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Article

Investigating Bacterial Bloodstream Infections in Dogs and Cats: A 4-Year Surveillance in an Italian Veterinary University Hospital

Raffaele Scarpellini ^{1,*}, Massimo Giunti ¹, Cecilia Bulgarelli ¹, Erika Esposito ¹, Elisabetta Mondo ¹, Fabio Tumietto ² and Silvia Piva ¹

¹ Department of Veterinary Medical Sciences, Alma Mater Studiorum—University of Bologna, Via Tolara di Sopra n 50, 40064 Ozzano dell'Emilia, Italy

² Unit of Antimicrobial Stewardship, Local Health Authority of Bologna; Bologna, Italy

* Correspondence: raffaele.scarpellin2@unibo.it; Tel.: +39 3450678239

Abstract: In small animal practice, blood cultures (BCs) are essential for diagnosing bacterial bloodstream infections (BSIs), guiding targeted antimicrobial therapy particularly in relation to the rise of multidrug-resistant (MDR) pathogens. This study analyzed 96 positive BCs from dogs and cats at the Veterinary University Hospital (VUH) of Bologna (2020–2024), assessing bacterial prevalence, antimicrobial resistance, and associated risk factors. *Escherichia coli* was the most common isolate (29/96), followed by *Streptococcus canis* (11/96). MDR percentage was 29.2% (28/96), with gram-negatives associated with higher rates ($p=0.040$). Nearly half of the cases (46.9%, 45/96) were suspected healthcare-associated infections (HAIs), significantly associated with the number of invasive devices used ($p=0.008$), and with the absence of co-positive samples ($p=0.012$). Empirical antibiotic therapy was administered in 94.8% (91/96) of cases, with ampicillin-sulbactam and marbofloxacin as the most used drugs. *In vitro* empirical therapy appropriateness was 76.9% (70/91). MDR was associated with inappropriate empirical therapy ($p<0.001$). Mortality within 30 days was 36.5% (35/96), significantly linked to antibiotic escalation ($p=0.006$). The findings highlight the need for systematic BC surveillance in veterinary settings to optimize treatment strategies (especially in countries with restrictions on antibiotic use in animals), to mitigate MDR spread and to protect public health.

Keywords: antimicrobial resistance; veterinary medicine; companion animals; bloodstream infections; blood cultures; antibiotic de-escalation; multi-drug resistance

1. Introduction

Blood cultures (BCs) play a critical role in diagnosing bacteremia and septicemia. These diagnostic tests are frequently used and cost-effective tools to identify the causative agents of bacterial bloodstream infections (BSIs), guiding a targeted antimicrobial therapy. BSIs are severe infections that can lead to sepsis, multiorgan failure and deaths, and can result from a variety of sources, including primary infections, ascending urinary infections, bacterial gut translocation or healthcare-associated sources such as indwelling catheters or surgical site infections [1]. Compared with human medicine, the management of BSIs in veterinary medicine presents more limitations, such as the lack of advanced monitoring and therapeutics, the access to long term organ support, and financial restrictions [2]. In small animal practice, some of the most common agents associated with bacterial BSIs are multi-drug resistant (MDR) pathogens such as methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), *Pseudomonas aeruginosa* and extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales [3]. They represent a threat not only for animals, but also for public health due to their potential zoonotic transmission. In the European Union, the significant therapeutic challenge they pose is exacerbated by the limitations on antibiotic use that veterinary medicine must

deal with [4], in order to preserve the efficacy of latest antibiotics for human medicine. For these reasons, an accurate and timely isolation and identification of the involved pathogens is crucial not only for the effective treatment and infection control, but also for mitigating the selective pressure with the use of the appropriate antibiotic drug. In this context, systematic data collection and analysis of BC through surveillance systems represent an effective tool for veterinary healthcare facilities (Morley). Surveillance of BCs allows to better understand the epidemiology of bacterial BSIs, to early detect potential outbreaks, to monitor and to compare AMR trends, to promote antimicrobial stewardship and to optimize the treatment considering local characteristics. The latter is specifically important for BSIs, given that they often require empirical treatment while waiting for the complete diagnosis. Nevertheless, data about BSIs and BCs in veterinary settings are still scarce and not assisted by national or international reference standards. The aims of this study were: i) to describe the prevalence, distribution and antimicrobial resistance percentage of bacterial pathogenic isolates from positive BCs collected from dogs and cats hospitalized at the Veterinary University Hospital (VUH) of the University of Bologna; ii) to determine potential risk factors associated with mortality, healthcare-associated infections, MDR pattern and inappropriate empirical therapy.

2. Materials and Methods

Study design. A perspective, observational, longitudinal study (from January 2020 to December 2024) was conducted at the Veterinary University Hospital of Bologna. Positive BCs obtained from dogs and cats as part of the bacteriological diagnostic routine were included in the study.

BCs processing

BCs were obtained by aseptically inoculating 5-10 ml of blood into commercial blood culture bottles (Signal Blood Culture System; Oxoid). Bottles were then incubated at $37\pm1^{\circ}\text{C}$ for a maximum of 7 days. Positive BCs, according to manufacturer's instructions, were then plated by streaking in aerobic, capnophilic and anaerobic conditions at $37\pm1^{\circ}\text{C}$ for 24-47 hours. Colonies were macroscopically evaluated and subsequently each isolate was identified using the matrix-assisted laser desorption-ionization time-of-flight mass spectrometry method (MALDI-TOF MS) (Biotyper, Bruker Daltonics, Billerica, MA), following manufacturer's instructions (Bruker Daltonik, Bremen, Germany). The species-level identification was confirmed when the ID score was >2 (green—high accuracy), or >1.8 (for *Staphylococcus* spp. isolates). BCs were considered contaminated and excluded from the analysis after a mutual agreement between the reporting laboratory and the clinical staff, considering both clinical and microbiological findings. Multiple isolates from the same patient obtained from a set of cultures were considered duplicates.

