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Antimicrobial Susceptibility Patterns of Bacteria Associated With Hepatobiliary Disease in Dogs and Cats (2010–2019)

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ABSTRACT

Background: Description of antibiotic susceptibility of isolates from dogs and cats with hepatobiliary disease is limited.

Objectives: To describe antibiotic susceptibility patterns of bacteria associated with hepatobiliary disease in dogs and cats over a 10 year-period.

Animals: Three hundred nine dogs and cats.

Methods: Bacterial species and antibiotic susceptibility data from positive bile and liver tissue cultures were reviewed from both a Veterinary Teaching Hospital and a private laboratory. Prevalence of multidrug-resistant (MDR) bacteria was assessed, along with its association with previous antibiotic administration.

Results: A total of 343 bacterial isolates were included from 310 cultures. Monobacterial cultures were more frequent (91%, 283/310). Gram-negative bacteria were predominant (67%, 227/340), with *Escherichia coli* (49%, 136/340), *Staphylococcus* spp. (14%, 47/340), and *Enterococcus* spp. (10%, 34/340) being the most prevalent isolates. Resistance of gram-negative bacteria were: amoxicillin-clavulanic acid (33%, 70/214), aminopenicillins (47%, 96/205), and fluoroquinolones (16%, 67/417); for gram-positive bacteria: amoxicillin-clavulanic acid (12%, 5/41), aminopenicillins (16%, 11/67), and fluoroquinolones (17%, 35/111). Resistance was significantly higher for aminopenicillins, first-generation cephalosporins, TMPS, tetracyclines, and fluoroquinolones during 2010–2014 compared to 2015–2019. MDR isolates comprised 40% (135/430) of all isolates, 30% (41/136) of *E. coli*, and 68% (23/34) of *Enterococcus* spp. A significantly higher incidence of MDR bacteria was observed in animals with previous antibiotic treatment (81%, 17/21) compared to those without (30%, 22/75; $p < 0.001$).

Conclusion and Clinical Importance: Conducting culture and sensitivity testing remains crucial in dogs and cats suspected of hepatobiliary infection to ensure effective treatment.

Abbreviations: MDR, multidrug resistant; PL, private laboratory; TMPS, sulfonamides associated with trimethoprim; VTH, Veterinary Teaching Hospital.

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1 | Introduction

Hepatobiliary bacterial infections can lead to severe illness in dogs and cats. Most of the time, infection is associated with other hepatobiliary conditions such as cholelithiasis, biliary mucocele, or pancreatitis [1–7]. The spectrum of bacteria species involved is well described, with *Escherichia coli* and *Enterococcus* spp. being the two most commonly identified bacteria species [1–4, 6, 8–13].

Documentation and surveillance of antibiotic resistance remain essential, especially to better guide clinicians in selecting empiric treatments, given the already limited antibiotic options in veterinary medicine. While patterns of antibiotic resistance in bacteria associated with hepatobiliary infections are described in previous studies, these reports remain rare and are based on only a limited number of isolates [2, 4, 11]. Multidrug resistant (MDR) bacteria are also reported in hepatobiliary infections in dogs and cats [2, 4, 11]. However, the frequency of MDR hepatobiliary bacteria, their antibiotic resistance patterns, and the potential factors associated with their occurrence are unknown.

Thus, the major aim of this retrospective study was to document bacterial species identified and their antimicrobial resistance pattern in dogs and cats with hepatobiliary infection over a long study period. Additional goals were (1) to compare antibiotic resistance between the first and second halves of the study period, (2) to characterized the MDR isolates, and (3) to evaluate the potential association between previous antibiotic administration and the isolation of MDR bacteria.

