ESCOLA UNIVERSITÁRIA VASCO DA GAMA

MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

SEPTIC PERITONITIS: CHARACTERIZATION OF BACTERIAL ISOLATES AND ITS RESISTANCE PROFILE IN DOGS AND CATS (20 CASES)

Diana Maria Gonçalves d'Oliveira Sérgio António

Coimbra, julho de 2020

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Aluna do Mestrado Integrado em Medicina Veterinária

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Ao meus queridos pais, por caminharem sempre a meu lado.

"(…) parecia que tinhamos chegado
ao fim da estrada e afinal era apenas
uma curva a abrir para outra
paisagem e novas curiosidades."

José Saramago

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Abbreviations

AMI - Amikacin
AMC - Amoxicillin-Clavulanic acid
AMR - Antimicrobial resistance
AMP - Ampicillin
AMS - Ampicillin-Sulbactam
AST - Antimicrobial Susceptibility Testing
AZI - Azithromycin
BEN - Benzylpenicillin
CIP - Ciprofloxacin
CUR - Cefuroxime
CUR-a - Cefuroxime Axetil
CHL - Chloranphenicol
CLI - Clindamycin
CMX – Co-trimoxazole
CPO - Cefpodoxime
CTA - Cefotaxime
CTF - Ceftiofur
CTZ - Ceftazidime
CVN - Cefovecin
CZO - Cefazolin
CXI - Cefoxitin
DOX - Doxycycline
ENR - Enrofloxacin
ERY - Erythromycin
FIP - Feline Infectious Peritonitis
FOS - Fosfomycin
FUS - Fusidic acid
GEN - Gentamicin
GI - Gastrointestinal
HB - Hepatobiliary
IMI - Imipenem
LEX - Cephalexin
MAR - Marbofloxacin
MER - Meropenem
MIC - Minimum Inhibitory Concentration
MUP - Mupirocin
MDR - Multidrug-Resistance
NIT - Nitrofurantoin

OXA - Oxacillin
PEN - Penicillin
PIT - Piperacillin-Tazobactam
PRA - Pradofloxacin
TET - Tetracyclin
TOB - Tobramicin
TRS - Trimethoprim-Sulfamethoxazole
SIRS - Systemic Inflammatory Response Syndrome
SD - Standard Deviation
SP - Septic Peritonitis
UG - Urogenital
VAN - Vancomycin

Septic peritonitis: Characterization of bacterial isolates and its resistance profile in dogs and cats (20 cases)

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Resumo

A Peritonite Séptica (PS) é um quadro clínico de elevado risco de vida e comumente fatal, que resulta da inflamação da cavidade peritoneal. A infeção ocorre maioritariamente por contaminação bacteriana e é classificada em primária, secundária e terciária.

O presente estudo teve como finalidade caracterizar retrospetivamente os isolados bacterianos provenientes de animais de companhia com peritonite séptica e os respetivos perfis de resistência a antimicrobianos, num Hospital de referência localizado em Barcelona, Espanha.

Esta análise incluiu uma amostra de 20 animais, dos quais 16 cães e 4 gatos diagnosticados e tratados entre os meses de Outubro de 2017 e Março de 2020 no Hospital Veterinari del Mar. Os doentes foram submetidos a avaliação clínica, exames complementares e subsequente intervenção cirúrgica. Os resultados dos testes de sensibilidade a antimicrobianos foram utilizados para analisar os perfis de resistência.

A Peritonite séptica secundária a contaminação de origem urogenital, gastrointestinal e hepatobiliar foi a forma mais comum neste estudo. Após o tratamento, sobreviveram 17 animais (85%), 16 cães e um gato sendo que os restantes 3 foram eutanasiados, nos dias seguintes à intervenção cirúrgica.

Escherichia coli (60%) foi a bactéria predominantemente isolada nos cães e gatos nos três tipos de origem. Dos 12 isolados de E. coli, seis apresentaram resistência para ampicilina e cinco para a doxiciclina e cefovecina. Três dos isolados demonstraram resistências simultâneas para ampicilina, amoxicilina com ácido clavulânico, cefalexina, cefuroxima, cefovecina, doxiciclina, todas as fluoroquinolonas testadas e trimetoprim-sulfametoxazol. Todos os testados apresentaram susceptibilidade para amicacina, imipenem e meropenem.

Os resultados obtidos confirmam a necessidade de se implementar as recomendações internacionais no âmbito da resistência aos antimicrobianos, de forma a reduzir a disseminação das resistências, um dos principais desafios das gerações futuras. A seleção de antimicrobianos com base nos testes de sensibilidade a antimicrobianos é essencial por promover o uso racional dos antimicrobianos e consequentemente diminuir os perfis de resistência.

Palavras-chave: *Peritonite séptica, Isolados bacterianos, Escherichia coli, Resistência a Antimicrobianos, Cães e Gatos.*

Abstract

Septic peritonitis (SP) is a life-threatening and frequently fatal situation that results from an inflammatory condition of the peritoneal cavity. This infection occurs mostly due to bacterial contamination and can be classified as primary, secondary or tertiary.

The present study aimed characterize retrospectively the bacterial isolates from small animals with septic peritonitis and respective antimicrobial resistance profiles, in a veterinary referral and emergency Hospital in Barcelona, Spain.

The assessment included a sample of 20 animals,16 dogs and 4 cats diagnosed and treated between the months of October of 2017 and March of 2020 at Hospital Veterinari del Mar. Patients were submitted to clinical evaluation criteria, complementary exams and surgical intervention. Antimicrobial susceptibility testing results were used to analyse resistant profiles.

Septic peritonitis secondary to urogenital, gastrointestinal and hepatobiliary leakage was the most common form in this study. After treatment, there was a survival of 17 animals (85%), 16 dogs and one cat and 3 cats were euthanised days after surgery.

Escherichia coli (60%) was the predominant bacteria isolated in both cats and dogs from all three sources. From 12 *E. coli* isolates, six showed resistance to ampicillin, five to doxycycline and cefovecin. Three of those present simultaneously resistance against ampicillin, amoxicillin-clavulanic acid, cephalexin, cefuroxime, cefovecin, all tested fluoroquinolones, doxycycline and trimethoprim-sulfamethoxazole. All tested isolates demonstrated susceptibility to amikacin, imipenem and meropenem.

Antimicrobial resistance profiles observed confirm the need to implement international recommendation in the scope of resistance to antimicrobials, to reduce the spread of resistance, one of the main challenges for future generations. Antimicrobial selection based on antimicrobial susceptibility testing is imperative to promote rational use of antimicrobials and consequently decrease resistant profiles.