Antimicrobial susceptibility testing (AST)

As part of routinary laboratory diagnostic, the AST was performed with the Kirby-Bauer disc diffusion method, according to the Clinical and Laboratory Standard Institute (CLSI) guidelines [5]. Overall, 15 antimicrobials from 6 antimicrobial classes were included in the final analysis (Table A1). All the discs were purchased from Oxoid (Oxoid, Milan, Italy). For every tested drug, each isolate was classified as susceptible (S), intermediate (I), or resistant (R) based on the CLSI veterinary breakpoints [6] or, when not specifically present, based on European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints for human medicine [7]. According to the National Reference Laboratory for AMR [8], antimicrobials known to exhibit expected resistance phenotypes in some bacterial species were not tested. For AST interpretation, intermediate isolates were classified as susceptible, as recommended by the EUCAST [9]. Isolates resistant to at least one drug were considered AMR, and isolates that not susceptible to at least one antimicrobial drug from three or more antimicrobial classes were considered as multidrug resistant (MDR), according with the definition given by Magiorakos et al. [10].

Data collection.

For each bacterial isolate, data about the patient signalment (species, age, presence of comorbidities) were extrapolated from the internal laboratory database, as well as hospitalization data when the blood sample was collected (previous hospitalization/surgery in the past 30 days,

length of hospitalization, hospitalization in the intensive care unit, surgery, use of invasive devices). Additionally, data about antimicrobial use in the previous 90 days, empirical antimicrobial treatment after BC collection and antimicrobial treatment after the AST result were recorded. Antimicrobial de-escalation was defined in case of a decrease in the number of antibiotics used or the narrowing of the spectrum of antimicrobial treatment, while escalation was defined in case of an increase in the number of antibiotics or the broadening of the spectrum, according with the definitions [11,12]. *In vitro* empirical appropriateness of the therapy was defined when the bacterial isolate was fully susceptible to at least one of the drug started empirically. When the specific drug used was not tested, the result from its prototype were used, according to the National Reference Laboratory for AMR [13]. Mortality ratio (natural death or euthanasia) within 30-days from the blood collection was also evaluated. Following the definition by Hacque *et al.* [14], the suspected healthcare-associated origin of the infection was defined if: i) the blood sample was collected after 48 hours or more from the hospitalization and no signs of systemic infection were present at the admission; ii) the patient was admitted with signs of systemic infection and had been hospitalized or underwent surgery in the previous 30 days for different reasons.

Data analysis

Descriptive statistics was performed considering bacterial species identified, co-presence of other positive samples (e.g. urines), resistance percentages towards each tested drug and AMR/MDR percentages, anamnestic and hospitalization data, mortality ratio, de-escalation and escalation frequency, *in vitro* empirical appropriateness of the therapy and suspected HAI origin. Differences in resistance percentages between gram-positives and gram-negatives bacteria were statistically evaluated using the Fisher exact test with a p-value ≤ 0.05 considered significant. The association between four outcomes (MDR, mortality ratio, appropriateness of the empirical therapy, HAI origin) and all the variables included in the study were evaluated using univariable logistic regression analysis, with a p-value of ≤ 0.05 considered significant. Normality and heteroskedasticity of data were assessed with the Shapiro–Wilk test and the Levene’s test. Significant results were included in the multivariate analysis model built up with a stepwise selection. Statistical analysis was performed with MedCalc (version v22.009).

3. Results

A total of 101 positive blood samples were recorded from 101 patients. Positivity rate was 17.7% ($n=101/571$). Five samples were excluded from the analysis because the AST was not performed. From the 96 samples included in the study, 22/96 (22.9%) were in co-presence with another positive sample (14 urines, 3 abdominal effusions, 2 uterine swabs, 2 abscesses, 1 synovial liquid). Seventy-four samples (77.1%) were collected from dogs, while 22 (22.9%) were from cats. Mean age was 9.4 years (SD 3.8). Sixty-four patients (66.7%) presented at least one comorbidity, of which the most frequent was cancer ($n=16$), and 35/96 (36.4%) had been hospitalized or underwent surgery in the previous 30 days. Ninety-four patients (98%) were hospitalized when the blood sample was collected, of which 77 (80.2%) in the Intensive Care Unit, and 19 (19.8%) had surgery. The mean length of hospitalization when blood was collected was 2.5 days (SD 2.3). At least one invasive device was used in 41 patients (57.3%), and nasogastric tube was the most common ($n=35/96$, 36.4%). Thirty-six patients (37.5%) were previously treated with antibiotics, including 8 cases (8.3%) with more than one. The most common antibiotic class previously used were potentiated penicillins ($n=17$, 17.7%), followed by fluoroquinolones ($n=13$, 13.5%).

All the positive BCs were monomicrobial. Ninety-six bacterial isolates were identified, of which 43/96 (44.8%) were gram-positives, and 53/96 (55.2%) were gram-negatives. The most isolated species were *Escherichia coli* ($n=29$), *Streptococcus canis* ($n=11$) and *Staphylococcus pseudintermedius* ($n=10$) (Table 1). Total MDR percentage was 29.2% ($n=28$). Non-intrinsic resistance percentages are shown in Table 2. The highest values were recorded for ampicillin (44.7%), tetracycline (43%) and enrofloxacin (29.2%). Gram-negatives bacteria were found to be statistically associated with a higher MDR percentage ($p=0.040$). Forty-five out of 96 cases (46.9%) were considered potential HAIs.