2 | Materials and Methods

2.1 | Criteria for Case Inclusion

Two data sources were searched for this study. The database of a Veterinary Teaching Hospital (VTH) from the National Veterinary College of Alfort and the database of a private laboratory (PL) VEBIO located in the same geographic area of France. The VTH database was searched for all dogs and cats with hepatobiliary cultures (positive and negative cultures), while only dogs and cats with a positive hepatobiliary culture were included from the PL. Data of animals with negative cultures were only used to calculate the prevalence of positive cultures at one center (VTH). For animals with positive culture, signalment (age, sex, and breed), bacteriology results and antimicrobial susceptibility testing results were collected. Antibiotic administration within 3 months before sampling were additionally registered for animals from the VTH (data unavailable for the PL). Figure 1 summarizes the process of animal's inclusion and the subsequent data collected and analyzed for this study. Animals were excluded if information on hepatobiliary culture result and/or antibiotic susceptibility testing were missing. For animals sampled more than once, only the results from the first sampling were included in the analysis (Table 1). Underlying diseases, results of bile cytology and/or hepatic histopathology were inconsistently available and were therefore not recorded. All cultures were performed on diseased animals at the discretion of the attending clinician.

2.2 | Bacteriologic Data

Specimens for culture included bile aspirates, liver tissue biopsies or a combination of both. Samples were obtained either

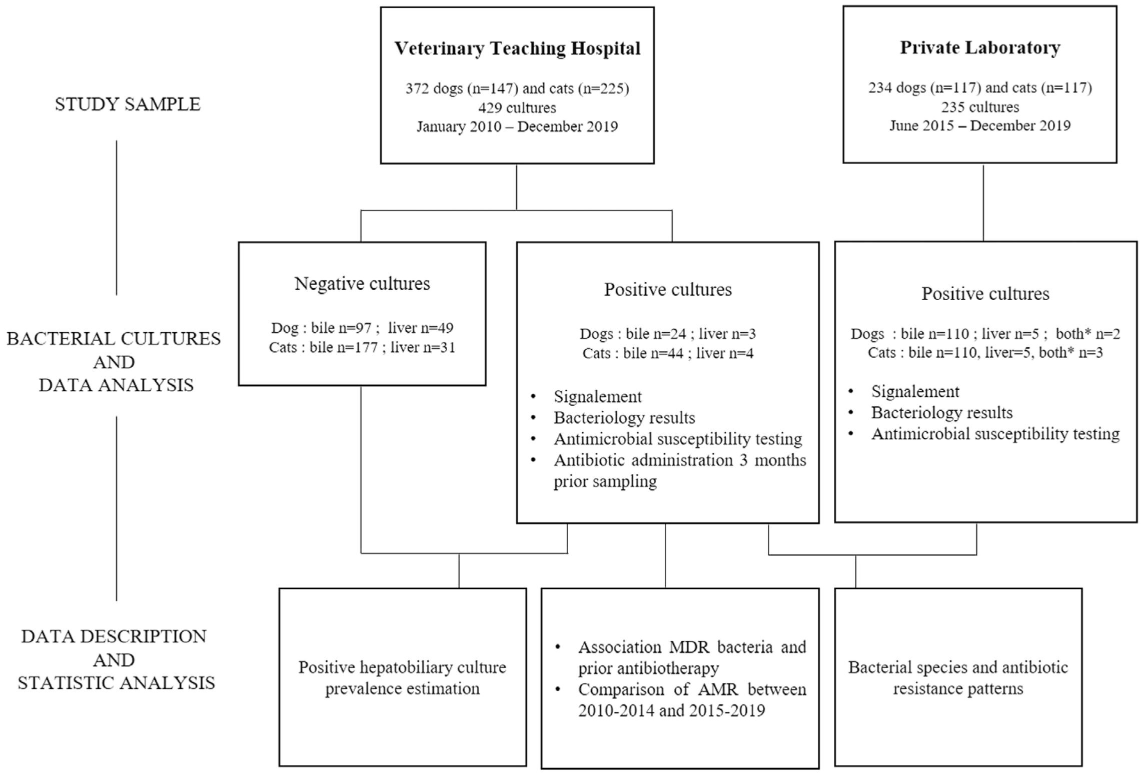

FIGURE 1 | Process of data collection and analysis for dogs and cats included from the VTH and the PL.

