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OVERVIEW ON ANTIMICROBIAL PRESCRIPTION HABITS IN CATS AT DIFFERENT SERVICES OF THE VETERINARY TEACHING HOSPITAL OF THE UNIVERSITY OF PARMA

Panoramica sulle abitudini di prescrizione antimicrobica nei gatti presso i diversi servizi dell'Ospedale Veterinario Universitario Didattico dell'Università di Parma

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Index

ABSTRACT

Antimicrobials are frequently administered for therapeutic and prophylactic purposes in companion animals. Their use is closely monitored as related to antimicrobial resistance both in human and veterinary medicine. This retrospective study aims to describe the antimicrobial prescription habits, at different services, in cats visited at the Veterinary Teaching Hospital of the University of Parma in 2021 and 2022. Overall, antibiotics were prescribed in the 43,8% (2021) and 35% (2022) of visited cats. The emergency service prescribed antibiotics in the 35,9% and the 25.9% of the cases, with the 9,1% and 11.4% of the prescriptions supported by culture and susceptibility testing (CST) in respectively 2021 and 2022; the primary care service prescribed no antibiotics. Ophthalmology prescribed topic antibiotics in the 87,2% and the 78,3% of cats, with only one CST in 2021 and not guided by CST in 2022. Internal medicine prescribed antibiotics in 41% and 27.9% of its patients, with 52,8% in 2021 and 61.2% of CST performed in 2022. Neurology prescribed antibiotics in the 23,3% and the 12.2% of cases, with no CST performed in 2021 and only 1 (9.1%) CST performed in 2022. Cardiology made one prescription (1,7%; 1.1%) supported by CST in both 2021 and 2022. In 2021 oncology and dermatology prescribed antibiotics in 10,3% and 23,5% of cats respectively, with 2 and 1 CST performed While in 2022 oncology and dermatology prescribed antibiotics in 3.2% and 6.2% of their patients, respectively, none guided by CST. Surgery (reproduction, soft tissue surgery and orthopedics) prescriptions were made for prophylactic use. Antibiotics were given only intraoperative in the approximately all of reproduction interventions (97,9% in 2021 and 97% in 2022), and in 59,4% in 2021 and 93,2% of soft tissue surgeries in 2022. Orthopedics routinely prescribed a 7-day course of antibiotic therapy. According to EMA's classification of antimicrobials for animals use, most of the prescriptions belonged to Category C "Caution" and D "Prudence" antibiotics. Category B "Restrict" antibiotics represented 14,9% of total antimicrobials prescribed in 2021 and 13,6% in 2022, guided by CST in 66,7% of cases in 2021 and 70% in 2022. In particular, the percentage of CST guided prescription was 90,7% and 90.6% in internal medicine, 100% and 50% in soft tissue surgery, 70% and 41,7% in emergency care, no CTS guided prescription was performed in neurology in 2021 and 14.3% in 2022. The low CST percentage performed at the neurology service is due to the inherent difficulty in sampling cerebrospinal fluid. This study shows that adhering to guidelines for the prudent use of antibiotics is feasible in feline medicine and surgery. The use of "Restrict" antibiotics was limited to a small number of selected feline patients in our hospital. The use of CST could strongly reduce the use of antibiotics; particular attention should be paid to use not-critical categories of antimicrobials for therapeutic and prophylactic use.

RIASSUNTO

Gli antimicrobici vengono spesso somministrati a scopo terapeutico e profilattico negli animali da compagnia. Il loro utilizzo è attentamente monitorato in relazione alla resistenza antimicrobica sia in medicina umana che veterinaria. Questo studio retrospettivo si propone di descrivere le abitudini di prescrizione antimicrobica, presso diversi servizi, nei gatti visitati presso l'Ospedale Veterinario Universitario Didattico dell'Università di Parma nel 2021 e nel 2022. Complessivamente, gli antibiotici sono stati prescritti nel 43,8% (2021) e nel 35% (2022) dei gatti visitati. Il servizio di pronto soccorso ha prescritto antibiotici nel 35,9% e nel 25,9% dei casi, con il 9,1% e l'11,4% delle prescrizioni supportate da esami culturali e test di sensibilità (Culture and Susceptibility Test - CST) rispettivamente nel 2021 e nel 2022; il servizio di medicina generale non ha prescritto antibiotici. Il servizio di oftalmologia ha prescritto antibiotici topici nell'87,2% e nel 78,3% dei gatti, con prescrizione guidata da un solo CST nel 2021 e non guidata da CST nel 2022. Il servizio di medicina interna ha prescritto antibiotici nel 41% e nel 27,9% dei suoi pazienti, di cui sono stati eseguiti il 52,8% nel 2021 e il 61,2% di CST nel 2022. Il servizio di neurologia ha prescritto antibiotici nel 23,3% e nel 12,2 % dei casi, senza CST eseguiti nel 2021 e solo 1 (9,1%) CST eseguito nel 2022. Il servizio di cardiologia ha effettuato una prescrizione (1,7%; 1,1%) supportata da CST sia nel 2021 che nel 2022. Nel 2021 i servizi di oncologia e dermatologia hanno prescritto antibiotici rispettivamente nel 10,3% e nel 23,5% dei gatti, con 2 e 1 CST eseguiti, mentre nel 2022 i servizi di oncologia e dermatologia hanno prescritto antibiotici rispettivamente nel 3,2% e nel 6,2% dei loro pazienti, nessuno guidato da CST. Le prescrizioni del servizio di chirurgia (riproduzione, chirurgia dei tessuti molli e ortopedia) sono state somministrate per uso profilattico. Gli antibiotici sono stati somministrati solo durante la chirurgia in quasi la totalità degli interventi di riproduzione (97,9% nel 2021 e 97% nel 2022), e nel 59,4% nel 2021 e nel 93,2% degli interventi chirurgici sui tessuti molli nel 2022. Il servizio di ortopedia ha prescritto abitualmente un ciclo di terapia antibiotica di 7 giorni. Secondo la classificazione EMA degli antimicrobici destinati all'utilizzo negli animali, la maggior parte delle prescrizioni apparteneva agli antibiotici della categoria C "Attenzione" e D "Prudenza". Gli antibiotici di categoria B "Limitati" hanno rappresentato il 14,9% (70/469) del totale degli antimicrobici prescritti nel 2021 e il 13,6% (57/420) nel 2022, guidati da CST nel 66,7% dei casi nel 2021 e nel 70% nel 2022. In particolare, la percentuale di prescrizione guidata da CST è stata del 90,7% e 90,6% in medicina interna, 100% e 50% in chirurgia dei tessuti molli, 70% e 41,7% in pronto soccorso, mentre nessuna prescrizione guidata da CST è stata eseguita in neurologia nel 2021 e il 14,3% nel 2022. La bassa percentuale di CST eseguita presso il servizio di neurologia è dovuta alla difficoltà intrinseca nel prelievo di liquido cerebrospinale. Questo studio dimostra che l'adesione alle linee guida per l'uso prudente degli antibiotici è fattibile nella medicina e chirurgia felina. L'uso degli antibiotici "Ristretti" è stato limitato nel nostro ospedale a un piccolo numero di pazienti felini selezionati. L'uso della CST potrebbe ridurre fortemente l'uso degli antibiotici; particolare attenzione dovrebbe essere prestata all'utilizzo di categorie non critiche di antimicrobici per uso terapeutico e profilattico.

1 | INTRODUCTION

1.1 Antimicrobial Classes

1.1.1 β-lactam antibiotics

β-lactam antibiotics work by preventing bacterial wall formation by interfering with the final step of peptidoglycan formation. This occurs through inhibition of the activity of transpeptidases, also known as *Penicillin Binding Proteins* (PBPs), whose main function is to catalyze the formation of cross-links between the polymeric units of glycopeptides that form the bacterial wall (Giguère et al., 2013). β-lactams exert a bactericidal action in particular against cells in the growth phase, in which peptidoglycan synthesis is strongly active (S. Carli et al., 2009). As far as bacterial resistance is concerned, this is mainly mediated by the production of β-lactamases, enzymes of microbial synthesis (S. Carli et al., 2009). In Gram-positive bacteria, particularly *S. aureus*, bacterial resistance is expressed through the production of β-lactamases that cleave the penicillin ring of most penicillins; in Gram-negative bacteria, resistance results rather from a low permeability of the bacterial cell wall of Gram-negative bacteria, a deficiency of PBPs and the production of a large variety of β-lactam enzymes (Giguère et al., 2013).

Penicillins

Penicillins are important antibacterial drugs, often used as drugs of first choice in animal infections due to their high power and low toxicity (Giguère et al., 2013).

Natural penicillins (Penicillin G): these include benzylpenicillin, with a bactericidal action restricted to Gram-positive cocci and bacilli, a few Gram-negative cocci and *Leptospira*. Those are sensitive to the inactivating action of penicillinases and are largely degraded in the stomach, which is why the drug is only available for parenteral and topical use (S. Carli et al., 2009).

Semisynthetic β-lactamase-resistant penicillins (Cloxacillin, Methicillin, Oxacillin): also considered as antistaphylococcal penicillins, they are resistant to penicillinases produced by *S. aureus* (Giguère et al., 2013). Oxacillin was proposed in the past as a possible antimicrobial drug in dog and cat's bite wounds (Elenbaas et al., 1982, 1984). These are also combined with resistance to acid pH, thus making them resistant to gastric acidity and therefore administrable orally. The prevalence of methicillin-resistant *S. aureus* (MRSA) is reported to be increasing, particularly in dogs and horses housed in hospital settings, as well as in farm livestock (Price et al., 2012).

Extended-Spectrum Penicillins (Aminobenzyl Penicillins: Ampicillin and Amoxicillin): these have lower efficacy than penicillin G against Gram-positive bacteria, however, they have a much higher efficacy against Gram-negative bacteria such as *E. coli, P. mirabilis* and *Salmonella* (Giguère et al., 2013). When combined with aminobenzyl penicillins, the broad-spectrum beta-lactamase inhibitors clavulanic acid and sulbactam exhibit notable synergism against beta-lactamase-producing bacteria (Giguère et al., 2013).

Although the serum peak concentrations from these formulations are low, the dosage interval is increased to 12 hours. Because it is better absorbed than ampicillin and is unaffected by food, amoxicillin is favored for oral administration (Giguère et al., 2013).

For mixed aerobic-anaerobic infections, as those brought on by cat bites, ampicillin or amoxicillin are the preferred medications. Since over 90% of *S. aureus*, *streptococci*, and *P. mirabilis*, almost 90% of *E. coli*, and 65% of *Klebsiella* are thought to be susceptible to urine concentrations of the antibiotic, ampicillin or amoxicillin is used to treat canine urinary tract infections (Mateus et al., 2011).

Antipseudomonal Penicillins (Piperacillin): they are the first effective against *P. aeruginosa* and *Proteus*, usually not sensible to ampicillin and similar, but they are sensible to penicillinases (S. Carli et al., 2009). Piperacillin is active against several anaerobes, including many *B. fragilis*, and inhibits over 95% of *P. aeruginosa* and many *Enterobacteriaceae*. The most effective broad-spectrum penicillin, piperacillin is also vulnerable to some common beta-lactamases including *S. aureus's* penicillinase (Giguère et al., 2013).

Cephalosporins

These compounds are obtained by addition to the basic nucleus of different side chains, but are sensitive to a variable extent to specific β-lactamases (cephalosporinases) (S. Carli et al., 2009). Cephalosporins were first launched (first generation) to treat penicillinase-resistant staphylococcal infections, and one benefit of these medications was that they also possessed a spectrum of activity against Gram-negatives that was comparable to that of extended-spectrum aminobenzyl penicillins (Giguère et al., 2013). The synthesis of β-lactamase is the most crucial of the main methods of resistance. Their significance stems from the wide variety of beta-lactamases that have been developed as a result of the widespread use of extended-spectrum cephalosporins, as well as the

fact that the genes for these beta-lactamases are frequently transmissible (Bush & Fisher, 2011; Bush & MacIelag, 2010).

First-Generation Cephalosporins (Cefazolin, Cephradine, Cefadroxil, Cephalexin): they present high activity against Gram-positive bacteria, such as *S. aureus* and *S. pseudintermedius,* which produce beta-lactamases; moderate activity against some non-transferable, beta-lactamase-producing, Gram-negative *Enterobacteriaceae* and fastidious Gram-negatives; and no activity against *Enterobacter spp*., *P. aeruginosa*, and *Serratia spp*. While rare in Gram-positive bacteria, acquired resistance is prevalent in Gram-negative bacteria. All cephalosporins are ineffective against methicillin-resistant *S. aureus* and methicillin-resistant *S. pseudintermedius* (Giguère et al., 2013). These medications are used in dogs and cats as well as humans who have undergone surgery for the prevention of surgical wound infections. Cefazolin (20-30 mg/kg IV) is the drug of choice as intraoperative medication recommended in most studies (Bassetti et al., 2015; Välkki et al., 2020).

One practical application of first-generation oral cephalosporins is the long-term (30 days) therapy of persistent *S. aureus* pyodermas in dogs. The preferred medication for *K. pneumoniae* urinary tract infections has been characterized as cephalexin, even though a fluoroquinolone is currently a superior option. Other applications include the treatment of abscesses and wound infections in dogs and cats brought on by susceptible organisms, in addition to skin and urinary tract infections brought on by susceptible organisms (Giguère et al., 2013).

Second-Generation Parenteral Cephalosporins (Cefoxitin, Cefmetazole, Cefotetan, Cefuroxime: these molecules are stable to a wide variety of β-lactamases, which contributes to their broad spectrum of antibacterial activity. For Gram-negative bacteria, antimicrobial activity is a little bit broader and stronger than that of cefazolin and other first-generation cephalosporins, and includes *Enterobacter spp*. and *Serratia spp*. There is slightly reduced activity against Gram-positive bacteria (Giguère et al., 2013).

The prohibitive cost of these medications restricts clinical applications in animals, although they may be similar to those found in human medicine, where cefoxitin is prized for its extensive efficacy against anaerobes, particularly *B. fragilis*, as well as against Enterobacteriaceae. Thus, indications include prophylaxis in colonic surgery or ruptured intestine, as well as the treatment of severe mixed infections with anaerobes (aspiration pneumonia, severe bite infections, gangrene, peritonitis, and pleuritis) (Giguère et al., 2013).

Third-Generation Parenteral Cephalosporins (Cefotaxime, Cefovecin, Ceftriaxone, Ceftiofur): they possess strong antibacterial properties and widespread resistance to beta-lactamases; they are particularly effective against the majority of Enterobacteriaceae. *Serratia* and *Enterobacter* are two exceptions. Staphylococci are moderately susceptible, enterococci are resistant, and streptococci are very susceptible (Giguère et al., 2013). Third-generation cephalosporins should only be used for severe, presumably life-threatening infections brought on by Gram-negative bacteria due to the propensity to select for resistant bacteria (Giguère et al., 2013).

Cefovecin is used as a single treatment for infections in dogs and cats that are brought on by highly susceptible bacteria, such as *S. pseudintermedius*, *S. canis*, and *P. multocida*, which are frequently found in skin infections, bite wounds, and abscesses. It is also efficient against enteric bacteria that cause urinary tract infections because of its urine excretion. Depending on the susceptibility and clinical factors, treatment can be repeated in cats and dogs twice to four times at 14-day intervals (Giguère et al., 2013). The benefit is that delivery by this manner increases the chances of compliance compared to owners trying to give amoxycillin-clavulanic acid pills twice day by mouth, increasing the likelihood of cure, especially in cats. According to one study, treatment failure caused by non-compliance was assessed to be 14% (Van Vlaenderen et al., 2011). According to a study, cefovecin and amoxycillin-clavulanic acid share a comparable range of clinical efficacy (Stegemann et al., 2007).

Antipseudomonal Parenteral Cephalosporins (Cefoperazone, Cefsulodin, Ceftazidime): These medications are mostly used to treat *P. aeruginosa* and other Gram-negative septicemias in neutropenic patients in human medicine, where their efficacy is significantly increased when combined with an aminoglycoside (Giguère et al., 2013).

Fourth-Generation Parenteral Cephalosporins (Cefepime, Cefpirome, Cefquinome): They exhibit improved staphylococci activity as well as strong activity against Enterobacteriaceae and *P. aeruginosa*. While being weak inducers of group 1 beta-lactamases, they are resistant to hydrolysis by many plasmid- or chromosomally mediated beta-lactamases. In human medicine, fourth-generation cephalosporins are used to treat simple skin or skin-related infections, bacterial meningitis, nosocomial or community-acquired lower respiratory diseases, and urinary tract infections (Giguère et al., 2013).

Beta-lactamase Inhibitors (Clavulanic Acid, Sulbactam)

The synthesis of β-lactamases plays a significant role in the constitutive or acquired resistance of bacteria to β -lactam antibiotics. β -lactamases' clinical significance has been linked, in particular, to how quickly plasmid-mediated resistance spreads among bacterial populations. Drugs like amoxicillin, which were once crucial, have significantly lost value as a result of this resistance (Giguère et al., 2013). The idea behind the use of β -lactamase inhibitors is that because they have a high affinity for β -lactamases but little antibacterial activity on their own, they can be given along with a β -lactam that would be very effective against the pathogen if it weren't for its β -lactamases. The inhibitors (clavulanic acid, sulbactam, and tazobactam) exhibit a high level of substrate specificity for several β -lactamases. Due to the irreversible nature of their binding to these inhibitors, the active β -lactams (amoxycillin, piperacillin, etc.) can kill the organism as β -lactamase is in fact absent (Giguère et al., 2013).

In domestic animals, clavulanic acid-amoxicillin is a useful additive as an oral antibiotic. It increases the effectiveness of amoxicillin against fastidious organisms, Enterobacteriaceae, and anaerobic bacteria that produce β -lactamases as common opportunist pathogens. Because some *Proteus, Klebsiella*, and *E. coli* are only vulnerable to urine concentrations of the combination, it can be advised for the empirical treatment of urinary tract infections in dogs and cats. Amoxycillin-clavulanic acid is an extremely popular antibiotic in the care of companion animals, and many of these practices employ it as a "first-line" antibiotic (Mateus et al., 2011; Murphy et al., 2012). Other uses include treating *S. aureus*-caused skin and soft tissue infections, infections following bite wounds caused by a mix of bacteria, including anaerobes, anal sacculitis, gingivitis, and urinary tract infections caused by common opportunist bacteria (*S. aureus, E. coli, Proteus, Klebsiella*) (Giguère et al., 2013). Anyway, the threat to inhibitor-potentiated β-lactams as well as third-generation cephalosporins is growing due to the emergence of ESBLs (extended spectrum beta-lactamases) in companion animals (Shaheen et al., 2011; So et al., 2012; Sun et al., 2010) and the rise in methicillin-resistant *S. aureus* and *S. pseudintermedius*.In contrast to earlier research that suggested 100% susceptibility rates (Pedersen et al., 2007; Pellerin et al., 1998), *S. (pseud)intermedius* susceptibility to amoxicillin-clavulanic acid ranged from 98,79% to 100% (dermatological and aural isolates, respectively) in this study (Kroemer et al., 2014). For *E. coli, P. mirabilis*, and *P. multocida*, susceptibility to amoxicillin-clavulanic acid ranged between 74,92% and 100%, and it depended on the source of the isolate (Kroemer et al., 2014).

Sulbactam-ampicillin expands and restores the antibacterial action of ampicillin to include common bacteria that have developed β -lactamases, similar to how amoxicillin-clavulanic acid does (Giguère et al., 2013). Between 52,77% and 65,52% of *E. coli* and *P. mirabilis* strains were thought to be ampicillin susceptible, respectively. Penicillin continues to be largely effective against *P. multocida* (>89,54%) (Kroemer et al., 2014).

Carbapenems

They are highly effective against a range of Gram-positive and Gram-negative bacteria and resistant to several beta-lactamases, giving them the broadest spectrum of activity of any antibiotic. Human medicine traditionally views carbapenems as a "last resort" drug, but in recent years, the prevalence of carbapenemase- and metallo-beta-lactamase-resistant Gram-negative bacteria has increased noticeably, so that they almost rival the ESBLs in their emergence, but are more serious (Bush, 2010; Bush & Fisher, 2011). Several dangerous illnesses, such as intra-abdominal infections, severe lower respiratory tract infections, septicemia, life-threatening soft tissue infections, and osteomyelitis, are among the conditions for which they are successfully employed in human patients (Giguère et al., 2013). They should only be used rarely in veterinary medicine due to the development of resistant nosocomial infections in small animal intensive care units (Giguère et al., 2013).

Monobactams

Aztreonam's potential rests in its ability to replace the more toxic aminoglycosides in combination therapy, such as with erythromycin in mixed infections with Gram-positive bacteria or with clindamycin or metronidazole in dangerous mixed anaerobic infections. As a relatively safe drug in human medicine, aztreonam is successfully used on its own in a variety of infections involving Gram-negative bacteria (urinary tract, lower respiratory tract, and septicemia), including critically ill, immunocompromised patients (Giguère et al., 2013).

1.1.2 Peptide Antibiotics

Polymyxins (Polymyxin E or Colistin, Polymyxin B)

Due to their systemic toxicity, they were mostly used topically (polymyxin B) or orally (colistin). However, more recent research indicates that they are much less dangerous than previously thought, and there is significant interest in employing these antibiotics to treat infections caused by Gram-negative bacteria that are resistant to carbapenems (Lim et al., 2010).

Polymyxins bind lipopolysaccharides (LPS, endotoxin) by direct contact with the anionic lipid A region, which causes the outer membrane of Gram-negative bacteria to become disorganized. According to Coyne and Fenwick (1993), this activity neutralizes LPS's endotoxin potential. Gram-negative bacteria may develop resistance to colistin and polymyxin B through shared pathways. Although it is uncommon, *P. aeruginosa* can develop acquired resistance. According to Hariharan et al. (2006), *P. aeruginosa* isolates from animals are still frequently sensitive to polymyxin B. The most significant resistance mechanism entails alterations to the bacterial outer membrane, mostly through the change of lipopolysaccharide (LPS) (Falagas et al., 2010). The development of an efflux pump/potassium system and subsequent alterations to the bacterial outer membrane are examples of further resistance mechanisms. First-exposure adaptive resistance occurs, similar to what happens with aminoglycosides (Tam et al., 2005). Polymyxins have a weak affinity for plasma proteins but a strong affinity for muscle tissue; as a result of the tissue affinity, accumulation happens with chronic dosages. Although polymyxins are well tolerated when administered orally or locally, systemic usage results in nephrotoxic, neurotoxic, and neuromuscular blocking effects (Giguère et al., 2013). Age (geriatric), prior renal insufficiency, hypoalbuminemia, and concurrent use of non-steroidal anti-inflammatory medications or vancomycin are risk factors for nephrotoxicity. According to multiple research projects, renal failure is dose-dependent and can be predicted by either the daily dose or the total cumulative dose (Yahav et al., 2012). Polymyxins are used to treat localized cases of bacterial keratitis, otitis externa, and other skin infections in dogs and cats that are brought on by sensitive Gram-negative bacteria (Giguère et al., 2013).

Glycopeptides (Vancomycin, Teicoplanin, Avoparcin)

Glycopeptide antibiotics prevent the production of peptidoglycans in bacterial cell walls. They have a cleft in their three-dimensional structure that can only accommodate peptides with a very particular configuration only present in the cell walls of Gram-positive bacteria (Giguère et al., 2013). Antibiotic resistance is relatively rare, although it does happen occasionally in *Enterococcus spp*., particularly *E. faecium*. VanA resistance is linked to a plasmid-mediated transposable element and encodes resistance to all glycopeptides. Teicoplanin is unaffected by VanB resistance, while vancomycin is; it is chromosomally based and ordinarily not transferrable. VanC resistance is a lower-level, non-transferable resistance found in *E. Gallinarum* (Giguère et al., 2013).

Vancomycin must be delivered slowly and diluted intravenously because it significantly irritates tissues when injected. Humans experience a histamine-like reaction after receiving an IV injection quickly (red-neck syndrome). In humans, the medication is ototoxic, especially in patients receiving high doses or those who have renal insufficiency. Additionally, vancomycin may be nephrotoxic. Vancomycin has few indications for usage in animals, especially considering that it is a "last resort" medication in human medicine (Giguère et al., 2013).

Teicoplanin exhibits high effectiveness against Gram-positive bacteria such as *L. monocytogenes, C. difficile, C. perfringens, S. aureus*, including methicillin-resistant strains, and streptococci (where it is more active than vancomycin). Teicoplanin is used in human medicine to treat serious infections brought on by Gram-positive bacteria when a bactericidal medication is necessary or there is drug resistance. Use hasn't been widely accepted in veterinary medicine (Giguère et al., 2013).

Bacitracin interferes with the regeneration of bacterial cell-wall peptidoglycan by complexing directly with the pyrophosphate transporter and preventing the dephosphorylation process. Gram-positive bacteria are killed by it thanks to the bactericidal effect, but Gram-negative organisms are barely affected. Resistance develops slowly. Bacitracin is typically exclusively used topically for the treatment of superficial infections of the skin and mucosal surfaces since it is highly nephrotoxic following parenteral use (Giguère et al., 2013). Since 2006, the use of bacitracin and other antibiotic growth promoters has been prohibited in the European Union (Castanon, 2007).

1.1.3 Lincosamides

The lincosamides attach to the 50S ribosomal subunit and prevent peptidyl transferases from functioning, which prevents protein synthesis. Because of their impermeability and methylation of the lincosamides' ribosome binding site, many Gram-negative bacteria are resistant. Particularly when it comes to anaerobes and *S. aureus*, clindamycin is many times more effective than lincomycin. They are inactive against the majority of Gram-negative bacteria (Giguère et al., 2013). Lincosamides alone can cause resistance to develop, but cross-resistance between Macrolides, Lincosamides, and Streptogramin group B antibiotics (MLSB resistance) is more prevalent. There are two types of this cross-resistance: constitutive resistance (MLSBc), where bacteria exhibit high levels of resistance to all MLSB antibiotics, and dissociated inducible cross-resistance (MLSBi), where bacteria that are resistant to macrolides but initially fully susceptible to clindamycin quickly develop resistance to lincosamides when exposed to macrolides (Giguère et al., 2013). Particularly with oral usage, anorexia, vomiting, and diarrhea have occasionally happened in dogs and cats. When clindamycin capsules are administered without food or drink, cats may develop esophagitis, esophageal ulcerations, and occasionally strictures (Beatty et al., 2006). Infections of the soft tissues or wounds caused by Gram-positive cocci or anaerobic bacteria are treated in dogs and cats with lincosamides, including abscesses, osteomyelitis, periodontal disease, and osteomyelitis (Giguère et al., 2013).