Keywords: Septic peritonitis, Bacterial isolates, *Escherichia coli*, Antimicrobial resistance, Dogs and Cats

1. Background

Septic peritonitis (SP) is a serious progressive condition and a recurrent cause of sepsis which affects both cats and dogs (Bentley, Mayhew, Culp, & Otto, 2011). It results from the inflammation of the peritoneal cavity due to bacterial contamination and may be classified as primary, secondary and tertiary (Adams, Doyle, Bray, & Burton, 2014; Bentley, 2016; Ludwig, 2013; Marshall et al., 2017; Ragetly, Bennett, & Ragetly, 2011). Primary or spontaneous bacterial peritonitis has an unrecognizable intraperitoneal origin, with no suspicious history of abdominal trauma (Barfield et al., 2014; Greene & Marks, 2012) neither loss of gastrointestinal integrity (Sartelli, 2010). Translocation of bacteria throughout the intestinal wall by lymphogenous or hematogenous routes may be the origin of this peritonitis (Bentley, 2016; Ragni & House, 2009). It is described as an uncommon condition in animals, nevertheless occurs mostly in cats, caused by the well-known feline coronavirus, that causes feline infectious peritonitis (Ruthrauff, Smith, & Glerum, 2009). Secondary peritonitis results from an intraperitoneal and identified source (Culp, Zeldis, Reese, & Drobatz, 2009) and is the most common form in cats and dogs (Bentley, 2016; Culp & Holt, 2010; Ludwig, 2013; Nelson & Couto, 2014; Ragetly et al., 2011). Septic leakage occurs mostly to a primary cause due to intestinal foreign body perforation, neoplasia or drug induced ulceration (Culp & Holt, 2010). Also it may be caused by pyometra, renal abscesses (urogenital tract), gallbladder rupture (hepatobiliary tract), bite and gunshot wounds (trauma) or even by dehiscence of the sutures and peritoneal dialysis (Nelson & Couto, 2014). Tertiary or recurrent septic peritonitis, mainly described in humans, is a persevering form following the management of a primary or secondary peritonitis (Culp & Holt, 2010) and is usually caused by different microorganisms when compared to the previous conditions (Bentley, 2016; Ragetly et al., 2011).

This condition can lead to a life-threatening organ dysfunction, as the host reacts to pathogen in a deregulated way (Gauthier, Bersenas, & Mathews, 2017; Thompson, Venkatesh, & Finfer, 2019), which results in a direct response named *systemic inflammatory response syndrome* (SIRS). This is a systemic reaction involving inflammatory mediators (complement and coagulation cascade), humoral and cellular reactions (Brady, Otto, Winkle, & King, 2000; Pierrakos & Vincent, 2010), secondary to a septic (fungi, anaerobic bacteria, viruses) or nonseptic (burns, ischemic organ necrosis, neoplasia) condition. According to Bone et al. (1992), both sepsis and SIRS may be described as an inflammatory process, even though sepsis is a clinical expression of SIRS.

Left shift leukocytosis or leukopenia, anaemia and ionized hypocalcemia are frequent findings in animals with bacterial peritonitis (Luschini, Fletcher, & Schoeffler, 2010; Ragetly et al., 2011; Swayne, Brisson, Weese, & Sears, 2012). First-line diagnostic techniques are based on abdominal radiography and ultrasonography and cytologic analysis of the peritoneal effusion which allows to observe degenerated or toxic neutrophils and intracellular or extracellular bacteria. Bacterial culture confirms definitive diagnosis. The level of glucose and lactate in both blood and effusion are also important diagnostic parameters of SP (Bentley, 2016; Bonczynski, Ludwig, Barton, Loar, & Peterson, 2003; Culp & Holt, 2010; Greene & Marks, 2012; Levin, Bonczynski, Ludwig, Barton, & Loar, 2004; Martiny & Goggs, 2019; Parsons, Owen, Lee, Tivers, & Gregory, 2009; Scotti et al., 2017).

Patient approach in this life-threatening situation encompasses fast medical and surgical intervention in order to stabilize the patient and to identify, control and eliminate the source of infection as well intensive postoperative monitoring (Cioffi, Schmiedt, Cornell, & Radlinsky, 2011; Ludwig, 2013; Ragetly, Bennett, & Ragetly, 2011; Staatz, Monnet, & Seim, 2002). Although surgery is the definitive treatment for this condition (Greene & Marks, 2012; Kalafut, Schwartz, Currao, Levien, & Moore, 2016; Ragetly et al., 2011), exploratory laparotomy should only be performed once hypovolemia, cardiovascular status and all metabolic disorders have been restored (Bentley, 2016; Mcgrotty & Doust, 2004; Ragetly et al., 2011).

Early diagnosis and surgical treatment are necessary in animals with septic peritonitis to prevent or minimize the progression of hypovolemia, acid base disturbances and electrolyte abnormalities, all of which will rapidly progress and increase morbidity and mortality (Swann and Hughes 2000). Medical therapy includes intrusive fluid therapy for a suitable resuscitation and improvement of tissue perfusion, pain management, gastrointestinal protectants, blood transfusion (blood products for coagulation abnormalities, bleeding ulcers and peritonitis induced by trauma), oxygen and nutritional supplementation (Culp & Holt, 2010; Greene & Marks, 2012; Mcgrotty & Doust, 2004; Ragetly et al., 2011; Rhodes et al., 2017). Nevertheless, as cases of septic peritonitis are usually high in contamination and laboratories results may delay, prompt empirical broad-spectrum antibiotic therapy is imperative (Marshall et al., 2017).

Bacterial isolates namely, *Staphylococcus epidermidis* are commonly isolated in cases of primary bacterial peritonitis while *Escherichia coli* is the most frequent isolated bacteria in secondary peritonitis (Culp & Holt, 2010; Greene & Marks, 2012). In addition, in this last condition polymicrobial and mixed isolates are frequently reported (Culp & Holt, 2010; Lanz, Ellison, Bellah, Weichman, & VanGilder, 2001) due to bacterial synergism among species as *E.coli* and *Bacteroides spp.* (Ragni & House, 2009).

Antimicrobial agents have been assuming an important role and impact in veterinary medicine shortly after its discovery in 1928 by Sir Alexander Fleming (Keir & Dickinson, 2014). Its suitable empirical administration has aimed to prevent and cure animals and people with infectious diseases and had important contributed in reducing mortality and morbidity rates (Abelson, Buckley, & Rozanski, 2013; Espinasse, 1993).