TABLE 1 | Characterization of positive hepatobiliary cultures in dogs and cats included from the VTH and PL from 2010 to 2019.

Category (number)	Dogs	Cats	Total
Animals sampled with positive culture[a]	144	165	309
Positive samples[a]	144	166[b]	310
Bile cultures	134	154	288
Liver biopsy cultures	8	9	17
Combined bile and liver biopsy culture	2	3	5
Isolates	158	182	340
Monomicrobial cultures	132/144 (92%)	151/166 (91%)	283/310 (91%)

[a] Inclusion of the first culture if animals were sampled more than once.
[b] Only one isolate included for one cat with both positive bile and liver culture.

through ultrasound guidance or during laparotomy. Bile aspirates were collected in syringes and hepatic biopsies were placed in an appropriate sterile transport media for bacterial culture, allowing for both aerobic and anaerobic testing. All samples were inoculated within 12 h after collection for both aerobic and anaerobic cultures. All specimens obtained at the VTH were cultured at the university's microbiology laboratory. The samples collected from the additional PL were primarily sourced from a private veterinary referral center, with a few additional samples collected from primary care practices, all within the same geographic area of France. Cultures were incubated and examined daily for growth for 5 days for the aerobic cultures and for 7 days for the anaerobic cultures. Isolates were considered as contaminants at the discretion of the laboratory staff based on bacterial species identification, amount and growth rate over time, and correlation with cytological examination performed on the same samples (if available). Isolates considered as contaminants were not included in the study. All identified bacterial species were recorded and only those isolated more than five times have been detailed in the results.

For both laboratories, the AFNOR NF U47-107 standard was followed for performing antibiotic susceptibility tests [14]. Antimicrobial susceptibility testing was performed using the Disk Diffusion method for the university laboratory (VTH). For the PL, the Disk Diffusion method was used until 2018, after which the Broth Microdilution method was implemented. The national veterinary standards from the CASFM-VET (updated annually) were used for the interpretation of results [15]. When antibiotics or bacteria were not included in the CASFM-VET documents, the laboratories referred to the human CASFM standards (before 2013) [16]. All « *intermediate* » results were classified as « *susceptible* » to prevent artificially increasing the size of the resistant sample group. Bacteria were thus classified as « *susceptible* » or « *resistant* » to tested antimicrobial agents. All microbiological results (bacterial species identification and antimicrobial susceptibility testing) were reviewed by a veterinary specialist in bacteriology. Susceptibilities to the most commonly used antimicrobials agents in veterinary practice were recorded for the study: aminopenicillins (ampicillin and amoxicillin), amoxicillin-clavulanic acid, first generation cephalosporins (cefalexin), third generation cephalosporins (ceftiofur), tetracyclines (doxycycline and tetracycline), fluoroquinolones (enrofloxacin and marbofloxacin), and sulfonamides associated with trimethoprim (TMPS). Given the natural resistance of enterococci to cephalosporins and TMPS, the in vitro susceptibility results for these antibiotics were excluded for the calculation of bacterial resistance [17]. MDR bacteria were defined as isolates being resistant to at least one agent from three or more antimicrobial classes [18].

2.3 | Statistical Analysis

Association between MDR bacteria and antimicrobial administration within 3 months before culture and comparison of bacterial resistance between two time periods (at VTH: period 1 from 2010 to 2014 and period 2 from 2015 to 2019) were evaluated by using a chi-squared test (or Fisher's exact test, when $n < 5$). Values of $p < 0.05$ were considered significant.

3 | Results

3.1 | Study Sample

The retrospective analysis of both laboratory databases allowed the inclusion of 309 animals (144 dogs and 165 cats) for 310 positive cultures during the study period (Figure 1, Table 1). Considering both positive and negative cultures from the VTH, the overall prevalence of positive cultures was 17.5% (15.6%, 27/173 in dogs and 18.8%, 48/256 in cats; Figure 1). These included 19.9% positive bile cultures (19.8%, 24/121 in dogs and 19.9%, 44/221 in cats) and 9% positive liver cultures (5.8%, 3/52 in dogs and 11.4%, 4/35 in cats).