An empirical treatment after the BC was started in 91 patients (94.8%). The three most used drugs were ampicillin-sulbactam, empirically started in 51 cases (55.4%), marbofloxacin in 42 (45.6%) and piperacillin-tazobactam in 29 (31.5%). In 40 patients (41.2%), the empirical treatment included more than one drug, with ampicillin-sulbactam/marbofloxacin as the most used combination (n=23 cases). Considering the AST results, empirical antibiotic therapy was considered appropriate *in vitro* in 70/91 cases (76.9%) (Table 3). The mortality ratio within 30 days was 36.5% (n=35/96). In 24 cases, the patients died before the execution of the AST. After the AST results, antimicrobial treatment was switched in 31/66 (47%) patients, of which 11 (16.7%) were escalations and 20 (30.3%) were de-escalations.

In the multivariate analysis, mortality within 30 days was significantly associated with the escalation of the therapy (p=0.006). Suspected HAIs were associated with the number of invasive devices (p=0.008), and with the absence of co-positive samples (p=0.012). Multi-drug resistance was associated with the escalation of the therapy (p=0.012) and with an inappropriate empirical treatment (p<0.001). No direct association between mortality, MDR and potential HAIs was found.

Table 1. Distribution of the 96 analyzed isolates based on bacterial species identification, and number of multidrug resistant (MDR) isolates for each species.

Bacterial species	total isolates	number of non-intrinsic MDR isolates (%)
<i>E.coli</i>	29	11 (37.9)
<i>Enterobacter spp.</i>	6	4 (66.7)
<i>Enterococcus spp.</i>	4	0 (0)
<i>S.canis</i>	11	0 (0)
<i>S.gallolyticus</i>	4	0 (0)
<i>K.pneumoniae</i>	4	3 (75)
other gram-negatives	8	2 (25)
<i>S.pseudintermedius</i>	10	5 (50)
<i>S.aureus</i>	5	1 (20)
other gram-positives	10	2
<i>P.aeruginosa</i>	5	0

Table 2. Resistance percentages of the 96 isolates included in the study for each tested antibiotic. Overall results are shown, as well as results of gram-positives and gram-negatives isolates. The p-value to evaluate the differences between gram-positives and gram-negatives obtained with the Fisher's exact test is shown. Values considered significant (p<0.05) are put in bold.

Antibiotic Drug	n. of tested isolates	n. of non intrinsic resistance (%)	n. of resistant gram-positive isolates resistant (%)	n. of gram-negative isolates resistant (%)	p-value
Amikacin	72	0 (0)	0/17 (0)	0/57 (0)	NA
Gentamicin	76	8 (10.5)	3/24 (12.5)	5/52 (9.6)	0.983
Ampicillin/Penicillin G (for <i>Staphylococcus</i> spp. isolates)	76	34 (44.7)	17/43 (39.5)	17/33 (51.5)	0.297
Oxacillin/Cefoxitin (for <i>Staphylococcus</i> spp. isolates)	19	7 (36.8)	NA	NA	NA
Amoxicillin-clavulanic acid	81	21 (25.9)	9/43 (20.9)	12/38 (31.6)	0.275
Ampicillin-sulbactam	81	18 (22.2)	8/43 (18.6)	10/38 (26.3)	0.405
Piperacillin-tazobactam	96	12 (12.5)	8/43 (18.6)	4/53 (7.5)	0.103
Cefazolin	76	23 (30.3)	8/38 (21.1)	15/38 (39.5)	0.080
Ceftiofur	86	23 (26.7)	8/39 (20.5)	15/47 (31.9)	0.139

Ceftazidime (for <i>P.aeruginosa</i> isolates)	6	0(0)	NA	NA	NA
Tetracycline	93	40 (43)	19/43 (44.2)	21/50 (42)	0.832
Enrofloxacin	96	28 (29.2)	9/43 (20.9)	19/53 (35.8)	0.110
Trimethoprim-sulfamethoxazole	89	23 (25.8)	7/41 (17.1)	16/48 (33.3)	0.081
Non-intrinsic multi-drug resistance	96	28 (29.2)	8/43 (18.6)	20/53 (38)	0.040*

Table 3. Distribution of empirical antibiotic treatment in the 96 patients included in the study according to the antibiotic used. The number of cases in which the antibiotic was considered *in vitro* appropriate is also shown.

Antibiotic	n.of patients empirically treated	<i>In vitro</i> appropriateness	%
Ampicillin-sulbactam	51	35	68.6%
Marbofloxacin	42	18	42.9%
Piperacillin-tazobactam	29	22	75.9%
Other	6	1	16.7%
Total	91	70	76.9%

4. Discussion

In this study, we report the results of a 4-years surveillance on positive BCs from dogs and cats admitted to an Italian VUH. BCs are considered the gold standard for diagnosing BSIs [15]. Despite that, studies that specifically provide data from bacterial BCs are lacking in small animal practice. In our research, we registered a positivity rate of 17.7%. Such value aligns with the few previous veterinary reports, in which it ranged from 20 to 24.4% [16–20]. Positivity rate variability is mainly due to differences in local infection prevalence, diagnostic protocols or sampling methods. Additionally, the overall accuracy of the test can be affected by the risk of contamination, timing, blood volume and differences in sampling protocols and techniques [18]. In this sense, the collection of a set of three BCs per patient (instead of one) has been demonstrated to be effective to enhance sensitivity and specificity[21,22], with increases of the detection rate up to 19% in dogs [23]. In the present study, the direct impact of this diagnostic aspect was not assessed, because it was not part of the aims, and it was not systematically performed by clinicians. Nevertheless, when it was executed, it was used as one of the discriminatory factors to exclude potential contaminants [24].

The co-positivity of other samples (mainly urines) for the same bacterial agent is a quite common phenomenon [18,25]. However, it is well established that BCs cannot be replaced by other culture methods, such as urine culture, for screening or diagnosis of BSIs [26]. The majority of the samples (77.1%) were from dogs, that compared to cats tended to be more frequently visited and sampled in the VUH where the study was conducted, as reported by our previous work [27].