Microbial culture and antimicrobial susceptibility testing (AST) in veterinary medicine are of paramount relevance allowing the pathogens identification and promoting appropriate antimicrobial use (Pierce-Hendry & Dennis, 2010). Antimicrobial susceptibility assays (Ogeer-gyles, Mathews, & Boerlin, 2005; Pierce-Hendry & Dennis, 2010) allow the determination of minimum inhibitory concentration (MIC), categorizing the microorganism in susceptible, intermediate or resistant to a specific antimicrobial agent. In septic peritonitis peritoneal effusion analysis may aim selecting suitable empiric antibiotherapy, until bacterial susceptibility results are available (Ragni & Moore, 2010).

Empiric administration of antibiotics must be wisely chosen as imprudent use enhances selection of resistance strains (Greene & Boothe, 2012) by pathogenic bacteria and conventional microbiota (Espinasse, 1993). In addition, hospitalization, invasive procedures, changes in animals

microbiota or weak immune system may promote bacteria to acquire and disseminate adaptive resistance mechanisms against antimicrobial substances and subsequently potentiating multidrug-resistant (MDR) organisms (Dickinson, Summers, Wignal, Boag, & Keir, 2013; Karczmarczyk, Abbott, Walsh, Leonard, & Fanning, 2011; Umber & Bender, 2009).

According to the author's knowledge, evaluation of antimicrobial resistance in septic peritonitis is scarce (annex 1).

This retrospective study aimed to characterize microbial isolates found in cases of septic peritonitis in cats and dogs, in a referral practice Hospital in Barcelona (Hospital Veterinari del Mar) and display its resistance profiles against commonly used antimicrobial substances.

2. Materials and Methods

2.1. Sample Characteristics

This study involved a cohort of 20 animals in total,16 dogs, and 4 cats, that were hospitalized and treated between October 2017 and March 2020 at Hospital Veterinari del Mar (Barcelona, Spain), a referral and emergency Hospital.

All the enrolled animals were diagnosed with septic peritonitis. Bacterial peritonitis (primary and secondary) was detected by physical examination along with abdominal leakage or certain anatomical findings at the moment of the ultrasound exam and during exploratory laparotomy. Lastly, peritoneal effusion samples were collected, and diagnosis has been reached by cytologic identification and positive bacterial culture.

This retrospective study was approved by EUVG Scientific Council in regard to EC679/2016 General Data Protection Regulation and furthermore applicable legislation.

2.2. Procedures

Data was retrieved from medical records from the Hospital database and included patient characterization (specie, breed, gender, reproductive status, lifestyle, age, weight), physical examination (capillary refill time, heart and respiratory rates, rectal temperature, abdominal pain and systolic blood pressure), ultrasound and blood testing, including haematological findings (blood count, serum biochemistry and ionogram). Peritoneal effusion samples of all animals were examined through cytology identification and further microbial cultures and antimicrobial susceptibility testing was performed with Vitek® at Iddex and Echervane laboratories (Barcelona, Spain). Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) interpretative criteria (CLSI, 2017). All cases of primary and secondary bacterial peritonitis were compared in distinct categories enrolling animal characterization, criteria values that were significant in cases of septic peritonitis, origin of source and bacterial isolates and their susceptibility to antimicrobials. Outcome was also considered (alive, death or euthanized).

All animals were treated empirically with appropriate broad-spectrum antimicrobials when diagnosed with SP and started it prior to surgery according to morphology and gram stain of the sample, patient age, clinical and pathological condition, source and target site.

2.3. Data Analysis

The registry database was firstly performed through software Microsoft Excel® 2020. Descriptive statistical analysis was carried out with R Core Team® 2019 (Vienna, Austria) where it was compared variables as age, body weight and further clinical parameters expressed as mean ± standard deviation (SD) by specie.

3. Results

3.1. Sample Characteristics

Twenty animals,16 canines and 4 felines diagnosed with septic peritonitis met criteria inclusion in the study and were treated between October 2017 and March of 2020 at Hospital Veterinari del Mar. Animal census variables included breed, gender, reproductive status, lifestyle, age, body weight and clinical parameters suggestive for systemic inflammatory response syndrome (annex 2) and are summarized in Table 1.

Table 1. Characterization of the target population (*n*=20) enrolling demographic and clinical data.

		Dogs	**Cats**
n		**16**	**4**
Breed ***n* (%)**	Mixed Breed	**6** (37.5%)	**4** (100%)
	Pure	**10** (62.5%)	**-**
Gender and Reproductive status ***n* (%)**	**Males**	**5 (31.3%)**	**2 (50%)**
	Intact	2 (12.5%)	**-**
	Neutered	3 (18.8%)	2(50%)
	Females	**11 (68.8%)**	**2 (50%)**
	Intact	5 (31.3%)	**-**
	Neutered	6 (37.5%)	2 (50%)
Lifestyle ***n* (%)**	Indoor	-	3 (75%)
	Outdoor	-	1 (25%)
	Mixed	16 (100%)	**-**
Age (years)	M ± SD	8.62 ± 2.98	9.5 ± 5.19
	Min-Max	5-13	5-14
Weight (Kg)	M ± SD	13.57 ± 12.28	2.84 ± 0.39
	Min-Max	1.25 - 47	2.6 – 3.5
Temperature (ºC)	M ± SD	38.8 ± 1.21	38.23 ± 0.66
	Min-Max	36.6 - 41	37.4 – 38.9
HR (bpm)	M ± SD	129.2 ± 34.5	202.5 ± 12.58
	Min-Max	70 - 180	190 – 220
RR (brpm)	M ± SD	29.12 ± 10.27	32 ± 8.64
	Min-Max	20 - 60	20 - 40
WBC (x 10^9/L)	M ± SD	11.84 ± 10.2	16 ± 12.7
	Min-Max	0.19 - 28.96	7.5 - 34.9

HR – Heart rate; RR – Respiratory rate; WBC – White Blood Cell count; Min– Minimum; Max – Maximum; M ± SD – Mean ± standard deviation

Animals (*n*=20) were from nine distinct breeds (*n*=10) and mixed-breed dogs (*n*=6): two Yorkshire Terriers and one each of 8 traditional breeds such as Bichon Frise, Chihuahua, Chinese Crested Dog, English Bulldog, Labrador Retriever, Miniature Pinscher, Schnauzer and a Spanish Water Dog. All felines (*n*=4) were domestic mixed-breed cats. Age ranged from 5 years to 14 years old for feline patients and from 5 years to 13 years old for dogs.

3.2. Characterization Of Septic Peritonitis Cases

The source of abdominal contamination was identified in all 19 cases of secondary peritonitis (95%) which included the urogenital tract (7/20, 35%), the gastrointestinal system (6/20, 30%) and the hepatobiliary tract (6/20, 30%). Primary peritonitis occurred in one case (5%). Peritoneal effusion samples were assayed to identify culture growth and results were all positive. Survival outcome was positive for dogs (16/16 100%) and poor for cats (1/4, 25%). All cases are depicted in Table 2.