Data regarding age, sex, and breed were available for 128, 74, and 133 dogs respectively. Median age of dogs was 9 years (range 6 months to 15 years) and male:female ratio was 1:1 (37 males and 37 females). The most represented breeds were Yorkshire Terrier (14%, 19/133), Cocker Spaniel (6%, 8/133), Jack Russel Terrier (5%, 7/133), and Shetland Sheepdog (5%, 7/133). The overall prevalence of Terrier breeds was 35% (46/133). Of the 165 cats included, information regarding age, sex, and breed was available for 147, 150, and 97 cats respectively. Median age of cats with positive culture was 11 years (range 1–20 years) and male:female ratio was 1.7 (61 males and 36 females recorded). Most cats were Domestic Shorthair cats (69%, 104/150), followed by Persian (5%, 7/150), Chartreux (4%, 6/150), and Birman (4%; 6/150).

3.2 | Culture Results

Culture inoculates were mainly of bile-aspirate origin rather than liver tissue biopsies and most of the cultures (91%) yielded a pure growth bacteria species in both dogs and cats (Table 1). Two isolates were identified in all polymicrobial cultures, except in four cultures (one from a dog and three from cats) where three isolates were identified.

For 12 animals, cultures were conducted on both bile and liver tissue biopsy samples. Among these, four (one dog and three cats) had a negative bile culture but a positive liver culture. For the remaining eight animals, seven (five dogs and two cats) had positive bile cultures, while one cat had both a positive bile culture and a concurrent positive liver culture. For this latter, since the same bacteria were isolated from both bile and liver culture, only one isolate was considered and included in the data analysis.

Bacterial species identified from the 340 isolates are detailed in Table 2. Almost all of isolates were aerobic bacteria; anaerobic bacteria (*Clostridium* spp. and *Bacteroides* spp.) were identified in only two dogs. Gram-negative bacteria were the most common identified bacteria in both dogs and cats. Common isolates included *E. coli* (40%), *Staphylococcus* spp. (14%), and *Enterococcus* spp. (10%) in both dogs and cats.

3.3 | Antimicrobial Susceptibility

Antimicrobial resistances for both laboratories are reported in Tables 3 and 4. For gram-negative bacteria, more than 20% were resistant to all antibiotic classes included in the study, except for fluoroquinolones and third-generation cephalosporins (in both dogs and cats). Notably, approximately 30% of all gram-negative isolates exhibited resistance to both aminopenicillins and amoxicillin-clavulanic acid. Resistance to aminopenicillins, amoxicillin-clavulanic acid, and fluoroquinolones was observed more frequently in dogs than in cats.

For gram-positive bacteria, the results vary by species (Table 4). In cats, approximately 20% resistance is observed for most antibiotics, with lower resistance rates for TMPS (7%) and fluoroquinolones (11%). In comparison, in dogs, the observed resistance to aminopenicillins, amoxicillin-clavulanic acid, and cephalosporins is lower (0%–15%), while over 20% resistance is observed for TMPS, tetracyclines, and fluoroquinolones.

Antibiotic resistances of *E. coli* and *Enterococcus* spp. strains are detailed in Table 5. *E. coli* isolates exhibited lower resistance rates than those observed for all gram-negative species combined. In

TABLE 2 | Distribution of the most frequent hepatobiliary bacteria species isolated in dogs and cats included from the VTH and PL from 2010 to 2019.

