

Hospital and Ambulatory-Associated Animal Infections and Susceptibility to Antibiotics from 2019 to 2021

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Abstract

Introduction: The only academic veterinary hospital and diagnostic laboratory in the State of Oregon receives samples for bacteriologic diagnosis and patients from the whole state and neighbor states. Infectious diseases diagnosed from 2019 to 2021 were evaluated for antimicrobial susceptibility by site of infection and isolated microbes. **Methods:** Bacteria were cultured and identified using standard laboratory methods. Antibiotic susceptibility was determined by using the Kirby-Bauer assay. **Results:** The analysis revealed the most common bacteria causing infection in diverse anatomic sites. The study also evaluated the percent of antibiotic susceptibility among the bacteria isolated. In 2019, a very common infection, such as peritonitis, the organisms isolated from the abdominal fluid were 66.7% susceptible to amikacin and 83% of them susceptible to enrofloxacin. In 2021, however, the organisms isolated from the abdominal fluid were susceptible to amikacin in 42.9% of the cases and 57.1 % susceptible to enrofloxacin. In urine infection 71% of the bacteria were susceptible to trimethoprim/sulfamethoxazole in 2019, 63.9% in 2020 and 65.7% in 2021. Some pathogens, like Enterobacter, were susceptible to amikacin in 58.8% of the cases in 2019, 50% in 2020 and 25% in 2021. Escherichia coli's susceptibility to 3rd generation cephalosporins was 65.4% to cefovecin and cefpodoxime, and 21.8% to ceftiofur in 2019, 68.4% and 75.4% to cefovecin and cefpodoxime and 7% ceftiofur in 2020, and 61.6%, 72.6% and 13.7% for the three antimicrobials in 2021. Of note is the low percent of susceptibility among pathogens isolated from many infectious sites, creating the need for veterinarians to make difficult decisions. **Conclusion:** The increased resistance of bacteria to antibiotics is now present in the veterinary world. The increased

contact and interaction between animals and humans have extended the limited choice of antibiotics to treat infections in domestic animals, and therefore antibiotic stewardship programs must be implemented in veterinary practice.

Keywords

Antibiotic Susceptibility, Sites of Infection, Prevalence, Pathogens, Animal Infections

1. Introduction

Antibiotic resistance is now considered an emergency around the world, with many bacterial and fungal pathogens showing increasing resistance to almost all if not all of the available therapy [1]. Domestic animals live in close contact with humans and a number of studies have documented the transmission of pathogens between owners and their pets [2] [3]. Therefore, bacterial resistance to antibiotics in isolates causing animal infections has become an important issue of public health.

Animal infections are increasingly caused by multi-drug resistance bacteria. Several recent reports examining the resistance of Gram-positive and Gram-negative bacteria in animals [4] [5] have pointed out the existence of antibiotic resistant pathogens. One of the major concerns for veterinarians is that animal infections have a reduced repertoire of antibiotics that can be utilized due to restrictions related to food-associated animals or increased toxicities related to metabolism differences from humans. In the past, outbreaks of *Staphylococcus pseudintermedius*, *Enterobacter sp* have been associated with increased antibiotic resistance and the passage of genetic material among the hospital isolated bacteria [6]-[8].

The importance of collecting and analyzing data through the adoption of continual surveys and further dissemination of the information to veterinary communities is crucial for the practice of medicine. The up-to-date knowledge about antibiotic resistance in different areas of the world, provided by public health or related entities, would allow veterinarians to make decisions regarding drug prescriptions based on recent information. The gathering of antibiotic susceptibilities and resistance among pathogens causing animal infections would also allow for a more integrative approach to human medicine, and the realization that on many occasions humans and their pets can exchange microbes, increasing the chances of spreading antibiotic-resistant bacteria.

Hospital acquired infections are mainly caused by antibiotic resistant bacteria. In animals with a history of recent hospitalization, it should be carefully considered. Surgical site infections are in many cases a catastrophic complication that leads to high morbidity and mortality [5]. The evidence is particularly noticeable in orthopedic surgeries, which is a field that is commonly reliant on implants. In addition, those patients normally stayed in the hospital for prolonged periods of

time, which is a documented cause of an increase chance of infection [6]. *Staphylococcus aureus*, *Staphylococcus pseudintermedius* are a common cause of those infections [7]-[10], but recently, we evidenced extended-spectrum beta-lactamase (ESBL)—positive bacteria, more frequently *Escherichia coli*, becoming increasingly prevalent [9]. Over the years, the resistance to antibiotics such as quinolones and cephalosporins has become increasingly more common in bacteria isolated from humans and animals [11].

In this retrospective study the aim was to examine and analyze the antibiotic susceptibility pattern associated with sites of infection and the bacterial agents causing them, during the years of 2019 to 2021 in a teaching veterinary hospital in Oregon. The cases were both diagnosed on ambulatory animals, hospital admitted animals, as well as material collected and submitted by community clinics and field veterinarians. The results point to a potential gravity of the problem involving antibiotic resistance animals and potentially humans.

2. Methods and Materials

Surveillance and reporting:

The site of infections, infecting microbes and the susceptibility to antibiotics were identified for clinical samples sent to the Diagnostic Laboratory of the Carlson College of Veterinary Medicine (OVDL). The information was then put together and analyzed. No patient identification was obtained. Only one episode of infection per infection site was computed in the survey, to avoid duplication.

