Antimicrobial resistance evaluation of bacteria isolated from infections in small animals in the Umarama region, Paraná

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ABSTRACT.- Souza M.M., Bordin J.T., Pavan A.C.L., Rodrigues R.G.A., Sfaciotte R.A.P., Vignoto V.K.C., Ferrante M. & Wosiacki S.R. 2020. Antimicrobial resistance evaluation of bacteria isolated from infections in small animals in the Umarama region, Paraná. Pesquisa Veterinária Brasileira 40(10):804-813. Departamento de Medicina Veterinária, Universidade Estadual de Maringá, Estrada da Paca s/n, Jardim São Cristóvão, Umuarama, PR 87507-190, Brazil. E-mail: srwosiacki@uem.br

Bacterial resistance is shown to be an inevitable side effect due to the excessive use of antibiotics, becoming a significant concern worldwide. Knowledge of regional bacterial resistance profiles enables the development of site-specific infection control practices, making conscious and moderate use of commercially available antibiotics. The aim of this study was the retrospective evaluation of the antimicrobial resistance profile of bacteria isolated from companion animal infections in the region of Umuarama/PR, from 2013 to 2017. This research was performed by analyzing the database belonging to the “Laboratório de Microbiologia Animal” at the “Universidade Estadual de Maringá” (UEM). Staphylococcus spp. represented 45.53% of the bacteria isolated from clinical infections in small animals in the period and place evaluated, followed by enterobacteria (34.04%), non-fermenting Gram-negative bacilli (NFGNB, 11.06%) and Streptococcus/Enterococcus (9.36%). A high number of antimicrobial resistance to antibiotics used in veterinary medicine was found. The lowest resistances associated with the best impact factor values were found for aminoglycosides, especially amikacin, chloramphenicol, and fluoroquinolones (norfloxacin and ciprofloxacin). Intermediate results were found for sulbactam-associated ampicillin, ceftriaxone, amoxicillin-clavulanic acid, and enrofloxacin. According to the number of resistant antimicrobial drugs, 64.26% (151/235) of the isolates were classified as multidrug-resistant, being 15.32% extensively resistant. Considering the resistance to antimicrobial classes, 68.94% (162/235) of the isolates were classified as multiresistant, being 19.15% extensively resistant. No bacterial strains were characterized as pan-resistant, but ten bacteria were resistant to all classes tested, with isolated susceptibility to certain drugs. Through the evaluation of resistance profiles found in the period and place studied and relevant literature, it is clear that there is a growing increase in the number of multiresistant bacteria among domestic animals which characterizes a serious risk to public health. The therapeutic arsenal is becoming increasingly diminished, and there is more difficulty in empirical drug selection, making antimicrobial susceptibility testing essential for more specific selection in antimicrobial therapy. Educational...
RESUMO.- [Avaliação da resistência antimicrobiana de bactérias isoladas de infecções em pequenos animais na região de Umuarama, Paraná.] A resistência bacteriana, mostra-se como um efeito colateral inevitável pelo excessivo uso de antibióticos, tornando-se alvo de grande preocupação mundial. O conhecimento dos perfis de resistência bacteriana regionais possibilita o desenvolvimento de práticas de controle de infecções específicas para cada localidade, fazendo uso consciente e moderado dos antibióticos disponíveis no mercado. O objetivo deste estudo foi a avaliação retrospectiva do perfil de resistência antimicrobiana de bactérias isoladas de infecções de animais de companhia na região de Umuarama/PR, no período de 2013 a 2017. Esta pesquisa foi realizada por meio da análise do banco de dados pertencente ao Laboratório de Microbiologia Animal da Universidade Estadual de Maringá (UEM). Os *Staphylococcus* spp. representaram 45,53% das bactérias isoladas de infecções clínicas em pequenos animais no período e local avaliado, seguido por enterobactérias (34,04%), bacilos Gram-negativos não fermentados (BGNNF, 11,06%) e *Streptococcus/Enterococcus* (9,36%). Um número elevado de resistência antimicrobiana frente aos antibióticos utilizados na medicina veterinária foi encontrado. As menores resistências associadas aos melhores valores do fator de impacto foram encontrados para aminoglicosídeos, em especial amicacina, cloranfenicol, fluoroquinolonas (norfloxacina e ciprofloxacina). Já resultados intermediários foram encontrados para ampicilina associada a sulbactam, ceftriaxona, amoxicilina com ácido clavulônico e enrofloxacina. Conforme o número de drogas antimicrobianas resistentes, foram classificados como multirresistentes 64,26% (151/235) dos isolados, sendo 15,32% extensivamente resistentes. Já considerando a resistência a classes de antimicrobianos, os 68,94% (162/235) dos isolados foram classificados como multirresistentes, sendo 19,15% extensivamente resistentes. Nenhum isolado bacteriano foi caracterizado como pan-resistente, porém 10 bactérias foram resistentes a todas as classes testadas, com susceptibilidade isolada a determinadas drogas. Por meio da avaliação dos perfis de resistência encontrados no período e local estudados e de literatura pertinente, percebe-se que há um aumento crescente no número de bactérias multirresistentes entre os animais domésticos o que caracteriza um grave risco à saúde pública. O arsenal terapêutico está se tornando cada vez mais diminuto e há mais dificuldade na seleção empírica de drogas, tornando essencial a realização de testes de susceptibilidade antimicrobiana para uma seleção mais específica na terapêutica antimicrobiana. Medidas educativas sobre o uso consciente dos antibióticos, controle de infecções e prevenção de zoonoses específicas para as localidades precisam ser instituídas para conhecimento dos profissionais do setor da saúde e acesso geral da população.