Bacterial species	Percent of canine isolates	Percent of feline isolates	Percent of all isolates
Gram-negative bacteria	**68% (107/158)**	**66% (120/182)**	**67% (227/340)**
Escherichia coli	43% (68/158)	37% (68/182)	40% (136/340)
Klebsiella spp.	8% (12/158)	3% (6/182)	5% (18/340)
Enterobacter spp.	3% (5/158)	4% (8/182)	4% (13/340)
Pseudomonas spp.	3% (5/158)	3% (6/182)	3% (11/340)
Proteus spp.	3% (5/158)	2% (4/182)	3% (9/340)
Salmonella sp.	0% (0/158)	3% (6/182)	2% (6/340)
Pasteurella spp.	1% (1/158)	3% (6/182)	2% (7/340)
Others	6% (10/158)	9% (16/182)	8% (26/340)
Gram-positive bacteria	**33% (52/158)**	**34% (62/182)**	**33% (114/340)**
Staphylococcus spp.	12% (19/158)	15% (28/182)	14% (47/340)
Enterococcus spp.	13% (20/158)	8% (14/182)	10% (34/340)
Streptococcus spp.	8% (12/158)	5% (9/182)	6% (21/340)
Others	1% (1/158)	6% (11/182)	4% (12/340)

TABLE 3 | Antimicrobial resistances of gram-negative bacteria identified in dogs and cats included from the VTH and PL from 2010 to 2019.

Antibiotic	Percent of canine isolates	Percent of feline isolates	Percent of all isolates
Amoxicillin/clavulanic acid	36% (36/100)	30% (34/114)	33% (70/214)
Aminopenicillin	52% (50/97)	43% (46/108)	47% (96/205)
First generation cephalosporins	33% (32/98)	30% (33/109)	31% (65/207)
Third generation cephalosporins	17% (17/101)	17% (19/110)	17% (36/211)
TMPS	24% (24/99)	25% (28/114)	24% (52/213)
Tetracyclines	34% (35/102)	33% (38/114)	34% (73/216)
Fluoroquinolones	19% (38/200)	13% (29/217)	16% (67/417)

TABLE 4 | Antimicrobial resistances of gram-positive bacteria identified in dogs and cats included from the VTH and PL from 2010 to 2019.

Antibiotic	Percent of canine isolates	Percent of feline isolates	Percent of all isolates
Amoxicillin/clavulanic acid	6% (1/17)	17% (4/24)	12% (5/41)
Aminopenicillin	15% (5/33)	18% (6/34)	16% (11/67)
First generation cephalosporins	0% (0/16)	17% (3/18)	8% (3/34)
Third generation cephalosporins	7% (1/15)	17% (3/18)	12% (4/33)
TMPS	34% (10/29)	7% (3/43)	18% (13/72)
Tetracyclines	41% (21/51)	23% (14/60)	32% (35/111)
Fluoroquinolones	23% (23/98)	11% (13/115)	17% (36/213)

TABLE 5 | Antimicrobial resistances of *Escherichia coli* and *Enterococcus* spp. isolates identified in dogs and cats included from the VTH and PL from 2010 to 2019.

Antibiotic	Percent of *Escherichia coli* isolates	Percent of *Enterococcus* spp. isolates
Amoxicillin/clavulanic acid	24% (32/135)	23% (3/13)
Aminopenicillin	39% (51/132)	30% (9/30)
First generation cephalosporins	19% (26/135)	—
Third generation cephalosporins	10% (14/135)	—
TMPS	21% (28/133)	—
Tetracyclines	26% (35/134)	47% (16/34)
Fluoroquinolones	11% (29/256)	22% (14/63)

contrast, *Enterococcus* spp. strains demonstrated, as observably, higher resistance rates to all antibiotics (when comparable) compared to all gram-positive species combined.

Antibiotic resistances of isolates cultured from the VTH were compared between the two halves of the study period (Table 6). Bacterial resistances were more frequently observed during 2010–2014 than during 2015–2019. Throughout the entire study period, resistance rates exceeded 20% for all antibiotics tested, with the exceptions of amoxicillin-clavulanic acid (18%) and both first and third-generation cephalosporins (15% and 6%, respectively). Resistances to aminopenicillin, first-generation cephalosporins, TMPS, tetracyclines, and fluoroquinolones were significantly less frequent during the second half of the study period (Table 6).