Microbiological methods of identification:

Microbial identification was performed by established phenotypic methods and criteria using agar plates and traditional biochemical tests as described previously [12]. All staphylococci were tested for methicillin-resistance using disk diffusion susceptibility to oxacillin/cefoxitin and/or the presence of penicillin binding protein 2 (PBP2') as determined by latex agglutination (Denka Seiken Kit, Hardy Diagnostics, Santa Maria, CA). Briefly, Coagulase positive *Staphylococcus* isolates were tested for penicillin binding protein 2 by latex agglutination (Oxoid™ PBP2' Latex Agglutination Test Kit) or by oxacillin disk (OX, 1 mg) \leq 17 mm for *Staphylococcus intermedius* group and cefoxitin disk (FOX, 30 mg) \leq 21 mm for *Staphylococcus aureus*. Coagulase negative *Staphylococcus* isolates were tested using FOX \leq 24 mm.

The laboratory reports all the identified organisms and sometimes gives specific recommendations regarding clinical importance. Notes were provided pointing out the probability that the isolates are colonizers or contaminants and all the analyzed data related to potential pathogens. Isolates were identified with Vitek MS MALDI-TOF (Biomérieux). Isolates were cultured for 16 - 24 hours and were tested from non-selective media by manufacturer recommendation.

Antibiotic susceptibility testing:

The isolated bacteria underwent antibiotic susceptibility testing using the Kirby-Bauer disk diffusion and minimum inhibitory concentration (MIC) was determined for the antibiotic-resistant bacteria. All isolates that did not have an

antibiotic susceptibility test were excluded from the study. Isolates were identified by various biochemical methods including Tryptic Soy Iron Agar (TSI, Remel), Detection of pyrrolidonyl arylamidase (PYR, Hardy Diagnostics), Christiansen's Urea (Remel), Simmons Citrate (Remel), Motility Test Medium with TTC (Hardy Diagnostics), and Coagulase (Rabbit Coagulase Plasma, BD BBL).

Antibiotic sensitivity testing (AST) was performed only on organisms for which interpretive criteria were available. The OVDL used Kirby-Bauer disk diffusion for AST and analysis is performed using the BIOMICV3 (Giles Scientific, Santa Barbara, CA) which utilizes the most current CLSI (Clinical and Laboratory Standard Institute) information and is updated annually. For the Kirby-Bauer assay, the concentration of antibiotics on the discs were amikacin (AN), 30 mg, amoxicillin/clavulanic acid (AMC), 20 mg/10 mg, ampicillin (AM), 10 mg, ceftiofur (CEF), 30 mg, cefpodoxime (CPD), 10 mg, cephalotin (CF), 30 mg, chloramphenicol (C), 30 mg, clindamycin (CM), 2 mg, enrofloxacin (ERO), 5 mg, gentamicin (GM), 10 mg, marbofloxacin (MAR), 5 mg, orbifloxacin (ORB), 10 mg, penicillin G (P), 10U, polymyxin B (PB), 300U, tetracycline (TE), 30 mg, tobramycin (TM), 10 mg, trimethoprim/sulfamethoxazole (SXT), 23.75 mg/1.25 mg, imipenem (IMP), 10 mg, azithromycin (AZT), 15 mg, ceftaroline (CPT), 30 mg, doxycycline (D), 30 mg, erythromycin (E), 15 mg, nitrofurantoin (NF), 300 mg, rifampin (RA), 5 mg, streptomycin (S), 10 mg, carbenicillin (CB100), 100 mg, ciprofloxacin (CIP), 5 mg, ceftiofur (CTF), 30 mg, piperacillin (PIP), 100 mg, ticarcillin/clavulanic acid (TCC), 75 mg/10 mg, and vancomycin (VA), 30 mg.

The minimum inhibitory concentration (MIC) was reported when available. All isolates were initially tested on 17 antibiotics at standard concentrations unless otherwise noted (amikacin (AN), amoxicillin/clavulanic acid (AMC), ampicillin (AM), ceftiofur (CEF), cefpodoxime (CPD), cephalotin (CF), chloramphenicol (C), clindamycin (CM), enrofloxacin (ERO), gentamicin (GM), marbofloxacin (MAR), orbifloxacin (ORB), penicillin G (P), polymyxin B (PB), tetracycline (TE), Tobramycin (TM), Trimethoprim/sulfamethoxazole (SXT). For *Pseudomonas* sp., imipenem (IMP) was added. Chloramphenicol was not reported for urinary tract infections. No cephalosporins were reported for enterococci. Ampicillin, penicillin, and amoxicillin were reported as "resistant" for methicillin resistant staphylococci regardless of the disk diffusion results. If drug resistance was observed, additional panels of antibiotics were tested. Methicillin-resistant staphylococci were tested against 7 additional antibiotics (azithromycin (AZT), ceftaroline (CPT), doxycycline (D), erythromycin (E) nitrofurantoin (NF), rifampin (RA), streptomycin (S)). Resistant Gram negative organisms were tested against 9 additional antibiotics (AZT, carbenicillin (CB100), ciprofloxacin (CIP), ceftiofur (CTF), IMP, NF, piperacillin (PIP), ticarcillin/clavulanic acid (TCC). Aminoglycoside-resistant enterococci were tested against high level gentamicin (120 mg) and streptomycin (300 mg). For monitoring purposes vancomycin (VA) was tested on resistant Gram positive organisms but was not reported [13].

