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Streptococcus spp. in Equines - Infection and Antimicrobial Susceptibility Profiles

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ABSTRACT

***Background*:** Empirical antimicrobial prescribing is commonly used in equine veterinary. Therefore, professionals can obtain information about antimicrobial susceptibility profile of the bacterial strains based on veterinary literature. Considering equine infections, *Streptococcus* spp. are important pathogens that can cause serious damage in horses. Therefore, the aim of this study was to describe the antimicrobial susceptibility testing (AST) and infection profiles of *Streptococcus* spp. strains isolated from equines with infectious diseases subjected to microbiological analysis.

***Materials, Methods & Results*:** Veterinarians sent 13 samples and culture in Blood and MacConkey Agar were performed. After the incubation period, suspected colonies, which showed significative growth, were analyzed by Gram-staining, biochemical tests, and subjected to confirmatory identification in Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry. *In vitro* AST analysis were performed by disc diffusion method, in accordance with the veterinarians' request. The antimicrobials tested in this study were: ceftiofur, gentamicin, ampicillin, enrofloxacin, amikacin, penicillin, trimethoprim-sulfamethoxazole, ciprofloxacin, doxycycline, vancomycin and metronidazole. The samples included uterine exudate, hock fistula, osteosynthesis exudate, exudate from the guttural pouch, and were originated from animals located in different and distant geographical regions in the cities of Porto Alegre, Pelotas, and Bagé, Rio Grande do Sul, Brazil. *Streptococcus dysgalactiae*, *Streptococcus equi* and *Streptococcus thoraltensis* were the *Streptococcus* species identified in the samples. *S. dysgalactiae* was the mainly species found in the uterus samples, while *S. thoraltensis*, an unusual *Streptococcus* species, was identified as etiological agent of endometritis in 2 of the analyzed animals. On the other hand, *S. equi* was found in both the guttural pouch, representing the etiological agent of the strangle case, and in the osteosynthesis exudate, as infectious agent of post-osteosynthesis surgery. The majority of streptococci strains were susceptible to ceftiofur drug. Amikacin and ciprofloxacin, however, were the drugs for which the strains were mainly resistant according to the results.

***Discussion*:** The present study provided the AST and infection profile of *Streptococcus* species related to equine infectious diseases. *S. dysgalactiae* is considered an unusual bacterium isolated from horses that can be related to endometritis, *S. equi* is the causative agent of strangles, and *S. thoraltensis* is unusual in equines. Generally, the observed susceptibility to ceftiofur of the strains analyzed was in agreement with previous results reported in the literature. However, ceftiofur is a third-generation cephalosporin and is considered a critically important antibiotic for human health and its use in veterinary medicine should be cautious. Considering the resistance profile found, *Streptococcus* spp. can be intrinsically resistant to low drug concentrations of aminoglycosides. Moreover, the emergence and spread of fluoroquinolones resistance may also be due to the acquisition of resistance via horizontal gene transfer. Therefore, the present study described both infection and antimicrobial susceptibility patterns of *Streptococcus* strains related to equine infectious diseases. Considering the findings, the results found in this study might contribute to the decision-making by veterinarians to further equine treatments.

Keywords: antimicrobial susceptibility, pattern, AST, drug, resistant bacteria, horses, veterinarians.

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INTRODUCTION

Streptococcus spp. are Gram-positive and catalase-negative cocci that show considerable ecological and genetic diversity [18]. In horses, *Streptococcus* spp. may be present in both healthy [7] and diseased animals, as involved in many clinical cases promoting serious damage to the animals' quality of life [1,9,14].

From a veterinary point of view, in many clinical situations it is not possible to obtain samples for culture and AST. Therefore, empirical antimicrobial prescription is performed [8]. Thus, the knowledge of anticipated drug efficacy and potential antimicrobial resistance is essential. This knowledge can be obtained from the veterinary literature, considering both the bacteria and the variability in antimicrobial susceptibility patterns in different geographic regions [13]. Using this information to practice, evidence-based antimicrobial administration can help for choosing the most appropriate drug and consequently for delaying the selection of drug resistant bacteria [2].

Streptococcus species are routinely involved in a high range of horses' diseases, and, in some cases, a complete microbiological diagnostic practice is restricted, either due to recurrent or non-responsive infectious cases [8]. Moreover, the differences of AST patterns among *Streptococcus* from different sites of infection in horses is still not fully known. Therefore, the aim of the present study was to describe the AST and infection profiles of *Streptococcus* spp. from different equines' infectious diseases subjected to microbiological analysis.