1.1.4 Macrolides

By reversibly interacting with the ribosome's 50S subunits, macrolides prevent the production of new proteins. The process of transpeptidation and translocation is inhibited, which results in the premature separation of incomplete polypeptide chains. Although macrolides are mostly bacteriostatic substances, they have the potential to be bactericidal in high quantities and against a small number of extremely susceptible bacteria (Giguère et al., 2013). The majority of bacterial resistance to macrolide action can be attributed to three main mechanisms: rRNA methylation, active efflux, and enzymatic inactivation. The majority of resistant isolates exhibit the two pathways of rRNA methylation and active efflux. Since the majority of macrolide resistance genes are linked to mobile components, they can travel between bacterial strains, species, and ecosystems (Giguère et al., 2013).

Macrolides Approved for Veterinary Use (Erythromycins, Tylosin)

Erythromycins are produced as a complex of six components (A to F), but only erythromycin A has been developed for clinical use. Gastric acids have a significant propensity to degrade the erythromycin base, so orally administered erythromycin needs an enteric coating to get around this (Giguère et al., 2013). All macrolides have an irritating side effect that causes intense pain upon intramuscular (IM) injection, thrombophlebitis and periphlebitis following intravenous (IV) injection, and an inflammatory response after intramammary delivery. Most animal species treated with erythromycin experience dose-related gastrointestinal problems, such as nausea, vomiting, diarrhea, and intestinal discomfort. Erythromycin must be diluted and infused slowly when given intravenously to avoid side effects (Giguère et al., 2013).

Tylosin and erythromycin both have a similar range of activity. It is more effective against a variety of *Mycoplasma spp*. but less effective against bacteria. Its toxic effects are generally similar to those reported for erythromycin. Dogs with infections from wounds, tonsillitis, tracheobronchitis, pneumonia, staphylococci, streptococci, anaerobes, and *Mycoplasma* have all responded well to tylosin treatment. Tylosin is frequently effective in treating cats' upper respiratory tract infection complex, probably as a result of its ability to inhibit *Chlamydophila* and *Mycoplasma* growth (Giguère et al., 2013).

Advanced-Generation Macrolide Antibiotics (Roxithromycin, Clarithromycin, Azithromycin)

The ability of macrolides to combat both established and newly discovered human pathogens, such as *Campylobacter spp*., *Helicobacter spp*., *Legionella spp*., as well as intracellular pathogens that have emerged as a result of the AIDS epidemic, such as *Bartonella spp.* and *Mycobacterium spp*., has sparked interest in them.

When compared to erythromycin, roxithromycin has a better pharmacological profile with a longer half-life and greater oral bioavailability, enabling once- or twice-daily treatment. In terms of action against bacteria, clarithromycin is roughly twice as effective as erythromycin on a weight basis. Azithromycin has a significantly longer half-life and is more effective than erythromycin against Gram-negative bacteria (Giguère et al., 2013). Newer macrolides, which have evolved from erythromycin, are more acid stable, cause fewer gastrointestinal side effects, have higher bioavailability after oral administration, have significantly longer serum half-lives, and cause higher

tissue concentrations, making single or twice daily dosing appropriate. Newer macrolides are often well tolerated in humans and cause fewer gastrointestinal problems, and the limited data for dogs and cats indicate that this is also the case for these species (Giguère et al., 2013). *Babesia gibsoni* was successfully eradicated from persistently infected dogs when azithromycin and atovaquone were used (Birkenheuer et al., 2004). When given to affected dogs, azithromycin decreased the bacterial load, or treated bouts of acute arthritis, but it did not completely eradicate *Borrelia burgdorferi* (Straubinger, 2000). When compared to doxycycline, azithromycin gave a comparably rapid remission of clinical symptoms in cats with *Chlamydophila felis* infections, but was proved unsuccessful in treating infection, in contrast to doxycycline (Owen et al., 2003). Gastric ulcers in dogs caused by *Helicobacter spp*. have been successfully treated with clarithromycin, amoxicillin, and a proton pump inhibitor (Pires Anacleto et al., 2011).

1.1.5 Aminoglycosides

The aminoglycosides are bactericidal antibiotics primarily used to treat severe infections brought on by staphylococci and aerobic Gram-negative bacteria (Giguère et al., 2013). Aminoglycosides must penetrate bacteria to assert their effect. Penetration can be enhanced by the presence of a drug, such as a beta-lactam antibiotic, that interferes with cell wall synthesis. Gram-negative aerobic bacteria that are susceptible actively pump the aminoglycoside into the cell; this is initiated by an oxygen-dependent interaction between the antibiotic cations and the negatively charged ions of the bacterial membrane lipopolysaccharides. Once inside the bacterial cell, aminoglycosides bind to the 30S ribosomal sub-unit and cause a misreading of the genetic code, interrupting normal bacterial protein synthesis. As a result, the cell membrane becomes more permeable, which promotes the uptake of more antibiotics, further cell damage, and ultimately cell death (Giguère et al., 2013). The antibacterial effect of the aminoglycosides is directed predominantly towards aerobic, Gram-negative bacteria. They are not active against facultative anaerobes or aerobic bacteria under anaerobic conditions because their uptake requires oxygen. The bactericidal action of the aminoglycosides on aerobic Gram-negative bacteria is markedly influenced by pH, being most active in an alkaline environment. Increased local acidity related to tissue injury or bacterial death may explain the failure of aminoglycosides to kill ordinarily susceptible infections (Giguère et al., 2013). The most common mechanism of resistance is enzymatic modification of the aminoglycosides themselves (Becker & Cooper, 2013). Most therapeutically important resistance to aminoglycosides

is generated by plasmid-mediated enzymes, broadly classified as phosphotransferases, acetyltransferases, and adenyltransferases. Resistance to aminoglycosides is plasmid-mediated and is transferable between bacteria: in this way, a single type of plasmid may confer cross-resistance to multiple aminoglycosides and to other unrelated antimicrobials as well (Giguère et al., 2013). Resistance was noticed increasingly as these antibiotics were used more frequently in clinical settings, and toxicological liabilities, particularly ototoxicity and nephrotoxicity, became more pronounced (Becker & Cooper, 2013). Gentamicin has two effects on the renal tubules: the death of tubular epithelial cells, primarily in the proximal segment, which is accompanied by a significant inflammatory component, and the non-lethal, functional change of essential cellular elements involved in water and solute transport (Lopez-Novoa et al., 2011). Gentamicin causes tubular epithelial cells to undergo apoptosis (Li et al., 2009; Mouedden et al., 2000) and necrosis (Edwards et al., 2007) in experimental mice models. Gentamicin causes mesangial contraction in the glomerulus (Martínez-Salgado et al., 2007), which lowers Kf (ultrafiltration coefficient) and glomerular filtration rate (GFR). High-dose treatments with gentamicin have been shown to cause a diffuse swelling of the filtration barrier linked to neutrophil infiltration, a slight increase in size, and changes to the roundness and density of the glomeruli (Stojiljkovic et al., 2008). In the end, proteinuria is caused by the loss of glomerular filtration barrier selectivity brought on by the neutralization of its negative charges, especially when tubular reabsorption is compromised, as in tubular necrosis (Lopez-Novoa et al., 2011). Aminoglycosides also cause ototoxicity. The propensity of a drug to cause cochlear damage (amikacin, kanamycin, neomycin) or vestibular damage (streptomycin, gentamicin) varies. Both vestibular (balance) and cochlear (hearing) functioning appear to be similarly impacted by tobramycin. Furosemide, ethacrynic acid, and possibly other loop diuretics might enhance the ototoxic effects of aminoglycosides (Giguère et al., 2013).Aminoglycoside use for empirical and directed therapy has been made significantly safer by the use of better dose regimens and therapeutic drug monitoring. However, there is still concern about the clinical toxicity of the traditional aminoglycosides, which can cause serious side effects (Becker & Cooper, 2013).

Gentamicin is a common medication in small animal practice due to the widespread susceptibility of common bacterial pathogens in dogs and cats. It is used with excellent efficacy in the treatment of respiratory tract, skin and soft tissue, ocular (superficial infections), and gastrointestinal tract infections. Gentamicin-susceptible organisms frequently cause post-surgical infections in dogs (Gallagher & Mertens, 2012). For local treatment of musculoskeletal infections, gentamicin-

impregnated polymethyl methacrylate beads and regional intravenous gentamicin perfusion can be employed (Vnuk D et al., 2012). Gentamicin is particularly helpful for topical therapy of canine otitis externa due to its effectiveness against *Staphylococcus pseudintermedius* and *P. aeruginosa* (Malayeri et al., 2010).

1.1.6 Tetracyclines

They are effective against both Gram-positive and -negative bacteria, mycoplasmas, some mycobacteria, the majority of pathogenic alpha-proteobacteria, and a number of protozoan and filarial parasites (Giguère et al., 2013).

Tetracyclines are typically utilized as inhibitors of protein synthesis. They prevent aminoacylated transfer RNA (AA-tRNA) from attaching to their docking site (A-site) on the bacterial 30S ribosome by attaching to the 16S RNA (rRNA) and S7 protein of the ribosome. The synthesis of peptides is stopped as a result. They work as antiparasitic agents by preventing the synthesis of proteins in endosymbionts or organelles with genomes (Giguère et al., 2013). Tetracycline resistance can be caused by a variety of mechanisms, including:

- Energy-dependent efflux systems, the majority of which are antiporters that exchange an extracellular H+ for a cytoplasmic drug-Mg2+ complex;
- Ribosomal protection proteins that release tetracyclines from their binding site close to the ribosomal AA-tRNA docking site;
- Ribosomal 16S RNA mutation at the primary tetracyclines binding site;
- Stress-induced down-regulation of the porins that allow the drug to cross the outer Gram-negative wall. By far the most prevalent methods are the first two (Giguère et al., 2013).

In a study, most bacterial species encountered during the investigation were highly susceptible to doxycycline (>91% susceptibility), although *P. mirabilis* and *E. coli* were not among them. As might be predicted given its natural resistance to tetracycline compounds, *P. mirabilis* was consistently resistant. The frequency of strains displaying sensitivity to doxycycline was lower than that reported elsewhere (72,96% versus 81,8-86%) (Authier et al., 2006; Hariharan et al., 2006; Pedersen et al., 2007) even though some *E. coli* isolates were responsive (Kroemer et al., 2014). Tetracyclines are comparatively safe from a toxicologic standpoint. They are irritants that can lead to tissue injury at the injection site and vomiting following oral administration. Acute cardiac toxicity is linked to their

capacity to bind calcium. They also cause osteoclasts to undergo apoptosis, which may result in long-term bone toxicity. Primary teeth and, to a lesser extent, permanent teeth turn yellow after administration to growing puppies or pregnant females. Tetracyclines' bacteriostatic activity, which makes treatment time longer than with bactericidal medications, is one disadvantage compared to a number of other antimicrobial drugs (Giguère et al., 2013). Tetracyclines, particularly doxycycline, are recommended as first-line antibiotic therapy for canine and feline clinical haemoplasmosis (Tasker, 2022); additionally, it has been documented that doxycycline effectively treated *M. haemocanis* infection in a splenectomized dog, as evidenced by clinical remission and a constant lack of *M. haemocanis* DNA by qPCR (Pitorri et al., 2012). To manage ehrlichial infections in companion animals, tetracyclines continue to be the antibiotic of choice, although their indiscriminate use raises the danger of antimicrobial resistance. The recommended antibiotic is still doxycycline, however alternative tetracyclines are equally useful for treating dogs and cats (Diniz & Moura de Aguiar, 2022).

Doxycycline in also used in the treatment of *Filaria*'s infection as it destroys the endosymbiont *Wolbachia pipientis*, which is crucial for the nematode's development and reproduction as well as its ability to evade the host immune system (McHaffie, 2012). The doxycycline regimen will kill these commensal bacteria, and without these bacteria, the nematode parasite will eventually perish (for adult worms, this will take around a year) (Prichard, 2021). A month-long course of doxycycline therapy also stops microfilariae from becoming contagious larvae if ingested by a mosquito (McCall et al., 2014), which may lessen the spread of *D. immitis* resistant to macrocyclic lactones (Prichard, 2021). Studies showed a variety of mechanisms by which tetracyclines stem the progression of osteoarthritis, including MMP inhibition, anti-inflammatory properties, and nitric oxide pathway inhibition. Doxycycline and minocycline limit the activity of cartilage degrading enzymes in laboratory and animal studies. Such activities might represent promising osteoarthritis therapy options (Platt et al., 2021).

1.1.7 Fenicolates (Chloramphenicol, Thiamphenicol, Florfenicol)

Chloramphenicol is a powerful inhibitor of microbial protein synthesis. It inhibits peptidyl transferase and prevents the transfer of amino acids to developing peptide chains, blocking the production of proteins. It binds permanently to a receptor site on the 50S subunit of the bacterial ribosome. Additionally, chloramphenicol suppresses the production of mitochondrial protein in mammalian bone marrow cells in a dose-dependent way (Giguère et al., 2013).

Chloramphenicol is effective against a variety of Gram-positive and many Gram-negative bacteria; it is typically bacteriostatic in these situations. Enzymatic inactivation of the drug through acetylation by chloramphenicol acetyltransferases (CATs) is the mechanism of bacterial resistance that is most frequently observed (Giguère et al., 2013).

Chloramphenicol's primary side effects on humans are bone marrow depression, which can result in either a dose-dependent anemia from the inhibition of protein synthesis or an individualized, non-dose-dependent aplastic anemia. Chloramphenicol toxicity in animals is dose and treatment duration dependent, with cats being more susceptible to toxicity than dogs. Unless the animals have substantially compromised renal function or decreased hepatic microsomal enzyme activity, administration for fewer than 10 days with the maintenance dose is unlikely to result in toxicity in either dogs or cats (Giguère et al., 2013).

Multi-resistant *Staphilococcus aureus* (MRSA) and multi-resistant *Streptococcus pseudintermedius* (MRSP) infections have caused a rise in the use of chloramphenicol in dogs and cats, although this medication has greater side effects (mostly gastrointestinal) than alternative therapeutic options such doxycycline, clindamycin, and amikacin (Bryan et al., 2012).

Thiamphenicol is a significant importance molecule since it does not produce irreversible bone marrow aplasia in humans due to the absence of the p-nitro group, even if it may cause dose-dependent bone marrow suppression more frequently than chloramphenicol. Anyway, it appears underutilized in the treatment of many infections caused by susceptible organisms (Giguère et al., 2013).

Florfenicol has the same modes of action as chloramphenicol and is a powerful inhibitor of microbial protein synthesis. Like thiamphenicol, florfenicol does not produce idiopathic aplastic anemia in people, but it can decrease bone marrow in animals in a dose-dependent manner (Giguère et al., 2013). With excessive or protracted florfenicol dosing, bone marrow suppression that could be lethal

has been recorded, due to the inhibition of protein synthesis in erythroid cells, although the study was carried out on alpacas (Holmes et al., 2012).

Florfenicol is less likely to generate resistance in bacteria that produce CAT enzymes, but researchers are discovering new methods by which bacteria are becoming resistant to chloramphenicol and florfenicol (Tao et al., 2012).

1.1.8 Sulfonamides

Sulfonamides prohibit para-aminobenzoic acid (PABA) from incorporating into the folic acid molecule, hence interfering with the formation of folic acid in bacterial cells. The distinction between bacterial and mammalian cells in the supply of folic acid determines their selective bacteriostatic effect: mammalian cells utilize preformed folic acid, whereas susceptible bacteria must generate it. If animals are to be treated with sulfonamides, any tissue exudates and necrotic tissue should be removed because the bacteriostatic activity can be reversed by an excess of PABA (Giguère et al., 2013).

Sulfonamides are broad-spectrum antimicrobial drugs that can inhibit bacteria, toxoplasma, and other protozoal agents like coccidia (Giguère et al., 2013). Sulfonamides may make harmful bacteria more resistant to antibiotics and hasten the spread of antibiotic resistance, which raises the risk of infection and mortality in humans (Chen & Xie, 2018). The slow and gradual development of chromosomal mutation to resistance is caused by a reduction in drug penetration, the formation of an insensitive dihydropteroate enzyme, or an excess of PABA. It is well known that bacteria obtained from animals, especially farm animals, are highly resistant to sulfonamides; this indicates that the medicine has been used extensively for a long time (Giguère et al., 2013).

The more frequent side effects are hematological problems (thrombocytopenia, anemia, leukopenia), dermatologic responses, and alterations of the urinary tract (crystalluria, hematuria, or even blockage) (Giguère et al., 2013). Hypersensitivity responses are a typical type of adverse reaction to sulfonamides. Antimicrobial sulfonamides actually impact about 7% of human patients exposed to these substances, making it one of the most often documented allergies in electronic health records (Chow & Khan, 2022). The syndrome affects dogs and manifests as fever, arthropathy, epistaxis, hepatopathy, different skin eruptions, uveitis, and keratoconjunctivitis sicca (Trepanier, 2004). For the past three decades, treating a variety of frequently occurring infections has mostly

relied on the combination of trimethoprim and sulfamethoxazole. In a study (Kroemer et al., 2014), the susceptibility to trimethoprim/sulfamethoxazole ranged from 80,74% to 100% for all identified bacterial strains. *P. mirabilis* showed a susceptibility of about 50%, which is less than the percentages found by Pedersen et al. (2007), Authier et al. (2006) and Hariharan et al. (2006).

1.1.9 Fluoroquinolones

The fluoroquinolones that are now marketed for use in veterinary medicine are usually well absorbed orally, penetrate almost all body tissues, and have longer elimination half-lives, allowing for every 24- or 48-hour dosage. The drawback of this class of medications is the potential for rather quick selection of resistance in some infections (Giguère et al., 2013). The newest generation of drugs exhibit excellent bactericidal activity against select Gram-positive bacteria and mycoplasms at low minimum inhibitory concentrations (MICs), whereas fluoroquinolones were initially formulated for the Gram-negative aerobic spectrum. *Streptococci* and *Enterococci* frequently develop resistance to them, and they have little impact on anaerobic bacteria (Farca et al., 2007). Fluoroquinolones prevent the transcription and replication of bacterial DNA, which ultimately results in cell death. They either stop DNA-gyrase from working or stop it from separating from the DNA. The topoisomerase inhibitors interact with DNA to produce their bactericidal effects. Because bacterial gyrase differs from mammalian topoisomerase so much, fluoroquinolones are 1000 times more selective against bacteria than the human or animal counterpart to that enzyme (Ezelarab et al., 2018). Fluoroquinolones are generally safe. Toxic effects are often modest and restricted to gastrointestinal problems such as nausea, vomiting, and diarrhea when administered at therapeutic levels (Giguère et al., 2013). Cats receiving high dosages of enrofloxacin (20 mg/kg every 24 hours) have been linked to retinal degeneration (Gelatt et al., 2001; Wiebe & Hamilton, 2002). After stopping enrofloxacin therapy, vision may or may not return. High dosages of enrofloxacin that result in high plasma concentrations, fast IV administration, persistent treatment, and senior age all appear to be risk factors for cats. Fluoroquinolones frequently cause neurotoxic side effects in humans, and enrofloxacin has also been known to cause neurotoxic side effects in horses, dogs, and cats, that include seizures, ataxia, sleeplessness, restlessness, somnolence, and tremors (Giguère et al., 2013). In epileptic dogs, enrofloxacin has been linked to an increase in seizure frequency and potency (Vancutsem et al., 1990). Fluoroquinolone resistance can be brought on by active drug transport out of the bacteria, decreased permeability, target protection, or changes in the DNA-gyrase or

topoisomerase IV coding genes on the bacterial chromosomes (Farca et al., 2007; Giguère et al., 2013)A plasmid-mediated quinolone resistance gene (*qnr*) was just recently discovered; it was first found in clinical isolates of *Klebsiella pneumoniae* (Martínez-Martínez et al., 1998) and later in *E. coli* (Kirchner et al., 2011; M. Wang et al., 2003). According to a study conducted by Kroemeret al. (2014), there was no difference in the prevalence of marbofloxacin and enrofloxacin susceptibility for strains of *P. multocida*, *S. (pseud)intermedius*, and *S. aureus* isolated from dermatological disease cases compared to the populations of *E. coli* and *P. mirabilis*. In contrast, marbofloxacin susceptibility rates for cutaneous isolates of *P. aeruginosa* were significantly greater than those for enrofloxacin (88,37% versus 4,65%). Mekić et al., in 2011 also noted the high prevalence of enrofloxacin resistance in *P. aeruginosa* linked with otitis, indicating 99% of resistant strains of *P. aeruginosa*. Another study indicated that 12 of 59 recently obtained canine *E. coli* isolates were resistant to ciprofloxacin and enrofloxacin, indicating that some *E. coli* have gained high level resistance to fluoroquinolones (Boothe et al., 2006; Gottlieb et al., 2008). According to the findings of these studies, marbofloxacin had good efficacy against the isolated strains and is still a successful antibiotic for treating otitis, urinary, respiratory, and dermatological infections in companion animals. The disparities in susceptibility seen for fluoroquinolones highlight the necessity of choosing an antibiotic medication in a systematic manner, which should frequently involve diagnostic tests like susceptibility testing (Gottlieb et al., 2008; Kroemer et al., 2014). Boothe et al., in 2006 further highlights the significance of treating most vulnerable isolates with a high dose of fluoroquinolones. Due to their ability to penetrate almost every tissue in the body, fluoroquinolones can be used to treat infections such as mastitis and prostatitis brought on by susceptible bacteria, as well as urinary tract infections, pneumonia brought on by *Bordetella bronchiseptica*, rhinitis, deep and superficial pyoderma, otitis media and externa, and wound infections, as well as peritonitis (when combined with metronidazole) (Giguère et al., 2013). An upper respiratory infection in cats caused by *Chlamydophila felis* and *Mycoplasma spp*. was treated with pradofloxacin in one research, and the clinical symptoms significantly improved (Hartmann et al., 2008). Treatment of experimentally *Mycoplasma hemofelis*-infected cats with pradofloxacin and enrofloxacin is also successful (Dowers et al., 2009).

1.1.10 Nitroimidazoles

The nitroimidazoles were originally widely used in veterinary treatment, but because they may be carcinogenic, they are no longer allowed to be used on animals meant for human consumption in the US, Canada, and the EU. Due to metronidazole's high effectiveness against anaerobes and protozoa, it is still utilized in human medicine and in companion animals (Giguère et al., 2013). Nitroimidazoles undergo reduction of the nitro group after entering the cell, creating a number of unstable intermediates, including antimicrobial compounds. Reduction takes place in anaerobic environments. Numerous DNA strands are severely broken by nitroimidazoles, and DNA-ase 1 is inhibited (Giguère et al., 2013). In bacteria that are typically sensitive, metronidazole resistance is uncommon (Lofmark et al., 2010). Resistance results in decreased drug activation inside the cells. Susceptibility testing is necessary for individuals with clostridial diarrhea because equine and canine isolates of *Clostridium difficile* and *Clostridium perfringens* have been identified as being resistant to metronidazole (Gobeli et al., 2012; Magdesian et al., 2006). Nitroimidazoles have been found to be mutagenic and carcinogenic in several in vitro experiments and laboratory animals. Without any reports of cancer-related morbidity, metronidazole is still used directly in humans (Giguère et al., 2013). According to reports on metronidazole side effects in dogs and cats, these include vomiting, hepatotoxicity, neutropenia, and neurologic symptoms like rigidity, tremors, vertical nystagmus, paresis, ataxia, and seizures (Caylor & Cassimatis, 2001; Olson et al., 2005). Among the anaerobic diseases treated with metronidazole in small animals are bacterial stomatitis, osteomyelitis, hepatitis, pneumonia, lung abscesses, clostridial enteritis, and peritonitis. Metronidazole seems to be an effective treatment for *Giardia* in cats, while fenbendazole may be more effective and have less side effects when treating *Giardia* in dogs (Scorza & Lappin, 2004). In combination therapy, metronidazole is used to treat *Helicobacter*-associated gastritis in canines and felines. While there is clinical improvement, infection is not completely eradicated by such therapy (Khoshnegah et al., 2011; Leib et al., 2007).

1.1.11 Rifamycins

Rifampicin is a broad-spectrum antibiotic that has activity against both facultative anaerobic and numerous Gram-positive and Gram-negative aerobic bacteria. Gram-negative bacteria should be thought of as resistant unless otherwise proven by susceptibility tests. Rifampicin inhibits bacterial RNA polymerase by attaching to conserved amino acids in the active center of the enzyme and preventing transcription from starting. Mammalian polymerase is unaffected at therapeutic concentrations. Mutations of these aminoacids are primarily to blame for the bacterial resistance to rifampicin. Rifampicin is provided along with other antimicrobials since these mutations commonly arise with high frequency (Giguère et al., 2013). When dogs with methicillin-resistant *Staphylococcus pseudintermedius* infections get rifampicin monotherapy, rifampicin resistance emerges quickly (Kadlec et al., 2011). Combining topical antimicrobials with low-dose rifampicin appears to be a rather safe and efficient single-agent systemic antibiotic for canine meticillin-resistant multidrug resistant staphilococcal pyoderma (Harbour et al., 2022). In another research, rifampicin therapy used to treat an acute *Ehrlichia canis* infection sped up hematological recovery but had inconsistent success in eradicating the infection (Theodorou et al., 2013).

1.2 Antimicrobial Prophylaxis for Surgery

The Centers for Disease Control (CDC) defines surgical site infections (SSI) as infections that develop following surgery in the area of the body where the surgery was performed. SSI continue to be an important factor contributing to patient mortality and morbidity (Bratzler et al., 2013). Antimicrobial prophylaxis (AMP) is crucial for SSI prevention, especially when patient-related risk factors like comorbidities, concurrent remote body-site infections, prolonged preoperative hospitalization, and microbial colonization are present (Bassetti et al., 2015).