Table 2. Characterization of peritonitis cases according to source, bacterial isolates and outcome.

Source	*n (%)*	Microbial isolates	Outcome
Primary Peritonitis	**1** (5%)	*Staphylococcus epidermidis*	Euthanized
Secondary Peritonitis	**19** (95%)		
Urogenital	**7** (35%)		
	4	*Escherichia coli*	Alive
	1	*Enterobacter cloacae*	
	1	*Klebsiella pneumoniae*	
	1	*Pseudomonas aeruginosa*	
Gastrointestinal	**6** (30%)		
	4	*Escherichia coli*	Alive
	1	*Enterococcus avium*	
	1	*Serratia marcescens*	
Hepatobiliary	**6** (30%)		2 Alive; 2 Euthanized
	4	*Escherichia coli*	
	1	*Enterobacter cloacae*	Alive
	1	*Streptococcus canis*	

Escherichia coli isolates were found in the same quantity in all three origin source.

Peritoneal effusion sample results are often polymicrobial due to the underlying cause and the commensal microbiota. In these cases, all shown monomicrobial infection enrolled isolates such as *E. coli* (60%), *Enterobacter cloacae* (10%) and others presented in Figure 1.

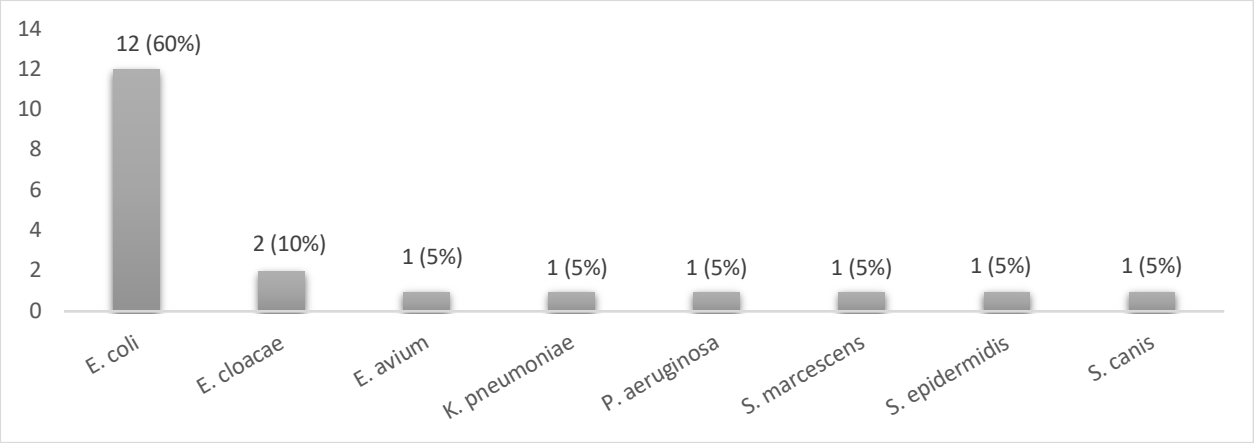

Figure 1. Percentages of microbial isolates from all animals (*n*=20) diagnosed with septic peritonitis.

3.3. Antimicrobial Susceptibility Profiles

Antimicrobial susceptibility results for each case were available and summarized in Table 3. From overall isolates (n=20), 38 antimicrobial substances were assayed, in different frequencies among isolates. Antimicrobial classes included beta lactams (e.g. cephalosporins, carbapenems) aminoglycosides, fluoroquinolones, macrolides, tetracyclines, sulfonamides, nitrofuran derivatives and steroid antibacterials. Regarding those isolates, resistance results against beta lactams were the following: ampicillin (12/20, 60%), ampicillin-sulbactam (1/2 , 50%), penicillin (1/3, 33%), amoxicillin-clavulanic acid (8/20, 40%), oxacillin (2/3, 66%), cefoxitin (1/1, 100%), piperacillin-tazobactam (1/20, 5%). First to third generations cephalosporins presented the following results: cephalexin (5/20, 25%), cefuroxime (4/14, 29%), cefpodoxime (6/17, 35%), cefotaxime (3/6, 50%), ceftiofur (2/6, 33%), ceftazidime (3/17, 18%), cefovecin (8/20, 40%) and cephamycin substance was cefoxitin with a high susceptibility (5/6, 83%) except for one *E. coli*; Most of all isolates shown high susceptibility profiles against Carbapenems, nevertheless imipenem showed 5% of resistance (*S. epidermidis*) and meropenem 7% (*E. avium*). Resistance values against fluoroquinolones substances (ciprofloxacin, enrofloxacin, marbofloxacin and pradofloxacin) ranged from 21% to 25%.

Aminoglycosides resistance results were associated to gentamicin (2/20, 10%), tobramycin (5/14, 36%) and amikacin (3/20, 15%); resistance to protein synthesis inhibitors such as erythromycin (1/3, 33%), azithromycin (1/3, 33%), clindamycin (1/3, 33%), tetracyclin (5/10, 50%), doxycycline (7/20, 35%), chloramphenicol (1/10, 10%) were also available; trimethoprim-sulfamethoxazole from sulfonamides presented 30% of resistance towards isolates (6/20); In nitrofuran derivates, only one isolate showed resistance (1/4) and to steroid antibacterial, fusidic acid, the only one tested (*S. epidermidis*) found to be resistant. Vancomycin and mupirocin were effective against *E. avium*, *S. canis* and *S. epidermidis*. Fosfomycin and co-trimoxazole were both effective against *E. coli* isolated from dogs, D06 and D12, in which one was isolated from gastrointestinal source and another from urogenital source, respectively.

Table 3. Summary of isolates and its antimicrobial susceptibility in dogs and cats.