MATERIALS AND METHODS

Study design and sampling procedures

All the 13 samples included in this study were collected from horses' infectious sites (10 uterine, 1 hock fistula exudate, 1 osteosynthesis exudate, and 1 guttural pouch exudate) were analyzed and submitted to the Veterinary Bacteriology Laboratory at the Federal University of Rio Grande do Sul, Brazil (LaBacVet - UFRGS). The veterinarians required bacterial identification and further AST analysis (Table 1).

Microbiological analysis

All Samples (swabs) were cultured in Blood Agar Base1 supplemented with 5% sheep blood and MacConkey agar[1], followed by incubation at 37°C up to 48 h in aerobic and microaerophilic conditions. After incubation, cultures were assessed and bacterial isolates with significative growth were further analyzed by Gram-staining and submitted to biochemical tests. In addition, each isolated bacterium was subjected to Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS), using Microflex LT instrument and MALDI Biotyper 3.1 software[2], for species identification.

Antimicrobial susceptibility test (AST)

In vitro antimicrobial susceptibility test (AST) was performed using Kirby & Bauer disc diffusion method [5], considering the antimicrobial drugs required by the veterinarians' requests. The following antibimicrobial drugs[3] used tested: ceftiofur (30 µg), gentamicin (10 µg), ampicillin (10 µg), enrofloxacin (5 µg), amikacin (30 µg), penicillin (10 µg), trimethoprim-sulfamethoxazole (25 µg), ciprofloxacin (5 µg), doxycycline (30 µg), vancomycin (30 µg), and metronidazole (4 µg). The interpretation of the tests was based on CLSI-VET2018 [6] and BrCAST [3].

Statistical analysis

Descriptive statistics, including absolute and relative frequencies, were performed. Considering that the most prevalent infection site was the uterine, Fisher's exact test was used to analyze whether there was an association between the bacteria and the resistance to amikacin and gentamicin, which were tested for all isolates recovered from uterus samples. Data collection and analysis were performed with Microsoft Excel[4] and R software 4.1.0. The level of significance was set at $P < 0.05$.

Table 1. Description of the analyzed samples from equines located in different geographical regions of Rio Grande do Sul state, Brazil.

Sample ID	*Streptococcus* spp.	City	Anatomical Site
1	*S. thoraltensis*	Bagé	Uterus
2	*S. thoraltensis*	Bagé	Uterus
3	*S. dysgalactiae*	Porto Alegre	Uterus
4	*S. equi*	Porto Alegre	Osteosynthesis
5	*S. dysgalactiae*	Pelotas	Fistula in the hock
6	*S. dysgalactiae*	Bagé	Uterus
7	*S. dysgalactiae*	Bagé	Uterus
8	*S. dysgalactiae*	Bagé	Uterus
9	*S. equi*	Porto Alegre	Guttural pouch
10	*S. dysgalactiae*	Bagé	Uterus
11	*S. dysgalactiae*	Bagé	Uterus
12	*S. dysgalactiae*	Bagé	Uterus
13	*S. dysgalactiae*	Bagé	Uterus

RESULTS

Only *Streptococcus dysgalactiae*, *Streptococcus equi* and *Streptococcus thoraltensis* were the species identified (Figure 1). The average score-values of the strains identified in the MALDI-TOF MS Microflex ranged from 2.33 to 2.98, which represents a high level of reliability for an accurate species identification.

The samples were originated from equines located in different and distant geographical regions, from the cities of Porto Alegre, Pelotas, and Bagé, which comprises, respectively, the Metropolitan, Southeast, and Southwest regions, from Rio Grande do Sul state, Brazil (Figure 2).

Considering the infection sites, the suspicious infectious diseases of the horses' cases were: endometritis, strangles, infectious in the hock, and infectious behind osteosynthesis surgery.

In the suspected cases of endometritis, *S. dysgalactiae* was the mainly species found in uterus samples, while *S. thoraltensis*, an unusual *Streptococcus* species, was identified as etiological agent of endometritis in 2 of the analyzed animals (Figure 3). Additionally, *S. dysgalactiae* was also identified as etiological agent in the animal with hock fistula (Figure 3). Nevertheless, *S. equi* was found in both the guttural pouch, being the etiological agent for a strangle case, and in the osteosynthesis exudate, as infectious agent of post-osteosynthesis surgery (Figure 3).