The presence of comorbidities was common (66.7%), confirming that BSIs are frequent in patients with other chronic or underlying diseases. Specifically, cancer was the most frequent concurrent disease, remarking that immunocompromised patients such as oncologic ones should be specifically monitored, especially in large facilities such as VUHs. The high proportion of patients of this study that were previously hospitalized (36.4%) or hospitalized in Intensive Care Unit (80.2%) is not surprising, considering that VUHs generally present a conspicuous number of high-risk or referred patients, more susceptible to pathogens able to cause BSIs, both community-acquired and healthcare-associated (HA BSIs). In detail, we estimated a high frequency of suspected HA BSI (46.9 %) in our study. Despite such value could have been overestimated due to the assigned definition and the methodology used, it is remarkable to notice that HA BSIs represents an emerging concern for small animal practice. The increasing quality of veterinary healthcare assistance is leading to more attention to the problem, but also to a more frequent use of invasive procedures, devices and to a longer length of hospitalization that are well-recognized risk factors for the development of HAIs [28–30]. The mean length of hospitalization when the BC was taken (2.5 days) is in line with this

consideration, indicating that in some cases the BC was not collected, or was negative, at the hospital admission. Similarly, in 57.3% of the patients at least one invasive device was applied, with the nasogastric tube as the most prevalent. Inadequate food intake during hospitalization is a common issue in companion animals, and nasogastric or esophagostomy feeding tubes are frequently used. If from one side enteral nutrition has been associated with improved outcome in several disease such as pancreatitis or septic peritonitis [31,32], from the other the risk of complications, such as tube dislodgement, stoma site or systemic infections, are present [33].

Considering previous antimicrobial use, our reported percentage (37.5%) is in line with previous studies from Europe, highlighting as previous antimicrobial therapy does not necessarily affect BC positivity [18,34]. Potentiated penicillins and fluoroquinolones are confirmed to be the most commonly used drugs in small animal practice, including critical patients [35–38].

The higher proportion of gram-negative bacteria (55.2%) compared to gram-positive bacteria (44.8%) is consistent with our previous findings [27]. *E. coli* emerged as the most frequently isolated species, followed by *S. canis* and *S. pseudintermedius*. These pathogens are commonly reported as infectious agents in small animal practice, including in bloodstream infections [15,18]. Notably, we observed a relatively high proportion of *Enterobacter* spp. cases associated with a potential HA BSIs (n=6, 100%). While 3 of them were associated with an outbreak, the others were not chronologically close to each other, suggesting the persistence of this pathogen within the hospital. The proportion of other bacteria frequently associated with human BSIs as HAI agents, such as *K. pneumoniae* and *A. baumannii* [39], was instead relatively low. Almost one third of the isolates (28.9%) was classified as MDR, in line with other reports [18,40]. Gram-negatives were significantly associated with higher MDR percentages ($p = 0.040$). Due to their dynamic structure and their tendency to acquire multiple resistance mechanisms through plasmid conjugation, gram-negative bacteria such as Enterobacterales develop and disseminate multi-drug resistance more frequently [41,42]. This is the main reason why they are predominant in the list of critical pathogens redacted by the WHO and recently updated [43].

We also observed a high percentage of patients in which the empirical treatment was started after the blood collection (95.8%) including a 41.2% with two or more different antibiotics. Again, this is not surprising, given that BCs are generally collected in patients in severe conditions, in which empirical treatment is necessary and, if appropriate, lifesaving. A study by Black et al. [38] on 74 dogs admitted in Intensive Care Unit showed similar results in terms of empirical treatment (94%) and *in vitro* appropriateness (75%). In our case, the most common drugs used were ampicillin-sulbactam, marbofloxacin and piperacillin-tazobactam, that had the highest *in vitro* empirical appropriateness (75.9 %). Piperacillin-tazobactam is a potentiated ureidopenicillin categorized as class A (Avoid) by the European Medicines Agency (EMA)[4], and whose use in veterinary medicine had been allowed in the EU until the official prohibition with the 2022/1255 UE regulation. Its regulation is an example of the dilemma that veterinary clinicians are dealing with in Europe. Indeed, while the most powerful antibiotics are restricted or banned for use in animals, the prevalence of pathogens resistant to the allowed drugs is rising, with a consequent narrowing of the treatment options. If from one side this choice can be considered ethical to preserve the most effective and recent antibiotic for human medicine, from the other it implies for veterinarians an increasing risk of therapeutic failure, with all the reputational, economic and emotional consequences. It is also important to remember that, if overused, important antibiotics such as piperacillin-tazobactam could drive the selection of High Priority pathogens, such as Carbapenem-Resistant Enterobacterales (CRE)[44]. A balanced approach, for example, allowing class A antibiotics only in very specific cases and with an AST to support the decision, could be desirable. Nevertheless, in cases such as severe BSIs where rapid, empirical treatment is needed, this kind of regulation could not be applied. However, compared with piperacillin-tazobactam, a similar *in vitro* empirical appropriateness (68.6%) was shown by ampicillin-sulbactam, a potentiated aminopenicillin that belongs to the class C (Caution) EMA category, whose use is permitted in animals in the EU. This suggests it could be a good empirical first choice to treat BSIs in small animals, with less risks of MDR selection for public health.