Animal Code	Sample/Source	Microbial Culture	Antimicrobial Susceptibility Testing (AST)
			AMP AMS PEN AMC OXA PIT LEX CZO CUR CUR-A CPO CXI CTA CTF CTZ CVN IMI MER CIP ENR MAR PRA GEN TOB AMI ERY AZI CLI VAN MUP TET DOX CHL TRS FOS CMX NIT FUS
D01	Abdominal Effusion/GI	*E. coli*	
D02	Abdominal Effusion/UG	*E. coli*	
D03	Abdominal Effusion/GI	*S. marcescens*	
D04	Abdominal Effusion/GI	*E. avium*	
D05	Abdominal Effusion/UG	*K. pneumoniae*	
D06	Abdominal Effusion/GI	*E. coli*	
D07	Intestinal Effusion/GI	*E. coli*	
D08	Abdominal Effusion/GI	*E. coli*	
D09	Abdominal Effusion/HB	*E. cloacae*	
D10	Biliary Effusion/HB	*E. coli*	
D11	Abdominal Effusion/UG	*E. coli*	
D12	Urine/UG	*E. coli*	
D13	Kidney Abcess/UG	*E. coli*	
D14	Abdominal Effusion HB	*S. canis*	
D15	Gallbladder, Bilis/HB	*E. coli*	
D16	Bladder/UG	*E. cloacae*	
C01	Biliary Effusion/ HB	*E. coli*	
C02	Duodenum, Jejunum, Ileon, Liver, Pancreas/HB	*E. coli*	
C03	Unknown	*S. epidermidis*	
C04	Pyometra/UG	*P. aeruginosa*	

Not tested | Susceptibility | Resistance | Intermediate

GI- Gastrointestinal; UG- Urogenital; HB- Hepatobiliary; AMP- Ampicillin; AMS- Ampicillin-Sulbactam; PEN- Penicillin; AMC- Amoxicillin-Clavulanic acid; OXA- Oxacillin; PIT- Piperacillin-Tazobactam; LEX- Cephalexin; CZO- Cefazolin; CUR-Cefuroxime (A-axetil); CPO- Cefpodoxime; CXI- Cefoxitin; CTA- Cefotaxime; CTF- Ceftiofur; CTZ- Ceftazidime; CVN- Cefovecine; IMI- Imipenem; MER- Meropenem; CIP- Ciprofloxacin; ENR- Enrofloxacin; MAR- Marbofloxacin; PRA- Pradofloxacin; GEN- Gentamicine; TOB- Tobramycin; AMI- Amikacin; ERY- Erythromycin; AZI- Azithromycin; CLI- Clindamycin; VAN- Vancomycin; MUP- Mupirocin; TET- Tetracycline; DOX- Doxycycline; CHL- Chloramphenicol; TRS- Trimethoprim-Sulfamethoxazole; FOS- Fosfomycin; CMX- Cotrimoxazole; NIT- Nitrofurantoin; FUS- Fusidic acid

In addition, in all tested substances (n=38) only 4 shown complete susceptibility to the assayed isolates.

Escherichia coli was the most frequently isolated (12/20, 60%). Bacteria isolates as *E. cloacae,* collected within urogenital source from a dog (D16), presented high resistance pattern up to 81% (13/16) of the assayed antimicrobials, namely ampicillin, amoxicillin-clavulanic acid, cephalexin, cefpodoxime, ceftiofur, ceftazidime, cefovecin, ciprofloxacin, enrofloxacin, marbofloxacin, gentamicin, doxycycline, trimethoprim-sulfamethoxazole and intermediate result to nitrofurantoin (6%). *P. aeruginosa* collected within same source yet from a cat (C04) shown a substantial resistant pattern as well (9/16, 56%), as presented resistance to the same antimicrobials tested in *E. cloacae*, differentiate only on the intermediate substance (enrofloxacin) and shown susceptibility against the remaining tested fluroquinolones and gentamicin. *S. epidermidis*, collected in a cat (C03) with primary septic peritonitis, presented resistance to antimicrobials up to 35% (10/29) enrolling beta lactamics, imipenem, tobramycin, erythromycin, azithromycin, tetracyclin and fusidic acid. *E. avium,* collected within gastrointestinal source from a dog (D04) shown resistant profile to 7/27 antimicrobials (26%) such as oxacillin, cefuroxime, cefpodoxime, cefovecin, meropenem, amikacin and clindamycin, yet with intermediate results (15%) against to cefotaxime, enrofloxacin, tobramycin and doxycycline. Regarding *S. marcescens,* collected within gastrointestinal source from a dog (D03) present resistance to 5/22 (23%) including ampicillin and amoxicillin-clavulanic acid, tobramycin, amikacin and tetracyclin and its intermediate results (18%) was against cephalexin, pradofloxacin, doxycycline and chloramphenicol. *S. canis* shown a high susceptibility profile up to 93% (25/27) among all tested antimicrobial classes except to aminoglycosides. *K. pneumoniae*, found in dog (D05) with secondary septic peritonitis due to urogenital leakage, presented resistance only to one antimicrobial (1/22, 5%), ampicillin and another *E. cloacae,* collected within hepatobiliary tract from a dog (D09) present resistance up to 14% (3/22) in aminopenicillins and intermediate results (9%) to cephalexin and cefovecin.

Out of all total of *Escherichia coli* isolates, 2 samples collected from dogs (D08, D10) were highlighted as extended-spectrum beta-lactamases (ESBL) according to laboratory results. Overall *E. coli* resistance percentages are depicted in Table 4. Focusing in *E. coli* resistance profiles, phenotypical characterization shown that 3 isolates were found to be resistant simultaneously to ampicillin, amoxicillin-clavulanic acid, cephalexin, cefuroxime, cefovecin, all tested fluoroquinolones, doxycycline and trimethoprim-sulfamethoxazole (one intermediate). Furthermore, *E. coli* isolates shown to be susceptible to imipenem (12/12, 100%), meropenem (8/8, 100%), ceftiofur (4/4, 100%), fosfomycin (3/3, 100%), co-trimoxazole and nitrofurantoin (2/2, 100%), cefuroxime-axetil (1/1, 100%). These isolates were classified as intermediate and susceptible to amikacin, in 92% and 8% respectively.

Table 4. Percentages of *E. coli* resistance profiles (n=12).

Antimicrobial Substances	Antimicrobial Classes	Resistant Number Isolates (%)
Ampicillin	Aminopenicillins	6 (50%)
Doxycycline	Tetracyclines	5 (42%)
Cefovecin	Cephalosporins (3^{rd} generation)	5 (42%)
Trimethoprim-Sulfamethoxazole	Sulfonamides	4 (33%)
Ciprofloxacin	Fluoroquinolones	4 (33%)
Enrofloxacin	Fluoroquinolones	4 (33%)
Marbofloxacin	Fluoroquinolones	4 (33%)
Tetracycline	Tetracyclines	3 (50%)
Pradofloxacin	Fluoroquinolones	3 (38%)
Cefuroxime	Cephalosporins (2^{nd} generation)	3 (38%)
Cefpodoxime	Cephalosporins (3^{rd} generation)	3 (30%)
Amoxicillin-Clavulanic acid	Aminopenicillins (+ beta lactamase inhibitor)	3 (25%)
Cephalexin	Cephalosporins (1^{st} generation)	3 (25%)
Tobramycin	Aminoglycosides	2 (25%)
Cefotaxime	Cephalosporins (3^{rd} generation)	2 (22%)
Ceftazidime	Cephalosporins (3rd generation)	2 (17%)
Ampicillin-Sulbactam	Aminopenicillins (+ beta lactamase inhibitor)	1 (50%)
Cefoxitin	Cephamycin	1 (33%)
Chloramphenicol	Amphenicols	1 (16%)
Piperacillin-Tazobactam	Carboxypenicillin and ureidopenicillin (+ beta lactamase inhibitor)	1 (12%)
Gentamicin	Aminoglycosides	1 (8%)

Note: E. coli isolates were not tested equally for overall substances (Table 3).