Data analysis:

The site of infections, bacterial isolate(s), and the susceptibility to antibiotics

were identified from the cases. The information was assembled and analyzed. Only one episode of infection was included in the survey, to avoid duplication. For the analysis, we counted each patient as a separate event. In cases where two microorganisms were isolated from the same patient, it was considered one episode of infection. If the same organism was isolated more than once, the most resistant isolate was included. Descriptive analysis of the extracted data of interest was standardized. The sensitivity and specificity of the tests applied were evaluated using positive and negative controls as reference standards. The t-test was used to compare infections from year-to-year.

3. Results

Sites of infection:

The number of positive cultures per year was 481 for 2019, 272 for 2020 and 327 for 2021. In 2019 the most common infections were by order, urinary tract infection, surgical site infection, bone infection, and abscess material. In 2020, the most common infection sites were urinary tract, non-surgical wound, surgical site infection, blood and abscess. During the year of 2021, the most frequent infections were from the urinary tract, surgical site/wound, bone infection, abscess and material from masses/subcutaneous nodules. **Table 1** shows the breakdown by site of infection of samples submitted during the years 2019 to 2021.

Table 1. Number of microbiological diagnosed infections by site of infection and animal species.

Infection Site	Species	Number of Cases per Year		
		2019	2020	2021
Abdominal fluid	Canine	5	-	6
	Equine	4	2	1
	Total number of cases	9	2	7
Abscess material	Bovine	-	1	-
	Camelid	-	1	-
	Canine	17	3	1
	Caprine	1	5	5
	Equine	10	4	11
	Feline	-	-	1
	Swine	1	-	1
	Total number of cases	29	14	19
Adipose tissue	Feline	2	-	-
	Total number of cases	2	0	0
BALF	Canine	13	10	3
	Feline	4	3	-
	Total number of cases	17	13	3
Bile fluid	Canine	2	2	5
	Caprine	1	-	-
	Total number of cases	3	2	5

Continued

Blood	Canine	4	8	4
	Equine	7	11	3
	Total number of cases	11	19	7
Bone	Canine	6	8	13
	Equine	-	-	2
	Total number of cases	6	8	15
Catheter	Equine	-	-	1
	Total number of cases	0	0	1
Colon swab	Canine	-	-	1
	Total number of cases	0	0	1
Cyst fluid	Canine	-	-	2
	Total number of cases	0	0	2
Ear swab	Canine	18	4	1
	Caprine	-	-	3
	Feline	-	1	3
	Total number of cases	18	5	7
Esophageal swab	Canine	-	2	-
	Swine	2	-	-
	Total number of cases	2	2	0
Eye swab	Equine	-	-	1
	Total number of cases	0	0	1
Foot swab (wound)	Canine	-	4	6
	Total number of cases	0	4	6
Gallbladder	Canine	-	-	3
	Total number of cases	0	0	3
Joint fluid	Canine	13	10	-
	Equine	-	3	1
	Total number of cases	13	13	1
Liver tissue	Canine	-	1	4
	Feline	3	-	-
	Total number of cases	3	1	4
Lung biopsy	Canine	-	1	2
	Total number of cases	0	1	2
Lymph node tissue	Canine	1	4	-
	Total number of cases	1	4	0
Mass material	Canine	-	4	5
	Caprine	-	-	2
	Equine	-	5	4
	Feline	-	-	2
	Total number of cases	0	9	13

Continued

Nasal material	Canine	1	-	6
	Caprine	1	-	-
	Equine	18	-	4
	Feline	-	1	-
	Total number of cases	20	1	10
Peritoneal swab	Canine	1	-	1
	Equine	-	-	4
	Total number of cases	1	0	5
Pleural fluid	Canine	2	2	3
	Total number of cases	2	2	3
Semen	Canine	-	1	-
	Total number of cases	0	1	0
Skin	Canine	-	1	4
	Caprine	-	-	1
	Swine	2	-	-
	Total number of cases	2	1	5
Spinal fluid	Canine	2	1	-
	Total number of cases	2	1	0
Surgical site/wound	Canine	26	23	33
	Caprine	1	-	1
	Equine	20	11	22
	Feline	2	-	-
	Total number of cases	49	34	56
Tracheal material	Canine	2	-	-
	Caprine	1	-	-
	Equine	10	10	8
	Feline	-	1	-
	Total number of cases	13	11	8
Umbilical swab	Bovine	2	-	2
	Equine	2	-	5
	Total number of cases	4	0	7
Urine	Camelid	-	2	-
	Canine	98	62	100
	Caprine	3	4	-
	Equine	4	9	1
	Feline	8	6	11
	Swine	1	-	-
	Total number of cases	114	83	112
Uterine swab	Canine	1	-	1
	Equine	3	-	1
	Feline	-	1	-
	Total number of cases	4	1	2

Continued

Vaginal swab	Canine	1	-	5
	Caprine	-	-	2
	Swine	-	-	1
	Total number of cases	1	0	8
Infection Sites—Others				
Swabs	Bovine	-	1	-
	Canine	44	24	5
	Caprine	-	-	1
	Equine	15	11	2
	Feline	1	-	2
	Total number of cases	60	36	10
Total number of cases per year		386	268	323

Pathogens associated with infections:

As shown in **Table 2**, a large number of pathogens have been associated with infections in 2019. Of note are the number of *Staphylococcus* cases in bone infections and surgical infections and the number of ear infections in dogs caused by *E. coli*, probably secondary to contamination by scratching the ears with *E. coli*-contaminated paws. The same pattern was observed in 2020 and 2021, with the isolation of *E. coli* and other intestinal organisms such as *Enterobacter sp* and *Enterococcus faecalis* from ear infections. A similar occurrence was observed in an eye infection caused by *Enterococcus faecalis* in 2021.