After the AST results, antimicrobial treatment was switched in 31/66 (47%) patients, of which 11 (16.7%) were escalations and 20 (30.3%) were de-escalations. Antibiotic de-escalation is an antimicrobial stewardship strategy aimed at reducing the emergence of AMR, and collateral damages from the empirical use of broad-spectrum antibiotics [45]. Nevertheless, its efficacy to reduce MDR rates is not still well weighted [46]. Some authors suggest that ultra-short treatment with no de-escalation, in some cases could be more beneficial than unnecessary prolonged treatment with de-escalation [47]. In small animal practices, few studies have assessed the frequency of its application, with ranges from 12% to 63% [16,48]. In our case, we did not detect any association between de-escalation and mortality, inappropriate therapy or MDR. In contrast, we found a statistically significant association between mortality and escalation ($p=0.006$) and between MDR and escalation ($p=0.012$). Antibiotic escalation is normally executed when the pathogen shows multiple resistances or in case of non-responsive patients, with an already compromised conditions that often, despite the antibiotic shift, leads to death. This could suggest that in most cases, antibiotic escalation does not furnish any benefit. To avoid unnecessary shifts, a study from human medicine proposed the use of the “Escalation Antibigram”, a model to inform empiric treatment changes in nonresponsive patients, using data from gram-negative BSIs [49].

Notably, we did not find any correlation between mortality and MDR, *in vitro* appropriateness of the therapy and HAIs. Although this finding could be due to the relatively small sample size, other studies in small animal practice described similar results [2,40,50–52]. Indeed, mortality is influenced by a plethora of other factors, such as patient’s response, bacterial load therapy timing, route of administration and doses [2,53]. On the other hand, we found a strong correlation between MDR and inappropriate therapy, that can be easily explained by the fact that bacteria resistant to multiple drugs are more likely empirically treated with one ineffective agent. Similarly, the association between potential HA BSIs and the number of invasive devices ($p=0.008$) find its explication in the fact that invasive devices, such as nasogastric tubes or indwelling catheters, are the main route of HAIs infection [14]. For this reason, they should be removed as soon as possible and managed with caution and an evidence-based approach. For example, in human medicine, regular intravenous catheter substitutions are not recommended [54,55].

Additionally, we found the patients with co-positive samples were less likely to develop suspected HA BSIs ($p=0.012$). The presence of another positive sample (e.g. urine) for the same pathogen indicates that probably the BSIs was a consequence of the expansion of the infection from the site of origin (e.g. the urinary tract). Conversely, HA BSIs often are caused by bacteria that directly infect the bloodstream through indwelling catheters or other invasive devices, without the involvement of other districts.

This study has some limitations. First, the sample size was relatively small, affecting the statistical power of the analysis. Second, the *in vitro* appropriateness of the therapy did not consider many other pharmacokinetics and clinical factors (dose, duration, timing) that could have influenced the efficacy of the treatment. Third, the methodology used to perform the AST (Kirby-bauer) was semi-quantitative, with a subsequent loss in sensitivity and accuracy of the test.

In conclusion, this research aimed to add novelty to the prevalence, the resistance rates and associated factors of bacterial BSIs in small animal medicine. Our findings want to emphasize the need for surveillance plans, antimicrobial stewardship programs and improved infection control protocols also in companion animals. While the progress made by human medicine should be used as a reference, also the specific differences, such as the limitations on antibiotic use, should be also considered to optimize BSIs management in this field. This is specifically important considering the deep interconnection between animal and human health, and the risk of interspecific transmission.

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Abbreviations

The following abbreviations are used in this manuscript:

BC	Bood Culture
BSI	Bloodstream Infection
AMR	Antimicrobial Resistance
MDR	Multidrug Resistance
HAI	Healthcare-Associated Infection
VUH	Veterinary University Hospital
EUCAST	European Committee on Antimicrobial Susceptibility Testing
CLSI	Clinical and Laboratory Standard Institute
AST	Antimicrobial Susceptibility Testing

Appendix A

Table A1. List of tested antimicrobials divided for antimicrobial class.

Antimicrobial class	Antimicrobial drug
Aminoglycosides	Amikacin 30 µg
Penicillins +/- beta-lactamases inhibitors	Gentamicin 10 µg (120 µg for <i>Enterococcus</i> spp. isolates)
	Ampicillin 10 µg
	Penicillin G 10 units (for <i>Staphylococcus</i> spp.)
	Oxacillin 1 µg (for <i>Staphylococcus pseudintermedius</i> and <i>Staphylococcus schleiferi</i>)
	Amoxicillin- clavulanate 30 µg
	Ampicillin-sulbactam 20 µg
	Piperacillin-tazobactam 110 µg
Cephalosporins	Cefazolin/cephalothin 30 µg
	Cefoxitin 30 µg (for <i>Staphylococcus</i> spp. other than <i>S.pseudintermedius</i> and <i>S.schleiferi</i>)
	Ceftiofur 30 µg
	Ceftazidime 30 µg (for <i>Pseudomonas aeruginosa</i>)
Tetracyclines	Tetracycline 30 µg
Fluoroquinolones	Enrofloxacin 5 µg
Sulfonamides + dihydrofolate reductase inhibitors	Trimethoprim- sulfamethoxazole 1.25/23.7 µg

References

1. Martinez, R.M.; Wolk, D.M. Bloodstream Infections. *Microbiol Spectr* **2016**, *4*, 4.4.42, doi:10.1128/microbiolspec.DMIH2-0031-2016.

2. Keir, I.; Dickinson, A.E. The Role of Antimicrobials in the Treatment of Sepsis and Critical Illness-related Bacterial Infections: Examination of the Evidence. *J Vet Emergen Crit Care* **2015**, *25*, 55–62, doi:10.1111/vec.12272.

3. *Antimicrobial Therapy in Veterinary Medicine*; Dowling, P.M., Prescott, J.F., Baptiste, K.E., Eds.; 1st ed.; Wiley, 2024; ISBN 978-1-119-65459-9.