4. Discussion

Septic peritonitis is a common and life-threatening condition that affects not only humans but also small animals as cats and dogs. As a response to infection, systemic inflammation occurs reflecting altered physical and clinical signs, usually non-specific and ambiguous (Culp & Holt, 2010; Mcgrotty & Doust, 2004). When infection is suspected, prudent empiric antimicrobial therapy must be administrated in order to reduce microbial growth and promote a prompt therapeutic approach, enhancing survival and decreasing mortality and morbidity rates (Marshall et al., 2017). According to Boothe (2009), risk of therapeutic failure in patients with sepsis due to postponement of antimicrobial administration may rise up to 11% per hour without treatment. Nevertheless, microbial culture and susceptibility profile evaluation are essential to the adequate antimicrobial selection (Papich, 2013). In addition we must keep in mind that antimicrobial resistance is increasing in a serious and dangerous way, affecting directly animal survival and threatening one health approach (Dargatz, Erdman, & Harris, 2017; Marques et al., 2016). In the present study, bacterial isolates namely, *Staphylococcus epidermidis* was found in a cat with primary septic peritonitis and *Escherichia coli, Enterobacter cloacae, Enterococcus avium, Klebsiella pneumoniae, Pseudomonas aeruginosa and Streptococcus canis* were found in cases of secondary bacterial peritonitis in both cats and dogs. Isolates characterization showed to be similiar to previous studies (Adams et al., 2014; Barfield et al., 2014; Bentley, Otto, & Shofer, 2007; Costello, Drobatz, Aronson, & King, 2004; Culp & Holt, 2010; Culp et al., 2009; Dickinson et al., 2013; Greene & Marks, 2012; Lanz et al., 2001; Marshall et al., 2017; Mueller, Ludwig, & Barton, 2001; Scotti et al., 2017; Swayne et al., 2012). Moreover, polymicrobial infections as *E. coli* and *Bacteroides spp* are common, yet, all isolates in this study found to be monomicrobial (Cioffi et al., 2011; Costello et al., 2004; Culp & Holt, 2010; Culp et al., 2009; Dickinson et al., 2013; Lanz et al., 2001; Marshall et al., 2017; Mueller et al., 2001; Ruthrauff et al., 2009). Also, according to studies the most common source of septic peritonitis (Table 2) is the gastrointestinal tract and in this study was almost equitable among sources (Barfield et al., 2014; Bentley, 2016; Bentley et al., 2007; Costello et al., 2004; Kalafut et al., 2016; Mueller et al., 2001).

According to previous studies (Barfield et al., 2014; Cioffi et al., 2011; Dickinson et al., 2013; Kalafut et al., 2016; Lanz et al., 2001; Marshall et al., 2017; Mueller et al., 2001; Papich, 2013; Scotti et al., 2017; Swayne et al., 2012) *Escherichia coli* was the most recurrent isolate (12/20, 60%) from sources as gastrointestinal, urogenital and hepatobiliary tract. *Enterobacter cloacae* was an isolate in two (10%), *P. aeruginosa* was one of the isolates (5%), thought it showed an interesting resistance pattern along with previous mentioned bacteria. Although it was not possible to characterize genotypically characterize the studied isolates, susceptibility testing results showed a substantial resistance profiles among the isolated bacteria (Table 3).

Pseudomonas aeruginosa, member of *Pseudomonadaceae* family presents resistance to aminoglycosides, fluroquinolones, beta lactams and macrolides. In this study, bacteria were found in a cat with primary bacterial peritonitis and the source site is unknown. In addition, its profile shown resistance to beta lactams (ampicillin, penicillin, amoxicillin-clavulanic acid, oxacillin), tobramycin (aminoglycoside), macrolides, tetracyclin, fusidic acid and to one carbapenem, imipenem. Although

there are reports affirming that *P. aeruginosa* has susceptibility to a few effective and active substances (Papich, 2013), in this study it was susceptible to cephalosporins, carbapenems as meropenem, fluoroquinolones, gentamicin and amikacin. This bacterium has great adaptive resistance mechanisms such as biofilm production, enhancing and perpetuate infections (Pang, Raudonis, Glick, Lin, & Cheng, 2019).

Enterobacteriaceae family are inherently resistant to beta lactamics due to production of enzymes that hydrolyzes the ring and turn the antimicrobial unsuccessful, aminoglycosides, fluoroquinolones and macrolides. *E. cloacae, K. pneumoniae* and *S. marcescens* in this study found to be resistant to substances such as ampicillin, amoxicillin-clavulanic acid (except *K. pneumoniae*), cephalexin (except *K. pneumoniae*). *S. marcescens* showed resistance to amikacin and tetracyclin and intermediate results to cephalexin, doxycycline and tobramycin. *E. cloacae* isolates showed resistance both to cefovecin and trimethoprim-sulfamethoxazole, one of them to cefpodoxime, ceftazidime, fluoroquinolones, gentamicin and other for cefotaxime. Nevertheless, some were found to be susceptive to cephalosporins, amoxicillin-clavulanic acid and all to imipenem.