Based on the level of surgical contamination projected by the National Research Council wound classification system (*Table 1*), recommendations for antimicrobial prophylaxis for surgery in veterinary medicine are provided. Antimicrobial drugs should be administered during surgical operations which frequently include patients undergoing clean-contaminated or contaminated procedures, in order to prevent certain post-operative infections. For the majority of clean surgical operations, there is little chance of infection, hence prophylactic antimicrobials are not necessary. However, it is advised to administer preventive antibiotics during clean procedures when placing implants or when an infection would have disastrous consequences (such as a total hip replacement) (Giguère et al., 2013).

Table 1. Classification of wounds and associated risk of surgical site infection; Adapted from Giguère et al., 2013.

CLASSIFICATION	CRITERIA	APPROXIMATE RISK (%)
CLEAN	• Non-traumatic, no inflammation encountered • Primarily closed • No break in aseptic technique • Respiratory, alimentary, biliary and genitourinary tract	< 5
CLEAN-CONTAMINATED	• Urgent case that is otherwise clean • Elective opening of respiratory, gastrointestinal, biliary or genitourinary tract with minimal contamination and no encounter with infected urine or bile • Minor break in technique	5 - 10
CONTAMINATED	• Non-purulent inflammation • Gross spillage from gastrointestinal tract • Entry into biliary or genitourinary tract in presence of infected material • Major break in technique • Penetrating trauma <4 hours old • Chronic open wounds	10 - 20
DIRTY	• Purulent inflammation (e.g. abscess) • Preoperative perforation of respiratory, gastrointestinal, biliary or genitourinary tract • Penetrating trauma >4 hours old	> 20

The most important criteria for selecting a good preventive antimicrobial medication should be efficacy and safety. Because they run the danger of encouraging bacterial resistance, broad-spectrum antibiotics should be avoided (Bassetti et al., 2015). In order to reduce the appearance of bacterial isolates that are resistant to these first-line therapeutic treatments, it is best to avoid using newer broad-spectrum medications during surgical prophylaxis (Bratzler & Houck, 2005).

Usually, cephalosporins are the most commonly used antibiotics according to the bibliography both in human and veterinary medicine; Ideally, the prophylactic medication should be administered within 30 minutes of the surgical incision (Weber et al., 2008).

1.3 Antimicrobial Usage in Veterinary Medicine

1.3.1 History and Current Use of Antimicrobial Drugs

Soon after the Second World War, the use of antimicrobial medications in agriculture and veterinary care revolutionized the way many animal diseases were handled (Prescott, 2017). *Table 2* provides a wide overview of the most important aspects of the development of animal antibacterial medication use. The following key details about the history of animal antibacterial usage are illustrated in the table:

- Antimicrobial drug resistance quickly developed following their introduction;
- In order to combat resistance, new classes of antimicrobials were typically developed, or novel antibiotics within an existing class were isolated from nature and developed as synthetic analogs;
- Animals were treated with antibacterial medications that were also utilized in human therapy (Prescott, 2017).

Table 2. Historical timeline of important events in the use of antimicrobial drugs; adapted from Prescott (2017)

FEATURE OF PERIOD	ANTIMICROBIAL DRUG DEVELOPMENT	IMPORTANT EVENTS
1925–1935 ANTISEPTIC ERA	Discovery of sulfonamides	Discovery of penicillin, first beta-lactam, by Alexander Fleming
1936–1940 ANTISEPTIC, SULFONAMIDE PERIOD	Penicillin efficacy shown in humans	Sulfonamides introduced into food animal use
1950-1960 "WONDER DRUG" ERA	Discovery bacitracin, chloramphenicol, neomycin, polymyxin, streptogramins, tetracycline antibiotics Discovery of erythromycin, first macrolide; introduction of neomycin, aminoglycoside, for topical or intestinal infections in animals	Penicillin, streptomycin released from military use for civilian population and animal use; widespread use in animals by 1950, largely empirical Tetracyclines, chloramphenicol used therapeutically in animals; widespread, largely empirical, use
1961–1965 EMERGING RESISTANCE PERIOD	Methicillin and other penicillinase-resistant penicillins Introduction of spiramycin, a macrolide, into animal use	Discovery of transmissible, plasmid- or "R" factor-based, multiple drug resistance in *Enterobacteriaceae*
1966–1975 NEW DRUG ANALOG PERIOD	Cephalothin, first-generation cephalosporin Ampicillin, first broad-spectrum penicillin, used in food and companion animals Flavomycin introduced as growth promoter	New drug analogs successfully address resistance problem Transmissible, multiple-drug resistant, serious *Salmonella* infections, transmission from calves to human in UK Because of transmissible resistance, Swann Report in UK removes drugs important in

	Other first-generation cephalosporins introduced Trimethoprim-sulfonamide combination	human medicine as feed antibiotics, allows their veterinary prescription-only use therapeutically in food animals FDA report (1972) suggests stopping feed use of subtherapeutic penicillin, tetracyclines; not implemented
1976–1980 TISSUE DRUG RESIDUE PROBLEMS IN FOOD ANIMALS; EARLY PHARMACOKINETIC PERIOD	Cexotin, first extended-spectrum (secondgeneration) Cephalosporin Introduction in Europe of avoparcin, glycopeptide, for growth promotion in food animals	Chloramphenicol use in food animals banned in USA and Denmark because of potential human toxicity through residues, followed by other countries Transmissible, multiple-drug resistant, *Salmonella typhimurium* spreads from calves to humans Focus on pharmacokinetics and drug metabolism in food animals
1981–1985 PHARMACOKINETIC, DRUG DOSAGE PREDICTION PERIOD	Cefotaxime, antipseudomonal cephalosporin, and other third-generation cephalosporins Broad-beta-lactamase inhibitors combined with aminopenicillins, e.g., sulbactam ampicillin used in food animals	Antimicrobial drug dosage prediction based on pioneering pharmacokinetic approach in food animals
1986–1995 INCREASING RESISTANCE PROBLEMS IN HUMANS: MRSA AND VRES EMERGES	Quinolones, fluoroquinolones introduced into human medicine Azithromycin and other improved macrolides introduced into human medicine Fluoroquinolones introduced for selected use in food animals in Europe	Development of Food Animal Residue Avoidance Database (FARAD) in USA Moratorium on sulfamethazine use in dairy cows in USA Moratorium on most use of aminoglycosides in food animals in USA because of kidney residues First fluoroquinolone, enrofloxacin, introduced into food animal (poultry) use in USA, with severe restrictions; includes resistance monitoring through National Antimicrobial Resistance Monitoring System Animal Medicines Use Clarification Act in USA allows veterinary prescription extra-label use of certain approved drugs

1996–2005 RESISTANCE CRISIS IN MEDICINE; INCLUDES PENICILLIN-RESISTANT STREPTOCOCCUS PNEUMONIAE	Oral, third-generation cephalosporins in human medicine may partially drive resistance crisis Effective antivirals introduced into human medicine	VRE emergence linked to avoparcin use in food animals in Europe; ban of avoparcin and four other growth promoters in Europe WHO (1998) recommends withdrawal of growth promoting antimicrobials if significant for human medicine Global emergence of multidrug-resistant *S. typhimurium* WHO Global Strategy for Containment of Antimicrobial Resistance calls for prescription-only use of antimicrobials in food animals, national usage and resistance monitoring, phasing out of growth promoters if drugs important for humans Withdrawal of fluoroquinolones for use in poultry in USA because of emerging resistance in *C. jejuni* Spread of multidrug resistance including cephalosporinase (CMY2) genes among certain *Salmonella* serovars Development of prudent use guidelines by practitioner specialty groups at national levels
2005–2010 RESISTANCE CRISIS IN MEDICINE CONTINUES; MRSA AND MRSP EMERGE IN ANIMALS, SPREAD PARTLY BY PEOPLE	No new antimicrobial drugs introduced for food animals Voluntary ban on use of ceftiofur in pigs in Denmark	Resistance crisis in medicine focuses intense effort on improved infection and antimicrobial drug use control by physicians; some benefits observed Veterinary “prudent” or “judicious” use approaches increasingly replaced by emerging concept of stewardship Global spread of food animal-associated MRSA, driven by zinc oxide use in food animals MRSP emerges in dogs, clonal spread in Europe and North America
2011–2016 RESISTANCE CRISIS IN MEDICINE REACHES HIGHEST POLITICAL LEVELS UNITED NATIONS AFFIRMS GLOBAL COLLECTIVE ACTION	No new important antimicrobials introduced	WHO Options for Action promotes development of national action plans incorporating human and animal health sectors Human medicine antibiotic resistance threats identified by microorganism level in USA, most unrelated to animal use Consumer demands for “antibiotic-free” animal production

		United Nations High-Level Meeting on the antimicrobial resistance threat unprecedented meeting
2017– STEWARDSHIP ERA	Intense activity to find alternatives to antibiotics or "animal only" antibiotics for food animals	Political will to address the resistance crisis continues in place Anticipated enhancement of surveillance, stewardship, and innovation as global response to antimicrobial resistance crisis Innovation in numerous fields relating to use of antibiotics anticipated Increasing adoption of a "One Health" approach to resistance

1.3.2 Antimicrobial Resistance

Antibiotic-resistant bacteria are rapidly emerging and spreading over the world as a result of widespread antibiotic abuse (C.-H. Wang et al., 2020). The global public health dilemma of antimicrobial resistance threatens our ability to effectively treat bacterial illnesses (McEwen & Collignon, 2018). Many infectious diseases that could formerly be successfully treated with any one of several drug classes have developed resistance to the majority of these medications, and in some cases, nearly all of them (Laxminarayan et al., 2013). Antibiotics and synthetic antimicrobial agents are under the greatest threat, but antifungals, antiparasitics, and antivirals are also in danger (World Health Organization (WHO), 2015).

Microorganisms under antimicrobial selection pressure improve their fitness through the process of Darwinian selection by acquiring and expressing resistance genes, which they then transfer with other bacteria (McEwen & Collignon, 2018). Thus, the abuse and misuse of antibiotics are significant contributors to the phenomenon of resistance; the other major contributors include elements that encourage the local and worldwide spread of resistant bacteria and their genes. Poor infection control, environmental contamination, and the geographical spread of diseased people and animals are a few of them (Burow & Käsbohrer, 2017; McEwen & Collignon, 2018). Antimicrobial resistance is damaging to health since it lessens the efficiency of antimicrobial therapy and has a tendency to

make infections more serious, more common, and more expensive (Barza, 2002). The majority of hospital infections in people with increased patient mortality are caused by "ESKAPE" pathogens, which include *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* species (Rice, 2008).

Guardabassi et al. (2004), who emphasized rises in resistance recorded in North America and Europe during the 1990s, brought up concerns related to rising resistance among bacteria that infect dogs. The authors noted that multidrug-resistant isolates of *Acinetobacter baumannii, Escherichia coli*, and *Salmonella enterica* serovars were becoming more extensively recognized as nosocomial infections in hospitalized dogs. The authors noted that this was an issue, particularly in intensive care units, and hypothesized that this might be due to the overuse of broad-spectrum antibiotics in these settings (Lloyd, 2007).

According to Haulisah et al. (2022), the most common pathogens isolated from diseased cats and dogs from 2015 to 2017 *were S. pseudintermedius, E. coli, S. intermedius, P. mirabilis, K. pneumoniae*, and *S. canis*. These bacteria have also relatively high levels of resistance to commonly prescribed antibiotics: for *K. pneumoniae* (>80%) and *E. coli* (>40%) isolates recovered from cats, high levels of resistance to enrofloxacin and marbofloxacin were found, and high resistance levels for *S. pseudintermedius* isolates from cats were recorded against azithromycin (90%). Also, *P. mirabilis* isolates from dogs exhibited high levels of tetracycline (100%) and cephalexin (79,2%) resistance, or *E. coli* isolates showed high resistance to cephalexin (82,1%) and amoxicillin/clavulanic acid (76,5%), as well as enrofloxacin, marbofloxacin, tetracycline, and trimethoprim/sulfamethoxazole, indicating that treating *E. coli* infections in local dogs will likely be challenging (Haulisah et al., 2022).

1.3.3 Antimicrobial Stewardship

The European Union (EU) is actively supporting and working with international organizations like the Codex Alimentarius Commission, the World Health Organization (WHO), the World Organization for Animal Health, the Food and Agriculture Organization, and others to ensure the creation and implementation of global strategies and measures intended to prevent the growth and spread of antimicrobial resistance (AMR) (Commission Notice EU (2015/C 299/04), 2015). Antimicrobial resistance can arise through the use of antimicrobials in any setting (such as human and veterinary care). The risk rises if these antibiotics are administered incorrectly, for example by administering them in large quantities or to non-susceptible microorganisms, or by using them frequently or for improperly long lengths of time. In cases where it is necessary to use antimicrobials to safeguard animal health and welfare, the following principles should be followed (Commission Notice EU (2015/C 299/04), 2015):

- The prescription and dispensation of antimicrobials must be justified by a diagnosis in accordance with the current status of scientific knowledge;
- Where it's necessary to prescribe an antimicrobial, the prescription should be based on a diagnosis made following clinical examination of the animal by the prescribing veterinarian. Where possible, antimicrobial susceptibility testing should be carried out to determine the choice of antimicrobial;
- Antimicrobial metaphylaxis should be prescribed only when there is a real need for treatment;
- Routine prophylaxis must be avoided;
- The use of broad-spectrum antimicrobials and antimicrobial combinations should be avoided;
- A number of compounds on the WHO list of critically important antimicrobials are only authorized in medicinal products for human use;
- The off-label use (cascade) of the compounds for non-food-producing animals (e.g. pets and animals used for sports) should be avoided and strictly limited to very exceptional cases, and only when laboratory antimicrobial susceptibility tests have confirmed that no other antimicrobial would be effective;
- The perioperative use of antimicrobials should be minimized by using aseptic techniques;

- When possible, alternative strategies for controlling disease that have been proven to be equally efficient and safe (e.g. vaccines) should be preferred over antimicrobial treatment.

Reducing the total usage of antimicrobial agents and rationalizing the use of the most expensive medications (such as carbapenems, fourth generation cephalosporins, and glycopeptides) are two of the most effective ways to manage AMR in human hospitals. The most powerful veterinary antimicrobials are frequently utilized as empiric first-line medications in primary care, including the treatment of minor or self-limiting infections; these drugs include β-lactamase-resistant penicillins, cephalosporins, and fluoroquinolones (Guardabassi & Prescott, 2015). The majority of first-generation cephalosporins, such as cephalexin, and aminopenicillins with β-lactamase inhibitors (mostly amoxicillin/clavulanic acid) make up about three-fourths of the total sales of antimicrobial tablets for companion animals in Europe (European Medicines Agency (EMA), 2014).

The phrase "antimicrobial stewardship" refers to the dynamic and multifaceted strategies needed to maintain the clinical efficacy of antibiotics while improving drug selection, dosing, duration, and route of administration and reducing the development of resistance and other side effects (Guardabassi & Prescott, 2015). Every small animal clinic, regardless of kind or size, ought to have some kind of antimicrobial stewardship program (ASP) in place as a component of a formal infection control program run by an infection control practitioner (ICP) (Canadian Committee on Antibiotic Resistance, 2008). A local antimicrobial policy (LAP) for prudent antimicrobial usage is the most fundamental type of ASP. Different categories of antimicrobial prescription and use should be defined in an LAP. Generally, three types are advised (Keuleyan & Gould, 2001):

1. First-choice medications that are unrestrictedly prescribed;
2. Restricted medications that may be administered for particular conditions determined by the LAP with the ASP coordinator's help;
3. Reserve medications that can only be prescribed with approval from the national expert committee or the ASP coordinator.

On the responsible use of antimicrobials, to encourage ethical antimicrobial prescribing practices the British Small Animal Veterinary Association (BSAVA) and Small Animal Medicine Society (SAMSoc) created the Protect and ProtectMe recommendations (BSAVA/SAMSoc, 2013, 2018). The ProtectMe poster promoted the creation of a practice-specific policy for empirical antibacterial use

and suggested suitable antibacterials for the most commonly encountered infectious conditions (BSAVA/SAMSoc, 2018). Based on these recommendations (BSAVA/SAMSoc, 2013, 2018):

- The BSAVA strongly advises against using any antibiotics with restricted usage in human medicine, such as imipenem and vancomycin;
- The BSAVA advises using antibiotics like third- or fourth generation cephalosporins or fluoroquinolones only when culture and sensitivity test results show they are effective and other agents are ineffective. The choice of antibiotic should not be based on ease of administration;
- To reduce the usage of antibacterials, the BSAVA advises veterinary doctors to adopt good cleanliness and biosecurity;
- The BSAVA advises veterinary surgeons to notify the Veterinary Medicines Directorate (VMD) of antibacterial treatment failure (where culture and sensitivity results showed a specific authorized antibacterial was used correctly but follow-up culture and sensitivity identified persistent infection).

Another association that has dedicated itself to drafting guidelines on the prudent use of antibiotics in veterinary medicine is also the Federation of European Companion Animal Veterinary Associations (FECAVA), which has proposed in 2018 a series of posters such as FECAVA Key Recommendations for Hygiene and Infection Control in Veterinary Practice, FECAVA Advice on Responsible use of Antimicrobials and FECAVA Recommendations for Appropriate Antimicrobial Therapy (FECAVA, 2018a, 2018b, 2018c).

1.3.4 The 5Rs Approach to Antimicrobial Stewardship

In both human and veterinary medicine, the idea and practice of antimicrobial stewardship are still developing, but it is an approach that emphasizes an active, dynamic process of continuous improvement contained in the concept of good stewardship practice (GSP) (Guardabassi & Prescott, 2015). In order to preserve the future effectiveness of antimicrobials, promote and safeguard human and animal health, and improve antimicrobial stewardship, a variety of integrated strategies and treatments are used. The "5R" method of responsibility, reduction, refinement, replacement, and review (*Table 3*) is employed to accomplish this (Page et al., 2014).

Table 3. 5Rs of Antimicrobial Stewardship; adapted from Page et al. (2014)

RESPONSIBILITY	The prescribing veterinarian acknowledges that using an antibiotic may have unfavorable effects on people and animals besides the recipient and accepts responsibility for the decision. The prescriber recognizes that an explicit risk assessment of the particular situation has determined that the advantages of such use, along with any risk management strategies suggested, will lessen the likelihood of any short- or long-term negative effects on the patient in question, other patients, or public health.
REDUCTION	Every opportunity should be used to reduce the use of antimicrobial agents. For instance, improved infection management, biosecurity, immunization, focused treatment of certain animals, or a shorter course of treatment can all result in a reduction in use.
REFINEMENT	To ensure that the likelihood of selecting antimicrobial resistance is minimized while the likelihood of clinical efficacy is maximized, each use of an antimicrobial agent should incorporate into the development of the dosage regimen all available information on the patient, the pathogen, the epidemiology, and the antimicrobial agent (especially species-specific pharmacokinetic and pharmacodynamic profiles). For instance, responsible use calls for using the appropriate medication at the appropriate time, dose, and (though this is frequently ambiguous) duration.
REPLACEMENT	When there is data to show the efficacy and safety of a different treatment whose benefit to risk balance is judged by the prescriber to be preferable to the intended use of an antimicrobial agent, the use of antimicrobial agents should be substituted.
REVIEW	To assess compliance with programs and ensure that antimicrobial use practices set or reflect current best practice, antimicrobial stewardship initiatives should be reviewed frequently and a method of continuous improvement should be adopted.

1.3.5 Categorisation of Critically Important Antimicrobials

In 2019, the Antimicrobial Advice Ad Hoc Expert Group (AMEG) created a list of Critically Important Antimicrobials (CIAs) and classified them. A list of factors to consider when prescribing antibiotics is included in the categorization, which takes into account all classes of antibiotics and includes criteria like the availability of substitute antibiotics in veterinary medicine and the influence of administration route on the development of antibiotic resistance. From A to D, there are now four categories in the classification: Avoid, Restrict, Caution and Prudence (European Medicines Agency (EMA) et al., 2019), as shown in *Figure 1*, *Figure 2*, *Figure 3*, *Figure 4*.

Category A ("Avoid"): covers antibiotic classes that are permitted in EU human medicine but not in veterinary medicine. In accordance with the "cascade" prescribed, these classes may occasionally be utilized in non-food producing animals. Animals raised for food cannot be exposed to these drugs under the prescription "cascade" in the absence of maximum residue restrictions. Regardless of the categorization of its parent (sub)class, every novel antibiotic drug that is approved for use in human medicine following the publication of the categorization shall by default be provisionally included in Category A (European Medicines Agency (EMA) et al., 2019).

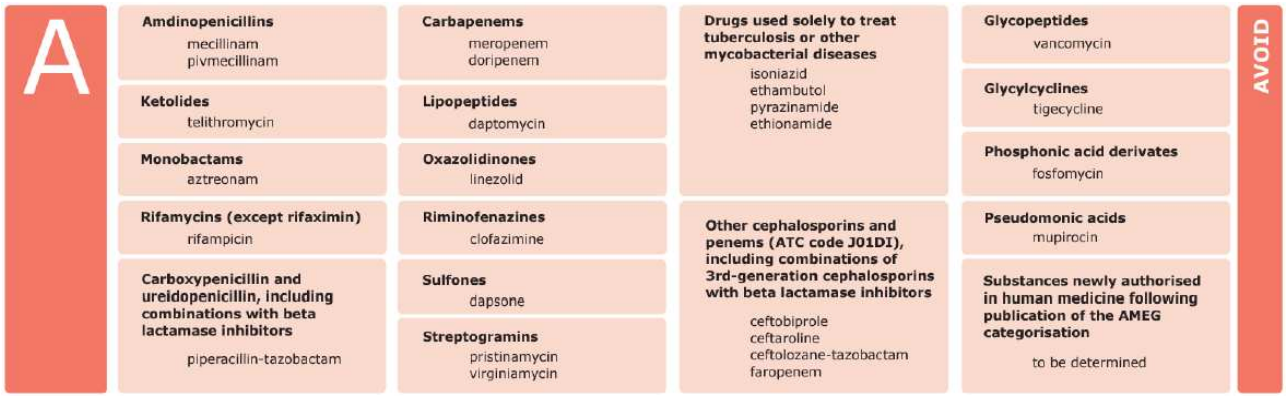


Figure 1. From EMA, Categorisation of antibiotics for use in animals for prudent and responsible use

Category B ("Restrict"): excludes macrolides and those classes listed in Category A, and includes the chemicals classified by the WHO as highest priority CIAs (HPCIAs). Quinolones, third and fourth generation cephalosporins, and polymyxins fall within this category. Specific limits are required for these antibiotics in order to reduce the risk to the public health posed by veterinary usage. When no other antibiotics in a lower category are available that could be clinically beneficial, these restricted antibiotics should only be used to treat clinical diseases. Use should, if possible, be based on the findings of antibiotic susceptibility testing, particularly for this category (European Medicines Agency (EMA) et al., 2019).

Figure 2. From EMA, Categorisation of antibiotics for use in animals for prudent and responsible use

Category C ("Caution"): has been added as an intermediate classification. This category includes specific antibiotic classes listed by the WHO in several categories, such as the HPCIA macrolides. In the EU, there are often alternatives for substances requested for inclusion in this category, however there are limited options in veterinary medicine for some purposes. This group also includes antibiotic classes that, via certain multi-resistance genes, may promote resistance to a drug in Category A. These antibiotics should be used only when no Category D drug is readily accessible that would be clinically efficacious (European Medicines Agency (EMA) et al., 2019).

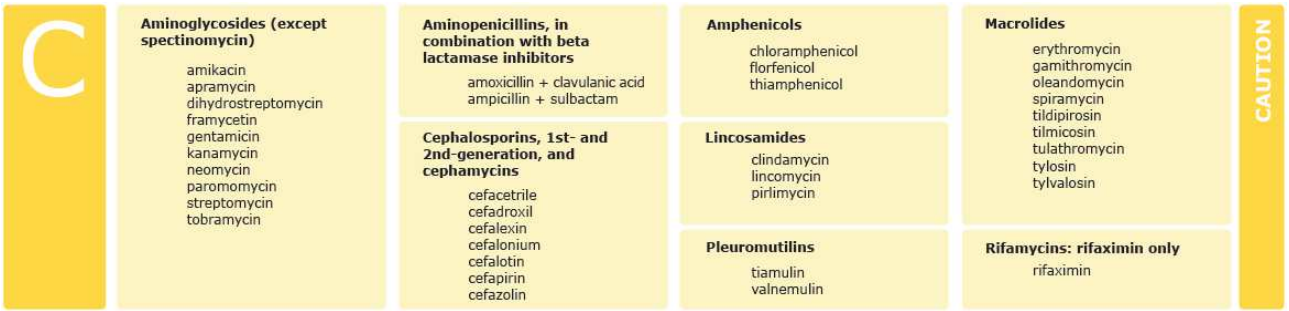


Figure 3. From EMA, Categorisation of antibiotics for use in animals for prudent and responsible use

Category D ("Prudence"): is the group with the lowest risk. Although the danger to human health associated with the use of the compounds in this category in veterinary medicine is regarded as low, several of the drugs in this category (aminopenicillins, natural penicillins, and isoxazolylpenicillin) are designated as WHO CIAs. It is known that these antibiotics do have an adverse effect on the emergence and dissemination of resistance, particularly through co-selection. As a result, even while there are no guidelines that are specific to avoid the use of Category D substances, it is generally advised that responsible usage principles be followed in daily life to reduce the risk associated with using these classes of substances as much as possible. Group therapy should only be used when individual therapy is impractical, and unnecessary use and lengthy treatment periods should be avoided (European Medicines Agency (EMA) et al., 2019).

