











Bacterial pattern of lower respiratory diseases in horses¹

Ana Luísa H. Albuquerque² , Lukas G. Albertino² , Thais G. Rocha³ ,
Wanderson A.B. Pereira² , Rogério M. Amorim² , Marcio G. Ribeiro² ,
Alexandre S. Borges²  and José P. Oliveira-Filho^{2*} 

ABSTRACT.- Albuquerque A.L.H., Albertino L.G., Rocha T.G., Pereira W.A.B., Amorim R.M., Ribeiro M.G., Borges A.S. & Oliveira-Filho J.P. 2025. **Bacterial pattern of lower respiratory diseases in horses.** *Pesquisa Veterinária Brasileira* 45:e07596, 2025. Departamento de Clínica Veterinária, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista “Júlio de Mesquita Filho”, Rua Prof. Doutor Walter Mauricio Correa s/n, Botucatu, SP 18618-681, Brazil. E-mail: jose.oliveira-filho@unesp.br

Bacterial infections of the lower respiratory tract in horses are often caused by multiple etiological agents. Transtracheal aspirate followed by bacterial culture is crucial for diagnosing bacterial causes of these infections. This retrospective study investigated bacterial cultures from transtracheal aspirates of 40 horses with lower respiratory diseases admitted to the Large Animal Hospital at FMVZ-Unesp, Botucatu/SP. Medical records of these horses were reviewed, and transtracheal aspirates were cultured and tested for antimicrobial sensitivity. Of the 40 cultures, 27 were mono-infections, and 13 were mixed infections. The most commonly isolated bacteria in mono-infections were *Streptococcus equi* (37%), *Rhodococcus equi* (26%), and α -hemolytic streptococci (11%). *Escherichia coli* was the predominant in mixed infections (69%). No strictly anaerobic bacteria were isolated. The most effective antimicrobials were azithromycin (92%), ceftiofur (72%), and gentamicin (72%), while higher resistance of isolates was noted for penicillin G (53%) and ampicillin (50%). Additionally, 36% of isolates were multidrug-resistant. This study underscores the diverse bacterial etiology and presence of multidrug-resistant isolates in lower respiratory infections in horses.

INDEX TERMS: Antibiotics, bacteria, bronchopneumonia, equids, transtracheal aspirates.

RESUMO.- [Perfil bacteriano de cavalos com doenças do trato respiratório inferior.] Infecções bacterianas do trato respiratório inferior em equinos são frequentemente causadas por múltiplos agentes etiológicos. A coleta de aspirado transtraqueal seguida por cultura bacteriana é crucial para o diagnóstico dos agentes bacterianos envolvidos com essa infecção. Este estudo retrospectivo investigou culturas bacterianas de aspirados transtraqueais de 40 cavalos com doenças do trato respiratório inferior admitidos na Clínica de Grandes Animais do Hospital Veterinário da FMVZ-Unesp, Botucatu/SP. Os prontuários médicos desses equinos foram revisados, e os aspirados transtraqueais foram cultivados

e testados para sensibilidade antimicrobiana. Das 40 culturas, 27 foram mono-infecções e 13 foram infecções mistas. As bactérias mais comumente isoladas em mono-infecções foram *Streptococcus equi* (37%), *Rhodococcus equi* (26%) e α -hemolytic streptococci (11%). *Escherichia coli* foi predominante nas infecções mistas (69%). Nenhuma bactéria estritamente anaeróbica foi isolada. Os antimicrobianos mais eficazes foram azitromicina (92%), ceftiofur (72%) e gentamicina (72%), enquanto maior resistência dos isolados foi observada para penicilina G (53%) e ampicilina (50%). Além disso, 36% dos isolados foram multirresistentes. Este estudo destaca a etiologia bacteriana diversificada e a presença de isolados multirresistentes em infecções respiratórias inferiores em equinos.

TERMOS DE INDEXAÇÃO: Antibióticos, aspirado transtraqueal, bactéria, broncopneumonia, equinos.