An interesting observation in 2020 was the isolation of *Acinetobacter baumannii* from the blood of a dog [14]. In case the pathogen was isolated in connection with a hospital stay, it should raise concern because of the ability of the pathogen to adapt to a hospital environment. The great majority of the urinary tract infections during the three years were caused by *E. coli*. Regarding cases of respiratory infection, besides the isolation of *Pasteurella*, which was a common isolate from dogs and cats, the isolation of *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* bring concerns about the source of infection and the possibility of co-habitation with an immunosuppressed individual. Other bacteria isolated from the respiratory tract are a small percentage of Gram-negative bacilli (Enterobacteriales), and pathogenic *Streptococcus*.

Table 2. Bacterial species associated with infection by infection site and bacteria for 2019, 2020, and 2021.

Infection Site	Bacteria	Number of Cases		
		2019	2020	2021
Abdominal fluid	<i>Enterococcus sp.</i>	1	1	
	Total number of cases	1	1	
Abscess material	<i>Enterococcus sp.</i>		2	1
	<i>Escherichia coli</i>	2	1	1

Continued

	<i>Pasteurella caballi</i>	1	2	
	<i>Staphylococcus aureus</i>	3		3
	<i>Staphylococcus pseudintermedius</i>	4	1	
	<i>Staphylococcus</i> sp.	2	2	4
	Total number of cases	12	8	9
Bacterial isolate - Source unknown	<i>Actinobacillus equuli</i> spp. <i>Equuli</i>	1	1	
	<i>Enterobacter</i> sp.	5	2	
	<i>Enterococcus</i> sp.	2	4	
	<i>Escherichia coli</i>	6	2	
	<i>Klebsiella</i> sp.	2	1	
	<i>Pasteurella canis</i>	2	1	
	<i>Staphylococcus aureus</i>	4	2	
	<i>Staphylococcus pseudintermedius</i>	9	6	
	<i>Staphylococcus</i> sp.	17	4	
	<i>Streptococcus</i> sp.	5	1	
	Unidentified species	1	3	
	Total number of cases	54	27	
BALF	<i>Escherichia coli</i>	3	3	
	<i>Pasteurella canis</i>	2	1	
	<i>Pseudomonas aeruginosa</i>	1	1	
	<i>Stenotrophomonas maltophilia</i>	1	1	
	Total number of cases	7	6	
Bile fluid	<i>Escherichia coli</i>	1		1
	Total number of cases	1		1
Bladder	<i>Escherichia coli</i>		1	2
	Total number of cases		1	2
Blood	<i>Escherichia coli</i>	4		2
	<i>Staphylococcus pseudintermedius</i>	1	1	
	<i>Staphylococcus</i> sp.	3	3	
	Unidentified species	1	6	2
	Total number of cases	9	10	4
Bone	<i>Staphylococcus pseudintermedius</i>	1	6	6
	<i>Staphylococcus</i> sp.	4	2	
	Total number of cases	5	8	6
Bronchoalveolar lavage	<i>Stenotrophomonas maltophilia</i>		3	1
	Total number of cases		3	1
Ear swab	<i>Escherichia coli</i>	1	1	4
	<i>Pseudomonas aeruginosa</i>	1		1
	<i>Staphylococcus</i> sp.	7	1	
	<i>Streptococcus</i> sp.	2		1
	Total number of cases	11	2	6

Continued

Foot swab	<i>Staphylococcus pseudintermedius</i>	3	1	
	Total number of cases	3	1	
Joint fluid	<i>Escherichia coli</i>	1	1	
	<i>Staphylococcus aureus</i>	1	4	
	<i>Staphylococcus pseudintermedius</i>	3	1	
	<i>Staphylococcus</i> sp.	3	1	
	<i>Streptococcus equi</i> subsp. <i>Zooepidemicus</i>	1	1	
	<i>Streptococcus</i> sp.	5	3	
	Total number of cases	14	11	
Liver	<i>Streptococcus</i> sp.	1	1	
	Total number of cases	1	1	
Lung swab	<i>Streptococcus</i> sp.	1	1	
	Total number of cases	1	1	
Mass material	<i>Escherichia coli</i>	1	2	
	<i>Staphylococcus aureus</i>	2	1	
	<i>Staphylococcus</i> sp.	1	2	
	Total number of cases	4	5	
Nasal material	<i>Streptococcus equi</i> subsp. <i>Zooepidemicus</i>	3	1	
	Total number of cases	3	1	
Surgical site/wound swab	<i>Enterobacter</i> sp.	2	2	3
	<i>Enterococcus faecalis</i>		1	2
	<i>Enterococcus</i> sp.	5	4	2
	<i>Escherichia coli</i>	7	3	4
	<i>Pasteurella canis</i>	3	2	2
	<i>Proteus mirabilis</i>	1	1	
	<i>Pseudomonas aeruginosa</i>	1	1	2
	<i>Staphylococcus aureus</i>	1	1	2
	<i>Staphylococcus pseudintermedius</i>	6	5	7
	<i>Staphylococcus</i> sp.	14	4	3
	<i>Streptococcus equi</i> subsp. <i>Zooepidemicus</i>		4	1
	<i>Streptococcus</i> sp.		1	6
	<i>Unidentified species</i>	2		1
Total number of cases	42	29	35	
Swab - Source unknown	<i>Staphylococcus</i> sp.	1	1	
	Total number of cases	1	1	
Tissue - Source unknown	<i>Escherichia coli</i>		3	2
	<i>Staphylococcus pseudintermedius</i>		1	1
	Total number of cases		4	3