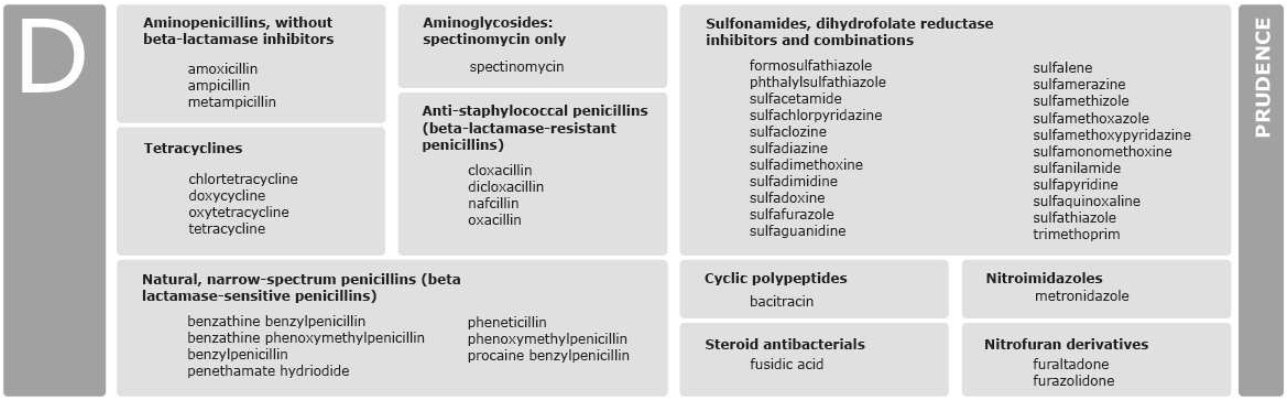


Figure 4 From EMA, Categorisation of antibiotics for use in animals for prudent and responsible use

When prescribing antibiotics, the way of administration should be considered along with the classification. From lowest to highest estimated influence on antibiotic resistance, a list of suggested administration methods and formulation types is provided by EMA (European Medicines Agency (EMA) et al., 2019):

- Local individual treatment (i.e. udder injector, eye or ear drops)
- Parenteral individual treatment (intravenously, intramuscularly, subcutaneously)
- Oral individual treatment (i.e. tablets, oral bolus)
- Injectable group medication (metaphylaxis), only if appropriately justified
- Oral group medication via drinking water/milk replacer (metaphylaxis), only if appropriately justified
- Oral group medication via feed or premixes (metaphylaxis), only if appropriately justified

1.3.6 Antimicrobials Reserved for Treatment in Humans

The European Commission published a list in 2022 about the commission implementing regulation (EU) 2022/1255 designating antimicrobials or groups of antimicrobials reserved for treatment of certain infections in humans, in accordance with Regulation (EU) 2019/6 of the European Parliament and of the Council and entered into force in Italy on February 9, 2023. The Regulation provides that all the antimicrobials (antibiotics, antiviral and antiprotozoal) listed in the annex cannot be used in veterinary medicines or medicated feed. Similarly, the use in animals of medicinal products for human use containing active ingredients listed in the same annex is also prohibited (under the conditions set out in articles 112, 113 and 114 of regulation (EU) 2019/6).

The list of reserved antimicrobials contains:

a) Carboxypenicillins
b) Ureidopenicillins
c) Ceftobiprole
d) Ceftaroline
e) Combinations of cephalosporins with beta-lactamase inhibitors
f) Siderophore cephalosporins
g) Carbapenems
h) Penems
i) Monobactams
j) Phosphonic acid derivates
k) Glycopeptides
l) Lipopeptides
m) Oxazolidinones
n) Macrocycles
o) Plazomicin
p) Glycylcyclines
q) Eravacycline
r) Omadacycline

1.4 Objective of the study

Before the discovery of antimicrobials, infections caused by microorganisms like bacteria, fungus, parasites, and viruses were a major cause of death (Hur et al., 2020). Antimicrobials save countless lives, but soon after they were introduced to clinical practice, resistance to these medications was found in clinical specimens (Aminov, 2010). AMR has dramatically increased over the past ten years, and it is now thought to be a major public health issue and an emerging global phenomenon (Roca et al., 2015). In addition to being able to acquire and transmit multidrug resistant infections to humans, companion animals can also operate as a reservoir for individuals who come into touch with them (Graveland et al., 2010; Guardabassi et al., 2004; Lloyd, 2007). In addition, AMR contributes to treatment failures in veterinary medicine as well as poor animal health and welfare outcomes (Hur et al., 2020). Implementing and monitoring antimicrobial stewardship programs require an understanding of antibiotic consumption patterns; in this context, one of the best strategies to lower AMR in a hospital setting is through antimicrobial stewardship (AMS), which has been proven to be successful (Arda et al., 2007; Baur et al., 2017; Cisneros et al., 2014; Pulcini et al., 2014).

In sight of this, the aim of this study focuses on describing the antibiotic prescriptions within the different services in the cats visited at the Veterinary Teaching Hospital (VTH) of the University of Parma in the two-year period 2021-2022. Within the study, the use of antibiotic molecules will be evaluated within the various services of the VTH, both in intensive care and in specialty services, associating the use or otherwise of the antibiotic with the presence of a diagnosis and possibly the use of a bacteriological examination to ensure prudent use of the drug into the facility.

2 | MATERIALS AND METHODS

The examination of antimicrobial drug usage practices is one of the fundamental tenets of antimicrobial stewardship (American Veterinary Medical Association (AVMA), 2020). Academic teaching hospitals have a number of advantages for such studies, including AMR surveillance systems, written biosecurity policies, searchable electronic medical record (EMR) systems, and personnel focused on AMR (Escher et al., 2011; Rantala et al., 2004; Wayne et al., 2011; Weese, 2006). This has led to a large portion of the documentation of AMD use, resistance patterns, and development of usage guidelines in companion animal medicine being concentrated in these institutions. However, the overwhelming majority of animals in veterinary teaching hospitals having complex or serious conditions for which AMDs have been previously prescribed poses a significant obstacle to research on AMD use in these facilities (Nienhoff et al., 2011; Weese, 2006; Weese et al., 2012).

The present study represents a retrospective study carried out considering a period of time ranging from January 2021 to December 2022, therefore taking into consideration a period of 2 years. The entire study was carried out within the VTH of the University of Parma taking into consideration the medical records of the structure.

2.1 Records Research

The study was conducted taking into consideration the use and prescription of antibiotics in cats, which were registered on the computer system at the Veterinary Teaching Hospital of the University of Parma. The management system provided by the VTH is the “Fenice” (5.0 version), developed by ZackSoft in 1999 and used in many Veterinary faculties in Italy. The data were collected by individually analyzing the medical records of each subject included in the study and entering the collected data in an Excel worksheet, kindly granted as a basic model by the University of Pisa which has conducted studies with similar purposes. To carry out the research, the medical records of all the cats visited at the VTH in the years 2021 and 2022 were analyzed; the cats were divided according to the different VTH service that compiled the medical record (Cardiology, Soft Tissue Surgery, Dermatology, Internal Medicine, Neurology, Ophthalmology, Oncology, Orthopedics, Reproduction, Emergency and Critical Care, General Medicine).

The main inclusion criteria applied concerns feline subjects visited at the VTH, who therefore presented medical records within the period of time considered within one of the specialist clinical areas offered by the structure.

The exclusion criteria were as follows: visits to the same subject following the first for the same pathology and with no changes in diagnosis and therapy (in this case only the first visit performed in chronological order will be recorded in the worksheet); medical records not complete in their entirety; animals that have been euthanized during a visit or during the first 24 hours of hospitalization; animals which arrived already dead at the emergency room visit; medical records regarding diagnostic imaging if only present without any anamnestic information (reports of ultrasounds, radiographs and CT scans).

2.2 Study Arrangement

The Excel worksheet utilized in the current study consists of a total of 48 cells on a row containing various information and filled in individually; in total, 22 cells could be filled in as an open table field (colored in yellow), while 26 cells could be filled in as a pre-filled table field with a dropdown menu (colored in red). The personal data of owners and animals were obtained through a file generated by “Fenice” which entered in chronological order, and within this in alphabetical order, the unique identification code, name and surname of the owner, name of the patient and month in which there was a medical record within the selected year. These data were then automatically inserted into the Excel worksheet to have a chronological list of patients to be analyzed during the study.

The main contents of the worksheet can be summarized as follows:

1. Patient identification
2. Antibiotic prescriptions
3. Sensitivity assessment

2.3 Patient Identification

A list of cell contents of the patient identification section is summarized in *Table 4* and shown graphically as in the Excel worksheet in *Figure 5*.

Table 4. Patient identification section in the Excel worksheet

PATIENT IDENTIFICATION	
Unique folder number	Month
Presence of prescription	Year
Surname of the owner	Service
Name of the patient	Hospitalization
Patient's race	Presence of diagnosis
Patient's age	Reason for visit or diagnosis
	Apparatus involved

Figure 5. Patient identification section in the Excel worksheet

The first cell of the row consists of the identification of the patient via a unique file number, i.e., a 10-digit code automatically generated by "Fenice" when a new patient is registered in it. The numerical code refers only to a single registered animal and can be used as an identifier in the case of subjects registered with the same name or in the case of homologies between owners. This is an open table field filled in automatically when the subjects taken from "Fenice" have been entered in the worksheet.

The second cell identifies cats that have received a prescription (antibiotic or not). It is an open table field cell in which a wording "yes/no AB" may be entered to indicate the presence of a non-antibiotic prescription.

The following 4 cells include general signaling information, such as the owner's surname and first name as found registered on "Fenice", the registered name of the corresponding pet, the breed and age of the cat. All 4 cells are open table field filled in by collecting the data from the medical records present on "Fenice".

The next 3 cells are open table fields and include temporal type information, such as the date in which a medical record has been compiled. The cell containing the date was filled in individually for each patient taking into consideration the date of the single visit in the case of outpatient and emergency room visits, while in the case of hospitalization of the animal was taken into consideration the date of the first visit after which the hospitalization has begun.

The service cell is an open table field which is filled in by assigning the medical service provided by the VTH to the patient. The services recorded in the worksheet are Cardiology, Soft Tissue Surgery, Dermatology, Internal Medicine, Neurology, Ophthalmology, Oncology, Orthopedics, Reproduction, Emergency and Critical Care, General Medicine.

The hospitalization cell is a pre-filled table field with a dropdown menu consisting of two options, “Yes” or “No”, depending on whether or not the animal has received a multi-day hospitalization. The information was obtained individually for each animal by analyzing the medical records. In the same way as the adjacent cell, the diagnosis presence cell has a pre-filled table field with a dropdown menu with also two options, "Yes" or "No", depending on whether a diagnosis of relative certainty or a certain reason for the visit is reported or not in the medical record of the patient.

The diagnosis or visit's purpose are listed in the next cell. It is an open table field that should be filled in by extrapolating the primary diagnosis made by the veterinarian from the animal's medical records, or by inputting the reason for accessing the VTH in the case of, for example, surgery or orthopedics. It may happen that, especially in the context of emergency medicine, a certain diagnosis is not formulated at the first recorded visit but rather a series of differential diagnoses; in this case, if the animal has been hospitalized, priority is given to the information contained in the discharge sheet as the diagnosis inserted in this document was formulated following in-depth examinations during hospitalization.

The next cell contains information about the apparatus involved in the pathology and is entered through a pre-filled table field with a dropdown menu with 13 different choices. A series of apparatus has been inserted within the drop-down menu: Cardiovascular, Gastrointestinal, Mammary Gland, Genitourinary, Lymphatic, Musculoskeletal, Ophthalmological, Ornithological, Respiratory, Systemic, Integumentary, Nervous and Healthy. All systems have a clear distinction, while in the case of the Systemic it can include for example

an endocrine pathology, blood diseases, or in the case of involvement of two or more organs like in the case of a polytrauma patient.

The last cell includes information regarding the patient's weight and is entered through an open table field. This information is obtained individually for each subject by searching for it within the medical records registered on “Fenice” when present. Information on weight was not always present and was mainly used to obtain the dosage of antibiotics when administered.

2.4 Antibiotic Prescriptions

The cells relating to the antibiotic prescription are made up of 11 cells repeated 3 times, in a way to be able to insert the information concerning 3 different antibiotics if administered in combination. The information contained in the cells is summarized in *Table 5* and shown graphically as in the Excel worksheet in *Figure 6*.

Table 5. Antibiotic prescription section in the Excel worksheet

ANTIBIOTIC PRESCRIPTION	
Antibiotic prescription or not	Unit of measure
Combination of multiple antibiotics	Frequency of administration
Class of antibiotic	Duration of therapy
Active principle	Drug registration
Route of administration	Type of medicine
Dosage	

Figure 6. Antibiotic prescription section in the Excel worksheet

The first cell provides a pre-filled table field with a dropdown menu with two options, "Yes" or "No", depending on whether or not an antibiotic has been prescribed or administered to a given animal. This cell is of fundamental importance as its compilation allows or not to continue with the compilation of the following cells. This is because, via the drop-down menu, selecting "No" in the event of no antibiotic prescription for the patient, the entire line following the cell becomes highlighted in black and impossible to fill in, determining the deadline for entering the data for that animal. On the contrary, by selecting "Yes" it will be possible to continue filling in the following cells with information on the antimicrobial utilized. A graphical representation is provided via *Figure 7*.

Figure 7. Antibiotic prescription field in the Excel worksheet filled in with Yes and No options.

The second cell instead requires you to enter whether there is an association between the prescribed antibiotics, i.e., to indicate whether a single antimicrobial or more than one has been prescribed or administered to the patient. The cell is made up of a pre-filled table field with a dropdown menu with two options, "Yes" or "No".

The next cell deals with the class of antibiotics used and provides a pre-filled table field with a dropdown menu containing 16 different classes of antimicrobials among the main ones used. Within the classes of antibiotics we find: Aminoglycosides (AMN), Cephalosporins (CEF), Enhanced Cephalosporins (CEF+), Macrolides (MAC), Nitroimidazoles (NITRO), Penicillins (PEN); Penicillins Associated With Beta-Lactam Inhibitors (PEN+), Fixed antibiotics combination (FIXED), Phenicolates (PHE), Polymyxins (POLY), Quinolones (QUI), Rifamycins (RIF), Sulfonamides (SULF), Tetracyclines (TTR), Lincosamides (LINC) and Piperacillin (PEN++). Then, the following cell is an open table field containing the name of the active principle of the antimicrobial utilized; in case of the combination of multiple drugs, the trade name of the product was used.

The cell about the route of administration is also a pre-filled table field with a dropdown menu containing 5 different ways of taking the drug. Among these are found: intravenous, intramuscular, oral, topical and subcutaneous route.

The drug dose cell is the one after this. It is an open table field that contains numerical data on the amount of antibiotic given to the animal. This information can either be obtained directly from the animal's medical records, in which the dosage of the drug is sometimes reported as mg/kg, or it can be calculated when the records show a prescription for an antibiotic, usually oral, expressed as a quantity of tablets and reporting the concentration of antibiotic in them.

The unit of measure cell is a pre-filled table field with a dropdown menu containing different units, such as: IU/Kg, mL/Kg, mg/Kg, mL, g/100Kg, mL/100Kg, gtt (for drops), cpr (for tablets), mg. However, only the most typical units of measurement were utilized in the study depending on the kind of medication: mg/kg for the majority of injectable and oral drugs; gtt primarily for ocular medications; and mg or mL for pediatric drugs when the concentration would have been too low. The intake frequency cell also has a pre-filled table field with a dropdown menu, where frequency is expressed as n/DIE; in this case the drop-down menu gave the possibility of reaching up to 10 daily doses.

The therapy duration cell is an open table field in which a numerical data is entered which corresponds to the duration of antibiotic intake expressed in days. Information on the duration of therapy is obtained individually for each subject from the medical records, which indicate the duration of the antibiotic prescription if this is carried out at home, or in the case of hospitalization, the number of days of therapy is obtained considering the period ranging from admission to discharge.

Finally, the last two cells report information on the registration of the drug and the type of drug. Both feature a pre-filled table field with a dropdown menu. In the drug registration cell, the drop-down menu presents 3 choices according to the type of antibiotic: Regulated for the Species, Not Regulated for the Species, Non-Veterinary. A drug regulated for the species means an antibiotic registered for use in dogs and cats; a drug not regulated for the species indicates a drug not registered for use in dogs and cats, but instead in other animal species such as livestock; a non-veterinary drug indicates a drug whose use is accepted only in humans, but

can be used in animals if it is prescribed in derogation according to the drug cascade (Regulation (EC) P8_TA(2018)0421 of 25 October 2018). While the cell on the type of drug presents a drop-down menu with 2 choices: veterinary or human, depending on whether the drug is registered for use in animals or only in humans.

For completeness, the worksheet ends with the last cell, which indicates whether the selected patient underwent surgical procedures or not. To obtain this information it is necessary to evaluate the medical records of each patient and to search for medical records belonging to surgical specialties, such as general surgery, orthopedics, endoscopy, ophthalmic surgery or oncological surgery. Once the information is found, a pre-filled table field with a dropdown menu is used to enter "Yes" or "No" in the cell.

2.5 Sensitivity Assessment

Within the fields of sensitivity assessment, information about culture and sensitivity tests can be found. The contents of this section are summarized in *Table 6* and shown graphically as in the Excel worksheet in *Image 8*.

Table 6. Sensitivity assessment section in the Excel worksheet

SENSITIVITY ASSESSMENT
Bacteriological examination
Outcome
Susceptibility test

Figure 8. Sensitivity assessment section in the Excel worksheet

Inside the first cell the presence of a bacteriological test or not for that patient is reported. It is a pre-filled table field with a dropdown menu with two choices, "Yes" or "No", depending on whether a culture test has been recorded on “Fenice”. In order to know this information, it is necessary to analyze the medical records of each patient individually.

The second cell instead reports the result of the bacteriological examination, using a pre-filled table field with a dropdown menu in which the choice can be "Positive", "Negative" or "-" to indicate None. The Positive or Negative option is indicated by analyzing the bacteriological test report individually for each patient and reporting the presence or absence of bacteria within the analyzed material.

The following cell indicates the presence of a sensibility test in case of bacteriological examination. Again, a pre-filled table field with a dropdown menu is included here, allowing you to choose "Yes," "No," or "-" to denote None. In the event that the bacteriological test is not performed, the option None is carried out as in the previous case. In the event of a positive bacteriological test, an antibiogram is always done, so in this case “Yes” is selected; in the event of a negative bacteriological test, no antibiogram can be performed, so “No” is instead selected.

2.6 Study Methodology

After collecting all the data and entering them into the Excel worksheet, the study continued through a descriptive statistical analysis of the collected data. The data from both years were entered into a series of Excel tables to extrapolate the collective data and percentages regarding the key information of the study. The data were sorted considering the objective of the study, i.e. the analysis of the use of antibiotics within the VTH of the University of Parma in conjunction with the analysis of the most used antibiotic molecules and the frequency of use of cultural and sensitivity tests.

We started by considering the general data on the study regarding number of total visits, number of visits of included patients, number of antimicrobial prescriptions and number of CSTs. For each of these data, the total number and the number associated with the percentage for each service offered by the VTH were considered for both 2021 and 2022. This is followed by the analysis of the data regarding the type of antibiotic prescription recorded on the worksheet, divided into monotherapy, empirical associations, fixed combinations and total antibiotics. Each of these divisions has been considered within the different services of the VTH for both the years 2021 and 2022. The analysis of data concerning CSTs follows, considering culture and susceptibility tests on the total antimicrobial prescriptions, positive

culture and susceptibility tests and culture and susceptibility tests on CIA molecules, all in the form of absolute number and percentage. The number of CIAs (piperacillin; quinolones; 3rd – 4th - 5th generation cephalosporins; polymyxins; macrolides) prescribed out of the total number of antibiotics was also analyzed, both as a total number and as a percentage. All this data was then analyzed within the different VTH services and percentages and absolute numbers extrapolated. Finally, the last table analyzed the antibiotic prescriptions considered within different systems, using the cells of the "System" worksheet and dividing the prescriptions through it. These data were extrapolated both as an absolute number and as a percentage. They were subsequently analyzed by associating the prescriptions within the different systems with the VTH services.

3 | RESULTS

3.1 Study Population

A total of 1.625 cases were collected in 2021 and 1.803 cases in 2022 for this study. A total of 1.072 cats visited in 2021 and 1.200 cats visited in 2022 met the inclusion criteria. Of these, 1.009 (94,1%) patients received a prescription in 2021 and among them 470 (43,8%) received an antimicrobial prescription, with 85 (18,1%) culture and susceptibility test performed; in 2022, 949 (79,1%) patients received a prescription and among them 420 (35%) received an antimicrobial prescription, with 73 (17,4%) culture and susceptibility test performed.

Within the cat population seen in 2021 and 2022, the patients split into several specialties, as shown below and in *Figure 9*:

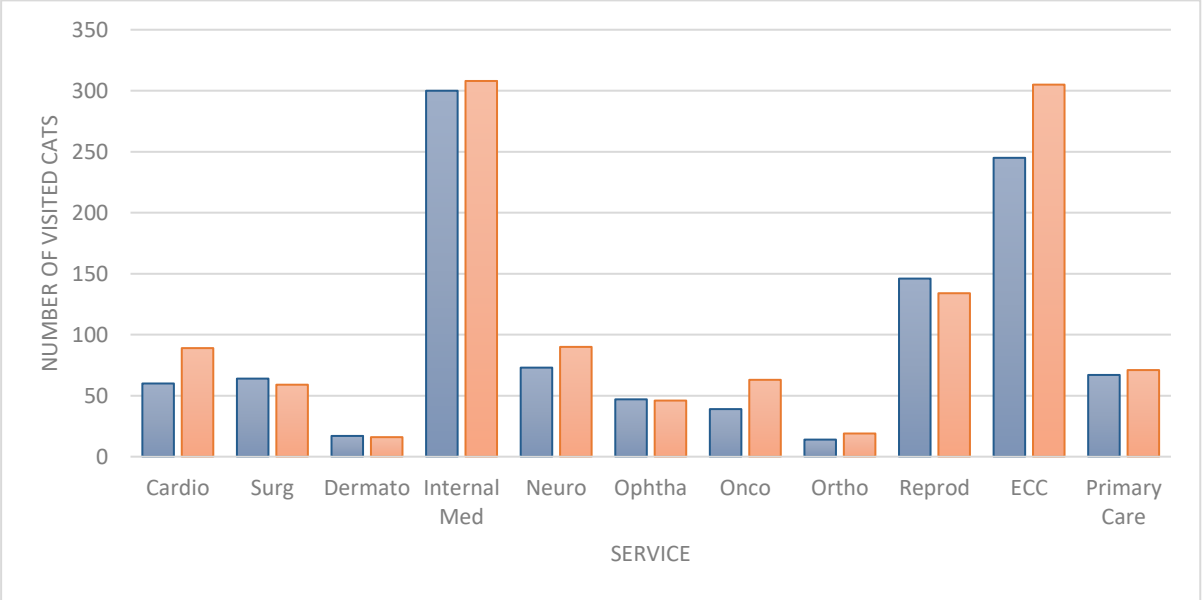


Figure 9: Number of visited cats in 2021 (Blue) and 2022 (Red) based on different services

Abbreviations: Cardio: Cardiology; Surg: Surgery; Dermato: Dermatology; Internal Med: Internal Medicine; Neuro: Neurology; Ophtha: Ophthalmology; Onco: Oncology; Ortho: Orthopedics; Reprod: Reproduction; ECC: Emergency and Critical Care; Primary Care.

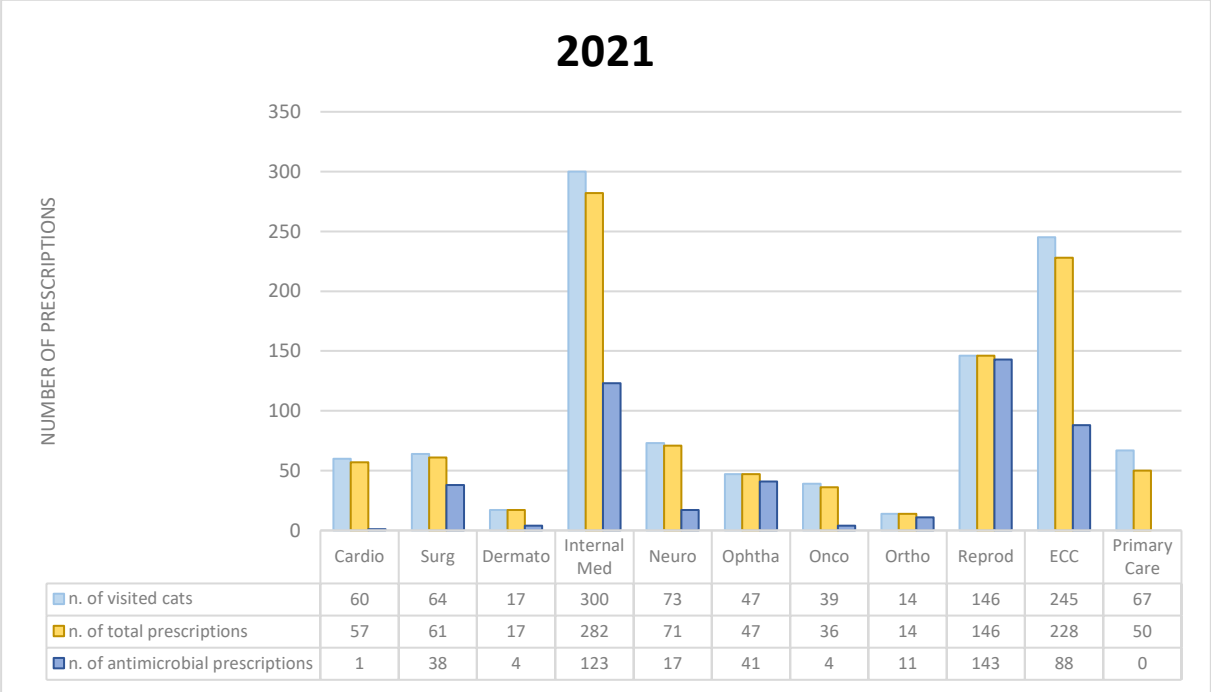
- The cardiology service handled 5,6% (60/1072) of the cases in 2021 and 7,4% (89/1200) in 2022;
- The surgery service handled 6% (64/1072) of the cases in 2021 and 4,9% (59/1200) in 2022;
- The dermatology service handled 1,6% (17/1072) of the cases in 2021 and 1,3% (16/1200) in 2022;

- The internal medicine service handled 28% (300/1072) of the cases in 2021 and 25,7% (308/1200) in 2022;
- The neurology service handled 6,8% (73/1072) of the cases in 2021 and 7,5% (90/1200) in 2022;
- The ophthalmology service handled 4,4% (47/1072) of the cases in 2021 and 3,8% (46/1200) in 2022;
- The oncology service handled 3,6% (39/1072) of the cases in 2021 and 5,3% (63/1200) in 2022;
- The orthopedics service handled 1,3% (14/1072) of the cases in 2021 and 1,6% (19/1200) in 2022;
- The reproduction service handled 13,6% (146/1072) of the cases in 2021 and 11,2% (134/1200) in 2022;
- The emergency and critical care (ECC) service handled 22,9% (245/1072) of the cases in 2021 and 25,4% (305/1200) in 2022;
- The primary care service handled 6,3% (67/1072) of the cases in 2021 and 5,9% (71/1200) in 2022.