According to the *in vitro* AST, the *Streptococcus* strains were susceptible at least to amikacin, ampicillin, ceftiofur, ciprofloxacin, doxycycline, enrofloxacin, gentamicin, penicillin, rifampicin, trimethoprim-sulfamethoxazole, and vancomycin (Figure 4). Interestingly, *S. thoraltensis* strains were susceptible to all the antimicrobial drugs tested. Precisely, ceftiofur was the antimicrobial drug with the highest number of strains that were characterized as susceptible by the *in vitro* tests, being *S. dysgalactiae* and *S. equi* strains the related to this susceptibility (Figure 4).

When analyzed the antimicrobial resistance profile of the strains, the analyzed bacteria showed resistance mainly to amikacin, ampicillin, ciprofloxacin, doxycycline, gentamicin, metronidazole, and penicillin (Figure 5). Among these antimicrobial drugs, amikacin and ciprofloxacin were the drugs for which the strains were mainly identified as resistant. No association was identified between the bacteria species and the *in vitro* resistance to amikacin and gentamicin ($P > 0.05$).

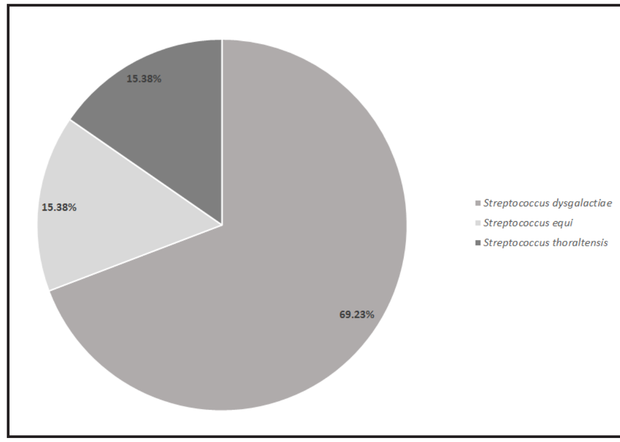

Figure 1. Proportion of *Streptococcus dysgalactiae*, *Streptococcus equi* and *Streptococcus thoraltensis* isolated from horses' infectious diseases in Rio Grande do Sul state, Brazil.

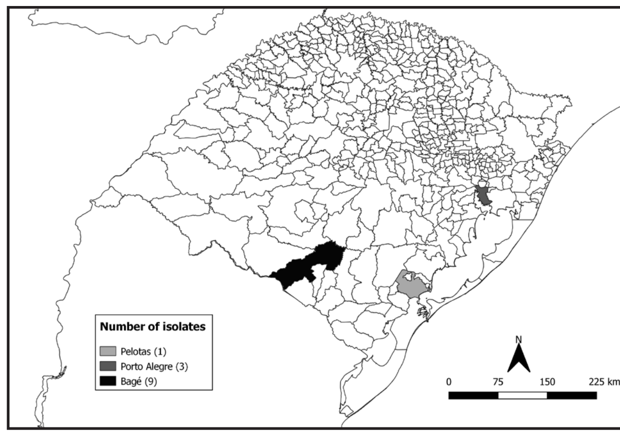

Figure 2. The isolates were originated from animals located in different and distant geographical regions in Rio Grande do Sul state, Brazil,

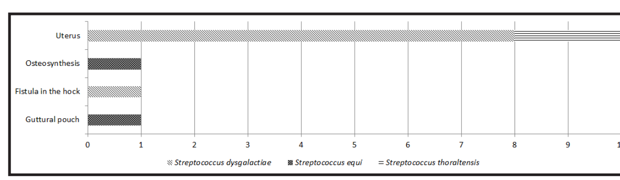

Figure 3. Number of isolates of *Streptococcus dysgalactiae*, *Streptococcus equi* and *Streptococcus thoraltensis* per site of infection from equines located in Rio Grande do Sul state, Brazil.