3.4 | Multidrug Resistant bacteria

Prevalence of MDR bacteria were 40% (135/340) in the study sample overall: MDR were isolated in 44% of dogs (69/158) and 36% of cats (66/182) having a positive culture. *E. coli* was the most common MDR bacterium identified in both dogs and cats, followed by *Enterococcus* spp. and *Staphylococcus* spp. in dogs and *Enterococcus* spp. and *Enterobacter* spp. in cats (Table 7). More than half of the MDR bacteria (64%, 87/135) were gram-negative isolates. Among *E. coli* and *Enterococcus* spp. isolates, 30% (41/136) and 68% (23/34) were MDR, respectively. Resistance patterns of identified MDR bacteria are summarized in Table 8.

3.5 | Association Between Previous Antibiotic Treatment and Isolation of MDR bacteria

Data regarding administration of antibiotics within 3 months before sampling were available for all cats and dogs recruited from the VTH. Among all MDR bacteria, 53% (21/39) of them were isolated from dogs and cats which had received antibiotics within the previous 3 months. Isolation of MDR bacteria was significantly more prevalent in dogs and cats that had received previous antibiotic treatment (81%, 17 out of 21) compared to those without previous antimicrobial treatment (30%, 22 out of 75; $p < 0.001$).

4 | Discussion

This study aimed to describe bacterial species associated with hepatobiliary infection in dogs and cats, and bacterial resistance patterns and their evolution between the first and second halves of the study period. The last objective was to investigate the prevalence of MDR bacteria within hepatobiliary infections and its association with antibiotic administration before sampling.

Based on results from the VTH laboratory, estimated prevalence of positive bile cultures was 20% for both dogs and cats and consistent with previous literature data [2–4, 8–11, 19]. Cultures from liver tissue biopsies were less frequently positive, as previously described [4, 9].

Among bacterial isolates identified in this study, over 20% of the gram-negative isolates showed resistance to most tested antibiotics (except for third-generation cephalosporins and fluoroquinolones), with a high resistance (> 30%) noted for amoxicillin-clavulanic acid. For gram-positive bacteria lower resistance rates to all antibiotics tested were observed compared to gram-negative bacteria, except for fluoroquinolones, where resistance levels were comparable between the two groups.

TABLE 6 | Antimicrobial resistances during different periods in dogs and cats included from the VTH 2010 to 2019.

Antibiotics	2010–2014	2015–2019	*p*	2010–2019
Amoxicillin/clavulanic acid	29% (7/24)	15% (10/69)	0.13	18% (17/93)
Aminopenicillin	48% (10/21)	15% (16/106)	0.002[a]	20% (26/127)
First generation cephalosporins	42% (8/19)	6% (3/53)	< 0.001[a]	15% (11/72)
Third generation cephalosporins	18% (2/11)	3% (2/58)	0.12	6% (4/69)
TMPS	53% (10/19)	19% (11/57)	0.005[a]	28% (21/76)
Tetracyclines	57% (13/23)	32% (21/66)	0.04[a]	38% (34/89)
Fluoroquinolones	41% (16/39)	21% (22/106)	0.01[a]	26% (38/145)

[a] Significative statistical difference between 2010–2014 and 2015–2019.

TABLE 7 | Distribution of the most frequent hepatobiliary MDR bacteria species isolated in dogs and cats included from the VTH and PL from 2010 to 2019.

Multiresistant bacterial species	Percent of canine isolates	Percent of feline isolates	Percent of all isolates
Escherichia coli	30% (21/69)	30% (20/66)	30% (41/135)
Enterococcus spp.	20% (14/69)	33% (22/66)	17% (23/135)
Staphylococcus spp.	14% (10/69)	14% (9/66)	11% (15/135)
Enterobacter spp.	3% (2/69)	8% (5/66)	7% (9/135)
Klebsiella spp.	9% (6/69)	11% (7/66)	7% (9/135)
Others	23% (16/69)	5% (3/66)	28% (38/135)

TABLE 8 | Antimicrobial resistances MDR bacteria identified in dogs and cats included from the VTH and PL from 2010 to 2019.