3.2 Antibiotic Prescription

3.2.1 Prescription Rate

Within the population analyzed, the frequency with which a specific service prescribed antibiotic drugs in the years 2021 and 2022 was taken into consideration, shown respectively in *Figure 10* and *Figure 11* in association with an overview of the total prescriptions carried out by the various services.



	Cardio	Surg	Dermato	Internal Med	Neuro	Ophtha	Onco	Ortho	Reprod	ECC	Primary Care
n. of visited cats	60	64	17	300	73	47	39	14	146	245	67
n. of total prescriptions	57	61	17	282	71	47	36	14	146	228	50
n. of antimicrobial prescriptions	1	38	4	123	17	41	4	11	143	88	0

Figure 10: Antimicrobials prescription rate in the year 2021 based on different services (on abscissae) compared with total prescriptions for the service.

Abbreviations: Cardio: Cardiology; Surg: Surgery; Dermato: Dermatology; Internal Med: Internal Medicine; Neuro: Neurology; Ophtha: Ophthalmology; Onco: Oncology; Ortho: Orthopedics; Reprod: Reproduction; ECC: Emergency and Critical Care; Primary Care.

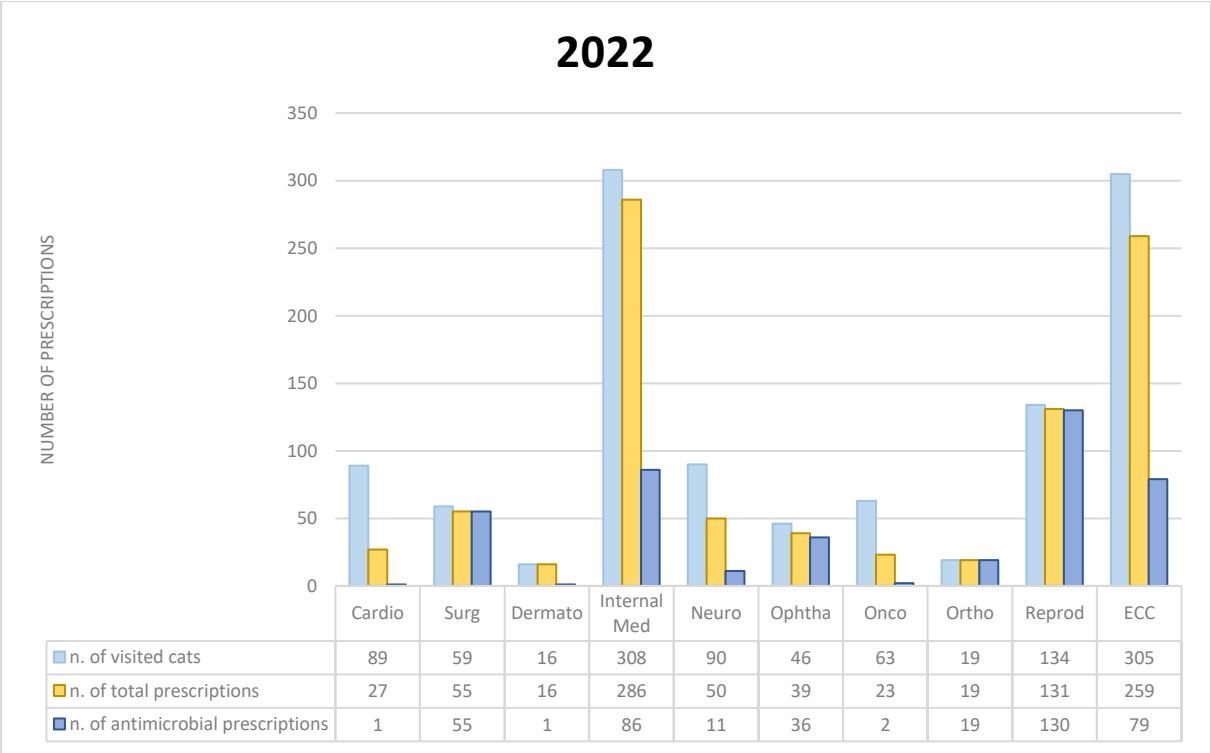


Figure 21: Antimicrobials prescription rate in the year 2022 based on different services (on abscissae) compared with total prescriptions for the service.

Abbreviations: Cardio: Cardiology; Surg: Surgery; Dermato: Dermatology; Internal Med: Internal Medicine; Neuro: Neurology; Ophtha: Ophthalmology; Onco: Oncology; Ortho: Orthopedics; Reprod: Reproduction; ECC: Emergency and Critical Care; Primary Care.

- The cardiology service prescribed antimicrobial drugs in 1,7% (1/60) of subjects in 2021 and in 1,7% (1/89) in 2022;
- The surgery service prescribed antimicrobial drugs in 59,4% (38/64) of subjects in 2021 and in 93,2% (55/59) in 2022;
- The dermatology service prescribed antimicrobial drugs in 23,5% (4/17) of subjects in 2021 and in 6,3% (1/16) in 2022;
- The internal medicine service prescribed antimicrobial drugs in 41% (123/300) of subjects in 2021 and in 27,9% (86/308) in 2022;
- The neurology service prescribed antimicrobial drugs in 23,3% (17/73) of subjects in 2021 and in 12,2% (11/90) in 2022;
- The ophthalmology service prescribed antimicrobial drugs in 87,2% (41/47) of subjects in 2021 and in 78,3% (36/46) in 2022;
- The oncology service prescribed antimicrobial drugs in 10,3% (4/39) of subjects in 2021 and in 3,2% (2/63) in 2022;

- The orthopedics service prescribed antimicrobial drugs in 78,6% (11/14) of subjects in 2021 and in 100% (19/19) in 2022;
- The reproduction service prescribed antimicrobial drugs in 97,9% (143/146) of subjects in 2021 and in 97% (130/134) in 2022;
- The emergency and critical care service prescribed antimicrobial drugs in 35,9% (88/245) of subjects in 2021 and in 25,9% (79/305) in 2022;
- The primary care service prescribed antimicrobial drugs in 0% (0/67) of subjects in 2021 and in 0% (0/71) in 2022.

3.2.2 Type of Prescription

Within the cases collected, the type of antibiotic prescription provided to the patient was considered, identifying it as antibiotic monotherapy, empirical association or fixed combinations. In 2021, 48,2% (223/463) of antibiotic prescriptions were detected in the form of monotherapy, 13,8% (64/463) as empirical combinations of drugs and 38% (176/463) as fixed combinations. While in 2022, 57,3% (240/419) of antibiotic prescriptions were prescribed as monotherapy, 7,6% (32/419) as empirical associations and 35,1% (147/240) as fixed combinations. These data are represented in *Figure 12*.

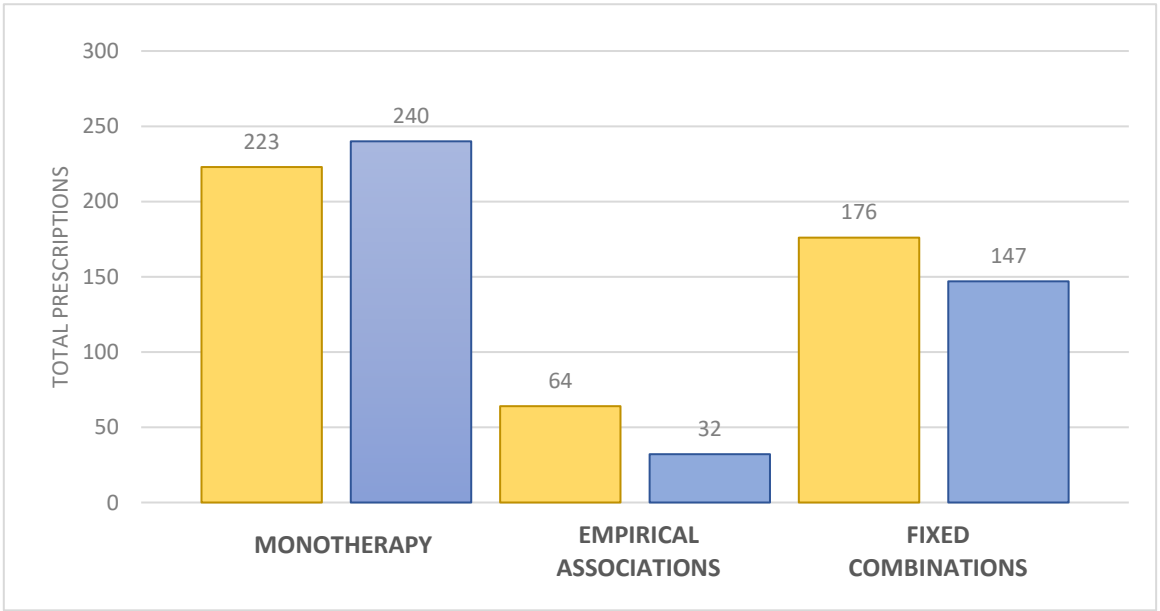
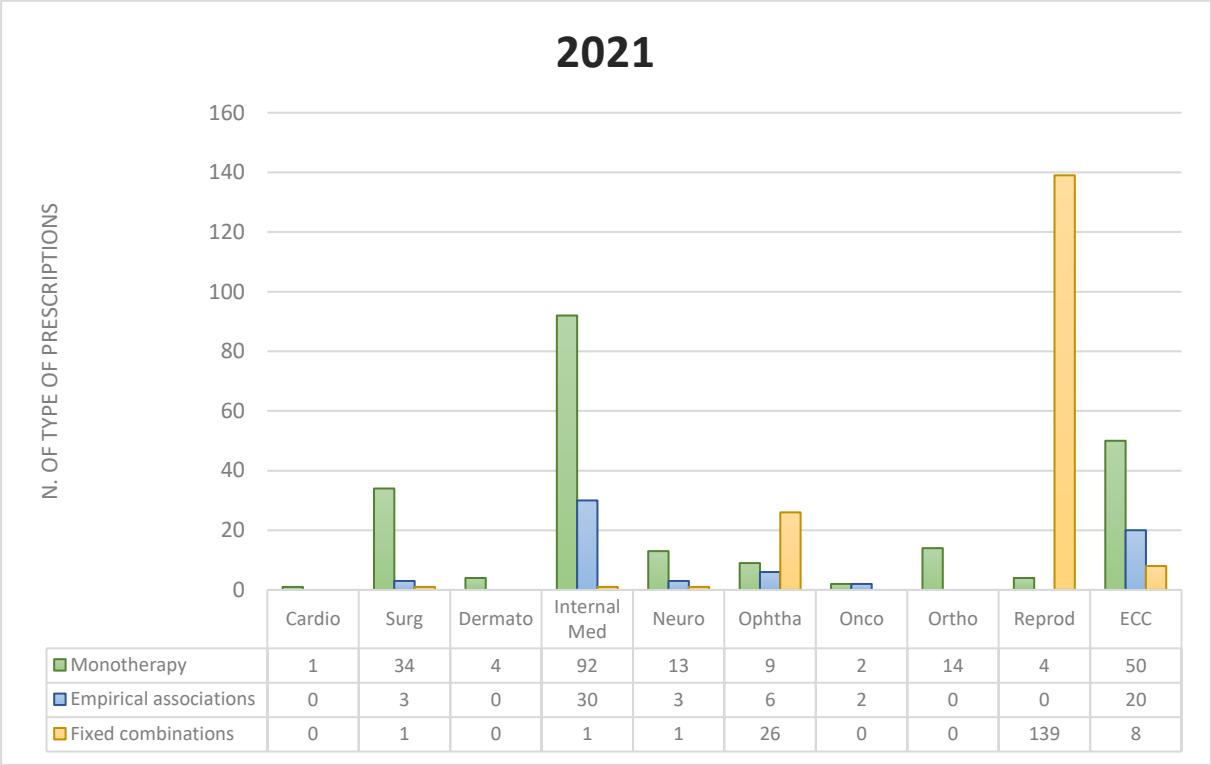


Figure 12: Type of antimicrobial prescription in years 2021 (yellow) and 2022 (blue) based on monotherapy, empirical associations or fixed combinations

Subsequently the data were analyzed considering the different services provided by the VTH, starting from 2021 as shown below and in *Figure 13*.



	Cardio	Surg	Dermato	Internal Med	Neuro	Ophtha	Onco	Ortho	Reprod	ECC
Monotherapy	1	34	4	92	13	9	2	14	4	50
Empirical associations	0	3	0	30	3	6	2	0	0	20
Fixed combinations	0	1	0	1	1	26	0	0	139	8

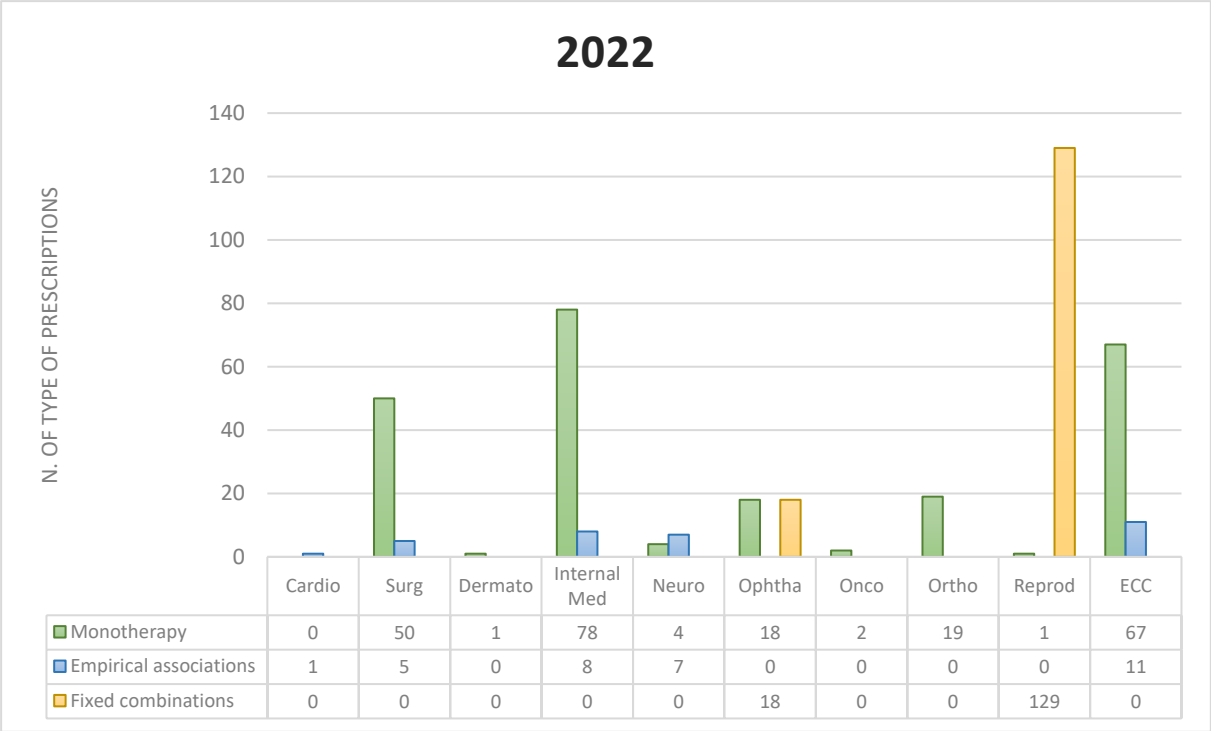
Figure 13: Type of antimicrobial prescriptions in 2021 based on different services (on the abscissae) of the VTH.

Abbreviations: Cardio: Cardiology; Surg: Surgery; Dermato: Dermatology; Internal Med: Internal Medicine; Neuro: Neurology; Ophtha: Ophthalmology; Onco: Oncology; Ortho: Orthopedics; Reprod: Reproduction; ECC: Emergency and Critical Care; Primary Care.

- The cardiology service prescribed 100% (1/1) of antibiotics as monotherapy;
- The surgery service prescribed 89,5% (34/38) of antibiotics as monotherapy, 7,9% (3/38) as empirical associations and 2,6% (1/38) as fixed combinations;
- The dermatology service prescribed 100% (4/4) of antibiotics as monotherapy;
- The internal medicine service prescribed 74,8% (92/123) of antibiotics as monotherapy, 24,4%;(30/123) as empirical associations and 0,8% (1/123) as fixed combinations;
- The neurology service prescribed 76,5% (13/17) of antibiotics as monotherapy, 17,6% (3/17) as empirical associations and 5,9% (1/17) as fixed combinations;
- The ophthalmology service prescribed 63,4% (26/41) of antibiotics as fixed combinations, 22% (9/41) as monotherapy and 14,6% (6/41) as empirical associations;

- The oncology service prescribed 50% (2/4) of antibiotics as monotherapy and 50% (2/4) as empirical associations;
- The orthopedics service prescribed 100% (14/14) of antibiotics as monotherapy;
- The reproduction service prescribed 97,2% (139/143) of antibiotics as fixed combinations and 2,8% (4/143) as monotherapy;
- The emergency and critical care service prescribed 64,1% (50/78) of antibiotics as monotherapy, 25,6% (20/78) as empirical associations and 10,3% (8/78) as fixed combinations;
- The primary care service didn't prescribed antibiotics.

Also in 2022, the type of prescription within the different services provided by the VTH were collected, as shown below and in *Figure 14*.



	Cardio	Surg	Dermato	Internal Med	Neuro	Ophtha	Onco	Ortho	Reprod	ECC
Monotherapy	0	50	1	78	4	18	2	19	1	67
Empirical associations	1	5	0	8	7	0	0	0	0	11
Fixed combinations	0	0	0	0	0	18	0	0	129	0

Figure 14: Type of antimicrobial prescriptions in 2021 based on different services (on the abscissae) of the VTH.

Abbreviations: Cardio: Cardiology; Surg: Surgery; Dermato: Dermatology; Internal Med: Internal Medicine; Neuro: Neurology; Ophtha: Ophthalmology; Onco: Oncology; Ortho: Orthopedics; Reprod: Reproduction; ECC: Emergency and Critical Care; Primary Care.

- The cardiology service prescribed 100% (1/1) of antibiotics as empirical associations;
- The surgery service prescribed 90,9% (50/55) of antibiotics as monotherapy and 9,1% (5/55) as empirical associations;
- the dermatology service prescribed 100% (1/1) of antibiotics as monotherapy;
- The internal medicine service prescribed 90,7% (78/86) of antibiotics as monotherapy and 9,3%;(8/86) as empirical associations;
- The neurology service prescribed 63,6% (7/11) of antibiotics as empirical associations and 36,4% (4/11) as monotherapy;
- The ophthalmology service prescribed 50% (18/36) of antibiotics as fixed combinations and 50% (18/36) as monotherapy;
- The oncology service prescribed 100% (2/2) of antibiotics as monotherapy;
- The orthopedics service prescribed 100% (19/19) of antibiotics as monotherapy;
- The reproduction service prescribed 99,2% (129/130) of antibiotics as fixed combinations and 0,8% (1/130) as monotherapy;
- The emergency and critical care service prescribed 85,9% (67/78) of antibiotics as monotherapy and 14,1% (11/78) as empirical associations;
- The primary care service didn't prescribed antibiotics.

3.2.3 Antimicrobial Prescriptions Classes

The information on the classes of antibiotics employed inside each service of the VTH was thereafter analyzed within the cases collected. Not all of the 15 antibiotic classes that were considered were employed.

In 2021, a total of 551 antimicrobial molecules were provided to the animals analyzed, and within these: 31,9% (176/551) were fixed composition antibiotics; 31,4% (173/551) were enhanced penicillins; 12,2% (67/551) were quinolones; 10,5% (58/551) were cephalosporins; 4,2% (23/551) were lincosamides; 3,3% (18/551) were tetracyclines; 3,3% (18/551) were nitroimidazoles; 2,4% (13/551) were aminoglycosides; 0,7% (4/551) were enhanced cephalosporins; 0,2% (1/551) was a polymyxin (Surolan). No macrolides, penicillins, phenicolates, sulphonamides or piperacillin were prescribed in that year. These data are represented in *Figure 15*.

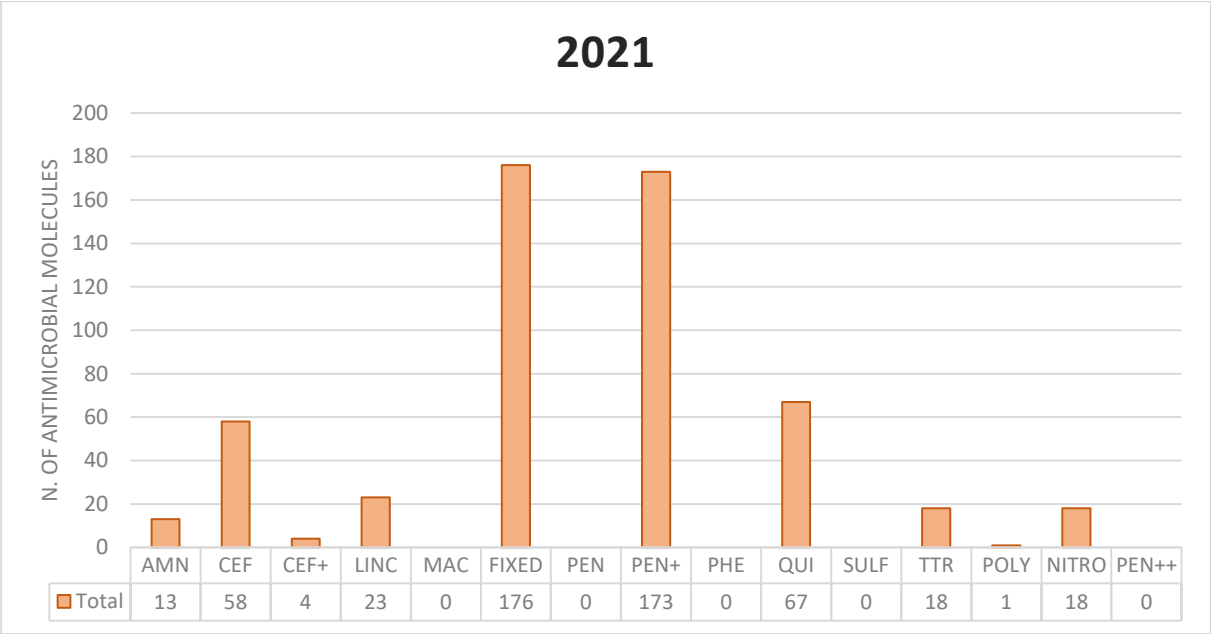


Figure 15: Number of antimicrobials prescribed in 2021 based on antimicrobial classes (on abscissae).

Abbreviations: AMN: Aminoglycosides; CEF: Cephalosporins, CEF+: Enhanced Cephalosporins; MAC: Macrolides; NITRO: Nitroimidazoles; PEN: Penicillins; PEN+: Penicillins Associated with Beta-Lactam Inhibitors; FIXED: Fixed combination; PHE: Phenicolates; POLY: Polymyxins; QUI: Quinolones; RIF: Rifamycins; SULF: Sulfonamides; TTR: Tetracyclines; LINC: Lincosamides; PEN++: Piperacillin.

The prescriptions are distributed within the VTH services, as shown below and in *Figure 16*:

- The cardiology service prescribed 100% (1/1) of enhanced penicillins;
- The surgery service prescribed 60,4% (29/48) of cephalosporins, 22,9% (11/48) of enhanced penicillins, 6,3% (3/48) of quinolones, 2,1% (1/48) of aminoglycoside, 2,1% (1/48) of enhanced cephalosporin, 2,1% (1/48) of lincosamides, 2,1% 1/48) of fixed composition antibiotics, and 2,1% (1/48) of nitroimidazoles;
- The dermatology service prescribed 25% (1/4) of lincosamides, 25% (1/4) of enhanced penicillins, 25% (1/4) of quinolones and 25% (1/4) of polymyxins (Surolan);
- The internal medicine service prescribed 51,6% (79/153) of enhanced penicillins, 28,1% (43/153) of quinolones, 8,5% (13/153) of tetracyclines, 8,5% (13/153) of nitroimidazoles, 2,6% (4/153) of lincosamides, and 0,7% (1/153) of fixed composition antibiotics;
- The neurology service prescribed 45% (9/20) of lincosamides, 20% (4/20) of quinolones, 10% (2/20) of cephalosporins, 10% (2/20) of enhanced cephalosporins, 5% (1/20) of fixed composition antibiotics, 5% (1/20) of enhanced penicillin, and 5% (1/20) of nitroimidazoles;

- The ophthalmology service prescribed 55,3% (26/47) of fixed composition antibiotics, 21,3% (10/47) of aminoglycosides, 8,5% (4/47) of enhanced penicillins, 8,5% (4/47) of quinolones, and 6,4% (3/47) of cephalosporins;
- The oncology service prescribed 50% (3/6) of enhanced penicillins and 50% (3/6) of quinolones;
- The orthopedics service prescribed 90,9% (10/11) of cephalosporins and 9,1% (1/11) of enhanced penicillins;
- The reproduction service prescribed 97,2% (139/143) of fixed composition antibiotics and 2,8% (4/143) of cephalosporins;
- The emergency and critical care service prescribed 61% (72/118) of enhanced penicillins, 8,5% (10/118) of cephalosporins, 7,6% (9/118) of quinolones, 6,8% (8/118) of lincosamides, 6,8% (8/118) of fixed composition antibiotics, 4,2% (5/118) of tetracyclines, 2,5% (3/118) of nitroimidazoles, 1,7% (2/118) of aminoglycosides, and 0,8% (1/118) of enhanced cephalosporins;
- The primary care service didn't prescribe any antibiotic.

