these antibiotic-resistant infections. At least 23,000 people die each year as a direct result of these antibiotic-resistant infections.
resistant infections. Many more die from other conditions that were complicated by an antibiotic resistant infection [8]. Several case studies have documented the presence of antibiotic-resistant bacterial strains in small animal veterinary medicine [9-11] including methicillin-resistant *Staphylococcus aureus* (MRSA). The resistant bacteria can be transferred between health care providers and patients and also between owners and their pets [12]. In the case of MRSA, veterinarians may also serve as reservoirs of resistant infections, and close contact between people and companion animals may also lead to the transfer of antimicrobial resistant bacteria in both directions. An effort is made to highlight the various factors that contribute to the emergence of antibiotic resistance in farm animals and to provide some insights into possible solutions to this major health issue [13,14]. The goal of the present study was to assess the current prescribing practices of therapeutic antibiotic in the veterinary clinics and analyze the antimicrobial resistance patterns which are essential to empower prudent use of antimicrobials and preserve the effectiveness of these important drugs in both human and veterinary medicine.

### Materials and Methods

Data collection from veterinary clinics: Data were collected through questionnaire-based survey electronically sent to nine small animals and mixed animal’s veterinary clinics. Three veterinary clinics responded to the online excel survey file completed. The three veterinary clinics included in the questionnaire-based analysis are; VC-1 is a teaching veterinary hospital at the College of Veterinary Medicine, VC-2 and VC-3 are private veterinary clinics. The questionnaires focused on the pharmacy database to get the list of antimicrobial agents used and the prescribing practices. Furthermore, personal comments on frequently used antibiotics, and clinical experiences were included.

Antibiotic Resistance: The antibiotic resistance data was collected from the diagnostic laboratory. Most of the samples submitted to the diagnostic laboratory were from veterinary clinic-1 (VC-1). A total of 88 recently submitted clinical samples were collected and analyzed against 43 antibiotics. The antibiogram tests were performed using Micro Scan auto Scan 4 (Biolog, Inc. Hayward, CA) which is a semi automated instrument that utilized micro dilution panels containing frozen conventional substrates for identification of bacterial isolates and their antimicrobial resistance level [15].

Data Analysis: The collected data was compiled, statistical analyzed and tabulated using Microsoft Excel 11.5.5 (Microsoft; Redmond, WA, USA)

### Results and Discussion

This study was intended to highlight the frequently used antimicrobials in animal clinics and analyze the antibiotic resistance patterns. Pharmacy data from veterinary clinics: In three of the veterinary clinical settings, the pharmacy database included 81 antibiotics (Figure 1). The antibiotics recorded in each veterinary clinic were 46, 10 and 25 from VC-1, VC-2 and VC-3, respectively. The common class of antibiotics recorded in all the veterinary clinics were, cephalosporin, amino glycoside, betalactam, quinolone, tetracycline and sulfonamide. The clinical cases frequently indicated to antibiotic treatments are summarized in (Figure 2). Based on the records, the major infections subjected to antibiotics treatment were dermatological infections (24%), respiratory infections (15%), ear infections (14%), and urinary infections (12%). Over all there are certain level of antibiotic awareness and antibiotic use guidelines available in the clinics.

**Antibiotic resistance data:** Randomly, 88 data records have been collected and analyzed from the recent clinical submissions for antibiotic tests. The veterinary diagnostic laboratory performs routine antimicrobial procedures supported by standard culture and semi automated Microscan procedures. The samples of origin were from 7 animal species out of which 66% of the samples were from canine (Figure 3). The rest samples were from avian, equine, feline, caprine, rabbit, and rat. About 9% records missed to register the animal source of the clinical specimen.

In general, 19 types of clinical specimens were submitted to the laboratory (Figure 4). The specimens were mainly from urine, (33%), ear swabs (11%), nasal swabs (6%), feces (6%), and vaginal swabs (5%). The antibiotic resistance test panel included 43 antibiotics, which is categorized to seven classes of antibiotics. Most of the antibiotics were categorized in betalactam, cephalosporin and fluoroquinolone classes.

Based on our records, most frequently isolated bacteria were *Staphylococcus spp*, *E. coli*, and *Pseudomonas* (Table 1). In the assessment of the antimicrobial results, the resistant and susceptibility were considered as variables. The antimicrobial resistance panels’ results are summarized in (Graph 1, A-E).

A total of 20 *Staphylococcus* isolates categorized in five *Staphylococcal* spp (*Staphylococcus xylosus, Staphylococcus sciuri, Staphylococcus hyicus, Staphylococcus cohnii and Staphylococcus aureus*) were identified and assessed the antimicrobial profile patterns. *Staphylococcus hyicus* is sensitive to all tested antibiotics in the antibiotics panel except levofloxacin and ofloxacin. *Staphylococcus cohnii, Staphylococcus aureus*, *Staphylococcus xylosus* were fully susceptible for some antimicrobial agents none of the isolates were full resistant to any of the antibiotics. *Staphylococcus sciuri* isolates were fully resistant for ten of the antibiotics (Graph 2,3).

*E. coli* is strongly susceptible to ciprofloxacin, levofloxacin, ticar/K clav and tabromycin. For the rest of antibiotics in the panel are differentially resistant/susceptible. The panel profile for *Pseudomonas* isolates looks partly similar to the *E. coli* pattern. All the *Pseudomonas* isolates were resistant to meropenem.