Escherichia coli also belongs to the *Enterobacteriaceae* family. Its isolates profiles shown resistances (Table 4) towards ampicillin (50%), cefovecin (42% and 58%, respectively), tetracyclin (50%), doxycycline (42%) and ampicillin-sulbactam (50%) and found to be 100% susceptive to, as example, carbapenems (imipenem and meropenem). Carbapenems were the most effective antimicrobials against the isolates. Consumption of carbapenems in human and veterinary hospitals are increasing which promotes resistant clones and consequently mutations (Haenni et al., 2017). Furthermore, assayed substances were effective against isolates as resistance frequency ranged only from 8% to 50%, depending on the agent (table 4). Two of the isolates were found to be extended-spectrum beta-lactamases (ESBL) worldwide known resistance mechanism of this family both from female dogs, one with HB origin and other GI and were susceptive only to imipenem and meropenem and one to piperacillin-tazobactam. Also, these substances are included in the *"avoid"* group, according to EMA, 2019, also reportedly last line antimicrobials, reserved to human medicine (Haenni et al., 2017). Along with associated gene mutations, previous mentioned members of *Enterobacteriaceae* family may produce extended-spectrum β-lactamases, remarkable mechanism responsible for therapy failure (Papich, 2013; Toombs-Ruane et al., 2017). Although ESBL-producing bacterium are associated with poor outcome due to its resistant mechanism, both animals in this study have survived. Resistances have been increasing and enhanced throughout the years and there are several possibilities for that: bacterium as *S. marcescens*, *Klebsiella* and *Escherichia coli* have been isolated from removed catheters from animals (Koenig, 2012), long-term therapy or antibiotherapy at borderline concentrations or below to MIC or doses, inappropriate use of empiric broad-spectrum antimicrobial substances and/or imprudent disinfection (Umber & Bender, 2009). Duration of the hospitalization may also contribute as animals are exposed to certain situations that may lead to resistance as well(Abelson et al., 2013). Some bacterium are inherently resistant (genes within the microorganism) or have been acquiring resistant mechanisms against antimicrobial substances (Papich, 2013b; Toombs-Ruane et al., 2017) due to previously mentioned situations, whereas genotypic detect presence or absence to certain

resistant determinants such as a particular β-lactamase, which phenotypic susceptibility testing, the one in this present study, only provide general information regarding resistance profiles. Therapeutic strategies, prompt and continuously disinfection of surfaces and clinicians, education and awareness of the appropriate guidelines(Umber & Bender, 2009) can be applied and aim the situation long-term.

Overall substances (n=38) frequency (n=424), isolates resistance to antimicrobials was 26% (110/424) mostly against ampicillin, amoxicillin-clavulanic acid, cefovecin, tetracyclin, doxycycline, oxacillin, ampicillin-sulbactam, cefoxitin, cefotaxime and fusidic acid, in which resistant patterns were similar in previous studies (Marshall et al., 2017; Mueller et al., 2001).

Survival rates for cats (1/4) and dogs (16/16) with septic peritonitis range from 25% to 100%, respectively. Conditions listed as reason for euthanasia in all 3 cats enrolled failure to antimicrobial therapy, dehiscence of suture or progressive refractory septic shock, a concomitant disease or poor prognostic.

Despite associated costs, molecular analysis should be considered at clinics and hospitals as may provide advantages over phenotypic susceptibility testing, since acquired resistances and adaptive mechanisms are emerging daily in both animal and human hospitals. Accordingly, microbial culture and antimicrobial susceptibility testing are essential for certain conditions (fatal conditions, history of prior antibiotherapy or nonresponsive therapy) improving efficacy and outcome, also promoting responsible antibiotic selection, administration (Black, Rankin, & King, 2009; Pierce-Hendry & Dennis, 2010) and consequently decreasing of resistance patterns.

Limitations of the present study included small sample size, different species in different total number and incomplete data from medical records. Nevertheless, dataset is valuable to study antimicrobial resistance trends in this condition, allowing to compare to future studies (retrospective and prospective) warning and encouraging sanitary strategies among the environment, animals and public health, improving One Health.

5. Conclusions

In conclusion, secondary septic peritonitis was the most common condition, with equitative source type distribution. *Escherichia coli was* the most prevalent isolate in which two were highlighted as extended-spectrum beta-lactamases and three of them demonstrate simultaneously resistance against ampicillin, amoxicillin-clavulanic acid, cephalexin, cefuroxime, cefovecin, all tested fluoroquinolones, doxycycline and trimethoprim-sulfamethoxazole. All tested isolates demonstrated susceptibility to amikacin and to imipenem and meropenem. According to the results, overall isolates shown high resistance patterns against ampicillin, tetracyclin and doxycycline.

Considering the small number of cases and previous retrospective studies in this matter, additional studies should be carried out in both Portugal and Spain, to trace the evolution and pattern of resistances in septic peritonitis in small animals, improving antimicrobial selection and promoting alternative antimicrobial protocols in order to decrease resistance profiles.

Veterinarians are imperative for the prevention of zoonotic diseases, and also to educate and alert animal guardians for this reality. It is essential to apply prudent sanitary and safety measures as antimicrobial resistances are rapidly increasing and threatening health worldwide.

6. References

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Annex

Annex 1. Studies enrolling sample isolates and antibiotherapy in cases of septic peritonitis