TERMOS DE INDEXAÇÃO: Resistência antimicrobiana, bactérias, infecção, pequenos animais, Brazil, antibióticos, multirresistência, cães, gatos.

INTRODUCTION

Antimicrobials include any natural substance of semi-synthetic or synthetic origin that kills or inhibits the growth of a microorganism, causing little or no damage to the host. Among these, the most widely used are antibiotics that were defined by Giguère (2013) as a low molecular weight substance produced by a microorganism that inhibits or kills other organisms at low concentrations. Since its discovery in 1928, when Alexander Fleming noted that staphylococci colonies were lysed on a Penicillium-contaminated plaque (Prescot 2013), they are widely used for the treatment or prevention of bacterial infections and are among the most important advances in modern medicine (Murphy et al. 2012).

As soon as penicillin was discovered, the presence of in vitro resistance was observed and it was said, in the early years of its application, that there was a need to be aware of the precautions to be taken when administering the drug due to possible undesirable reactions and even of antibiotic resistance (Pereira & Pita 2005).

Although numerous studies have provided substantial evidence of the spread of multi-resistant bacteria from animals to humans, current research indicates that humans can also transmit animal-resistant pathogens in a reverse zoonotic event called zoonathroponosis (Messenger et al. 2014). Pathogens acquired in the hospital environment by owners, for example, can be transmitted directly to the animal or indirectly through the domestic environment, becoming a critical route of transmission between them (Fernandes et al. 2018).

Resistant pathogen transfer rates at the human-animal interface as well as antibiotic resistance were increased in both companion animals (Aidara-Kane 2012) and humans. This increase was accompanied by the more frequent use of these broad-spectrum drugs in the treatment of patients without real certainty of this need as bacterial agent identification, and susceptibility tests are not performed most of the time (Guardabassi et al. 2010).

Antimicrobial resistance is defined as the condition in which a microorganism can survive when exposed to an antimicrobial to which it was initially susceptible (Barie 2012, WHO 2019) or organisms capable of multiplying in the presence of therapeutic or higher antimicrobial concentrations (Guardabassi et al. 2010). It is considered a complex phenomenon, especially in veterinary medicine, due to the number of species and the diversity of environments in which animals are reared, the differences in the bacteria involved, the range of pathogenicity mechanisms and the intricate epidemiology (Acar et al. 2012). Antimicrobial resistance is an inevitable side effect due to the excessive use of these drugs (Guardabassi et al. 2010) and is a subject of intense worldwide concern (Ventola 2015).

Antimicrobial susceptibility testing is considered to be one of the most important factors governing drug selection for veterinary clinical use and is used in the elaboration of local therapeutic guides (ANVISA 2017). Joseph & Rodvold (2008) described the "4 Ds of Ideal Antimicrobial Therapy": right Drug, right Dose, De-escalated to pathogen-directed therapy, and right Duration of therapy. De-escalating is referred to by Molina et al. (2012) as an intervention aimed at improving the...
use of antimicrobials and implying their discontinuation when there is no infection or restricting the spectrum of antimicrobial coverage according to clinical response and bacterial cultures.

The aim of this study was to retrospectively evaluate the antimicrobial resistance profile found in bacteria isolated from infections in small animals in the Umurama/PR region.

MATERIALS AND METHODS

This research was conducted through the analysis of the database belonging to the “Laboratório de Microbiologia Animal” of the “Universidade Estadual de Maringá” (UEM), located at the “Campus Regional de Umurama” (CAU), Fazenda, from 2013 to 2017.

The Laboratory database refers to the exam files containing animal identification items (name, ID number, species, race, sex, age, material collected and clinical diagnosis), identification of the bacteria (s) isolated (s) and results of the diameter of the bacterial growth inhibition halos obtained by disc diffusion or antibiogram technique.

The information obtained from the exam files was inserted into spreadsheets using Excel software, where it was separated by year of isolation, bacterial type, and place of infection. A total of 32 antibiotics in 11 classes were used. The interpretation of antimicrobial resistance/susceptibility was performed by CLSI (2008, 2013, 2018) and BrCAST (2019) standards, as shown in Table 1.

Following the guidance of BrCAST (2019), members of the old family Enterobacteriaceae were referred to as members of the new order Enterobacterales. The order Enterobacterales currently includes the families (Adeolu et al. 2016). Enterobacteriaceae, Erwiniaeae, Pectobacteriaceae, Yersiniaceae, Hafniaceae, Morganellaceae, and Budviciaceae.