Antibiotic resistance has the potential to affect people at any stage of life, as well as the healthcare, veterinary, and agriculture industries, making it one of the world’s most urgent public health problems. Each year in the U.S., at least 2 million people are infected with antibiotic resistant bacteria, and at least 23,000 people die as a result [16]. Antimicrobial resistance is a critical and emerging threat to public health. In response to the need for improved antimicrobial usage, guidelines have been developed to direct treatment of common companion animal infections. However, studies indicating low awareness of these guidelines among veterinarians suggest that poor concordance of usage patterns with guideline recommendations might be expected [17].

Therefore, clinicians should use a variety of tools when deciding whether or not to prescribe an antibiotic and which antibiotic to use. As in human medicine, there is likely overuse and in appropriate use of antibiotics in veterinary medicine. Veterinarians should engage in discussions regarding clinically applicable guidelines for judicious use of antibiotic. Introduction Antibiotic resistance is of considerations. Shea et al. (2011) published if there was documentation of confirmed, suspected or no evidence of infection in veterinary practices. Their results showed that in 17% of therapeutic antibiotic prescriptions there was confirmed infection, in 45% suspected infection, and in 38% there was no documented evidence of infection.

Amoxicillin clavulanate was the most frequently prescribed antibiotic followed by cefazolin/cephalexin, enrofloxacin, ampicillin/amoxicillin and doxycycline. Doxycycline was the most frequently prescribed with no documented evidence of infection, and amoxicillinclavulanate was the most frequently prescribed with either confirmed or suspected evidence of infection [18].

In order to effectively prevent and control resistance, medical communities need to monitor and limit antibiotic use. Once a resistant strain has emerged, re-developing susceptibility to antimicrobial therapy is a difficult and lengthy process. Therefore, efforts should be focused on preventing the emergence of resistant strains through prudent use of antibiotics [16].

Due to a large overlap in antibiotics used in human and in small animal veterinary medicine, and because of the close and continuous
<table>
<thead>
<tr>
<th>Bacteria isolated</th>
<th>Number of isolates</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus</em> spp</td>
<td>29</td>
<td>32.95</td>
</tr>
<tr>
<td><em>Escherichia</em> spp</td>
<td>29</td>
<td>32.95</td>
</tr>
<tr>
<td><em>Pseudomonas</em> spp</td>
<td>11</td>
<td>12.5</td>
</tr>
<tr>
<td><em>Klebsiella</em> spp</td>
<td>3</td>
<td>3.41</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>2</td>
<td>2.27</td>
</tr>
<tr>
<td><em>Salmonella</em> spp</td>
<td>2</td>
<td>2.27</td>
</tr>
<tr>
<td><em>Enterococcus</em></td>
<td>2</td>
<td>2.27</td>
</tr>
<tr>
<td><em>Enterobacter</em></td>
<td>2</td>
<td>2.27</td>
</tr>
<tr>
<td><em>Shigella</em> species</td>
<td>1</td>
<td>1.14</td>
</tr>
<tr>
<td><em>Proteus</em> spp</td>
<td>1</td>
<td>1.14</td>
</tr>
<tr>
<td><em>Morganella morgani</em></td>
<td>1</td>
<td>1.14</td>
</tr>
<tr>
<td><em>Kluyvera ascorbata</em></td>
<td>1</td>
<td>1.14</td>
</tr>
<tr>
<td><em>Fermenter species</em></td>
<td>1</td>
<td>1.14</td>
</tr>
<tr>
<td><em>Aeromonas hydrophila</em></td>
<td>1</td>
<td>1.14</td>
</tr>
<tr>
<td><em>Aeromonas hydrophila</em></td>
<td>1</td>
<td>1.14</td>
</tr>
<tr>
<td><em>Edwardsiella tarda</em></td>
<td>1</td>
<td>1.14</td>
</tr>
</tbody>
</table>

Table 1: Identified bacteria from the clinical specimen submitted for antibiotic resistance.

Figure 1: Antimicrobial classes listed from three veterinary clinics (number of antibiotics in each antibiotic class is in parentheses). VC-1: Veterinary clinic (mixed animals clinic; VC-2 and VC-3: small animals private veterinary clinics.

Figure 2: Percentage of infectious diseases commonly subjected to antibiotic treatment in the veterinary clinics.

Figure 3: Percentage of animal species (in %) where the clinical samples were collected.

Figure 4: Types of specimens submitted to the veterinary diagnostic laboratory for antibiotic resistance assay.
A: Staphylococcus xylosus (4), B: Staphylococcus sciuri (4), C: Staphylococcus hyicus (3), D: Staphylococcus cohni (3), E: Staphylococcus aureus (6)
R=Resistant, S= Susceptible, Number of isolates in parenthesis
Y-Axis = Number of isolates; X-Axis = List of antibiotics
Graph 2: Resistance/susceptibility patterns of nine E. coli isolates

Graph 3: Resistance/susceptibility patterns of nine Pseudomonas isolates
contact many pet owners have with their dogs and cats, it is especially important to extend the focus of antimicrobial research to include use in small animal Medicine [19, 20]. In our study 29 Staphylococcus isolated were identifies with certain degree of resistance level. Other studies have provided evidence also that microbe such as Staphylococci can be readily transferred in both directions [21, 22]. Domestic pets, livestock, wild birds, and other animals have recently been identified as carriers of Methicillin-resistant S. aureus (MRSA) in several countries and settings.

In conclusion, there is a need to empower multidisciplinary perspective to control the emerging antimicrobial resistance under One Health Initiative. Furthermore, antimicrobial stewardship, feasible guidelines and related proactive educational components to the clinicians and the users will be central to mitigate the unwanted consequence of the resistance problem and prevail the therapeutic use of the drugs.

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Conflict of Interest
No conflict of interest.

References