Study Number	Animals (*n*)	Species	Source/Type	Microbial Cultures	Antimicrobial Substances	References
1	38	Canines	**Secondary** -26 gastrointestinal (GI) -8 urogenital (UG) -2 hepatobiliary (HB) Polymicrobial in 25 canines (66%) Monomicrobial in 13 canines (34%)	*-Bacteroides spp, B. vulgatus, B. pyogenes, B. thetataiotaomicro B. stercoris; Campylobacter spp, C. bifementans, Citrobacter freundii, Corynebacterium, Coryneform bactéria, C. perfrigens, C. ramosum, C. tertium, C. septicum, C. sordellii; E. aerogenes E. avium, E. coli, E. durans, E. faecalis, E. faecium, E. gergoviae; Klebsiella pneumoniae; L. anguillarum, Listonella anguillarum; P. oris, P. melaninogenica, P. loescheii, Prevotella bivia, Proteus mirabilia; Staphylococcus intermedius, S. agalactiae, S. mutans, S. uberis, S. warneri, S. xylosus, S. suis, S. bovis, Streptococcus spp, Streptococcus beta*	- Amikacin, Ampicillin, Ampicillin+Sulbact; Cefazolin, Clindamycin; Doxycyclin; Enrofloxacin, Erythromycin Gentamicin; Trimethoprim+Sulfo.	(Marshall et al., 2017)
2	57	Felines	**Primary** **-**15 **Secondary** -27 gastrointestinal -3 urogenital -2 hepatobiliary -2 trauma -6 intra-ab. Abcess -1 recurrence -1 other Monomicrobial In primary (27%) In secondary (30%)	**Primary** *- Bacteroides spp; Corynebacterium, Clostridium spp; Enterococcus spp, E. coli; Fusobacterium spp; Peptostreptococcus, Porphyromonas; Prevotella; Mycoplasma; Staphylococcus, Streptococcus spp* **Secondary** *- Actinomyces, Aspergillus; Bacillus spp, Bacteroides spp; Clostridium spp; Enterococcus spp, E. coli, Eubacterium; Fusobacterium, Flavobacterium; Klebsiella spp; Lactobacillus; Mycoplasma; Pasteurella, Peptostreptococcus, Prevotella, Pseudomonas spp, Prphyromonas; Staphylococcus, Streptococcus spp; Tenerellla*	- Amikacin, Ampicillin, Ampicillin+Sulbact; Enrofloxacin; Ticarcillin+Clav	(Scotti et al., 2017)
3	29 6	Canines Felines	**Secondary** -24 gastrointestinal -7 urogenital -4 hepatobiliary	*- Adecarboxylata; Candida albicans, Candida krusei, Clostridium perfringens; Enterobacter aerogenes, Enterobacter cloacae, Enterococcus spp, E. coli; Klebsiella pneumoniae; Lecrercia; Proteus mirabilis; Staphylococcus spp., Staphylococcus lugdunensis, Staphylococcus pseudointermedius, Staphylococcus warneri, Streptococcus spp.; Yeast spp*	-Ampicillin, Ampicillin+Sulbact.; Cefazolin, Cefotaxime, Clindamycin; Enrofloxacin; Imipenem; Meropenem, Metronidazole; Vorizonazole	(Kalafut, Schwartz, Currao, Levien, & Moore, 2016)
4	34	Canines	**Secondary** -Iatrogenic/trauma -Gastrointestinal	*-Bacterioides; Candida albicans, Clostridium spp, Clostridium perfrigens; Enterococcus spp, Enterobacter spp Enterobacter cloacae, Enterococcus faecalis, E. coli; MRSA; Pseudomonas spp; Proteus spp; Serratia marcesens, Staphylococcus spp (coag-), Streptococcus spp*	-	(Barfield et al., 2014)
5	4	Canines	**Secondary** -20 gastrointestinal Polymicrobial in 1 (25%)	*- E. coli*	-Amoxi-Clav; Cefuroxime; Enrofloxacin; Metronidazole	(Adams, Doyle, Bray, & Burton, 2014)
6	81	Canines	**Primary** -1 **Secondary** (80) 3 sources Polymicrobials in 45 (56%) Monomicrobial in 36 (45%) 2 pathogenes in 18 (22%) 3+pathogenes in 18 (22%)	*-Clostridium; Enterococcus spp, E. coli; Streptococcus*	-Amoxi-Clav; Cefuroxime, Clindamycin, Chloramphenicol, Fluoroquinolone; Imipenem; Lincomicine; Metronidazole; -Trimethoprim+Sulf.	(Dickinson, Summers, Wignal, Boag, & Keir, 2013)
7	15	Canines	**Secondary** -13 gastrointestinal -1 urogenital -1 hepatobiliary Polymicrobials in 7 (47%) Monomicrobial in 8 (53%)	*- Bacterioides fragilis, Bacillus spp; Citrobacter, freundii; Enterococcus spp, E. coli; Klebsiella oxytoca, Klebsiella pneumoniae; Staphilococcus, Streptococcus spp intermedius*	-Amoxicillin-Clav., Ampiciliin; Cefazolin, Cefoxitin, Cephalotin Clindamycin; Enrofloxacin; Gentamicine; Kanamycin; Metronidazole; Tetracicline, Trimethoprim+Sulf.	(Swayne, Brisson, Weese, & Sears, 2012)
8	6 2	Canines Felines	**Secondary** -7 gastrointestinal -1 hepatobiliary Polymicrobial In 5 (75%) Monomicrobial in 2 (25%)	*- Bacterioides spp; Candida spp; Clostridium spp; Enterobacter spp, Enterococcus spp, E. coli; Proteus spp; Staphylococcus intermedius, Streptococcus spp*	- Amikacin, Ampicilin, Cefazolin; Enrofloxacin; Metronidazole; Ticarcilin	(Cioffi, Schmiedt, Cornell, & Radlinsky, 2011)

9	9	Feline	**Primary** Polymicrobial in 8 (89%) Monomicrobial in 1 (11%)	*-Actinomyces; Bacterioides; Fusobacterium; Morganella morganii; Peptostreptococcus*	-Amoxi-Clav, Ampicilin: Cefazolin, Ciprofloxacin, Chloramphenicol, Clindamycin; Enrofloxacin; Metronidazole; Penicilin; Timentin, Trimethoprim+Sulf.	(Ruthrauff, Smith, & Glerum, 2009)
10	43 14	Canines Felines	**Primary** Polymicrobial in 4 dogs (44%) in cats Monomycrobial in 5 dogs (56%) **Secondary** -23 GI (dogs) -8 UG(dogs) -6 HB (dogs) -2 Iatrogenic/trauma (dogs) -7 GI (cats) -2 UG (cats) -2 iatrogenic/ trauma Polymicrobial in 22 dogs (65%) in 2 cats (22%) Monomycrobial in 12 dogs (35%) in 7 cats (78%)	**Primary** *-Bacillus spp; Clostridium spp; Enterococcus spp; E. coli; Propriobacterium spp; Staphylococcus spp (dogs)* *- Clostridium spp; E. coli; Streptococcus spp (cats)* **Secondary** *-Acinetobacter spp; Bacterioides spp; Clostridium spp; Enterobacter spp, Enterococcus spp, E. coli; Paraeruginosa spp, Proteus spp; Serratia spp; Staphylococcus spp, Streptococcus spp*	-	(Culp, Zeldis, Reese, & Drobatz, 2009)
11	43	Canines	**Secondary** - 3 sources	*-Clostridium spp; Enterobacter cloacae spp, Enterococcus spp, E. coli; Staphylococcus spp*	-Aminoglycosides, Ampicilin; Cephalosporin; Enrofloxacine; Metronidazole	(Bentley, Otto, & Shofer, 2007)

Annex 2. Suggestive criteria for diagnosis of SIRS in cats and dogs

Clinical Criteria	Dogs (2 criteria required)	Cats (3 criteria required)
Heart rate (bpm)	>120	<140 or >225
Respiratory rate (brpm)	>20	>40
Temperature (ºC)	>39.2 or <38.1	>39.7 or <37.8
White blood cells (x 10^9/L)	>16, <6 or >3% bands	>19.5, <5 or >5% bands

Note. Adapted from Gauthier, V., Bersenas, A., & Mathews, K. A. (2017) Sepsis and Septic Shock. *Veterinary Emergency + Critical Care Manual 3rd Edition* (3rd Edition, pp. 629); Silverstein, D. (2015) Systemic Inflammatory Response Syndrome & Sepsis Part 1: Recognition and Diagnosis. *Today's Veterinary Practice*, *5*(1), 38–44; and Ragetly, G. R., Bennett, R. A., & Ragetly, C. A. (2011) Septic peritonitis: Etiology, pathophysiology, and diagnosis. *Compendium: Continuing Education for Veterinarians*, *33*(10), E1–E6.