Table 1. Antimicrobial susceptibility interpretation standard, in millimeters of growth inhibition halos, for bacteria of animal origin

| Antimicrobial                         | Staphylococcus spp. | Enterococcus spp. | Streptococcus spp. | Enterobacterales | Pseudomonas spp. | Acinetobacter baumanii |
|---------------------------------------|---------------------|-------------------|--------------------|-----------------|-----------------|----------------------|
| Penicillin G                          | ≥ 29<sup>a</sup>    | ≥ 15<sup>a</sup>  | ≥ 24<sup>a</sup>   | -               | -               | -                    |
| Ampicillin                            | 10 μg               | ≥ 17<sup>a</sup>  | ≥ 24<sup>a</sup>   | ≥ 17<sup>a</sup> | -               | -                    |
| Amoxicillin                           | 10 μg               | amp<sup>b</sup>   | amp<sup>b</sup>    | -               | -               | -                    |
| Amoxicillin + clavulanate             | 30 μg               | pen e oxa<sup>a</sup> | pen e oxa<sup>a</sup> | -               | ≥ 14<sup>b</sup> | -                    |
| Oxacillin                             | 1 μg                | ≥ 18<sup>a</sup>  | -                  | ≥ 20            | -               | -                    |
| Cefoxitin                             | 1 μg                | ≥ 22<sup>a</sup>  | IR<sup>a</sup>     | ≥ 19<sup>b</sup> | -               | -                    |
| Cephalothin/cefalexin                 | 30 μg               | pen e oxa<sup>a</sup> | IR<sup>a</sup>     | ≥ 14<sup>b</sup> | -               | -                    |
| Cefazidine                            | 30 μg               | IR<sup>a</sup>    | ≥ 22<sup>b</sup>   | ≥ 17<sup>b</sup> | -               | -                    |
| Cefotaxime                            | 30 μg               | IR<sup>a</sup>    | ≥ 23<sup>c</sup>   | -               | -               | -                    |
| Ceftiraxone                           | 30 μg               | pen e oxa<sup>a</sup> | IR<sup>a</sup>     | ≥ 24             | ≥ 25<sup>c</sup> | ≥ 21<sup>c</sup>     |
| Cefepime                              | 30 μg               | IR<sup>a</sup>    | ≥ 24<sup>b</sup>   | ≥ 27<sup>b</sup> | ≥ 21<sup>b</sup> | -                    |
| Aztreonam                             | 30 μg               | IR<sup>a</sup>    | ≥ 26<sup>b</sup>   | ≥ 16<sup>b</sup> | -               | -                    |
| Meropenem                             | 10 μg               | -                 | ≥ 22<sup>a</sup>   | ≥ 24<sup>b</sup> | ≥ 21<sup>b</sup> | -                    |
| Imipenem                              | 10 μg               | pen e oxa<sup>a</sup> | ≥ 21<sup>b</sup>   | ≥ 23<sup>c</sup> | ≥ 19<sup>c</sup> | ≥ 23<sup>c</sup>     |
| Vancomycin                            | 30 μg               | ≥ 18<sup>d</sup>  | ≥ 17<sup>a</sup>   | -               | IR<sup>a</sup>  | -                    |
| Teicoplanin                           | 30 μg               | van<sup>a</sup>   | ≥ 16<sup>b</sup>   | ≥ 17<sup>b</sup> | IR<sup>a</sup>  | -                    |
| Linezolid                              | 30 μg               | ≥ 21<sup>b</sup>  | ≥ 19<sup>a</sup>   | ≥ 19<sup>b</sup> | IR<sup>a</sup>  | -                    |
| Amikacin                              | 30 μg               | ≥ 18<sup>b</sup>  | IR<sup>a</sup>     | ≥ 17<sup>a</sup> | ≥ 17<sup>a</sup> | ≥ 19<sup>b</sup>     |
| Gentamicin                            | 10 μg               | ≥ 15<sup>a</sup>  | IR<sup>a</sup> ≥ 8<sup>b</sup> | ≥ 16<sup>a</sup> | ≥ 16<sup>a</sup> | ≥ 17<sup>b</sup>     |
| Streptomycin                          | 10 μg               | IR<sup>a</sup> ≥ 14<sup>b</sup> | -                 | ≥ 15<sup>a</sup> | ≥ 17<sup>b</sup> | -                    |
| Tobramycin                            | 10 μg               | ≥ 18<sup>b</sup>  | IR<sup>a</sup>     | ≥ 17<sup>a</sup> | ≥ 16<sup>b</sup> | -                    |
| Erythromycin                          | 15 μg               | ≥ 23<sup>a</sup>  | ≥ 23<sup>a</sup>   | ≥ 21<sup>a</sup> | IR<sup>a</sup>  | -                    |
| Azithromyx                            | 15 μg               | ≥ 18<sup>a</sup>  | -                 | IR<sup>a</sup>  | -               | -                    |
| Clindamycin                           | 2 μg                | ≥ 21<sup>a</sup>  | IR<sup>a</sup>     | ≥ 17<sup>a</sup> | IR<sup>a</sup>  | -                    |
| Rifampicin                            | 5 μg                | ≥ 20<sup>a</sup>  | ≥ 20<sup>a</sup>   | ≥ 21<sup>a</sup> | -               | -                    |
| Chloramphenicol                       | 30 μg               | ≥ 18<sup>a</sup>  | ≥ 18<sup>a</sup>   | ≥ 19<sup>a</sup> | ≥ 18<sup>a</sup> | -                    |
| Tetracycline                          | 30 μg               | ≥ 23<sup>a</sup>  | ≥ 19<sup>a</sup>   | ≥ 23<sup>a</sup> | ≥ 15<sup>a</sup> | -                    |
| Doxycycline                           | 30 μg               | ≥ 25<sup>a</sup>  | ≥ 16<sup>a</sup>   | ≥ 28<sup>a</sup> | ≥ 14<sup>a</sup> | -                    |
| Sulfamethoxazole + trimethoprim       | 23.7/1.25 μg        | ≥ 16<sup>a</sup>  | IR<sup>a</sup>     | ≥ 19<sup>a</sup> | ≥ 16<sup>a</sup> | -                    |
| Enrofloxacin                          | 10 μg               | ≥ 23<sup>a</sup>  | -                 | ≥ 23<sup>a</sup> | -               | -                    |
| Norfloxacin                           | 10 μg               | ≥ 17<sup>b</sup>  | ≥ 12<sup>a</sup>   | ≥ 12<sup>a</sup> | ≥ 22<sup>b</sup> | ≥ 17<sup>a</sup>     |
| Ciprofloxacin                         | 5 μg                | ≥ 21<sup>b</sup>  | -                 | ≥ 26<sup>b</sup> | ≥ 26<sup>b</sup> | ≥ 21<sup>b</sup>     |
| Levofloxacin                          | 5 μg                | ≥ 22<sup>b</sup>  | -                 | ≥ 17<sup>a</sup> | ≥ 23<sup>b</sup> | ≥ 23<sup>b</sup>     |