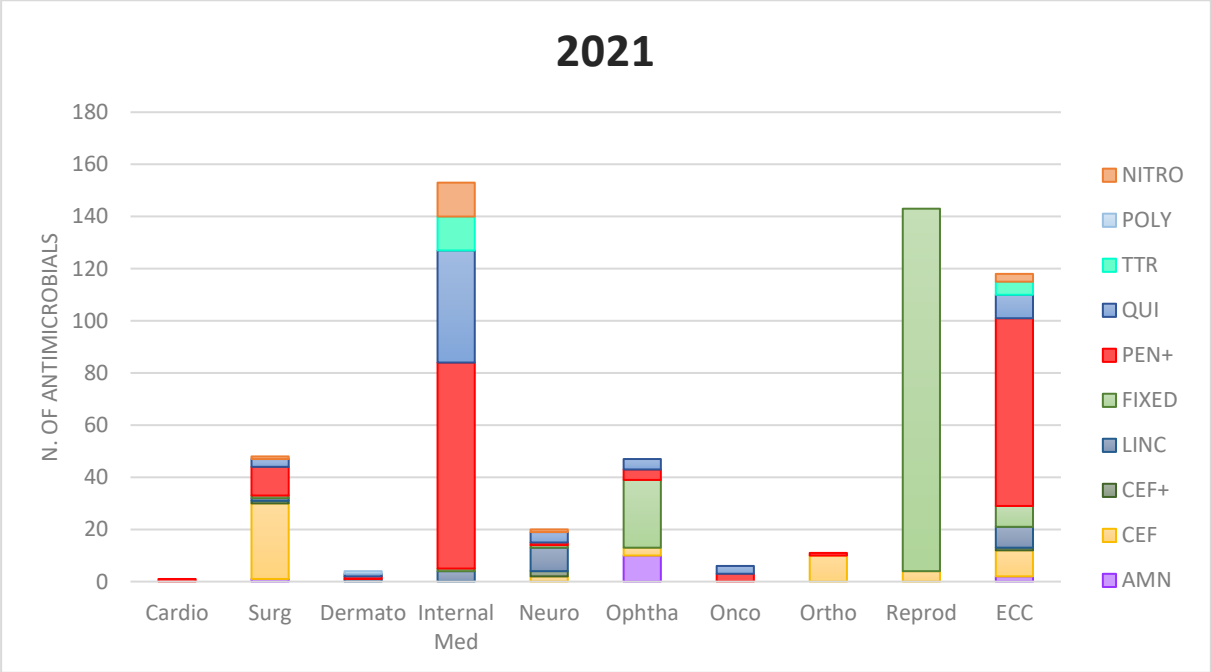


Figure 16: Number of antimicrobial prescriptions classes prescribed in 2021 based on different services (on abscissae) of VTH.

Abbreviations: AMN: Aminoglycosides; CEF: Cephalosporins, CEF+: Enhanced Cephalosporins; MAC: Macrolides; NITRO: Nitroimidazoles; PEN: Penicillins; PEN+: Penicillins Associated with Beta-Lactam Inhibitors; FIXED: Fixed combination; PHE: Phenicolates; POLY: Polymyxins; QUI: Quinolones; RIF: Rifamycins; SULF: Sulfonamides; TTR: Tetracyclines; LINC: Lincosamides; PEN++:Piperacillin.

In 2022, a total of 476 antimicrobial molecules were provided to the animals analyzed, and within these: 31,3% (149/476) were enhanced penicillins; 31,1% (148/476) were fixed composition antibiotics; 14,7% (70/476) were cephalosporins; 11,8% (56/476) were quinolones; 3,6% (17/476) were aminoglycosides; 2,9% (14/476) were tetracyclines; 2,3% (11/476) were lincosamides; 2,1% (10/476) were phenicolates; 0,2% (1/476) were piperacillin (PEN++). These data are represented in *Figure 17*.

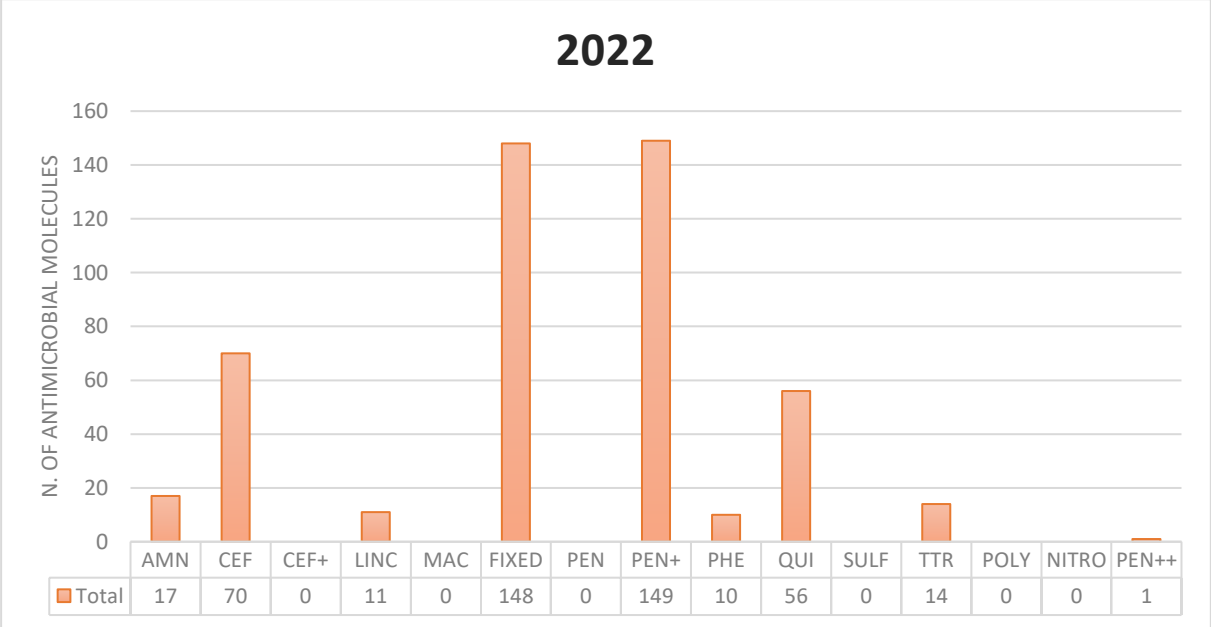


Figure 17: Number of antimicrobials prescribed in 2021 based on antimicrobial classes (on abscissae).

Abbreviations: AMN: Aminoglycosides; CEF: Cephalosporins, CEF+: Enhanced Cephalosporins; MAC: Macrolides; NITRO: Nitroimidazoles; PEN: Penicillins; PEN+: Penicillins Associated with Beta-Lactam Inhibitors; FIXED: Fixed combination; PHE: Phenicolates; POLY: Polymyxins; QUI: Quinolones; RIF: Rifamycins; SULF: Sulfonamides; TTR: Tetracyclines; LINC: Lincosamides; PEN++:Piperacillin.

The prescriptions are distributed within the VTH services, as shown below and in *Figure 18*:

- The cardiology service prescribed 50% (1/2) of quinolones and 50% (1/2) of tetraciclines;
- The surgery service prescribed 60,3% (47/78) of cephalosporins, 32,1% (25/78) of enhanced penicillins, 5,1% (4/78) of quinolones, 1,3% (1/78) of lincosamides, and 1,3% (1/78) of phenicolates;
- the dermatology service prescribed 100% (1/1) of enhanced penicillins;
- The internal medicine service prescribed 53,2% (50/94) of enhanced penicillins, 33% (31/94) of quinolones, 11,7% (11/94) of tetracyclines, 1,1% (1/94) of lincosamides, and 1,1% (1/94) of piperacillin (PEN++);

- The neurology service prescribed 44,4% (8/18) of lincosamides, 38,9% (7/18) of quinolones, 11,1% (2/18) of enhanced penicillins, and 5,6% (1/18) of cephalosporins;
- The ophthalmology service prescribed 50% (18/36) of fixed composition antibiotics, 33,3% (12/36) of aminoglycosides, 8,3% (3/36) of cephalosporins, 5,6% (2/36) of phenicolates, and 2,8% (1/36) of quinolones;
- The oncology service prescribed 100% (2/2) of enhanced penicillins;
- The orthopedics service prescribed 76% (19/25) of cephalosporins and 24% (6/25) of enhanced penicillins;
- The reproduction service prescribed 99,2% (129/130) of fixed composition antibiotics and 0,8% (1/130) of enhanced penicillins;
- The emergency and critical care service prescribed 68,9% (62/90) of enhanced penicillins, 13,3% (12/90) of quinolones, 7,8% (7/90) of phenicolates, 5,6% (5/90) of aminoglycosides, 2,2% (2/90) of tetracyclines, 1,1% (1/90) of lincosamides, and 1,1% (1/90) of fixed composition antibiotics;
- The primary care service didn't prescribe any antibiotic.

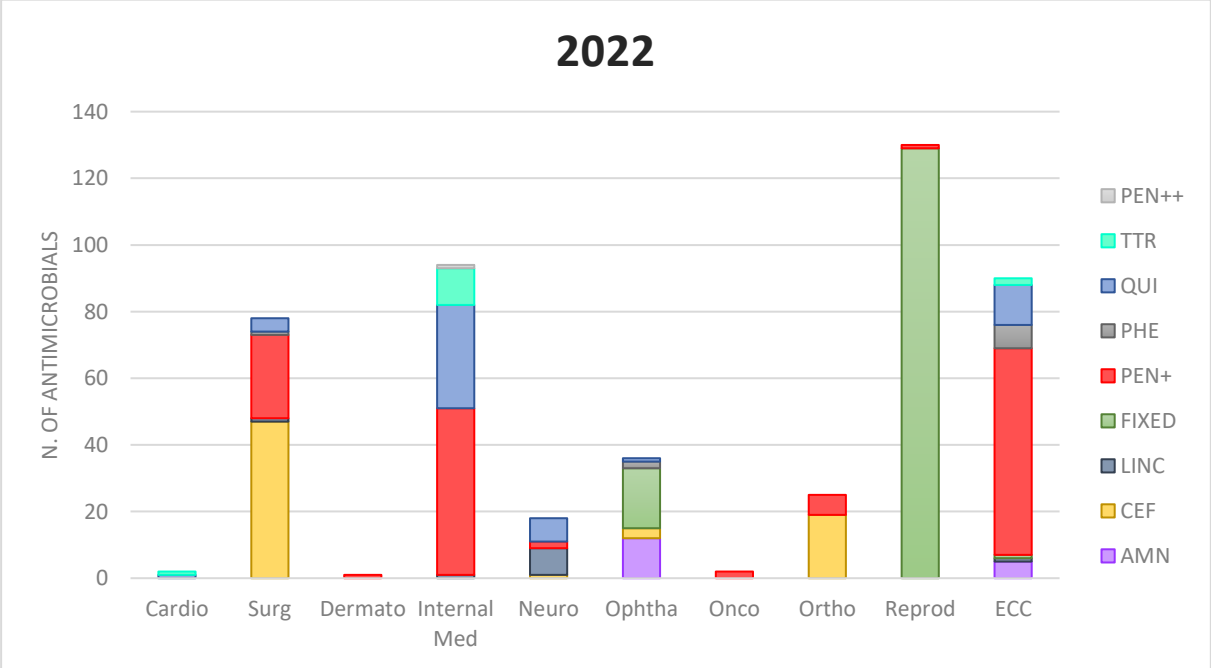


Figure 18: Number of antimicrobial prescriptions classes prescribed in 2021 based on different services (on abscissae) of VTH.

Abbreviations: AMN: Aminoglycosides; CEF: Cephalosporins, CEF+: Enhanced Cephalosporins; MAC: Macrolides; NITRO: Nitroimidazoles; PEN: Penicillins; PEN+: Penicillins Associated with Beta-Lactam Inhibitors; FIXED: Fixed combination; PHE: Phenicolates; POLY: Polymyxins; QUI: Quinolones; RIF: Rifamycins; SULF: Sulfonamides; TTR: Tetracyclines; LINC: Lincosamides; PEN++:Piperacillin.

Within these classes of antibiotics, a number of Critically Important Antimicrobials (CIAs) have also been prescribed, represented by category A and B within the EMA classification of antibiotics for veterinary use (European Medicines Agency (EMA) et al., 2019). The study therefore also took into consideration the number of CIAs prescribed by the various VTH services in both years taken into consideration. In 2021, out of the total antimicrobial prescriptions recorded, 4,9% (70/469) were CIAs, distributed among the various services of the VTH as shown in *Figure 19*. In 2022, out of the total antimicrobial prescriptions recorded, 13,6% (57/420) were CIAs, distributed among the various services of the VTH as shown in *Figure 20*.

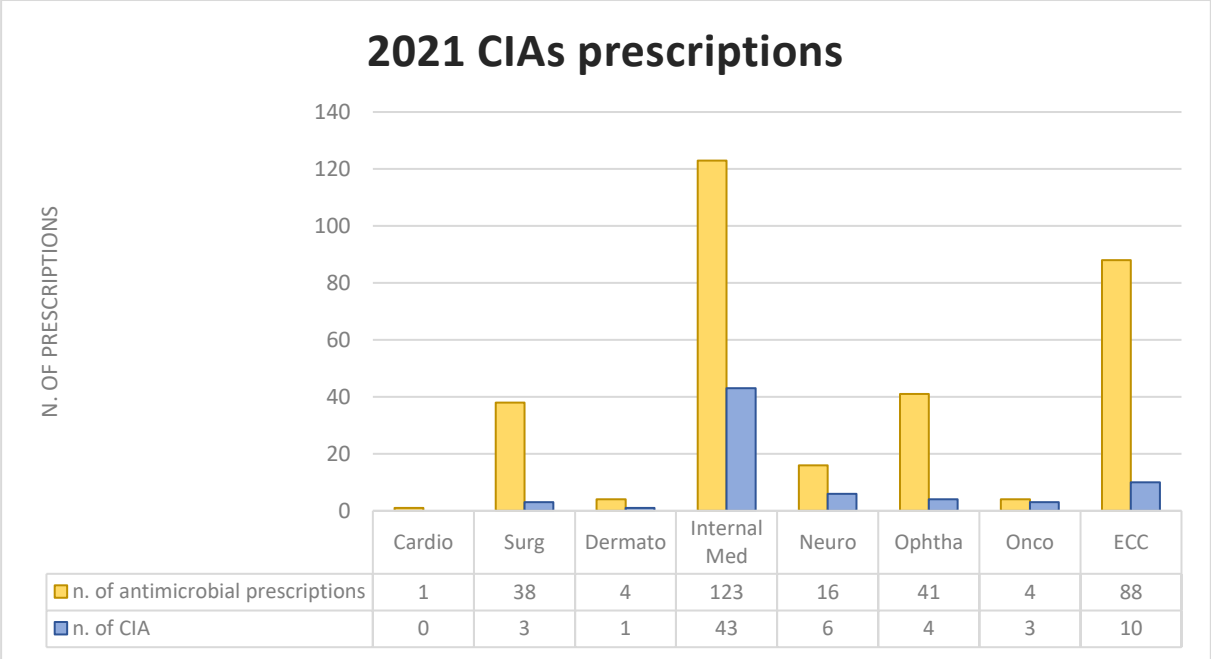


Figure 13: CIAs prescription rate in the year 2021 based on different services (on abscissae) of the VTH.

Abbreviations: Cardio: Cardiology; Surg: Surgery; Dermato: Dermatology; Internal Med: Internal Medicine; Neuro: Neurology; Ophtha: Ophthalmology; ECC: Emergency and Critical Care.

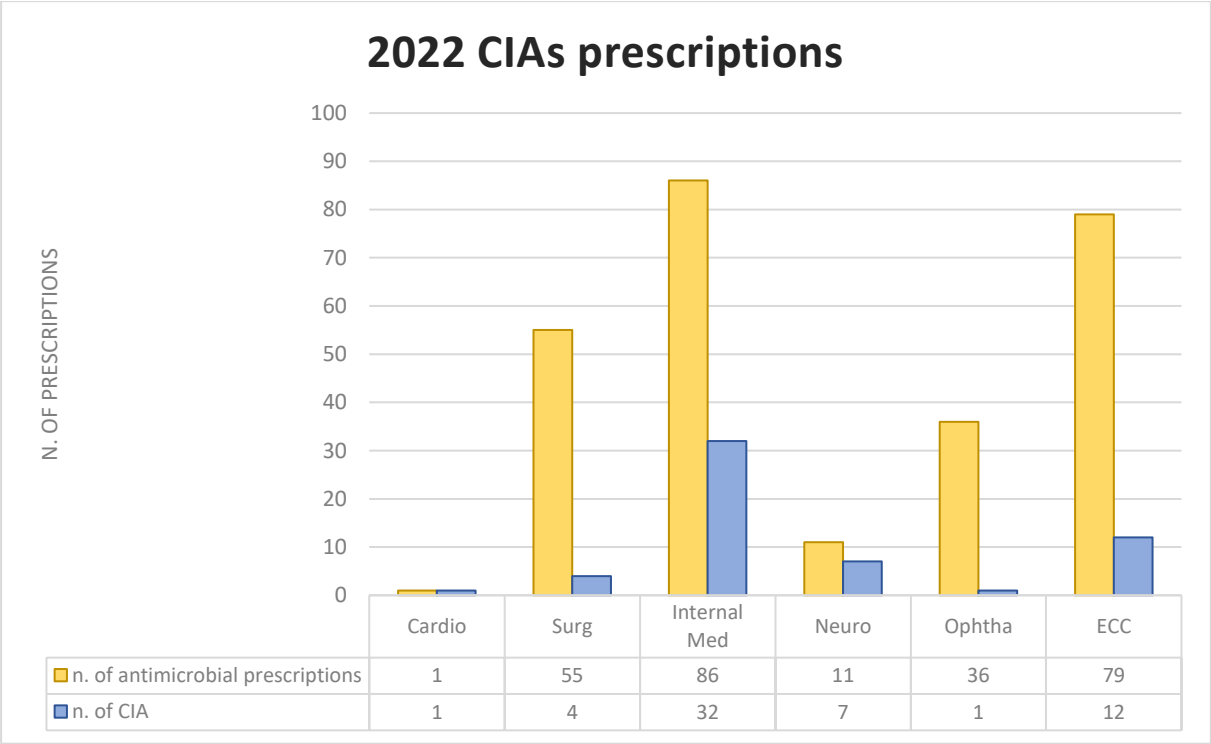


Figure 20: CIAs prescription rate in the year 2022 based on different services (on abscissae) of the VTH.

Abbreviations: Cardio: Cardiology; Surg: Surgery; Internal Med: Internal Medicine; Neuro: Neurology; Ophtha: Ophthalmology; ECC: Emergency and Critical Care.

An overview of the prescription rate in the years 2021-2022 within the Veterinary Teaching Hospital of the University of Parma based on the EMA classification of antimicrobials for use in animals is finally shown in *Table 7.*

Table 7. Overview on antimicrobial classes prescription over two years based on EMA's classification of antimicrobials for animals' use.

Abbreviations: AMN: Aminoglycosides; CEF: Cephalosporins, CEF+: Enhanced Cephalosporins; MAC: Macrolides; NITRO: Nitroimidazoles; PEN: Penicillins; PEN+: Penicillins Associated with Beta-Lactam Inhibitors; FIXED: Fixed combination; PHE: Phenicolates; POLY: Polymyxins; QUI: Quinolones; RIF: Rifamycins; SULF: Sulfonamides; TTR: Tetracyclines; LINC: Lincosamides; PEN++:Piperacillin.

		2021		2022	
		n.	*%*	*n.*	*%*
A	PEN++	0	0	1	0,2
B	CEF+	4	0,7	0	0
	POLY	1	0.2	0	0
	QUI	67	12,2	56	11,8
	MAC	0	0	0	0
C	AMN	13	2,4	17	3,6
	PEN+	149	31,3	173	31,4
	CEF	58	10,5	70	14,7
	PHE	0	0	10	2,1
	LINC	23	4	11	2,3
D	PEN	0	0	0	0
	TTR	18	3,3	14	2,9
	NITRO	18	3,3	0	0
	SULF	0	0	0	0
OTHERS	FIXED	176	31,9	148	31,1

3.3 Culture and Susceptibility Tests

3.3.1 Test Execution Rates

In 2021, within the cats included in the study the CSTs corresponded to 18,1% (85/470) of the total antimicrobial prescriptions (*Figure 21).*

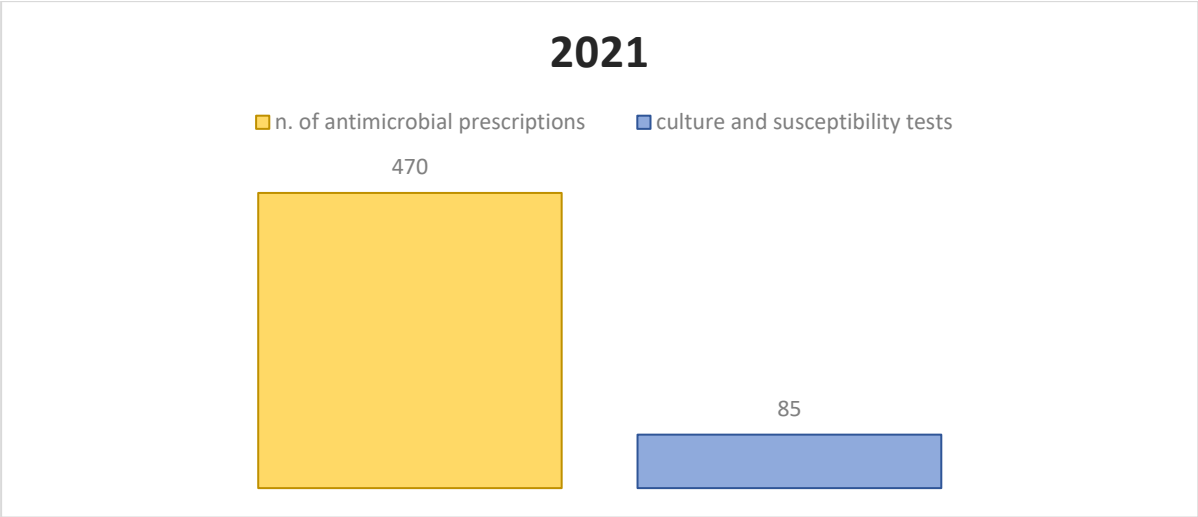


Figure 21: Antibiotics prescribed and CSTs carried out in the year 2021

Within the population, the CSTs were performed under the directive of a service of the VTH. Not all services required CSTs, while those involved are shown below and in *Figure 22*:

- The cardiology service performed 100% (1/1) CSTs on total antibiotic prescriptions;
- The surgery service performed 18,4% (7/38) CSTs on total antibiotic prescriptions;
- The dermatology service performed 25% (1/4) CSTs on total antibiotic prescriptions;
- The internal medicine service performed 52,8% (65/123) CSTs on total antibiotic prescriptions;
- The ophthalmology service performed 2,4% (1/41) CSTs on total antibiotic prescriptions;
- The oncology service performed 50% (2/4) CSTs on total antibiotic prescriptions;
- The emergency and critical care service performed 9,1% (8/88) CSTs on total antibiotic prescriptions;
- The neurology (0/17), orthopedics (0/11), reproduction (0/143) and primary care (0/0) services didn't perform any CSTs on total antibiotic prescriptions.

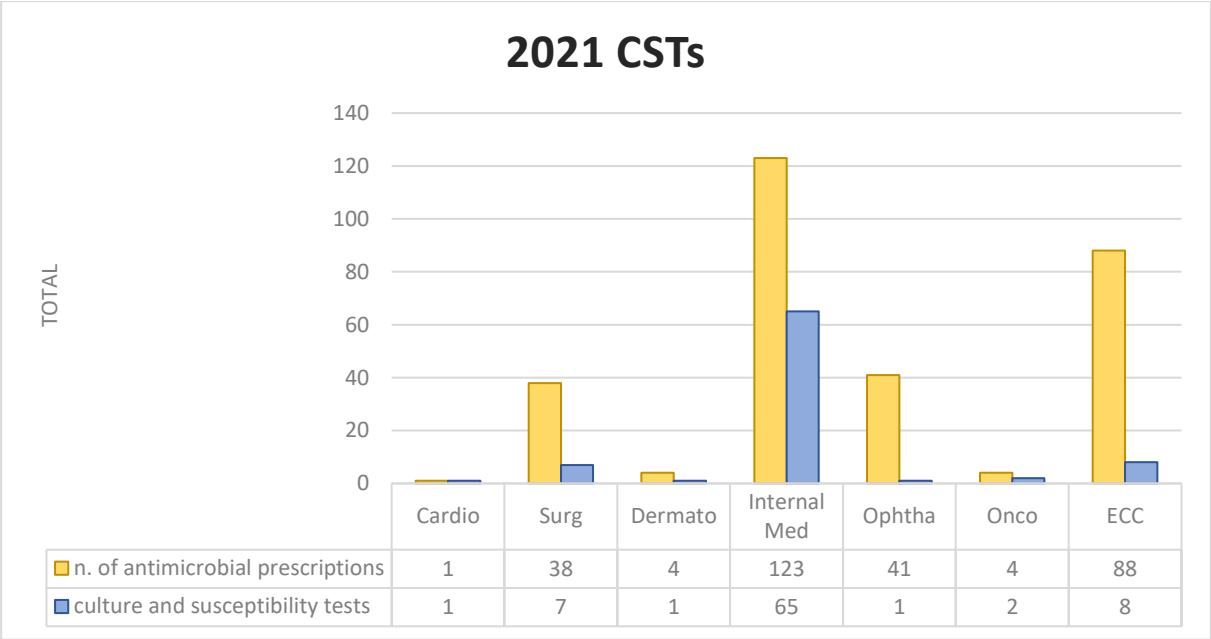


Figure 22: CSTs performed in different services of the VTH (on abscissae) in 2021 based on total antimicrobial prescriptions in that service.

Abbreviations: Cardio: Cardiology; Surg: Surgery; Dermato: Dermatology; Internal Med: Internal Medicine; Ophtha: Ophthalmology; Onco: Oncology; ECC: Emergency and Critical Care.

In 2022, within the cats included in the study the CSTs corresponded to 17,4% (73/420) of the total antibiotic prescriptions (*Figure 23*).