Continued

Tracheal material	<i>Actinobacillus equuli</i>		2	1
	<i>Enterobacter</i> sp.	2	1	
	<i>Klebsiella</i> sp.	1	1	
	<i>Pasteurella caballi</i>	1		1
	<i>Pasteurella</i> sp.	1	2	
	<i>Streptococcus equi</i> subsp. <i>Zooepidemicus</i>	1	4	2
	<i>Streptococcus</i> sp.	1		1
	Total number of cases	7	10	5
Urine	<i>Enterobacter</i> sp.	1	3	2
	<i>Enterococcus</i> sp.	11	12	4
	<i>Escherichia coli</i>	47	38	37
	<i>Klebsiella</i> sp.	2	1	
	<i>Proteus mirabilis</i>	7	5	9
	<i>Pseudomonas aeruginosa</i>	4	2	1
	<i>Pseudomonas</i> sp.	1	2	
	<i>Staphylococcus pseudintermedius</i>	5	6	5
	<i>Staphylococcus</i> sp.	12	7	14
	<i>Streptococcus equi</i> subsp. <i>Zooepidemicus</i>	1	7	
	<i>Streptococcus</i> sp.	11		5
	<i>Unidentified species</i>	3		1
	Total number of cases	105	83	78
Uterus swab	<i>Escherichia coli</i>		1	1
	Total number of cases		1	1

Antibiotic susceptibility associated with infection sites:

Serious infections usually require the use of antibiotics in a “blind” manner, until results of the bacteriological tests become available. **Table 3** shows the percentage of susceptible bacteria causing infection at different sites. When comparing the three years, it became clear that in certain infections, antibiotic resistance is becoming more prevalent. Of note are infections of the bones and infections of joints. Both sites are associated with infection by bacteria with high levels of resistance to antibiotics.

Much like human infections, infections caused by *Enterobacter spp* and *Klebsiella spp* are increasingly linked to antibiotic resistance. The level of resistance of *E. coli* strains to third generation cephalosporins is also of concern, as is the resistance among *Staphylococcus aureus* and *Staphylococcus pseudintermedius* strains (**Table 4**).

Comparing infection etiology and susceptibility among years:

The etiology of the infection when one looks at the three years of the investigation, did not vary much, except for ear infection that had a predominance of

Streptococcus in 2019 and *E. coli* in 2021. The susceptibility of infecting bacteria to antibiotics, and did not vary significantly from year-to-year (Table 3).

Table 3. Susceptibility to antibiotics by site of infection.

Site of Infection	Susceptibility to the 5 Most Active Antibiotics (% of the Total)		
	2019	2020	2021
Abdominal fluid	Gentamicin (83.3)	Ampicillin (100)	Amoxicillin/Cla (85.7)
	Enrofloxacin (83)	Clindamycin (100)	Chloramphenicol (71)
	Amikacin (66.7)	Amoxicillin/clav (100)	Enrofloxacin (57.1)
	Ceftiofur (50)	TMP/sulpha (50)	Amikacin (42.9)
	Ampicillin (50)	Penicillin (50)	Cefovecin (42.9)
Abscess material	Gentamicin (81)	Enrofloxacin (78)	Amikacin (57.1)
	Enrofloxacin (81)	Amikacin (71.4)	Enrofloxacin (57.1)
	Amikacin (76.2)	TMP/supha (64.3)	TMP/sulpha (57.1)
	Ceftiofur (42.9)	Ceftiofur (57.1)	Ceftiofur (42.9)
	Chloramphenicol (42.9)	Gentamicin (50)	Gentamicin (42.9)
BALF	Amikacin (82.9)	Chloramphenicol (76.9)	Enrofloxacin (100)
	Chloramphenicol (82.3)	Enrofloxacin (76.9)	TMP/sulpha (100)
	TMP/sulpha (82.3)	Gentamicin (76.9)	Chloramphenicol (100)
	Gentamicin (76.5)	TMP/sulpha (76.9)	Amikacin (66.7)
	Enrofloxacin (70.6)	Amikacin (69.2)	Gentamicin (66.7)
Blood	Amikacin (100)	TMP/supha (68.5)	Amikacin (85.7)
	Gentamicin (100)	Ampicillin (47.4)	Enrofloxacin (85.7)
	Enrofloxacin (100)	Amikacin (36.8)	Gentamicin (85.7)
	TMP/supha (100)	Enrofloxacin (36.1)	Chloramphenicol (71.4)
	Ceftiofur (63.3)	Gentamicin (26.3)	Ampicillin (71.4)
Bone	Chloramphenicol (100)	Amikacin (75)	Amikacin (73.3)
	Amikacin (66.7)	Rifampin (50)	Enrofloxacin (60)
	Enrofloxacin (66.7)	Clindamycin (50)	Gentamicin (60)
	Gentamicin (66.7)	Ceftaroline (50)	TMP/supha (60)
	TMP/sulpha (66.7)	Doxycycline (50)	Chloramphenicol (53)
Ear swab	Amikacin (77.8)	Amoxicillin/clav (80)	Amikacin (71.4)
	Gentamicin (50)	Tetracycline (80)	Enrofloxacin (71.4)
	Chloramphenicol (38.9)	Enrofloxacin (60)	Gentamicin (57.1)
	Enrofloxacin (33.3)	Amikacin (40)	TMP/sulpha (57.1)
	Cefovecin (27.8)	Cefovecin (40)	Cefpodoxime (42.9)
Joint fluid	TMP/sulpha (84.2)	Amikacin (61.5)	
	TMP/sulpha (84.2)	TMP/sulpha (53.5)	
	Enrofloxacin (52.6)	Gentamicin (46.2)	No cases
	Gentamicin (42.1)	Chloramphenicol (38.5)	
	Ampicillin (42.1)	Rifampin (23.1)	