<sup>a</sup>CLSI (2018), <sup>b</sup>BrCAST (2019), <sup>c</sup>CLSI (2013), <sup>d</sup>CLSI (2008); IR = intrinsic resistance.
The categories were organized into:

1) Gram-positive bacteria: Staphylococcus spp., Enterococcus spp., Streptococcus spp., Enterococcus/Streptococcus (when it was not possible to differentiate gender);

2) Gram-negative bacteria: order Enterobacterales and NFGNB (non-fermenting Gram-negative bacilli). Clinically significant NFGNB associated with severe nosocomial infections such as Pseudomonas spp. and Acinetobacter baumannii were reported separately from the other NFGNB, which were included in the "other" category.

Absolute and relative frequencies of antimicrobial resistance were determined using descriptive statistical analysis. The graphics were made by Excel software.

The impact factor of each antimicrobial drug used to help choose empirical antimicrobial therapy was calculated according to Rampacci et al. (2018) and referred to the results of the prevalence study and antimicrobial susceptibility test (antibiogram) with their frequency expressed as a percentage, according to the formula:

$$F = \left[ \frac{\%P_{(\alpha)} + \%S_{(\alpha)}}{100} \right] + \left[ \frac{\%P_{(\alpha)} + \%S_{(\beta)}}{100} \right] + \left[ \frac{\%P_{(\beta)} + \%S_{(\beta)}}{100} \right] + \left[ \frac{\%P_{(\beta)} + \%S_{(\gamma)}}{100} \right]$$

Where $F =$ impact factor of the evaluated antibiotic, $\%P =$ prevalence of bacteria isolated from each bacterial group, $\%S =$ percentage of antibiotic susceptibility of each bacterial group, \(\alpha\) = Staphylococcus, \(\beta\) = Enterococcus/Streptococcus, \(\gamma\) = Enterobacteriales, \(\gamma\) = NFGNB.

The Multiple Antibiotic Resistance Indexing (MAR), according to Krumperman (1983), was calculated by the number of antibiotics to which the isolate had resistance (including intermediate resistance) divided by the total number of antibiotics tested. Bacteria with an index equal to or greater than 0.2 (20% of the tested antibiotics) were considered multidrug-resistant.

Multidrug-Resistant Bacteria (MDR), according to Magiorakos et al. (2012), were detected by the calculation, through an index, considering the number of resistant classes in relation to the total number of tested classes. The class was considered resistant when one or more drugs were resistant. Values equal to or greater than 0.3 were considered multidrug-resistant, values equal to or greater than 0.8 as extensively drug-resistant, and values of 1.0 with resistance to all drugs tested were considered pandrug-resistant.

RESULTS AND DISCUSSION

During the study period, 235 bacterial strains collected from infections in small animals were isolated, 207 from dogs and 28 from cats. The records were evaluated regardless of age and gender, and all animals were from the Umuarama region and surrounding areas in the state of Paraná, Brazil.