INTRODUCTION

Horses develop lower respiratory diseases (LRD) from various pathogens (bacterial, viral, fungal, and parasitic) primarily transmitted through inhalation (Battistin et al. 2023, Calou

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² Departamento de Clínica Veterinária, Faculdade de Medicina e Zootecnia (FMVZ), Universidade Estadual Paulista “Júlio de Mesquita Filho” (Unesp), Rua Prof. Doutor Walter Mauricio Corrêa s/n, Botucatu, SP 18618-681, Brazil. *Corresponding author: jose.oliveira-filho@unesp.br

³ Departamento de Clínica e Cirurgia Veterinária, Faculdade de Ciências Agrárias e Veterinárias (FCAV), Universidade Estadual Paulista “Júlio de Mesquita Filho” (Unesp), Via de Acesso Prof. Paulo Donato Castellane s/n, Joticabal, SP 14884-900, Brazil.

2023). Predisposing factors include stress from weaning, shipment, extreme weather, geographic conditions, intense exercise, poor nutrition, and the introduction of new animals, as well as anatomical and physiological aspects in certain livestock species (Constable et al. 2017). In adult horses, lower respiratory infections often result from bacteria aspirated from the nasopharynx and oral cavity (Hoquet et al. 1985, Bailey & Love 1991), especially after strenuous exercise or during transportation when horses are cross-tied, increasing the risk of inhaling pharyngeal bacteria (Racklyeft & Love 2000).

Transtracheal aspirate for cytologic examination, combined with microbiological culture, is the preferred method for routinely diagnosing bacterial (Sweeney et al. 1991, Melo & Ferreira 2022, Battistin et al. 2023) and fungal (Battistin et al. 2023, Calou 2023, Melo et al. 2024) causes of pulmonary infections in horses. Multidrug resistance in animal-origin bacteria is a global public health concern.

Understanding bacterial agents and their antimicrobial sensitivity patterns in equine pulmonary infections across various regions can help adopt effective control measures and improve therapeutic strategies (Giguère et al. 2010). Fungi have been identified in the transtracheal aspirates of horses with LRD (Calou 2023), such as *Aspergillus fumigatus* have been documented as causative agents of pleural empyema (Battistin et al. 2023), pneumonia (Melo et al. 2024) and systemic aspergillosis (Busato et al. 2020), with or without concurrent bacterial infections in horses in Brazil, and this demonstrates the importance of fungi in the pathogenesis of lower respiratory diseases in horses. The aim of the study was to specifically investigate the bacterial culture findings and antimicrobial sensitivity profile from transtracheal aspirates of horses with LRD.

MATERIALS AND METHODS

Ethical approval. This research was approved by the Ethics and Animal Use Commission (CEUA) of the “Faculdade de Medicina Veterinária” (FMVZ) of the “Universidade Estadual Paulista ‘Júlio de Mesquita Filho’” (Unesp), Botucatu/SP, protocol number 214/2024 CEUA.

In this study, clinical and laboratory findings, including microbiological culture and *in vitro* antimicrobial sensitivity pattern, were obtained from 40 horses with lower respiratory disease diagnosed at the “Clínica de Grandes Animais” (Large Animal Internal Medicine Service) at the “Hospital Veterinário” (Veterinary Teaching Hospital) at the FMVZ-Unesp Botucatu (-22° 53' 22" S, 48° 29' 52" W), Brazil, were used.

Transtracheal aspirate samples were collected and plated on 5% defibrinated sheep blood agar, incubated aerobically and in a 5% CO₂ atmosphere at 37 °C for 96 h. The samples were also plated on MacConkey media and kept under the same aerobic conditions. Clinical specimens were cultured under anaerobic conditions on sheep blood agar at 37 °C for up to seven days if necessary. Microorganisms were identified using conventional phenotypic tests (Quinn et al. 2011).

The isolates were tested for antimicrobial sensitivity using the standard disk diffusion method, following Clinical Laboratory Standards Institute guidelines (CLSI 2024). Nine antimicrobials from seven distinct groups, recommended for treating bacterial pulmonary infections in livestock [1], were used: β-lactams (ceftiofur, 30 µg; penicillin G, 10 IU; ampicillin, 10 µg), fluoroquinolones (enrofloxacin, 5 µg), aminoglycosides (gentamicin, 10 µg), rifamycin (rifampicin, 5 µg), sulphonamides (sulfamethoxazole/trimethoprim, 25 µg),

tetracyclines (oxytetracycline, 30 µg), and macrolides (azithromycin, 3 µg). Bacteria were classified as multidrug-resistant if resistant to three or more different drug groups (Krumperman 1983).