4. European Medicines Agency *Categorisation of Antibiotics in the European Union*; European Medicines Agency: Amsterdam, 2019;
5. Clinical and Laboratory Standards Institute (CLSI) Performance Standards for Antimicrobial Disk and Dilutions Susceptibility Test for Bacteria Isolated from Animals, 4th Ed.; CLSI Supplement Vet08. 2018.
6. Clinical and Laboratory Standards Institute (CLSI) Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals. 6th Ed. CLSI Supplement VET01S 2023.
7. European Committee on Antimicrobial Susceptibility Testing (EUCAST) Breakpoint Tables for Interpretation of MICs and Zone Diameters. Version 15.0. 2025.
8. CRAB *Tabelle Resistenze Intrinseche Dei Batteri Di Interesse Veterinario*; Centro di Referenza Nazionale per l'Antibioticoresistenza: Rome, Italy, 2018;
9. Nabal Díaz, S.G.; Algara Robles, O.; García-Lechuz Moya, J.M. New Definitions of Susceptibility Categories EUCAST 2019: Clinic Application. *Rev Esp Quimioter* **2022**, *35*, 84–88, doi:10.37201/req/s03.18.2022.
10. Magiorakos, A.-P.; Srinivasan, A.; Carey, R.B.; Carmeli, Y.; Falagas, M.E.; Giske, C.G.; Harbarth, S.; Hindler, J.F.; Kahlmeter, G.; Olsson-Liljequist, B.; et al. Multidrug-Resistant, Extensively Drug-Resistant and Pandrug-Resistant Bacteria: An International Expert Proposal for Interim Standard Definitions for Acquired Resistance. *Clinical Microbiology and Infection* **2012**, *18*, 268–281, doi:10.1111/j.1469-0691.2011.03570.x.
11. Tabah, A.; Cotta, M.O.; Garnacho-Montero, J.; Schouten, J.; Roberts, J.A.; Lipman, J.; Tacey, M.; Timsit, J.-F.; Leone, M.; Zahar, J.R.; et al. A Systematic Review of the Definitions, Determinants, and Clinical Outcomes of Antimicrobial De-Escalation in the Intensive Care Unit. *Clin Infect Dis*. **2016**, *62*, 1009–1017, doi:10.1093/cid/civ1199.
12. Vallicelli, C.; Minghetti, M.; Sartelli, M.; Coccolini, F.; Ansaloni, L.; Agnoletti, V.; Bravi, F.; Catena, F. Antibiotic De-Escalation in Emergency General Surgery. *Antibiotics* **2022**, *11*, 1148, doi:10.3390/antibiotics11091148.
13. Centro Di Referenza Nazionale per l'Antibioticoresistenza LINEE GUIDA PER L'INTERPRETAZIONE DELLE PROVE DI SENSIBILITÀ AI CHEMIOANTIBIOTICI IN VITRO PER UN UTILIZZO NELLA TERAPIA CLINICA 2018.
14. Haque, M.; Sartelli, M.; McKimm, J.; Abu Bakar, M.B. Health Care-Associated Infections & an Overview. *IDR* **2018**, *Volume 11*, 2321–2333, doi:10.2147/IDR.S177247.
15. Tsuyuki, Y.; Kurita, G.; Murata, Y.; Takahashi, T. Bacteria Isolated from Companion Animals in Japan (2014–2016) by Blood Culture. *Journal of Infection and Chemotherapy* **2018**, *24*, 583–587, doi:10.1016/j.jiac.2018.01.014.
16. Saarenkari, H.K.; Sharp, C.R.; Smart, L. Retrospective Evaluation of the Utility of Blood Cultures in Dogs (2009–2018): 45 Cases. *J Vet Emergen Crit Care* **2022**, *32*, 141–145, doi:10.1111/vec.13144.
17. Moraes, R.; Ribeiro, D.; Melchert, A.; A, H.; Regalin, D.; Filho, R.; Giuffrida, R.; Takahira, R.; Okamoto, A.; Okamoto, P. **A Retrospective Description of Blood and Urine Alterations in 386 Male Cats with Urethral Obstruction in Botucatu, São Paulo, Brazil.** *Open Vet J* **2024**, *14*, 2901, doi:10.5455/OVJ.2024.v14.i11.19.
18. Ogrodny, A.J.; Mani, R.; Schmid, S.M.; Gould, E.N.; Fellman, C.L.; DeStefano, I.; Shropshire, S.; Haines, J.M.; Bolton, T.A.; Jablonski, S.A.; et al. Multi-Institutional Retrospective Study Investigating Blood Culture Protocols and Test Positivity in 701 Dogs. *Front. Vet. Sci.* **2023**, *10*, 1301018, doi:10.3389/fvets.2023.1301018.
19. Greiner, M.; Wolf, G.; Hartmann, K. A Retrospective Study of the Clinical Presentation of 140 Dogs and 39 Cats with Bacteraemia. *Journal of Small Animal Practice* **2008**, *49*, 378–383, doi:10.1111/j.1748-5827.2008.00546.x.
20. Greiner, M.; Wolf, G.; Hartmann, K. Bacteraemia and Antimicrobial Susceptibility in Dogs. *Veterinary Record* **2007**, *160*, 529–530, doi:10.1136/vr.160.15.529.
21. Lee, A.; Mirrett, S.; Reller, L.B.; Weinstein, M.P. Detection of Bloodstream Infections in Adults: How Many Blood Cultures Are Needed? *J Clin Microbiol* **2007**, *45*, 3546–3548, doi:10.1128/JCM.01555-07.
22. Tarai, B.; Jain, D.; Das, P.; Budhiraja, S. Paired Blood Cultures Increase the Sensitivity for Detecting Pathogens in Both Inpatients and Outpatients. *Eur J Clin Microbiol Infect Dis* **2018**, *37*, 435–441, doi:10.1007/s10096-018-3188-8.