Given the analysis of the data obtained, the most common microorganisms (54.89%, 129/235) in dogs and cats were Gram-positive bacteria. Of these, 82.17% (106/129) were identified as Staphylococcus spp., representing 45.10% of the total bacterial isolates studied (for further analysis Micrococcus spp. was included with Staphylococcus spp. for similarity). The second most prevalent genus of Gram-positive bacteria was Enterococcus (8.53%, 11/129), followed by Streptococcus (4.65%, 6/129). Five isolates were not differentiated between Enterococcus and Streptococcus genera. Of the 106 (45.1%) Gram-negative bacteria, Enterobacteriales were identified in 75.47% (80/106) of the isolates, their largest representative was Escherichia coli identified in 13.75% (11/80), while NFGNB were identified in 26 isolates, of which 73.08% (19/26) comprised Pseudomonas spp. Despite the low frequency, the identification of Acinetobacter baumannii and Burkholderia pseudomallei (Table 2) is noteworthy.

The highest prevalence of Gram-positive bacteria, especially Staphylococcus spp., is found in several studies (Pereira et al. 2009, Ishii et al. 2011, Sfaciotte et al. 2014, Kohl et al. 2016) of infections in dogs and cats. Sfaciotte et al. (2014) and Kohl et al. (2016) also identified Escherichia coli as the second most prevalent bacteria. Ishii et al. (2011) detected a high prevalence of Pseudomonas spp. in samples from wounds, followed by Escherichia coli. The prevalence of other bacteria was similar in studies conducted by Ishii et al. (2011), Kohl et al. (2016) and in the present work. Ishii et al. (2011) was also detected Acinetobacter spp.

Most cases of infections evaluated occurred in the integumentary system (98/235, 41.70%); followed by infections in the sensory system (83/235, 35.32%), divided into otological infections (69/235, 29.36%), ophthalmic and nasal infections (7/235, 2.98% each); in the urinary system (32/235; 13.62%); and in the female reproductive system collected from vaginal secretions (10/235, 4.26%); and other systems, with body fluids by CSF collection or abdominal aspiration from peritonitis (4/235, 1.70%). The origin of the materials was not identified in the eight-sample examination sheet (3.40%) (Table 3).

With a higher frequency of clinical infections, and more straightforward diagnosis and collection, samples of integumentary infections, urinary and otological infections, are often more sent to microbiology laboratories by clinicians, as also reported by Pereira et al. (2009), Ishii et al. (2011) and Kohl et al. (2016).

A high number of antimicrobial resistance to antibiotics used in veterinary medicine was found (Fig.1). In Staphylococcus spp. resistance/susceptibility to beta-lactam drugs is predicted by penicillin, oxacillin, and cefoxitin (CLSI 2018, BrCAST 2019). Penicillin resistance predicts resistance to penicillins and aminopenicillins, and these strains are considered susceptible to the association of aminopenicillins with beta-lactamase inhibitors (clavulanic acid and sulbactam). Oxacillin/cefoxitin resistance predicts resistance to all beta-lactam drugs, including penicillins, aminopenicillins, whether or not associated with inhibitors, cefalosporins, except the 5th generation, untested, and carbapenems. In this study, 24.30% (26/107) of Staphylococcus spp. were susceptible to all beta-lactams drugs, 37.38% (40/107) were resistant to penicillins and aminopenicillins but sensitive to associations with beta-lactamase inhibitors, cefalosporins, and carbapenems, and 38.32% (41/107) were resistant to all beta-lactams.

Staphylococcus spp. resistant to oxacillin/cefoxitin are reported as methicillin-resistant Staphylococcus spp. (MRS), often being studied for their great clinical and epidemiological importance. These strains are often associated with resistance to several other antimicrobial drugs (Bardiau et al. 2013, Haenni et al. 2014).

Bardiau et al. (2013) detected high resistance to penicillin, gentamicin, amikacin, erythromycin, tetracycline, ciprofloxacin, clindamycin, sulfis, and chloramphenicol in methicillin-resistant Staphylococcus pseudintermedius (MRSP) from dogs and cats from Belgium. All isolates were resistant to at least five of the fifteen tested antibiotics. One isolate was resistant to all tested antibiotics.

Haenni et al. (2014) in their study also with canine origin MRSP found high resistance to penicillin, tetracycline, erythromycin, enrofloxacin, amikacin, tobramycin and...
gentamicin, only chloramphenicol had a lower resistance (22%). In MSSP strains, low resistance was found for all drugs, especially enrofloxacin (9.4%), tobramycin and gentamicin (5.4% each).