Continued

Liver biopsy	Chloramphenicol (100)		Chloramphenicol (100)
	Amikacin (33.3)		Amoxicillin/clav (75)
	Amoxicillin/Clavulanate (33.3)	No cases	Amikacin (50)
	Enrofloxacin (33.3)		Cefovecin (50)
	Rifampin (33.3)		Enrofloxacin (50)
Nasal material.	Amikacin (70)	TMP/sulpha (100)	Ampicillin (70)
	Enrofloxacin (65)	Enrofloxacin (88.9)	TMP/sulpha (70)
	Ceftiofur (40)	Amikacin (77.8)	Amikacin (60)
	Gentamicin (40)	Gentamicin (77.8)	Enrofloxacin (60)
	TMP/supha (35)	Ampicillin (55.6)	Gentamicin (60)
Skin tissue.	Ampicillin (100)		Enrofloxacin (100)
	Ceftiofur (100)		TMP/sulpha (100)
	Gentamicin (100)	All susceptible	Amikacin (50)
	TMP/sulpha (100)		Ampicillin (50)
Surgical wound	Florfenicol (100)		Cefovecin (50)
	Amikacin (69.6)	TMP/sulpha (62.1)	TMP/sulpha (58.9)
	Enrofloxacin (67.4)	Amikacin (58.6)	Enrofloxacin (53.6)
	Gentamicin (56.5)	Enrofloxacin (55.2)	Amikacin (46.4)*
	TMP/sulpha (54.3)	Gentamicin (55.2)	Ampicillin (44.6)
Tracheal material	Chloramphenicol (52.2)	Amoxicillin (48.3)	Gentamicin (42.9)*
	Enrofloxacin (92.5)	TMP/sulpha (72.7)	Gentamicin (75)
	TMP/sulpha (84.6)	Ampicillin (63.6)	Ceftiofur (62.5)
	Ceftiofur (76.9)	Amikacin (54.5)	Amikacin (50)
	Amikacin (69.2)	Gentamicin (54.5)	Ampicillin (50)
Urine	Gentamicin (69.2)	Enrofloxacin (54.5)	Enrofloxacin (37.5)*
	Enrofloxacin (75.9)	Amikacin (68.7)	Gentamicin (68.6)
	Amikacin (75)	Gentamicin (65.1)	Amikacin (67.6)
	Gentamicin (71.4)	TMP/sulpha (63.9)	Amoxicillin/clav (67.6)
	TMP/supha (71.4)	Amoxicillin/cla (55.4)	TMP/sulpha (65.7)
Uterine swab	Amoxacillin/clav (60.7)	Enrofloxacin (53)	Enrofloxacin (57.1)
	TMP/sulpha (75)		Amikacin (50)
	Ampicillin (75)		Ampicillin (50)
	Enrofloxacin (50)	all isolates susceptible	Enrofloxacin (50)
	Amikacin (50)		Ceftiofur (50)
	Gentamicin (50)		TMP/supha (50)

Susceptibility to the remaining antibiotics was lower than the ones reported on the table. * $p < 0.05$ compared to 2020.

Table 4. Susceptibility to antibiotics by bacteria pathogens.

Bacteria	Antibiotics with Activity (% Susceptible Bacteria)		
	2019	2020	2021
<i>Actinobacillus equuli</i> ssp. Equuli	Enrofloxacin (100)	Enrofloxacin (100)	Amikacin (100)
	Gentamicin (100)	Amikacin (100)	Enrofloxacin (100)
	Ticarcillin (100)	Ceftiofur (100)	Ceftiofur (100)
	Ceftiofur (100)	Ticarcillin (100)	Gentamicin (100)
	Amikacin (66.7)	Gentamicin (100)	Penicillin (80)
<i>Enterobacter</i> sp.	Amikacin (58.8)	Amikacin (50)	Enrofloxacin (35.7)
	Enrofloxacin (52.9)	Enrofloxacin (40)	Amikacin (25)*
	TMP/supha (52.9)	Gentamicin (40)	Gentamicin (25)*
	Gentamicin (47.1)	TMP/supha (30)	TMP/supha (12.5)*
	Cefovecin (35.3)	Cefovecin (20)	Cefovecin (12.5)*
<i>Enterococcus</i> sp	Amikacin (79.3)	Amoxicillin (91.7)	Amoxicillin (72.7)*
	Penicillin (75.9)	Amikacin (79.2)	Ampicillin (72.7)
	Amoxicillin (72.4)	Doxycycline (75)	Penicillin (63.6)
<i>Enterococcus faecali</i>		Amoxicillin (100)	Penicillin G (100)
		Ampicillin (100)	Amoxicillin (94.7)
			Doxycycline (63.2)
<i>Enterococcus faeciu</i>			Doxycycline (63.6)
			Amikacin (45.5)
			Amoxicillin (36.4)
<i>E. coli</i>	Amikacin (98.7)	Amikacin (93)	Amikacin (93.2)
	Enrofloxacin (96.2)	TMP/supha (91.2)	Gentamicin (90.4)
	TMP/supha (85.9)	Gentamicin (89.5)	Enrofloxacin (83.2)
	Gentamicin (88.5)	Enrofloxacin (78.9)	TMP/supha (80.8)
	Cefovecin (65.4)	Cefovecin (68.4)	Cefpodoxime (72.6)
	Ampicillin (60.3)	Ampicillin (57.9)	Ampicillin (52.1)
<i>Klebsiella pneumoniae</i>	Enrofloxacin (70)	Amikacin (66.7)	Amikacin (71.4)
	TMP/supha (70)	Imipenem (66.7)	TMP/supha (71.4)
	Gentamicin (60)	Enrofloxacin (33.3)	Gentamicin (71.4)
	Ceftiofur (30)	Gentamicin (33.3)	Enrofloxacin (57.1)
	Cefpodoxime (30)		Cefovecin (42.9)
<i>Pasteurella canis</i>	Enrofloxacin (100)	Amikacin (100)	Amikacin (100)
	Gentamicin (100)	Ampicillin (100)	Ampicillin (100)
	TMP/supha (100)	Cephalothin (100)	Enrofloxacin (100)
	Amikacin (90)	Enrofloxacin (100)	TMP/supha (100)
	Cefovecin (90)	TMP/supha (100)	Cephalothin (50)
	Amoxicillin (80)		