Antibiotics	Percent of canine isolates	Percent of feline isolates	Percent of all isolates
Amoxicillin/clavulanic acid	70% (32/46)	52% (29/55)	60% (61/101)
Aminopenicillin	76% (41/54)	75% (43/57)	76% (84/111)
First generation cephalosporins	64% (27/42)	65% (31/48)	64% (58/90)
Third generation cephalosporins	38% (17/45)	34% (16/47)	36% (33/92)
TMPS	54% (28/52)	51% (29/57)	52% (57/109)
Tetracyclines	66% (38/58)	69% (45/65)	67% (83/123)
Fluoroquinolones	42% (54/129)	34% (42/123)	38% (96/252)

Enterococcus strains are naturally resistant to cephalosporins and TMPS and therefore were excluded from resistance calculations for these antibiotics [17]. This might explain the observed low resistance against cephalosporins. High antimicrobial resistance of gram-negative bacteria against amoxicillin-clavulanic acid and cephalosporins have already been described [2, 4, 11]. The largest retrospective study involved 58 bacterial species isolated from 238 dogs and cats with suspected hepatobiliary disease; it reported similar *E. coli* antibiotic resistance against amoxicillin-clavulanate (33%) and fluoroquinolones (18%), but higher resistance to first-generation cephalosporins (36%) comparing with our results [4]. For gram-positive bacteria, considerable variability regarding resistance against penicillins (0% to 30%–50%) have been reported in literature, while higher resistances against fluoroquinolones have been observed compared to our study (> 50%) [2, 11].

Earlier studies on hepatobiliary infections relied on a limited number of isolates with a focus on specific bacterial species, without considering all isolates. Other data about antimicrobial resistance are available, including reports on epidemiological surveillance of antimicrobial resistance in Europe. However, these studies included bacterial isolates from dogs and cats with various infections, with only few hepatobiliary isolates [20, 21]. In contrast to our study, these publications monitor antibiotic resistance by examining resistance patterns of *E. coli*, *Staphylococcus pseudintermedius*, and *Staphylococcus aureus* strains, which allows for only limited comparison with our findings. Regarding *E. coli*, these studies consistently found high antibiotic resistance to amoxicillin-clavulanic acid (< 20%, except in France where it ranged from 35% to 40%) and low resistance to third-generation cephalosporins (approximately less than 10%) and to fluoroquinolones (< 15% approximately). The resistance pattern of *E. coli* isolates observed in our study was similar, while higher antibiotic resistance for all gram-negative bacteria were observed. These results suggest that, despite prevalence of *E. coli*, other gram-negative bacteria might exhibit higher resistance rates, possibly due to strains of *Pseudomonas* spp. and *Proteus* spp. which are known for their resistance to specific antibiotics, such as fluoroquinolones and beta-lactams [22–24]. In our study, it was observed that enterococci antibiotic resistance was higher compared to resistances of all gram-positive bacteria. It is therefore likely that enterococcal strains contribute to the overall increase in resistance among gram-positive bacteria. Considering the observed low frequency of *S. aureus* and *S. pseudintermedius* isolates in our study, comparison with published epidemiological data were not conducted.

This study also compared antibiotic resistance between the first 4 years and the last 4 years of the study period for the VTH. We hypothesized that bacterial resistance would remain stable over the years. However, a significant decrease in the frequency of resistance to aminopenicillins, first generation cephalosporin, TMPS, tetracyclines, and fluoroquinolones was observed. It is noteworthy that three times more bacteria were cultured during the last 4 years of the study period. Therefore, it could have biased the comparison. This decrease in resistance could be due to changes in practice regarding rational use of antibiotics. Further studies examining the temporal evolution of antibiotic resistance would be valuable for better documenting this trend.