The Enterobacteriales order has intrinsic resistance to penicillin, but aminopenicillins, considered broad-spectrum penicillins, also show adequate Gram-negative action. Resistance to these drugs was found in 68.75% (55/80) of

### Table 2. Identification of isolated bacteria in small animal infections in the Umuarama region, Paraná

| Identification | n | %  |
|---------------|---|----|
| **Gram-positive** |  |  |
| Staphylococcus spp. | 106 | 45.10 |
| Micrococcus spp. | 1 | 0.43 |
| Enterococcus spp. | 11 | 4.68 |
| Streptococcus spp. | 6 | 2.55 |
| Streptococcus/Enterococcus | 5 | 2.13 |
| **Total** | 129 | 54.89 |
| **Gram-negative** |  |  |
| Enterobacteriales |  |  |
| Escherichia coli | 11 | 4.68 |
| Escherichia fergusonii | 6 | 2.55 |
| Salmonella spp. | 4 | 1.70 |
| Citrobacter spp. | 3 | 1.28 |
| Citrobacter braakii | 2 | 0.85 |
| Citrobacter freundii | 1 | 0.43 |
| Citrobacter youngae | 1 | 0.43 |
| Proteus mirabilis | 5 | 2.13 |
| Proteus spp. | 2 | 0.85 |
| Proteus vulgaris | 1 | 0.43 |
| Klebsiella pneumoniae | 3 | 1.28 |
| Klebsiella oxytoca | 1 | 0.43 |
| Klebsiella ozaeae | 1 | 0.43 |
| Morganella morganii | 4 | 1.70 |
| Enterobacter amnogenus 2 | 2 | 0.85 |
| Enterobacter sakazakii | 1 | 0.43 |
| Enterobacter cloacae | 2 | 0.85 |
| Enterobacter cancerogenus | 3 | 1.28 |
| Enterobacter spp. | 1 | 0.43 |
| Hafnia alvei | 3 | 1.28 |
| Serratia spp. | 2 | 0.85 |
| Serratia liquefaciens | 1 | 0.43 |
| Providencia spp. | 3 | 1.28 |
| Plesiomonas shigeloides | 1 | 0.43 |
| Yersinia frederiksenii | 1 | 0.43 |
| Yersinia enterococitica | 1 | 0.43 |
| Yersinia pseudotuberculosis | 1 | 0.43 |
| Yersinia intermedium | 1 | 0.43 |
| Yersinia spp. | 1 | 0.43 |
| Shigela boydii sorogrupo C | 1 | 0.43 |
| Ledercia adcarboxylata | 2 | 0.85 |
| Raistonia picketti va2 | 1 | 0.43 |
| Klyvera ascorbata | 1 | 0.43 |
| Not identified | 6 | 2.55 |
| **Total** | 80 | 34.04 |
| **Non-fermenting Gram-negative bacilli** |  |  |
| Pseudomonas aeruginosa | 19 | 8.09 |
| Acinetobacter baumannii | 2 | 0.85 |
| Burkholderia pseudomallei | 1 | 0.43 |
| Ochrobactrum anthropi | 1 | 0.43 |
| Chromobacterium violaceum | 1 | 0.43 |
| Brevundimonas vesicularis | 1 | 0.43 |
| Not identified | 1 | 0.43 |
| **Total** | 26 | 11.06 |

**TOTAL** 235 100%
the studied enterobacterial isolates. In the combination of amoxicillin with clavulanic acid, resistance was identified in 52.50% (42/80) of the isolates, whereas in the combination of ampicillin with sulbactam the resistance was only 28.95% (22/76). Resistance to cephalosporins was detected in 55.70% (44/79) of enterobacteria for first generation (cephalothin), 48.68% and 55.00% for the third generation (ceftriaxone and ceftazidime, respectively), and 56.06% for fourth generation (cefepine). Carbapenems, hospital drugs indicated for the treatment of severe infections by multiresistant enterobacteria, presented a resistance percentage of 7.50% (6/80) and 18.42% (14/76) for meropenem and imipenem, respectively.

Carvalho et al. (2016), studying E. coli from dog feces in Rio de Janeiro, Brazil, found a resistance percentage of 85.7% to ampicillin, 35.7% to amoxicillin with clavulanic acid, 33.3% to celexin, 16.6% to ceftazidime, 19.4% to cefotaxime, 21.4% to ceftriaxone and 9.5% to cefepine. Pseudomonas are susceptible only to third and fourth generations of cephalosporins and carbapenems. There were found 66.67% of resistance to ceftriaxone and cefotaxime and 16.67% to cefepine. Carbapenem resistance was found in 21.05% of isolates for meropenem and 16.67% for imipenem. Acinetobacter baumannii and Enterococcus spp. are intrinsically resistant to beta-lactams except for carbapenems. Both isolates of Acinetobacter baumannii were susceptible to meropenem, and one was resistant to imipenem, and of the three Enterococcus tested for imipenem, one was resistant. The second broad class broad-spectrum drug, fluoroquinolones, tested on all bacterial isolates, showed general resistance in the 30-40% range for the second generation, represented by enrofloxacin (42.56%), ciprofloxacin (36.45%) and norfloxacin (32.59%). The third generation represented by levofloxacin presented resistance in 29.71% of the isolates tested. Resistance values were similar between Staphylococcus spp. and enterobacteria, in pseudomonas as the resistance to enrofloxacin was 40% while the other fluoroquinolones had resistance in 26 and 29% in the isolates. The two Acinetobacter isolates were resistant to ciprofloxacin and levofloxacin, as well as all Streptococcus spp. Fifty percent of Enterococcus spp. were resistant to norfloxacin. Stefanetti et al. (2017) detected resistance to fluoroquinolones in 76% of isolates to enrofloxacin; Silva et al. (2014) by 20.1% for enrofloxacin and 18.8% for ciprofloxacin, representing 22.3% of fluoroquinolone-resistant bacterial isolates; Ishii et al. (2011) found resistance to enrofloxacin in 53.8% of isolates and norfloxacin in 41.3%. Scherer et al. (2018) detected 38.6% of resistance to enrofloxacin; Kohl et al. (2016) detected 30% of resistance to enrofloxacin in Gram-negative bacteria and 13.33% in Gram-positive bacteria; and Carvalho et al. (2016), 11.9% to ciprofloxacin.