23. Neumann, N.; Solis, S.A.F.; Crawford, S.; Rogovskyy, A.S. Are Multiple Blood Cultures Advantageous for Canine Patients? *J VET Diagn Invest* **2023**, *35*, 332–335, doi:10.1177/10406387231164095.
24. Hossain, B.; Islam, M.S.; Rahman, A.; Marzan, M.; Rafiqullah, I.; Connor, N.E.; Hasanuzzaman, M.; Islam, M.; Hamer, D.H.; Hibberd, P.L.; et al. Understanding Bacterial Isolates in Blood Culture and Approaches Used to Define Bacteria as Contaminants: A Literature Review. *Pediatric Infectious Disease Journal* **2016**, *35*, S45–S51, doi:10.1097/INF.0000000000001106.
25. Perry, K.M.; Lynch, A.M.; Caudill, A.; Vigani, A.; Roberston, J.B.; Vaden, S. Clinical Features, Outcome, and Illness Severity Scoring in 32 Dogs with Urosepsis (2017–2018). *J Vet Emergen Crit Care* **2022**, *32*, 236–242, doi:10.1111/vec.13158.
26. Barash, N.R.; Birkenheuer, A.J.; Vaden, S.L.; Jacob, M.E. Agreement between Parallel Canine Blood and Urine Cultures: Is Urine Culture the Poor Man's Blood Culture? *J Clin Microbiol* **2018**, *56*, e00506-18, doi:10.1128/JCM.00506-18.
27. Scarpellini, R.; Assirelli, G.; Giunti, M.; Esposito, E.; Mondo, E.; Piva, S. Monitoring the Prevalence of Antimicrobial Resistance in Companion Animals: Results from Clinical Isolates in an Italian University Veterinary Hospital. *Transboundary and Emerging Diseases* **2023**, *2023*, 6695493, doi:10.1155/2023/6695493.
28. Guardabassi, L. Veterinary Hospital-Acquired Infections: The Challenge of MRSA and Other Multidrug-Resistant Bacterial Infections in Veterinary Medicine. *The Veterinary Journal* **2012**, *193*, 307–308, doi:10.1016/j.tvjl.2012.04.005.
29. Vincze, S.; Stamm, I.; Kopp, P.A.; Hermes, J.; Adlhoch, C.; Semmler, T.; Wieler, L.H.; Lübke-Becker, A.; Walther, B. Alarming Proportions of Methicillin-Resistant *Staphylococcus Aureus* (MRSA) in Wound Samples from Companion Animals, Germany 2010–2012. *PLoS ONE* **2014**, *9*, e85656, doi:10.1371/journal.pone.0085656.
30. Stull, J.W.; Weese, J.S. Hospital-Associated Infections in Small Animal Practice. *Veterinary Clinics of North America: Small Animal Practice* **2015**, *45*, 217–233, doi:10.1016/j.cvsm.2014.11.009.
31. Freilich, L.; Jugan, M.C. Retrospective Evaluation of Enteral Nutrition Supplementation in 295 Hospitalized Dogs and Cats (2014–2023). *javma* **2024**, 1–7, doi:10.2460/javma.24.07.0494.
32. Taylor, S.; Chan, D.L.; Villaverde, C.; Ryan, L.; Peron, F.; Quimby, J.; O'Brien, C.; Chalhoub, S. 2022 ISFM Consensus Guidelines on Management of the Inappetent Hospitalised Cat. *Journal of Feline Medicine and Surgery* **2022**, *24*, 614–640, doi:10.1177/1098612X221106353.
33. Breheny, C.R.; Boag, A.; Le Gal, A.; Höim, S.; Cantatore, M.; Anderson, D.; Nuttall, T.; Chandler, M.L.; Gunn-Moore, D.A. Esophageal Feeding Tube Placement and the Associated Complications in 248 Cats. *Veterinary Internal Medicine* **2019**, *33*, 1306–1314, doi:10.1111/jvim.15496.
34. Greiner, M.; Wolf, G.; Hartmann, K. Bacteraemia in 66 Cats and Antimicrobial Susceptibility of the Isolates (1995–2004). *Journal of Feline Medicine and Surgery* **2007**, *9*, 404–410, doi:10.1016/j.jfms.2007.04.004.
35. Fidanzio, F.; Rega, M.; Bertini, S.; Carrillo Heredero, A.M.; Corsini, A.; Corti, F.; Crosara, S.; Quintavalla, C. Overview on Antimicrobial Prescription Habits in Cats at Different Clinical Services of the Veterinary Teaching Hospital of Parma. *BMC Vet Res* **2025**, *21*, 106, doi:10.1186/s12917-025-04602-5.
36. De Briyne, N.; Atkinson, J.; Borriello, S.P.; Pokludová, L. Antibiotics Used Most Commonly to Treat Animals in Europe. *Veterinary Record* **2014**, *175*, 325–325, doi:10.1136/vr.102462.
37. Yudhanto, S.; Hung, C.-C.; Maddox, C.W.; Varga, C. Antimicrobial Resistance in Bacteria Isolated From Canine Urine Samples Submitted to a Veterinary Diagnostic Laboratory, Illinois, United States. *Front. Vet. Sci.* **2022**, *9*, 867784, doi:10.3389/fvets.2022.867784.
38. Black, D.M.; Rankin, S.C.; King, L.G. Antimicrobial Therapy and Aerobic Bacteriologic Culture Patterns in Canine Intensive Care Unit Patients: 74 Dogs (January–June 2006). *J Vet Emergen Crit Care* **2009**, *19*, 489–495, doi:10.1111/j.1476-4431.2009.00463.x.
39. European Centre for Disease Prevention and Control *Point Prevalence Survey of Healthcare- Associated Infections and Antimicrobial Use in European Acute Care Hospitals – Protocol Version 6.1*; ECDC, 2023;
40. Schreiber, A.; Epstein, S.E.; Byrne, B.A.; Reagan, K.L. Survey of Bacterial Isolates and Their Antimicrobial Susceptibility Patterns from Dogs with Infective Endocarditis. *Pathogens* **2023**, *12*, 1011, doi:10.3390/pathogens12081011.