Chi-square or Fisher's exact tests compared outcomes versus age and sex and the proportion of LRD occurrence versus season. Analyses were performed using SPSS version 14, with statistical significance set at 0.05.

RESULTS

Of the 40 horses sampled, 16 (40%) were males, and 24 (60%) were females, with a mean age of 3.7-year-old (range: 2 months to 29 years). No significant association was found between outcome and age ($p = 0.2053$) or sex ($p = 1.000$). Although no significant association was observed between the occurrence of LRD and the seasons ($p = 0.0710$), cases were most commonly diagnosed in summer (17/40, 42%), followed by spring (28%, 11/40), autumn (15%, 6/40), and winter (15%, 6/40) seasons.

Clinical signs observed were tachypnea (14/40), respiratory distress (10/40), nasal discharge (9/40), cough (9/40), abnormal lung sounds (8/40), fever (6/40), and anorexia (4/40). Hyperfibrinogenemia and neutrophilic leucocytosis were observed in 48% and 35% of cases, respectively. Based on clinical, imaging, and laboratory findings, bronchopneumonia was diagnosed in 58% of cases, followed by rhodococcosis (23%), pleuropneumonia (7%), asthma (7%) and interstitial pneumonia (5%) (Table 1).

Transtracheal aspirates were performed after diagnosis, and cultures were processed immediately. Fifteen different bacteria were isolated from the 40 transtracheal aspirate cultures. Mono-infections were observed in 68% of the transtracheal aspirates and mixed infections in 32%. The main microorganisms isolated in mono-infections were *Streptococcus equi* (37%), *Rhodococcus equi* (26%), and α-haemolytic *Streptococcus* (11%), whereas *Escherichia coli* was the main bacteria isolated in mixed infections (69%). *Rhodococcus equi* was isolated exclusively in foals aged two to six months. No strictly anaerobic bacteria were isolated.

The most effective antimicrobials against the isolates were azithromycin (92%), ceftiofur (72%), and gentamicin (72%) (Table 2). In contrast, higher resistance rates of isolates were observed for penicillin G (52%), ampicillin (50%), enrofloxacin (38%), rifampicin (35%) and sulfamethoxazole/trimethoprim (35%). When the efficacy of antimicrobials against individual agents was analyzed, azithromycin and gentamicin were ineffective against α-hemolytic streptococci; gentamicin was ineffective against *S. equi*, tetracycline was ineffective against *E. coli*, and ceftiofur was ineffective against *Klebsiella pneumoniae*. Conversely, sulfamethoxazole/trimethoprim was effective against *R. equi* and *K. pneumoniae*, although it is not the antibiotic of choice for these diseases *in vivo*. Penicillin was effective against *Nocardia* sp. and *Pasteurella multocida*, while ampicillin was effective against *E. coli* and *Nocardia* sp. Multidrug-resistant bacteria were observed in 36% of the isolates.

The vast majority of cases (37/40) were discharged from the hospital (Table 1). Three horses died: one with bronchopneumonia (*E. coli*) after 11 days, one with bronchopneumonia (*Enterobacter cloacae* + *P. multocida*) after 16 days, and one foal with rhodococcosis (*R. equi*) after 27 days of disease progression.

DISCUSSION

This study evaluated the diversity of etiologies involved in LDR infections in horses, finding a predominance of streptococci, enterobacteria, and *Rhodococcus equi* in mono- and coinfections. A high frequency of multidrug-resistant isolates was observed, particularly against conventional antimicrobials like penicillin, ampicillin, and sulfamethoxazole/trimethoprim, commonly used in Brazil for treating these infections. These findings highlight the need to understand the etiological profiles and antimicrobial susceptibility patterns of lower respiratory bacterial infections in horses across different regions to improve therapeutic approaches and implement effective preventive and control measures.