Another objective of this study was to characterize hepatobiliary MDR bacteria in dogs and cats from referral centers. A very high prevalence of MDR bacteria was identified in both dogs and cats (40%), with almost 50% of the MDR species being either *E. coli* or *Enterococcus* spp. strains. More than 50% of multidrug-resistant (MDR) bacteria exhibited resistance to each of the antimicrobial agents tested, with the exception of third-generation cephalosporins and fluoroquinolones. Description of MDR bacteria in hepatobiliary isolates in companion animals is particularly scarce in veterinary literature [2, 4, 11]. Data are only available on the prevalence of MDR bacteria in various infection conditions in dogs and cats [25–27]. Published data from countries in the Americas (Canada, Colombia, and Argentina) reported rates of MDR bacteria in dogs and cats ranging from 10% to 20%, while a study conducted in Italy reported a rate of 45% [25–28]. These different results could reflect either varying antibiotic uses depending on the geographic area or only geographical differences. Therefore, comparison and data extrapolations should be conducted with caution. In this study, among animals that had undergone previous antibiotic treatment within the 3 months, a significantly higher incidence of MDR bacteria was found. Administration of antibiotics is known to induce a selective pressure, promoting emergence of antibiotic resistance [29]. Other previous studies have also evaluated this association but results are inconsistent [28, 30–32]. To our knowledge, this is the first report establishing association between previous antibiotic treatment and incidence of MDR bacteria in hepatobiliary infections in dogs and cats.

In humans with biliary infections, widespread emergence of antimicrobial resistance, particularly among *Enterobacteriaceae*, has been identified [33]. Resistances commonly concern penicillins, third-generation cephalosporins, and quinolones, and are attributed to extended-spectrum β-lactamases (ESBL) bacteria [34–36]. ESBL bacteria, which can inactivate penicillins and cephalosporins, cause challenges in antimicrobial therapy due to uncertain sensitivities. In veterinary literature, limited reports describe ESBL-producing *E. coli* strains in hepatobiliary infection cases [35, 36]. Considering the limited choice in antibiotics in veterinary medicine, additional studies evaluating this phenotype within hepatobiliary bacteria would be relevant.

This study has several limitations, primarily due to its retrospective nature. It would have been interesting to evaluate a potential association between the bacteriological results and any underlying diseases. Additionally, details on previous antibiotic use were only available for animals from the VTH, and information on administered drugs and duration of treatment were unknown. Furthermore, microbiology techniques differ from 2018 between the VTH and the PL and might affect the comparability of results. Finally, our results are specific to the studied geographic area and influenced by local factors including prescription practices; caution should thus be taken regarding extended extrapolation.

5 | Conclusion

This study is the largest one documenting antibiotic resistances of hepatobiliary bacteria isolated in dogs and cats over an extended time period. A concerning prevalence of resistance was observed to key antibiotics, especially for amoxicillin-clavulanic acid among gram-negative bacteria. Fortunately, resistance to fluoroquinolones was lower. Alarmingly, 40% of isolates were MDR, with a higher prevalence of MDR bacteria in animals recently treated with antibiotics. Additionally, the observed decline in resistance to several antibiotics over time suggests a potential shift in resistance patterns.

Present results highlight the importance of performing bacterial culture and antimicrobial susceptibility testing before initiating antibiotic treatment in cases of canine and feline hepatobiliary infection. It also raises awareness about the emergence of MDR strains. Further complementary studies analyzing the temporal evolution of antibiotic resistances, including the investigation of the ESBL phenotype, would be beneficial.

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Disclosure

Authors declare no off-label use of antimicrobials.

Ethics Statement

Authors declare no Institutional Animal Care and Use Committee (IACUC) or other approval was needed. Authors declare human ethics approval was not needed.

Conflicts of Interest

The authors declare no conflicts of interest.

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