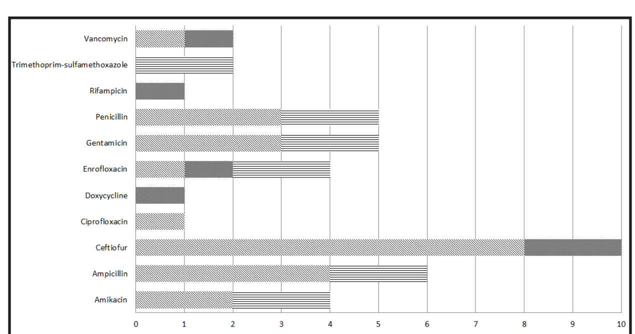

Figure 4. *In vitro* antimicrobial susceptibility profile of *Streptococcus* spp. isolates from equines located in Rio Grande do Sul state, Brazil.

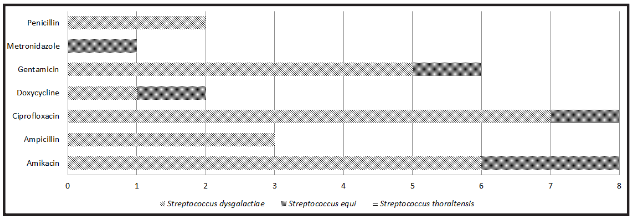

Figure 5. *In vitro* antimicrobial resistance profile of *Streptococcus* spp. isolates from equines located in Rio Grande do Sul state, Brazil.

DISCUSSION

The present study provided data about *Streptococcus* species related to equine infectious diseases, in addition to the elucidation of the susceptibility profile of the *Streptococcus* strains, which will contribute to the treatment choices of animals. *S. equi*, *S. dysgalactiae*, and *S. thoraltensis* were the species related to the analyzed horses' infectious diseases.

Streptococcus dysgalactiae was the most common streptococci found in the analyzed period, being mainly related as etiological agent of mare endometritis. Interestingly, *S. dysgalactiae* is considered an infrequent bacterium pathogen from horses in this context, being previously reported in aborted placenta [19] and involved in sporadic endometritis cases [17,18]. The analyzed *S. dysgalactiae* strains were susceptible to a significant range of antimicrobial drugs; nevertheless, many *S. dysgalactiae* strains showed a multidrug resistance profile, highlighting the importance of performing AST in order to determine the most adequate therapy treatment.

In the present study, *S. equi* was found in guttural pouch and in the osteosynthesis exudate, being susceptible to vancomicyn, rifampicin, enrofloxacin, doxycycline, and ceftiofur. *S. equi* is the causative agent of strangles, a highly contagious infection of the upper respiratory tract and associated with lymph nodes of horses [9]. Similar to the findings, a study in Colombia determined that *S. equi* strains were susceptible to penicillin and ceftiofur and were resistant to oxytetracycline and enrofloxacin [11].

Streptococcus thoraltensis is a less common *Streptococcus* species, being previously described mainly in swine [10,15]. Therefore, *S. thoraltensis* is an unusual bacteria related to infectious diseases in horses. Here, *S. thoraltensis* from mare endometritis cases were identified, being susceptible to all antibiotics according to the results. Although, in porcine, *S. thoraltensis* resistance to sulphadimethoxine and spectomycin was found [15].

Ceftiofur was the antimicrobial drug investigated in this study with the highest number of strains showing susceptibility profile, which is in agreement with previous results described in the literature [11,12]. However, ceftiofur is a third-generation cephalosporin that is considered a critically important antibiotic for human health according to the World Health Organization; thus, the use of such drug should be avoided in veterinarian practice [20].

Noteworthy, amikacin, member of the aminoglycoside class, and ciprofloxacin, member of quinolones class, were the antimicrobial drugs with the highest number of resistant *Streptococcus* strains, according to the results. The literature demonstrated that *Streptococcus* genera can be intrinsically resistant to low concentrations of aminoglycosides [4]. However, the combination with cell-wall-active antibiotics agents could result in a significant bactericidal synergy [4], making some aminoglycosides, such as amikacin, a significative choice in the equine clinic. Finally, the emergence and high spread of fluoroquinolones resistance may be due to the *Streptococcus* acquisition and selection of resistance mechanisms by horizontal gene transfer [16].

CONCLUSIONS

The present study described both infection and antimicrobial susceptibility profiles of *Streptococcus* strains related to equine infectious diseases. Also, *S. dysgalactiae* was highly frequent among the analyzed cases, showing a multidrug resistant profile. The acquired information will contribute to the decision-making by veterinarians for equine treatments.

MANUFACTURERS

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