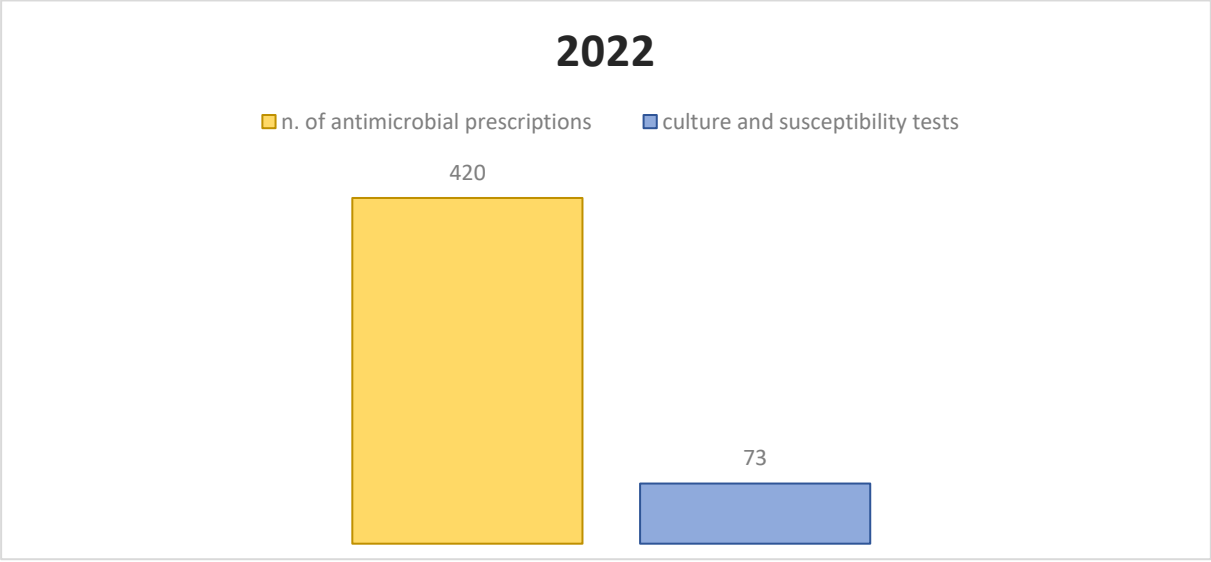


Figure 23: Antibiotics prescribed and CSTs carried out in the year 2022

Within the population, the CSTs were performed under the directive of a service of the VTH; those involved are shown below and in *Figure 24*:

- The cardiology service performed 100% (1/1) CSTs on total antibiotic prescriptions;

- The surgery service performed 16,4% (9/55) CSTs on total antibiotic prescriptions;
- The internal medicine service performed 61,6% (53/86) CSTs on total antibiotic prescriptions;
- The neurology service performed 9,1% (1/11) CSTs on total antibiotic prescriptions;
- The emergency and critical care service performed 11,4% (9/79) CSTs on total antibiotic prescriptions;
- The dermatology (0/1), ophthalmology (0/36), oncology (0/2), orthopedics (0/19), reproduction (0/130) and primary care (0/0) services didn't prescribe any CSTs.

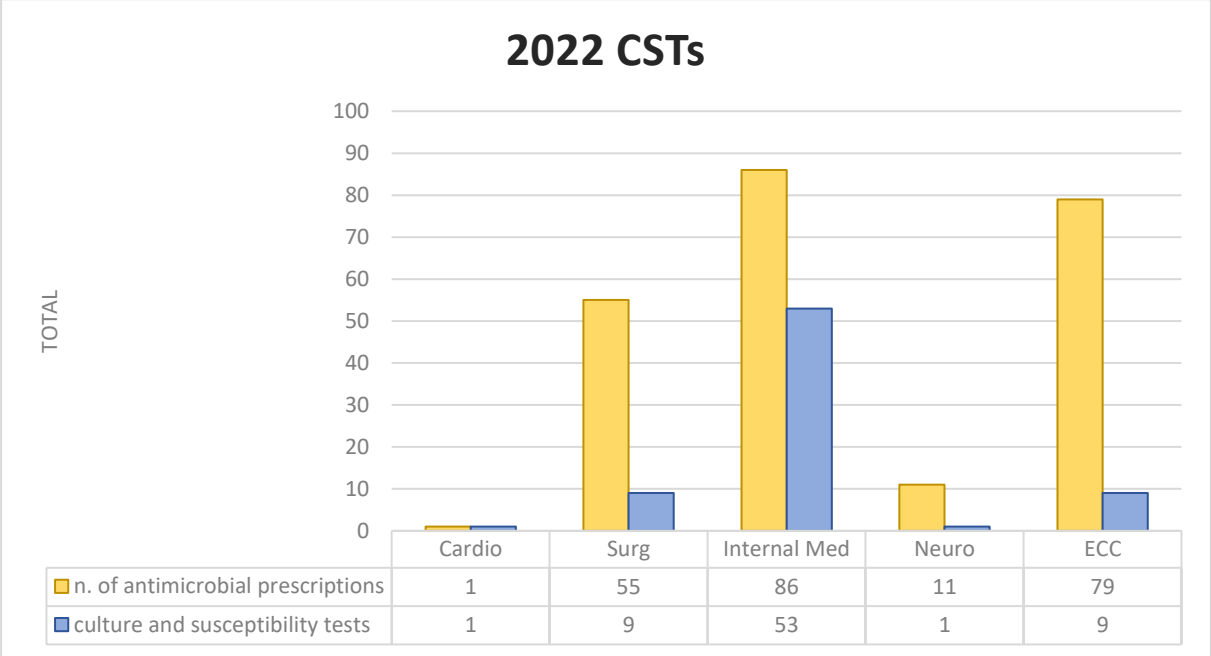


Figure 24: CSTs performed in different services of the VTH (on abscissae) in 2022 based on total antimicrobial prescriptions in that service.

Abbreviations: Cardio: Cardiology; Surg: Surgery; Internal Med: Internal Medicine; Neuro: Neurology; ECC: Emergency and Critical Care.

3.3.2 Positive Tests

Within the CSTs carried out, we investigated how many of these were positive and therefore the percentage of positive tests on the entire number of tests performed. In 2021, over the culture and sensibility tests performed, a total of 35,3% (30/85) tests resulted positive, as shown in *Figure25*. The number of positive tests within each VTH service was then analyzed:

- The cardiology service obtained 0% (0/1) of positive CSTs out of the total performed;
- The surgery service obtained (57,1% (4/7) of positive CSTs out of the total performed;
- The dermatology service obtained 100% (1/1) of positive CSTs out of the total performed;
- The internal medicine service obtained 27,7% (18/65) of positive CSTs out of the total performed;
- The ophthalmology service obtained 0% (0/1) of positive CSTs out of the total performed;
- The oncology service obtained 0% (0/2) of positive CSTs out of the total performed;
- The emergency and critical care service obtained 87,5% (7/8) of positive CSTs out of the total performed;
- The neurology, orthopedics, reproduction and primary care services didn't perform CSTs.

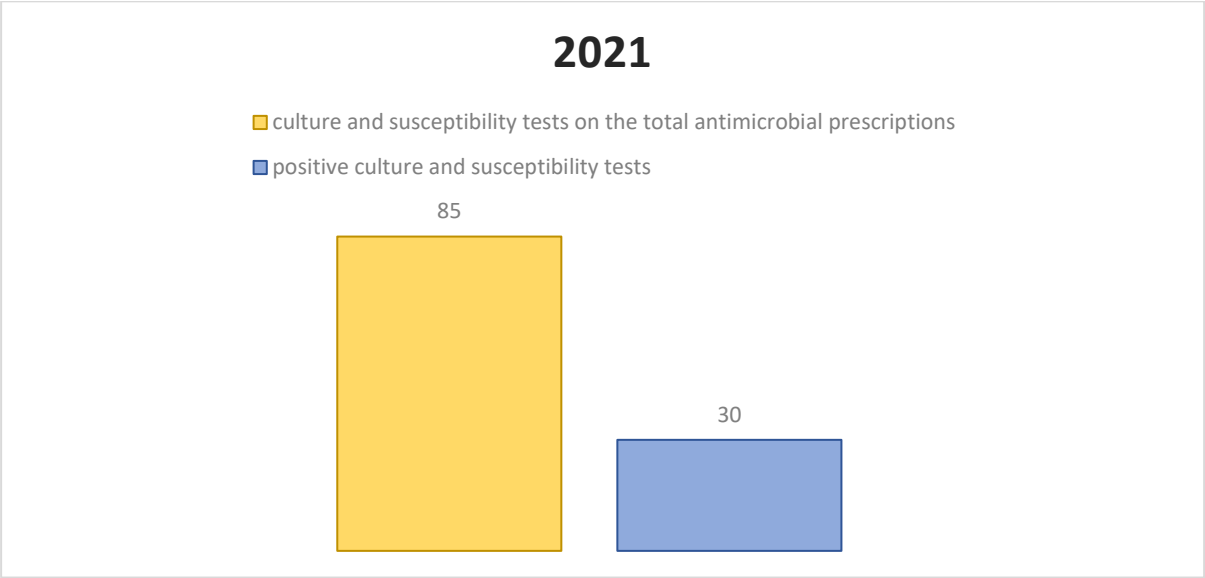


Figure 25: CSTs performed with positive outcome compared with total CSTs performed in 2021

In 2022, over the culture and sensibility tests performed, a total of 50,7% (37/73) tests resulted positive, as shown in *Figure 26*. The number of positive tests within each VTH service was then analyzed:

- The cardiology service obtained 100% (1/1) of positive CSTs out of the total performed;
- The surgery service obtained 66,7% (6/9) of positive CSTs out of the total performed;
- The internal medicine service obtained 45,3% (24/53) of positive CSTs out of the total performed;
- The neurology service obtained 0% (0/1) of positive CSTs out of the total performed;
- The emergency and critical care service obtained 66,7% (6/9) of positive CSTs out of the total performed;
- The dermatology, ophthalmology, oncology, orthopedics, reproduction and primary care services didn't perform any CSTs.

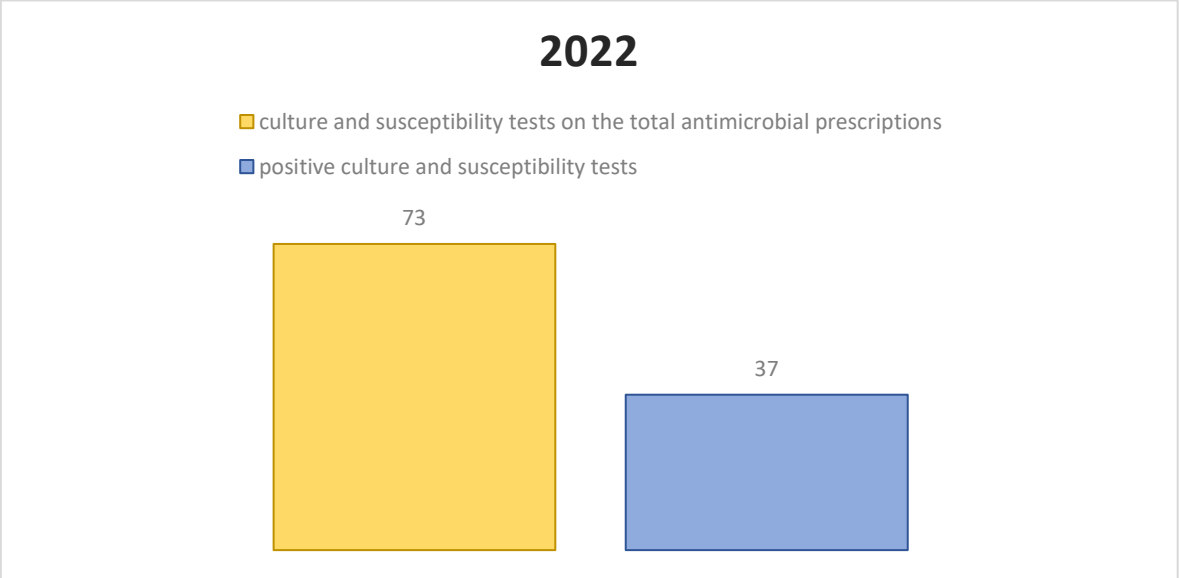


Figure 26: CSTs performed with positive outcome compared with total CSTs performed in 2022

3.3.3 Tests Performed on CIAs

Among the patients included in the study, considering the cases in which the animal was prescribed a CIA, the CSTs performed were finally analyzed. In 2021, within the CIAs prescriptions, 70% (49/70) of CSTs were performed. These were then analyzed in the context of the different VTH services, as shown below and in *Figure 27*:

- The surgery service performed 100% (3/3) of CSTs over the CIAs prescribed;
- The dermatology service performed 0% (0/1) of CSTs over the CIAs prescribed;
- The internal medicine service performed 90,7% (39/43) of CSTs over the CIAs prescribed;
- The neurology service performed 0% (0/6) of CSTs over the CIAs prescribed;
- The ophthalmology service performed 0% (0/4) of CSTs over the CIAs prescribed;
- The oncology service performed 0% (0/3) of CSTs over the CIAs prescribed;
- The emergency and critical care service performed 70% (7/10) of CSTs over the CIAs prescribed;
- The cardiology, orthopedics, reproduction and primary care services didn't prescribed CIAs and didn't perform any CSTs on them.

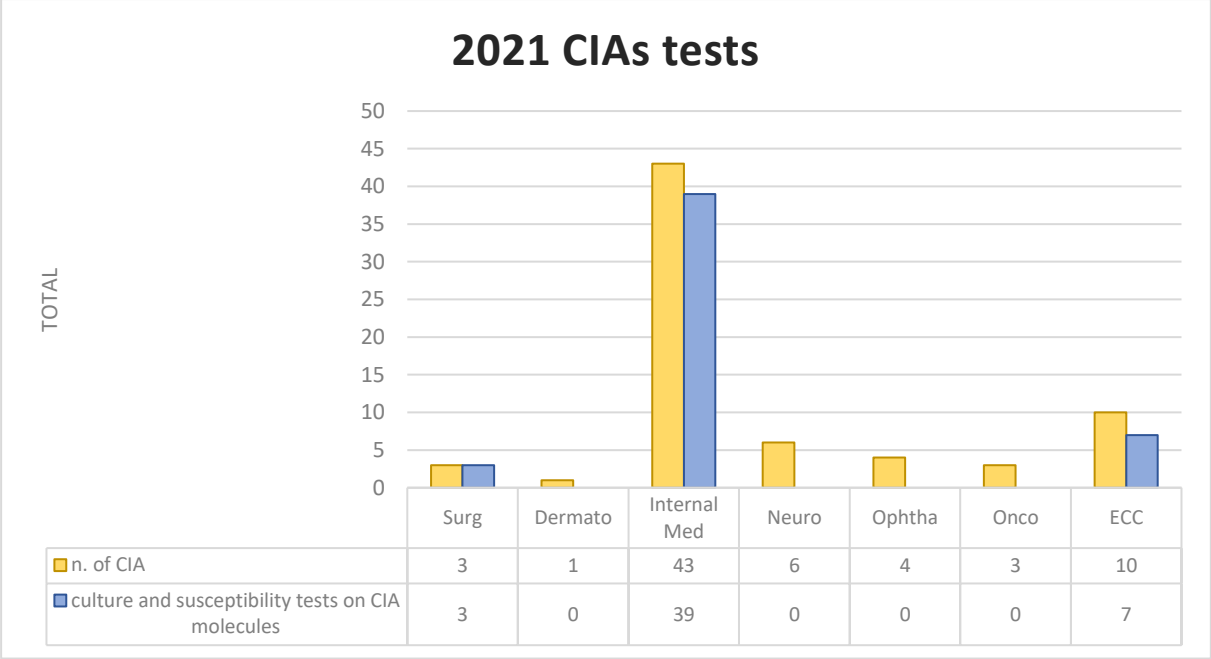


Figure 27: CSTs performed over CIAs prescriptions (on abscissae) in 2021.

Abbreviations: Surg: Surgery; Dermato: Dermatology; Internal Med: Internal Medicine; Neuro: Neurology; Ophtha: Ophthalmology; Onco: Oncology; ECC: Emergency and Critical Care.

In 2022, within the CIAs prescriptions, 66,7% (38/57) of CSTs were performed. These were then analyzed in the context of the different VTH services, as shown below and in *Figure 28*:

- The cardiology service performed 100% (1/1) of CSTs over the CIAs prescribed;
- The surgery service performed 50% (2/4) of CSTs over the CIAs prescribed;
- The internal medicine service performed 90,6% (29/32) of CSTs over the CIAs prescribed; of CSTs over the CIAs prescribed;
- The neurology service performed 14,3% (1/7) of CSTs over the CIAs prescribed;
- The ophthalmology service performed 0% (0/1) of CSTs over the CIAs prescribed;
- The emergency and critical care service performed 41,7% (5/12) of CSTs over the CIAs prescribed;
- The dermatology, oncology, orthopedics, reproduction and primary care services didn't prescribed CIAs and didn't perform any CSTs on them.

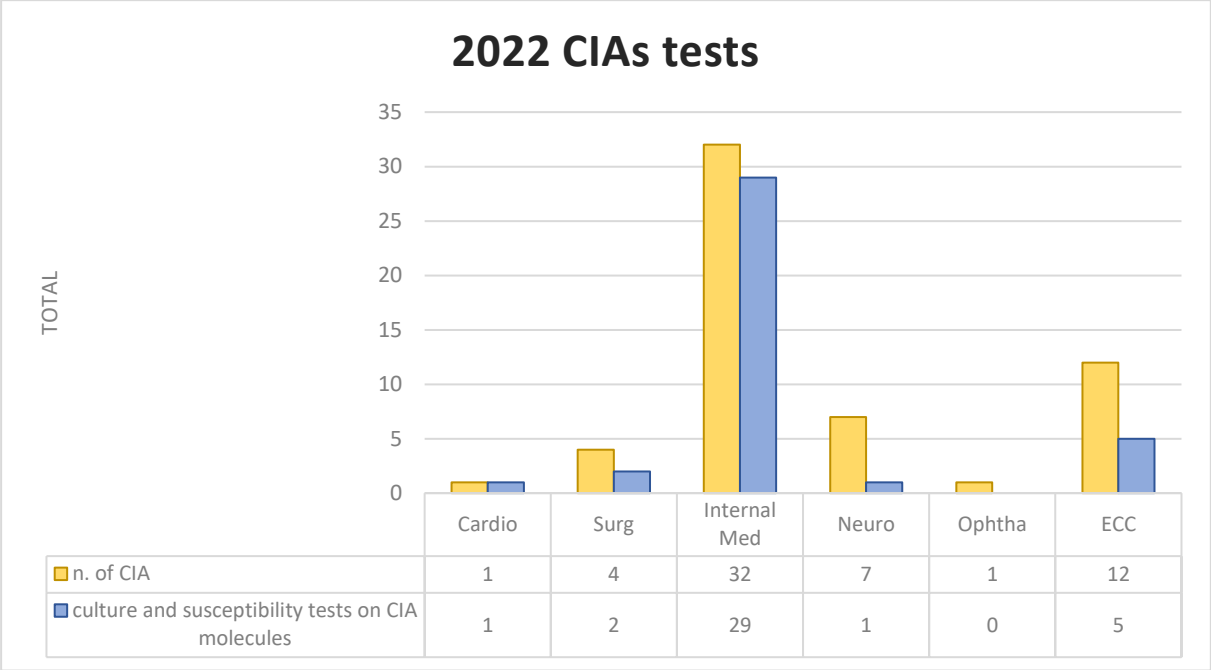


Figure 28: CSTs performed over CIAs prescriptions (on abscissae) in 2021.

Abbreviations: Cardio: Cardiology; Surg: Surgery; Internal Med: Internal Medicine; Neuro: Neurology; Ophtha: Ophthalmology; ECC: Emergency and Critical Care.

3.4 Antimicrobial prescriptions in different organic systems

Within the study, as a last phase, antibiotic prescriptions in the various organic systems in both 2021 and 2022 were analyzed. Antibiotic prescriptions were distributed in 2021 for 39,4% (185/470) in the genitourinary system, 0,2% (1/470) in the cardiocirculatory system, 7% (33/470) in gastrointestinal system, 0,2% (1/470) in lymphopoietic system, 11,3% (53/470) in musculoskeletal system, 10,2% (48/470) in the ophthalmic system, 2,3% (11/470) in the ENT system (Ear, Nose, Throat), 4,9% (23/470) in the respiratory system, 19,1% (90/470) in the systemic system, 1,7% (8/470) in the integumentary system, 3,6% (17/470) in the nervous system, no prescriptions for the mammary gland. These data are shown in *Figure 29*.

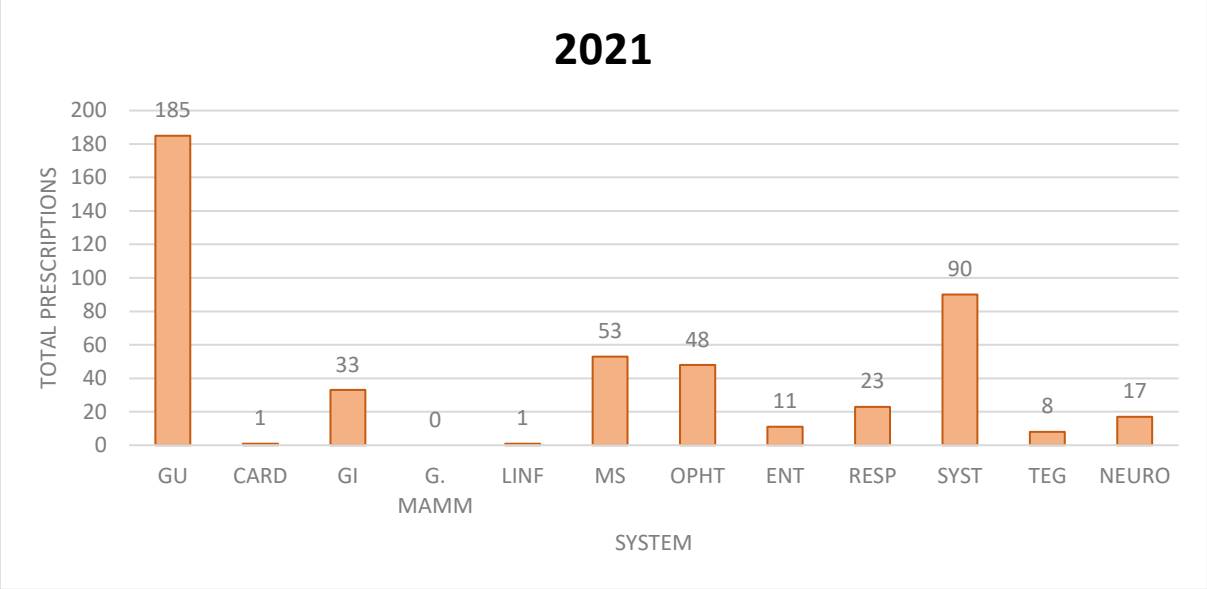


Figure 29: Antimicrobial prescriptions based on different organic systems in 2021

Abbreviations: GU: Genitourinary; CARD: Cardiovascular; GI: Gastrointestinal; G. MAMM: Mammary Gland; LINF: Lymphopoietic; MS: Musculoskeletal; OPHT: Ophthalmic; ENT: Ear-Nose-Throat; RESP: Respiratory; SYST: Systemic; TEG: Integumentary; NEURO: Nervous.

The antibiotic prescriptions were then divided, for each VTH service, within the different systems, as shown below and in *Figure 30*:

- The reproduction service prescribed 100% (143/143) of antimicrobial prescriptions for genitourinary system;
- The cardiology service prescribed 100% (1/1) of antimicrobials for cardiovascular system;

- The surgery service prescribed 28,9% (11/38) of antimicrobials for respiratory system, 23,7% (9/38) for musculoskeletal system, 15,8% (6/38) for gastrointestinal system, 13,2% (5/38) for genitourinary system, 7,9% (3/38) for ophthalmic system, 5,3% (2/38) for ENT system, 2,6% (1/38) for integumentary system, 2,6% (1/38) for nervous system;
- The orthopedics service prescribed 100% (11/11) of antimicrobials for musculoskeletal system;
- The neurology service prescribed 94,1% (16/17) of antimicrobials for nervous system and 5,9% (1/17) for musculoskeletal system;
- The internal medicine service prescribed 44,7% (55/123) of antimicrobials for systemic, 28,5% (35/123) for genitourinary system, 17,1% (21/123) for gastrointestinal system, 6,5% (8/123) for respiratory system, 1,6% (2/123) for musculoskeletal system, 0,8% (1/123) for lymphopoietic system, 0,8% (1/123) for ENT system;
- The ophthalmology service prescribed 100% (41/41) of antimicrobials for ophthalmic system;
- The oncology service prescribed 75% (3/4) of antimicrobials for systemic and 25% (1/4) for respiratory system;
- The dermatology service prescribed 75% (3/4) of antimicrobials for integumentary system and 25% (1/4) for ENT system;
- The emergency and critical care service prescribed 36,4% (32/88) of antimicrobials for systemic, 34,1% (30/88) for musculoskeletal system, 8% (7/88) for ENT system, 6,8% (6/88) for gastrointestinal system, 4,5% (4/88) for ophthalmic system, 4,5% (4/88) for integumentary system, 3,4% (3/88) for respiratory system, 2,3% (2/88) for cardiovascular system.