Continued

<i>Pasteurella multocida</i>	Ampicillin (100) TMP/supha (100) Amikacin (75) Enrofloxacin (75) Ceftiofur (75) Cephalothin (75)		One strain susceptible to Amikacin, Amoxicillin, Cephalothin, Enrofloxacin, Enrofloxacin, TMP/sulpha.
<i>Proteus mirabilis</i>	Amikacin (100) Amoxicillin (100) Cefovecin (100) Gentamicin (100) Enrofloxacin (90.9)	Amikacin (100) Amoxicillin (100) Gentamicin (100) Cefovecin (85.7) Enrofloxacin (85.7)	Amoxicillin (100) Amikacin (100) Cefovecin (90) Gentamicin (90) Enrofloxacin (80)
<i>P. aeruginosa</i>	Amikacin (100) Imipenem (80) Gentamicin (50) Enrofloxacin (50)	Amikacin (100) Enrofloxacin (66.7) Tobramycin (33.3) Imipenem (83.3)	Amikacin (100) Tobramycin (100) Gentamicin (100)
<i>S. aureus</i>	Amikacin (83.3) Enrofloxacin (75) TMP/supha (75) Gentamicin (66.7) Rifampin (58.3) Oxacillin (16.7)	Amikacin (69.2) Enrofloxacin (69.2) Gentamicin (69.2) TMP/supha (69.2) Rifampin (46.2)	Amikacin (90.9) Enrofloxacin (90.9) Ceftiofur (72.7) TMP/supha (72.7) Ampicillin (54.5)
<i>S. pseudintermedius</i>	Amikacin (94.6) Enrofloxacin (64.9) Gentamicin (62.2) Cefovecin (48.6) TMP/sulpha (45.9) Oxacillin (13.5)	Amikacin (80.6) Gentamicin (55.6) Rifampin (52.8) Ceftaroline (47.2) TMP/sulpha (47.2) Oxacillin (2.8)	Amikacin (74.4) Gentamicin (66.7) Enrofloxacin (64.1) TMP/sulpha (47.2) Cefovecin (53.8)
<i>S. schleiferi</i>	Amikacin (80) TMP/supha (80) Cefovecin (60) Gentamicin (60) Amoxicillin/Cla (60)	All susceptible	Amikacin (100) Gentamicin (100) TMX/sulpha (100) Enrofloxacin (66.7) Ceftiofur (66.7)
<i>S. epidemidis</i>			TMP/sulpha (85.7) Amikacin (71.4) Gentamicin (71.4) Cefovecin (42.9) Amoxicillin (42.9)
<i>Strepto equi Subp Zooepidemicus</i>	Ampicillin (100) Penicillin (100) TMP/supha (71.4)	Ampicillin (100) TMP/supha (84.3) Penicillin (69.2)	Penicillin (100) Ampicillin (90) Doxycycline (50)

Continued

<i>S. maltophilia</i>	Chloramphenicol (100)	Chloramphenicol (100)	Chloramphenicol (100)
	Enrofloxacin (100)	Enrofloxacin (100)	TMP/sulpha (100)
		TMP/sulpha (100)	Marbofloxacin (100)
			Enrofloxacin (50)
<i>Streptococcus sp.</i>	TMP/sulpha (82.9)	TMP/sulpha (57.7)	TMP/sulpha (75)
	Ampicillin (65.9)	Ampicillin (38.8)	Penicillin (62.5)
	Penicillin (48.8)	Penicillin (44.6)	Ampicillin (54.2)
	Enrofloxacin (43.3)	Gentamicin (30.8)	Clindamycin (45.8)
<i>A. baumannii</i>		Enrofloxacin (100)	
		Amikacin (50)	
	Not isolated	Gentamicin (50)	Not isolated
		Ceftiofur (50)	
		Ticarcillin (50)	

Susceptibility to the remaining antibiotics was lower than the ones reported on the table. *P < 0.05 compared to 2020.

4. Discussion

The estimated burden of bacterial infection in humans is many million cases every year. The collective knowledge about the susceptibility of bacteria isolated causing infections in animals is less understood. The existence of government sponsored program of antibiotic stewardship is important, and efforts to have it established are slowly showing success in the control of infections associated with healthcare in humans.

In the current retrospective analysis we carried out the evaluation of three years of infections identified at the Oregon Veterinary Diagnostic Laboratory. The laboratory is center of a large region, and diagnose animal infections from most of the State of Oregon. A broad look at the data determined that abscess, respiratory infection, nasal material, surgical infection, and urinary infection were the most common cases encountered during the three years studied. The results provided as infections per site and susceptibility to antibiotics by bacterial pathogen show important findings related to animal infections.