41. Impey, R.E.; Hawkins, D.A.; Sutton, J.M.; Soares Da Costa, T.P. Overcoming Intrinsic and Acquired Resistance Mechanisms Associated with the Cell Wall of Gram-Negative Bacteria. *Antibiotics* **2020**, *9*, 623, doi:10.3390/antibiotics9090623.
42. Breijyeh, Z.; Jubeh, B.; Karaman, R. Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to Resolve It. *Molecules* **2020**, *25*, 1340, doi:10.3390/molecules25061340.
43. World Health Organization *WHO Bacterial Priority Pathogens List, 2024: Bacterial Pathogens of Public Health Importance to Guide Research, Development and Strategies to Prevent and Control Antimicrobial Resistance.*; World Health Organization: Geneva, Switzerland., 2024;
44. Scarpellini, R.; Pulido-Vadillo, M.; Serna, C.; Gonzalez-Zorn, B.; Blanco, J.L.; Delgado-Blas, J.F.; Giunti, M.; Piva, S. High Frequency of Detection of NDM-Producing Enterobacterales Among Companion Animals Hospitalized in an Italian Veterinary Teaching Hospital. *Transboundary and Emerging Diseases* **2025**, *2025*, 2622185, doi:10.1155/tbed/2622185.
45. Tabah, A.; De Bus, L.; Leone, M. Antibiotic De-Escalation: Finally, Some Action and Not Only Words. *The Lancet Infectious Diseases* **2024**, *24*, 331–333, doi:10.1016/S1473-3099(23)00749-1.
46. on behalf of The Nine-I study Group; Rello, J.; Sarda, C.; Mokart, D.; Arvaniti, K.; Akova, M.; Tabah, A.; Azoulay, E. Antimicrobial Stewardship in Hematological Patients at the Intensive Care Unit: A Global Cross-Sectional Survey from the Nine-i Investigators Network. *Eur J Clin Microbiol Infect Dis* **2020**, *39*, 385–392, doi:10.1007/s10096-019-03736-3.
47. De Waele, J.J.; Schouten, J.; Beovic, B.; Tabah, A.; Leone, M. Antimicrobial De-Escalation as Part of Antimicrobial Stewardship in Intensive Care: No Simple Answers to Simple Questions—a Viewpoint of Experts. *Intensive Care Med* **2020**, *46*, 236–244, doi:10.1007/s00134-019-05871-z.
48. Smith, A.; Wayne, A.S.; Fellman, C.L.; Rosenbaum, M.H. Usage Patterns of Carbapenem Antimicrobials in Dogs and Cats at a Veterinary Tertiary Care Hospital. *Veterinary Internal Medicine* **2019**, *33*, 1677–1685, doi:10.1111/jvim.15522.
49. Teitelbaum, D.; Elligsen, M.; Katz, K.; Lam, P.W.; Lo, J.; MacFadden, D.; Vermeiren, C.; Daneman, N. Introducing the Escalation Antibigram: A Simple Tool to Inform Changes in Empiric Antimicrobials in the Nonresponding Patient. *Clinical Infectious Diseases* **2022**, *75*, 1763–1771, doi:10.1093/cid/ciac256.
50. Robbins, S.N.; Goggs, R.; Lhermie, G.; Lalonde-Paul, D.F.; Menard, J. Antimicrobial Prescribing Practices in Small Animal Emergency and Critical Care. *Front. Vet. Sci.* **2020**, *7*, 110, doi:10.3389/fvets.2020.00110.
51. Dickinson, A.E.; Summers, J.F.; Wignall, J.; Boag, A.K.; Keir, I. Impact of Appropriate Empirical Antimicrobial Therapy on Outcome of Dogs with Septic Peritonitis. *J Vet Emergen Crit Care* **2015**, *25*, 152–159, doi:10.1111/vec.12273.
52. Proulx, A.; Hume, D.Z.; Drobatz, K.J.; Reineke, E.L. In Vitro Bacterial Isolate Susceptibility to Empirically Selected Antimicrobials in 111 Dogs with Bacterial Pneumonia. *J Vet Emergen Crit Care* **2014**, *24*, 194–200, doi:10.1111/vec.12128.
53. Kumar, A.; Haery, C.; Paladugu, B.; Kumar, A.; Symeoneides, S.; Taiberg, L.; Osman, J.; Trenholme, G.; Opal, S.M.; Goldfarb, R.; et al. The Duration of Hypotension before the Initiation of Antibiotic Treatment Is a Critical Determinant of Survival in a Murine Model of *Escherichia Coli* Septic Shock: Association with Serum Lactate and Inflammatory Cytokine Levels. *J INFECT DIS* **2006**, *193*, 251–258, doi:10.1086/498909.
54. Rickard, C.M.; Webster, J.; Wallis, M.C.; Marsh, N.; McGrail, M.R.; French, V.; Foster, L.; Gallagher, P.; Gowardman, J.R.; Zhang, L.; et al. Routine versus Clinically Indicated Replacement of Peripheral Intravenous Catheters: A Randomised Controlled Equivalence Trial. *The Lancet* **2012**, *380*, 1066–1074, doi:10.1016/S0140-6736(12)61082-4.
55. Vendramim, P.; Avelar, A.F.M.; Rickard, C.M.; Pedreira, M. d. L.G. The RESPECT Trial—Replacement of Peripheral Intravenous Catheters According to Clinical Reasons or Every 96 Hours: A Randomized, Controlled, Non-Inferiority Trial. *International Journal of Nursing Studies* **2020**, *107*, 103504, doi:10.1016/j.ijnurstu.2019.103504.

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