The third primary class, aminoglycosides, showed resistance in 10.78% of the isolates to amikacin, 22.01% to gentamicin.
and 25% to tobramycin, maintaining similar values between *Staphylococcus* and enterobacteria. *Pseudomonas* showed 5.55% of resistance to amikacin and 17.65% of resistance to tobramycin. Both *Acinetobacter* isolates were susceptible to amikacin, and one was resistant to gentamicin. *Streptococcus* and *Enterococcus* are intrinsically resistant to aminoglycosides.

Resistance rates vary according to the type of microorganism studied and location. The lowest resistances found in this study for conventional antimicrobial drugs in veterinary medicine were for amikacin, gentamicin, and tobramycin. Unlike Stefanetti et al. (2017) in Italy, with resistance in 43% of *S. pseudintermedius* isolates for amikacin and 61% for gentamicin. Scherer et al. (2018) studying *S. pseudintermedius* in dogs with otitis externa in Minas Gerais, Brazil, found 27.3% of resistance to gentamicin. Kohl et al. (2016) in the state of Santa Catarina, Brazil, detected gentamicin resistance in 33.33% of Gram-negative and 12% of Gram-positive. Ishii et al. (2011) detected resistance to amikacin in 32.6%; gentamicin in 36.6% and tobramycin in 33.3% of bacterial isolates. Carvalho et al. (2016), studying *Escherichia coli* from dog feces in Rio de Janeiro, Brazil, detected resistance of 30.9% to gentamicin. Yadav et al. (2018) found 100% of isolates of *S. aureus* of canine origin susceptible to gentamicin and amikacin.

The resistance to tetracycline and doxycycline was observed in 62.56% and 56.63%, respectively, remaining in these proportions in staphylococci and enterobacteria. In enterococci and streptococci, the resistance to tetracycline was 85% and 73.68%, respectively. Potentiated sulfon resistance was found in 54.95% of the bacterial isolates tested, where the same patterns of tetracyclines were found in staphylococci and enterobacteria, increasing in enterococci and streptococci (66.67%). The resistance to phenicols, represented by chloramphenicol was 21.95%, being 16.04% in staphylococci, 30.77% in enterobacteria and 19.05% in enterococci and streptococci. Intrinsic resistance for these three classes is observed in NFGNB, including pseudomonas and acinetobacter.

Resistance to tetracyclines and potentiated sulfonamides was also found by Stefanetti et al. (2017) with 73% of resistance to doxycycline and 80% to sulfamethoxazole-trimethoprim; Scherer et al. (2018) with 61.4% to tetracycline and 63.6% to sulfamethoxazole + trimethoprim; and Ishii et al. (2011) with 75.6% to sulfamethoxazole + trimethoprim. While Silva et al. (2014) detected 4.5% of resistance to doxycycline and 29.9% to sulfamethoxazole + trimethoprim and Carvalho et al. (2016), 50% to tetracycline, 33.3% to doxycycline and 30.9% to sulfamethoxazole + trimethoprim. Carvalho et al. (2016), in *E. coli*, found chloramphenicol resistance in 23.8% of dog isolates.

**Table 4. Evaluation of multiresistance in bacteria isolated from infections in small animals in the region of Umuarama/PR, Brazil**

| Resistance | *Staphylococcus* spp. | *Enterococcus* / *Streptococcus* spp. | Enterobacterales | NFGNB | Total |
|------------|----------------------|--------------------------------------|-----------------|-------|-------|
|            | MDR | MAR | MDR | MAR | MDR | MAR | MDR | MAR | MDR | MAR | MDR | MAR |
| >0.3/>0.2  | 70  | 64  | 18  | 17  | 61  | 58  | 13  | 12  | 162 | 151 |
| >0.8       | 21  | 22  | 2   | 5   | 19  | 9   | 3   | 0   | 45  | 36  |
| 1          | 6   | 0   | 1   | 0   | 3   | 0   | 0   | 0   | 10  | 0   |
| MDR        | 49  | 42  | 16  | 12  | 42  | 49  | 10  | 12  | 117 | 115 |
| XDR        | 21  | 22  | 2   | 5   | 19  | 9   | 3   | 0   | 45  | 36  |
| Non-Resistant | 37 | 43  | 4   | 5   | 19  | 22  | 13  | 14  | 73  | 84  |
| TOTAL      | 107 | 107 | 22  | 22  | 80  | 80  | 26  | 26  | 235 | 235 |