One of the interesting observation is that the percentage of susceptible microbes to antibiotics in some of the infections is very low. As examples, bone-associated as well as joint fluid-associated infections were caused by microorganism with high degree of resistance to many classes of antibiotics. In the case of bone-associated infections, either post-surgery or secondary to an open fracture, a large percent of the infections were caused by *S.pseudintermedius*, possibly acquired in the hospital environment since their susceptibility pattern to antibiotics is quite difference from the susceptibility of *S.pseudintermedius* acquired from a community source, as can be observed in cases of community acquired skin infections [7] [15]. In regard to joint infections, the bacteria obtained from the joint fluid over the

three years showed to be quite resistant to the majority of the antibiotics, raising the questions about the source of those organisms, and the fitness of the pathogen to reach the joint space without breaching the skin. Those are infections difficult to treat, since they involve bones as areas with limited access for antibiotics, and tissue necrosis associated with decrease blood supply.

Among the common infections, infections in the urinary tract we commonly observed, both as uncomplicated as well as complicated infections. The presence of urinary stones for example, increased the chances of infection caused by *Staphylococcus pseudintermedius* and other skin or fur associated bacteria. The important aspect of the observation is that Gram-positive cocci belonging to the *S. aureus*, *S. pseudintermedius* and *Staphylococcus spp* as well as *Streptococcus spp*, are described to be able to form biofilm on the surface of different salt stones [8] [14] [16]. It has been described that bacterial reservoir of *S. aureus*, that evades the host immune system and antibiotics, are not only found in implants but also deep into the bone, due to invasion of the osteocyte canulo-cunicular network [17]. The presence of sporadic bacteremia with passage of a blood filtrate and bacteria to the inflamed joint, could also explain the findings [18].

Infections caused by organisms usually encountered in the intestinal tract, were commonly identified among the clinical samples from distant locations in the body. For example, bronchoalveolar lavage procedures were able to isolate many enterobacteria, which is not expected to be associated with oropharynx, as source of infection. Probably the animal contaminated the paws with feces and spread that for the oropharynx and high airways. Equally, ear infections were caused mostly by enteric and environmental bacteria, which call the attention for the animals scratching the ears, with contaminated paws a possible seeding mechanism.

Important information that can be obtained from the study is the percentage of susceptible bacteria isolated from each source. **Table 3** shows that depending on the site of infection, the susceptibility of the organisms isolated caused some concern. For example, the susceptibility of abscess material to antibiotic is in general poor, with exception of amikacin, TMP/SMX and enrofloxacin. If the infection is a close abscess, sulfonamides should have questionably activity, and probably should not be prescribed. In blood infections there was significant differences between susceptibility of pathogens from year to year. Another example was joint infections which showed low susceptibility to the great majority of the antibiotics during the years of 2019 and 2020. Third generation cephalosporins for example, should not be used as empirical therapy, since the great majority of joint infections are caused by Gram-positive cocci. In contrast, bacteria isolated causing skin infections were for the great majority susceptible to many classes of antibiotics. The observation of high levels of antibiotic resistance is also valid for bacteria isolated from livestock, either causing infection or just colonizing the host. In some locations bacteria isolated from livestock, specially *E. coli*, shows high level of resistance to many classes of antibiotics, impacting food security and potential dissemination or horizontal transfer of antibiotic resistant genes to different

environments [19].

Among the Gram-negative bacteria *Enterobacter sp* and *Klebsiella sp* are the pathogens that recently joined *Pseudomonas aeruginosa* as main challenges for therapy. The percent of these bacteria susceptible to the diverse classes of antibiotics is concerning low, not reaching even the level of 60%. Among the Gram-positive bacteria, *Enterococcus faecium* like in human-related infections, is quite resistant to the majority of antibiotics [20] [21]. In human hospitals, surveillance and active decontamination has reduced infections by *S. aureus*, although many without eradication of the strain [22].

5. Conclusions

In general, the pattern of susceptibility to antibiotics is quite similar for common pathogens in human and animal infections. The role of the animal microbiome is interesting, and thus far not well explored as a reservoir of antibiotic-resistant genes, between animals and co-inhabitant humans [23]-[25]. That observation can certainly apply to domestic animals, but is less likely for many of the wildlife, although a recent study from Sweden scientists identified antibiotic resistant genes in the teeth of 82 bears in the collection of the Swedish National Museum, dating back to 1842 [26]. The study also showed that between 1951 and 1970, the number of antibiotic-resistant genes doubled in comparison with those before the antibiotic era. Although antibiotic-resistant bacteria causing infection in wildlife can be investigated, much of the wildlife recovered from necropsy has had contact with soil and water that is potentially contaminated with antibiotics.

This study, looking at three recent years in a veterinary hospital and external patients from the State of Oregon, clearly shows that antibiotic resistance is a disseminated problem, affecting humans and animals. The establishment and inclusion of surveys of antibiotic resistance in animals in public health approaches to address the issue is certainly necessary.

Participation

AR: Analysis of the data, wrote part of the original draft. SS: Performed assays, wrote portions of the manuscript, and edited the manuscript, MM, performed the assays, and edited the manuscript. AM, performed assays, and edited the manuscript. AM, data analysis, edited the manuscript. LEB, the original idea, directed the study, wrote portions of the manuscript, and obtained funds for the study.

Conflicts of Interest

None of the authors have any conflict to declare.

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