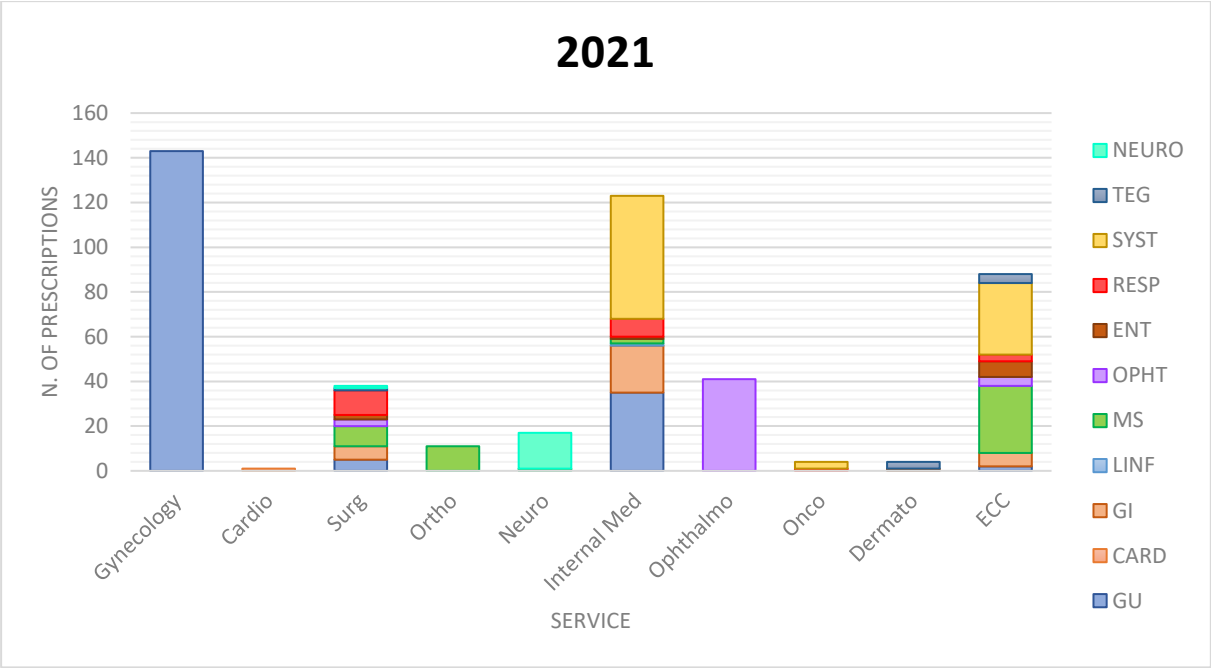


Figure 30: Antimicrobial prescriptions in different organic systems based on different VTH services (on abscissae) in 2021.

Abbreviations: GU: Genitourinary; CARD: Cardiovascular; GI: Gastrointestinal; G. MAMM: Mammary Gland; LINF: Lymphopoietic; MS: Musculoskeletal; OPHT: Ophthalmic; ENT: Ear-Nose-Throat; RESP: Respiratory; SYST: Systemic; TEG: Integumentary; NEURO: Nervous.

While in 2022, antibiotic prescriptions were distributed for 46,9% (197/420) in the genitourinary system, 0,2% (1/420) in the cardiocirculatory system, 7,4% (31/420) in gastrointestinal system, 0,2% (1/420) in the mammary gland, 6,9% (29/420) in musculoskeletal system, 8,8% (37/420) in the ophthalmic system, 1,4% (6/420) in the ENT system (Ear, Nose, Throat), 3,6% (15/420) in the respiratory system, 14,3% (60/420) in the systemic system, 7,6% (32/420) in the integumentary system, 2,6% (11/420) in the nervous system, no prescriptions for the lymphopoietic system. These data are shown in *Figure 31*.

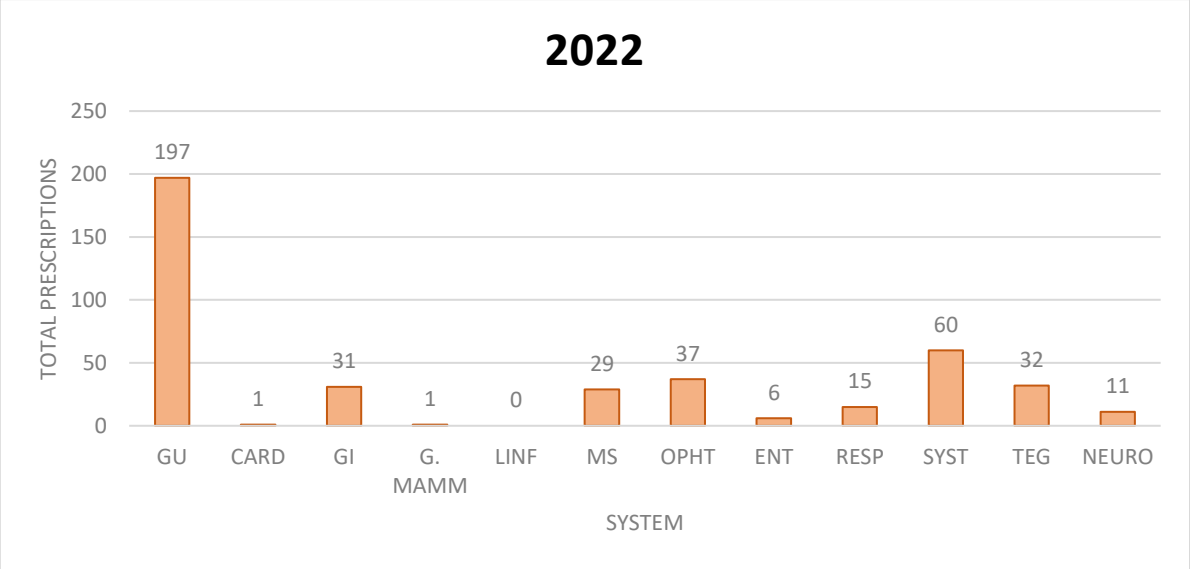


Figure 31: Antimicrobial prescriptions based on different organic systems in 2022.

Abbreviations: GU: Genitourinary; CARD: Cardiovascular; GI: Gastrointestinal; G. MAMM: Mammary Gland; LINF: Lymphopoietic; MS: Musculoskeletal; OPHT: Ophthalmic; ENT: Ear-Nose-Throat; RESP: Respiratory; SYST: Systemic; TEG: Integumentary; NEURO: Nervous.

The antibiotic prescriptions were then divided, for each VTH service, within the different systems, as shown below and in *Figure 32*:

- The reproduction service prescribed 100% (130/130) of antimicrobial prescriptions for genitourinary system;
- The cardiology service prescribed 100% (1/1) of antimicrobials for cardiovascular system;
- The surgery service prescribed 31% (17/55) of antimicrobials for gastrointestinal system, 29,1% (16/55) for genitourinary system, 16,4% (9/55) for integumentary system, 9,1% (5/55) for systemic, 5,4% (3/55) for musculoskeletal, 5,4% (3/55) for ENT system, 1,8% (1/55) for respiratory system, 1,8% (1/55) for mammary gland;
- The orthopedics service prescribed 100% (19/19) of antimicrobials for musculoskeletal system;
- The neurology service prescribed 100% (11/11) of antimicrobials for nervous system;
- The internal medicine service prescribed 48,8% (42/86) of antimicrobials for genitourinary system, 29,1% (25/86) for systemic, 11,6% (10/86) for gastrointestinal system, 9,3% (8/86) for respiratory system, 1,2% (1/86) for ENT system;
- The ophthalmology service prescribed 100% (36/36) of antimicrobials for ophthalmic system;

- The oncology service prescribed 100% (2/2) of antimicrobials for systemic;
- The dermatology service prescribed 100% (1/1) of antimicrobials for integumentary system;
- The emergency and critical care service prescribed 35,4% (28/79) of antimicrobials for systemic, 27,8% (22/79) for integumentary system, 11,4% (9/79) for genitourinary system, 8,9% (7/79) for musculoskeletal system, 7,6% (6/79) for respiratory system, 5,1% (4/79) for gastrointestinal system, 2,5% (2/79) for ENT system, 1,3% (1/79) for ophthalmic system.

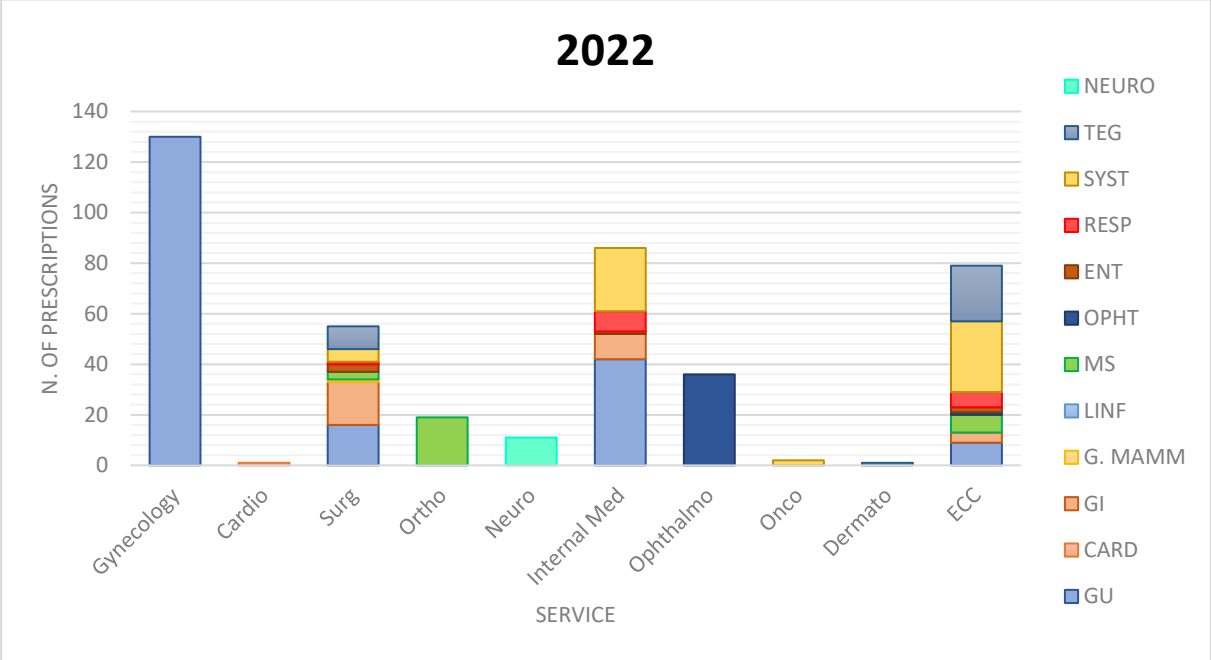


Figure 2: Antimicrobial prescriptions in different organic systems based on different VTH services (on abscissae) in 2021

Abbreviations: GU: Genitourinary; CARD: Cardiovascular; GI: Gastrointestinal; G. MAMM: Mammary Gland; LINF: Lymphopoietic; MS: Musculoskeletal; OPHT: Ophthalmic; ENT: Ear-Nose-Throat; RESP: Respiratory; SYST: Systemic; TEG: Integumentary; NEURO: Nervous.

4 | DISCUSSION

In this study, among the cats that received a visit in 2021 and 2022 in the VTH of University of Parma, it was observed an antibiotic prescription rate of 35% and 43,8% respectively.

Despite the fact that these numbers are not absolutely low, this antibiotic prescribing trend demonstrates some consideration by the VTH services of compliance with proposed guidelines to reduce the progression of antibiotic resistance, such as the EMA guidelines and the Protect/ProtectMe and FECAVA posters. Within the general vision of antibiotic prescribing in our Veterinary Teaching Hospital, soft tissue surgery, orthopedics and reproduction services were also included in this study. Within these services the use of antibiotic molecules is part of the perioperative routine, furthermore the orthopedic service routinely prescribes a 7-day postoperative antibiotic therapy. For this reason, the results obtained from the study could be considered distorted, given that these three services considerably increase the frequency of antibiotic prescription due to a prophylactic use. If we exclude the antibiotic prescriptions in cats that underwent surgery (soft tissue surgery service - 8,1% of total antimicrobial prescriptions in 2021 and 13,1% in 2022; orthopedics service - 2,3% in 2021 and 4,5% in 2022; reproduction service - 30,4% in 2021 and 30,9% in 2022) the VTH antibiotic prescription trend drops to 25,9% in 2021 and 18% in 2022. The data obtained are in line with those obtained from other studies, where antibiotic prescriptions in cats were around 36,5% (Beaudoin et al., 2023), 33,2% (Mateus et al., 2011), 20,6% (Buckland et al., 2016) and 18,1% (Hsieh et al., 2022). This information highlights the favorable results of the data collected at our facility, which are positioned in the average of these investigations of similar nature.

Considering the VTH's many services, encouraging data can be seen if they are contextualized to the type of service and the type of conditions seen. In the cardiology service (1,7% in 2021; 1,1% in 2022), the oncology service (10,3% in 2021; 3,2% in 2022), the dermatology service (23,5% in 2021; 6,3% in 2022) and the primary care service (0%) fewer antibiotic prescriptions were noted both in 2021 and 2022. In general, these services are recognized to be those where antimicrobial molecules are usually used less. For the primary care service, the 0% of antimicrobial prescriptions is a data to be seen as positive, indicating that in our facility a general visit does not justify the use of antibiotic molecules, instead these are usually prescribed following a specialistic visit to one of the VTH services. Compared to another study, the incidence of antimicrobial prescriptions in primary care services in Europe was of 10,3%

(Goggs et al., 2021). A service that also slightly differs from other research of a comparable nature is dermatology, where 36% (De Briyne et al., 2014), 30,6% (Goggs et al., 2021), 24,6% (Escher et al., 2011), and 12,5% (Hsieh et al., 2022) of the antibiotics used are claimed to fall under this service. However, it must be considered that no cases of pyoderma, which make up a significant portion of the cases considered by the prior studies, were recorded during our investigation and that bite wounds and abscesses are referred to the emergency care service rather than the dermatology department.

Based on the total prescriptions for each service, the reproduction service (97,9% in 2021; 97% in 2022), the soft tissue surgery service (59,4% in 2021; 93,2% in 2022), the orthopedics service (78,6% in 2021; 100% in 2022) and the ophthalmology service (87,2% in 2021; 78,3% in 2022) prescribed the greatest number of antibiotics. Almost all patients in these services have undergone surgery, meaning that almost all patients underwent routine perioperative antibiotic prophylaxis, as per guideline directive (Bassetti et al., 2015; Boothe & Boothe, 2015). Accordingly to this, reproduction, orthopedics and soft tissue surgery services have a very high number of antibiotic prescriptions, especially when compared to the total number of prescriptions. All surgical site infections (SSIs) prevention recommendations advise the use of antibiotic prophylaxis, indicating the correctness of the prophylaxis used in the surgical field in our facility. To increase the antibiotic's tissue concentration, it is recommended that antibiotics be administered within 60 minutes following the incision. In order to reach acceptable tissue concentrations, it is also advised to dosage antibiotics according to the patient's weight (Seidelman et al., 2023). All these procedures are adopted at the VTH, demonstrating adherence to the guidelines. In the case of the ophthalmology service, even here some patients underwent surgery with local antibiotic administration, but in general most of the antibiotics were administered for cases of herpesvirus conjunctivitis and corneal ulcers. In general, the use of antibiotics in the ophthalmic field recorded in the VTH seems higher than the use recorded in other studies, such as that of Escher et al. (2011) in which the use of antibiotics for the eye was very low and amounted to 3,87% of the total antibiotics used in patients enrolled in the study. Within the prescriptions from the ophthalmology service, most of these are topical formulations. This data reflects the prescribing habits in this service, considering that in the literature most therapies are administered topically (Austin et al., 2017; Høvding, 2008).

Internal medicine, emergency and critical care, and neurology services may be the most representative in this study because they specifically have the most discretion over whether or not to prescribe antibiotics. In fact, excluding services related to surgery, these three services are those which visited most cats and the most represented services in the results obtained, both in terms of prescription frequency and number of antibiotic classes prescribed to their patients. Observing our results, within the prescriptions for each service in 2021 and 2022, the internal medicine service had 41% and 27,9%, the ECC service had 35,9% and 25,9%, and the neurology service had 23,3% and 12,2%, respectively. A comparison could be made with Goggs et al. (2021) study, according to which the prescription of systemic antimicrobials in included cats consisted of 18,4% in specialistic fields (internal medicine, orthopedics surgery), 23,2% in emergency and critical care and 9,6% in primary care (community practice). In De Briyne et al. (2014), within the top five indications where antibiotics are said to be prescribed for use in cats, respiratory diseases (24%), urinary tract infections (16%) and periodontal diseases (14%) cover 54% of the total mentions, within which the use of CIAs covers respectively 16%, 62% and 38%. Also, in Escher et al. (2011), genitourinary (14%) and respiratory (13,6%) tract diseases represented the third and fourth most common cause of antibiotic prescription in an Italian study. Likewise in our study, pathologies of the genitourinary system covered 39,4% in 2021 and 46,9% in 2022 of the total antibiotic prescriptions within the various systems considered. However, unlike the previous study, pathologies of the respiratory system were observed to a much lower extent than the reported value, with an antibiotic prescription rate on the total of 4,9% in 2021 and 3,6% in 2022.

From what we could observe, in 2021 the most used antibiotic classes were fixed combinations (31,9%) and enhanced penicillins (31,4%), much higher than the value found for quinolones (12,2%) and cephalosporins (10,5%). A similar situation is observed in 2022, where enhanced penicillins (31,3%) and fixed combinations (31,1%) stand out over the underlying classes of cephalosporins (14,7%) and quinolones (11,8%). The class of enhanced penicillins is utilized in two main services of the VTH, namely that of the internal medicine service (51,6%-53,2%) and the emergency and critical care service (61%- 68,9%) for the two consecutive years. It is in fact usual to use intravenous ampicillin/sulbactam as first choice broad-spectrum antibiotic in the case of hospitalized patients or oral amoxicillin/clavulanic acid as home therapy. Enhanced penicillins are the most common prescribed antimicrobials in pets (del Solar Bravo et al., 2023;

Hsieh et al., 2022) and their use nearly doubled from 1995 to 2004 (Weese, 2006). In 2023, the prescription of enhanced penicillins was registered in 33,8% of feline patients in 14 American VTHs. Amoxicillin/clavulanic acid is generally the most prescribed antibiotic in veterinary medicine (Beaudoin et al., 2023; Mateus et al., 2011). The fixed combination of antibiotics has a marked use in the reproduction (97,2%-99,2%) and ophthalmology (55,3%-50%) services. This is explained by the fact that the reproduction service of the VTH uses rubrocillin as a perioperative antibiotic, based on the active molecules benzylpenicillin benzatinic and dihydrostreptomycin, motivated by the existence of an agreement between the University of Parma and the municipal shelter of Parma for which most of the interventions take place on colony cats. Administering rubrocillin in this context allows for a single intramuscular administration with a half-life of 48 hours. For the ophthalmology service, on the other hand, this usually prescribes Colbiocin as a topical antibiotic, a drug registered for human use based on chloramphenicol, colistimethate sodium and rolitetracycline.

The third class most commonly represented among the antibiotics used is that of the cephalosporins, in particular first generation cephalosporins. This class is widely used in surgery, especially cefazolin as the antibiotic of choice in the perioperative setting (Bassetti et al., 2015). It is therefore not surprising to observe that the services in which cephalosporins are most used are those of soft tissue surgery (60,4%-60,3%) and orthopedics (90,9%-76%). Outside of the surgical context, first generation cephalosporins have very limited use in cats (Hsieh et al., 2022), or are still used as a topical treatment in skin diseases (Escher et al., 2011). Finally, the class of quinolones assumes importance as this fall within the CIAs (Critically Important Antibiotics), and it is therefore an antibiotic which, following the guidelines of the EMA, should be limited in its use. Based on what was observed in our study, the use of quinolones is concentrated in the internal medicine service (28% in 2021 -33% in 2022), however remaining at values that can be defined as low compared to the antibiotic classes at the top of the ranking in this study. A quinolone was used in 49% of cases of genitourinary tract disease (De Briyne et al., 2014), in 62,7% of cases (Escher et al., 2011) and in 14.6% of cases in a more recent study (Goggs et al., 2021). These results justify the predominant use of this antibiotic class in the internal medicine and ECC services.

As far as the antibiotic classes are concerned, these are of great importance since in veterinary medicine a series of antibiotic classes are recognized to be used much more frequently than others and which, unfortunately, are seen to fall within antibiotic classes of crucial importance, the so-called CIAs (Critically Important Antibiotics). In our case piperacillin (EMA's category "Avoid"), quinolones, 3rd - 4th - 5th generation cephalosporins, polymyxins (EMA's category "Restrict") and macrolides (EMA's category "Caution") were considered as CIAs. In Australia, 14,5% of antibiotic prescriptions were observed in dogs, of which 3,8% were CIAs and 10,8% of antibiotic prescriptions in cats, of which 4,7% were CIAs; the most used antibiotics were cefovecin (3rd generation cephalosporin) and amoxicillin/clavulanic acid (enhanced penicillin) and the most used topical antibiotic was polymyxin B (polypeptide antibiotic) (Hur et al., 2020). While in Europe some years earlier, of the total antibiotics prescribed to cats 30% were CIAs and 70% were non-CIAs (De Briyne et al., 2014). Our study settles on a lower value of CIAs prescribed in feline patients, considering the data in Europe which may represent a situation closer to our context, or even better taking into consideration the value in Italy in 2011 of 38,3% (Escher et al., 2011), with critically important antibiotics prescribed for a total of 14,9% in 2021 and 13,6% in 2022, thus also demonstrating a certain reduction in their use.

The CIAs used in the VTH are almost quinolones (12,2% in 2021 and 11,8% in 2022), considering that enhanced cephalosporins are only 0,7% prescriptions in 2021 and 0% in 2022 and that there is only 0,2% piperacillin's prescription in 2022, while there are no prescriptions for the classes of macrolides and polymyxins. In 2023, Beaudoin et al. reported the use of quinolones in 23,8% of cats included in their study and the use of macrolides in 2,5% of cases. The internal medicine service has the most prescription of quinolones (28,1% in 2021 and 33% in 2022) and the prescription of piperacillin (1,1% in 2022). This is also the service in which the greatest number of CSTs on CIAs molecules have been performed (90,6%). CIAs molecules are represented also in emergency and critical care service, where 7,6% of antimicrobial prescriptions in 2021 and 13,3% in 2022 were quinolones, and 0,8% of antimicrobial prescriptions in 2021 were enhanced cephalosporins. This service performed CSTs over 70% of CIAs prescribed in 2021 and 41,7% in 2022. Based on this data, it seems like in the VTH the habit is to prescribe a mid-low number of quinolones and very few to none other CIAs. Also, the prescribing is usually accordant to a CST.

For the use of culture and susceptibility tests within the population analyzed, in our investigation it was discovered that 18,1% of feline patients in 2021 and 17,4% of feline patients in 2022 underwent CSTs. This data is of particular importance as this represents the only way to know if the antibiotic treatment to which an animal is subjected is valid, both as regards the need to administer an antibiotic to the patient and in terms of the validity of the chosen antibiotic. It is therefore possible to evaluate this data also in relation to other studies, in which the use of cultural and sensitivity tests in the populations taken into consideration was 38,5% (Beaudoin et al., 2023), 22,9% (del Solar Bravo et al., 2023) and 8,8% (Hsieh et al., 2022), demonstrating that within our structure the use of these tests falls little below the observed average. This is in any case a low value compared to the total number of antibiotics prescribed, indicating that in few cases it was really certain that the prescribed treatment was effective. However, it must be considered the economic and temporal aspect while discussing about CSTs. It may be that an owner refuses to perform a CST for economic reasons, or it may be that the patient's condition was so serious that a CST would not make sense in the context of the animal's welfare.

Considering both 2021 and 2022, the VTH services that performed most CSTs related to the total number of prescriptions are of the internal medicine service (52,8% and 61,6% respectively) and the emergency and critical care service (9,1% and 11,4% respectively). This data could be determined by the fact that among these services the major number of genitourinary, respiratory and systemic diseases were observed. For these diseases, the biological samples utilized for the CSTs are easy to collect, such as urine, blood and respiratory secretions. The gold standard for the diagnosis of bacterial urinary tract infections (UTIs) is quantitative urine culture prior to the start of antibiotic therapy (Lulich & Osborne, 2004). In a recent study, bacterial culture was performed in 56% of patients presented with clinical signs reconducting to urinary tract infections (Sørensen et al., 2018). The gold standard for diagnosing bacteremia might be regarded as blood culture. The successful management of bacteremic patients depends on the prompt detection and subsequent identification of bacteria; failure frequently leads to septicemia, which can be fatal (Neumann et al., 2023). For this reason, the majority of blood samples for CSTs are provided by the emergency and critical care service. Considering the samples utilized, it could be possibly explained why for the neurology service the number of CSTs was so low (0 and 1 CSTs performed); the cerebrospinal

fluid (CSF). is particularly difficult to collect, as the patient must be under general anesthesia for the collection, the site must be shaved and aseptically prepared due to the risk of contaminating the central nervous system with infectious pathogens; additionally, elevated intracranial pressure is a frequently reported contraindication to CSF collection (Di Terlizzi & Platt, 2009).

Among the limitations concerning this study, the retrospective nature of the same must be counted, in addition to the presence of medical records not correctly filled. During the study several services were considered within the same clinical case; it is not rare at the VTH that an animal is subjected to initial management by the emergency and critical care service and is subsequently referred to the more pertinent service for joint management of the case. In hospitalized cats it was also impossible to determine whether antibiotic administration began empirically before the results of any cultural examination or whether it began before the antibiogram results. Also, in this study nether the duration or the dosage of the antimicrobial treatment was taken into consideration, placing a major limit on the evaluation of correct management of antibiotic therapy and stewardship in the facility.

5 | CONCLUSIONS

This study demonstrates that it is possible to follow recommendations for the careful use of antibiotics in feline medicine and surgery. The use of "Restrict" antibiotics, as for the EMA's classification, in our facility was restricted to a few carefully chosen feline patients. Despite the overall positive results at our institution, there is always potential for advancement. On the other hand, we must give credit to the VTH since, given the enormous case series to which it is subjected, the antibiotic prescription rate is not a disappointing result and certainly predicts a large margin for improvement.

Our study was conducted in a university clinic setting not only because clinical data was easily accessible there, but also because we thought it was crucial to assess the prescribing practices of those practitioners who are influencing the same prescribing habits of upcoming practitioners. The study's ability to provide a comprehensive picture of antimicrobial prescription practices in companion animals and in Italy is, however, constrained by the study's focus on such a narrow context.

There is a definite need for more data on antimicrobial drugs prescribing habits in veterinary medicine, spanning all specialties and countries. The current study sought to quantify AMD prescribing and use in specialty, emergency, and primary care veterinary practice at the VTH of University of Parma in order to fill these knowledge gaps. Particular focus was placed on the prescription of a few AMDs that the EMA deemed as critically important. Adopting a comprehensive diagnostic strategy that includes laboratory testing is a critical move that will aid in either preventing excessive usage or making it easier to use certain criteria for judicious antimicrobial prescribing. Our data points out various areas that deserve additional prospective studies and support for bettering instruction on suitable diagnoses, antibiotic class, and dosage options, which would enhance adherence to antibiotic stewardship recommendations. Future efforts in this area might concentrate on establishing professional consensus regarding the kind, scope, and details of AMD prescribing data that should be gathered inside veterinary facilities.

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