NFGNB = Non-fermenting Gram-negative bacilli; MDR = multidrug-resistant bacteria, MAR = multiple antibiotic resistance indexing. XDR = extensively drug-resistant bacteria.
Antimicrobial resistance evaluation of bacteria isolated from infections in small animals in the Umuarama region, Paraná

Arias et al. (2013) report a gradual increase in multidrug resistance of antimicrobial agents in veterinary medicine. High rates of multiresistant bacteria were found by Arias et al. (2008) in bacteria isolated from infected and contaminated traumatic animal wounds, with a MAR Index ≥0.2 representing 95% of isolated bacteria, with a mean of 0.7. Three isolates presented MAR = 1.0. Säciotte et al. (2014) found similar values, where 89.4% of isolates were considered multiresistant, with a mean MAR index of 0.65 and a maximum of 1.0. Kohl et al. (2016) detected multiresistant strains in 33.3% to 100% of the groups evaluated per period, between Gram-positive and -negative bacteria, with MAR averages between 0.2 and 0.52. Corroborating the study by Arias et al. (2013), who at the Veterinary Hospital of Londrina-Paraná, found high multidrug resistance in all isolates evaluated. All three studies found higher MAR values in Gram-negative bacteria.

Verifying the distribution of the MAR index and evaluating the resistance profile in the period studied (Fig.2), the average curve remains constant from 2013 to 2015, with a low for the following two years. It is also observed that both the average and the median presented values above 0.2 in all evaluated years. The distribution of the MAR index according to the site of infection (Fig.3) shows superior position measurements for urinary tract infections, respiratory infections represented by nasal samples and body fluids, although the latter two, by low sampling, may not represent the real importance of this resistance.

One of the pillars of prudent antimicrobial therapy is the use of evidence-based antimicrobials (Guardabassi & Prescott 2015). The prevalence of each microorganism associated with its susceptibility underlies the calculation of the impact factor of each antimicrobial, according to Hall et al. (2013) and Rampacci et al. (2018), considered the most accurate indicator of antimicrobial efficacy, according to which the higher the impact factor, the greater the predicted clinical efficacy. The drugs used in antimicrobial therapy in animals with the highest impact factor (Fig.4) were amikacin, followed by gentamicin, chloramphenicol, tobramycin, for topical use only, norfloxacin and ciprofloxacin. Intermediate results for sulbactam-associated ampicillin, ceftiraxone, clavulanic acid amoxicillin, and enrofloxacin. The worst results were found for azithromycin, streptomycin, and penicillin, where we can mention the short spectrum of these antibiotics.

Ishii et al. (2011) point out the difficulty of the empirical selection of antimicrobial drugs with the emergence of antimicrobial resistance. Knowledge of bacterial resistance profiles in the region is essential so that antibiotic prescriptions are not based on resistance patterns described in the literature that may not reflect the reality of the site, as observed in this study. According to Guardabassi & Prescott (2015), these studies increase the possibilities of developing infection control practices, specific to each location, making conscious and moderate use of available antibiotics.

CONCLUSIONS

**Staphylococcus** spp. represented 45.53% of bacteria isolated from clinical infections in small animals in the region of Umuarama/PR, Brazil.
According to the number of resistant antimicrobial drugs, 64.26% (151/235) of the isolates were classified as multidrug-resistant, being 15.32% extensively resistant. Considering the resistance to antimicrobial classes, 68.94% (162/235) of the isolates were considered multiresistant, being 19.15% extensively resistant. No bacterial isolates were characterized as pan-resistant, but ten bacteria were resistant to all classes tested, with isolated susceptibility to certain drugs.

Resistance to all antimicrobial drugs used in veterinary medicine was found. The lowest resistances associated with the best impact factor values were found for amikacin, gentamicin, chloramphenicol, tobramycin, norfloxacin, and ciprofloxacin. Intermediate results were found for sulbactam-associated ampicillin, ceftriaxone, clavulanic acid amoxicillin, and enrofloxacin.

By evaluating the antimicrobial resistance profiles found at the time and place studied, it can be concluded that there is an increasing in the number of multidrug-resistant agents among domestic animals, which becomes a serious public health risk for all those come into direct or indirect contact with them - including through the environment - especially their tutors and healthcare professionals such as the veterinarian.

It can also be seen that the therapeutic arsenal is becoming smaller and smaller, and there is more difficulty in the empirical selection of drugs to be instituted in clinical treatment. Making it essential to perform bacterial identification tests and their awareness of their sensitivity to a more specific selection of drugs to be used, and educational measures on the conscious use of antibiotics, infection control, and prevention of site-specific zoonoses need to be instituted for knowledge of health professionals and general access of the population.

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