In this study, clinical outcomes were not associated with animal age or season, although bacterial LRD has been predominantly observed during cold or dry periods when aerosolization facilitates pathogen transmission (Constable et al. 2017). The main clinical signs observed in the horses were consistent with those commonly seen in respiratory diseases (Constable et al. 2017, Melo & Ferreira 2022). In horses, radiographs may reveal extensive interstitial and/or bronchointerstitial patterns, although diffusely distributed nodular infiltrates may also be present (Lakritz et al. 1993). Thoracic ultrasonography can detect abscesses, pleural effusion, comet tails, pleuritis, fibrin, gas, hydrothorax, lung shadowing, consolidation, atelectasis, and increased pulmonary volume (Morresey et al. 2014). Alongside physical examination, thoracic radiography and ultrasonography were essential for diagnosing bronchopneumonia, pleuropneumonia, interstitial pneumonia, asthma, and abscesses, demonstrating their value in providing early and detailed information on LRD in the horses studied. As observed in this study, leukocytosis

with neutrophilia and hyperfibrinogenemia are common hematological findings in equine lower respiratory infections (Reuss & Giguère 2015, Constable et al. 2017).

Streptococcus equi was the predominant agent recovered in pure cultures, while *Escherichia coli* was primarily detected in association with other microorganisms in aspirate samples. These findings align with previous studies that identified streptococci, enterobacteria, and certain actinomycetes as the most prevalent agents in lower respiratory infections in horses in other countries (Bailey & Love 1991, Ensink et al. 1993, Racklyeft & Love 2000, Reuss & Giguère 2015). Mixed pulmonary infections were also common, likely due to the synergistic interaction between bacteria in the upper respiratory tract of horses (Reuss & Giguère 2015).

Streptococcus species are Gram-positive bacteria commonly found on the skin, conjunctiva, and mucous membranes (particularly the upper respiratory tract) of livestock, and they can cause opportunistic infections in various organs and tissues (Racklyeft & Love 2000, Constable et al. 2017). In horses, *S. equi* subsp. *equi* and subsp. *zooepidemicus* are closely linked to strangles, a prevalent upper respiratory tract disease (Quinn et al. 2011). The high detection rates of *S. equi* and α -hemolytic streptococci, especially in mono-infections in horses with LRD, may be due to the opportunistic nature of these pathogens. Being present in the upper respiratory tract, they may infect the lungs secondary to certain predisposing conditions (Racklyeft & Love 2000, Constable et al. 2017). In this sense, *S. equi* subsp. *zooepidemicus* was an opportunistic pathogen identified in the lung fragments of a horse that died from bronchopneumonia secondary to the inhalation of an *Araucaria angustifolia* pine branch in southern Brazil (Molossi et al. 2022).

Table 1. Lower respiratory diseases (LRD) secondary to bacterial agents and outcome among 40 horses

LRD (number of cases)	Bacterial agents (number of isolates)	Outcome (successful treatment)
Bronchopneumonia (23/40)	<i>Streptococcus equi</i> (7) α -haemolytic <i>Streptococcus</i> (1) <i>Alcaligenes faecalis</i> (1) <i>Enterobacter aerogenes</i> (1) <i>Escherichia coli</i> (1) <i>Pasteurella multocida</i> (1) <i>Staphylococcus</i> sp. (1) <i>Enterobacter cloacae</i> / <i>P. multocida</i> (1) <i>E. coli</i> / <i>Klebsiella pneumoniae</i> (1) <i>E. coli</i> / <i>Edwardsiella tarda</i> (1) <i>E. coli</i> / <i>Salmonella</i> spp. (1) <i>E. coli</i> / <i>Staphylococcus</i> sp. (1) <i>E. coli</i> / <i>S. equi</i> (1) <i>E. coli</i> / α -haemolytic <i>Streptococcus</i> (1) <i>S. equi</i> / <i>E. cloacae</i> (1) <i>S. equi</i> / <i>Mannheimia haemolytica</i> (2)	91% (21/23)
Rhodococcosis (9/40)	<i>Rhodococcus equi</i> (7) <i>R. equi</i> / <i>E. coli</i> (2)	89% (8/9)
Pleuropneumonia (3/40)	<i>Actinobacillus lignieresii</i> (1) <i>S. equi</i> (1) α -haemolytic <i>Streptococcus</i> (1)	100% (3/3)
Asthma (3/40)	<i>Nocardia</i> sp. (1) <i>S. equi</i> (1) α -haemolytic <i>Streptococcus</i> (1)	100% (3/3)
Interstitial pneumonia (2/40)	<i>S. equi</i> (1) <i>E. coli</i> / <i>K. pneumoniae</i> (1)	100% (2/2)

Enterobacteria are a diverse group of bacteria that are part of the enteric microbiota of animals and are also found in the environment, feces, water, and contaminated farm utensils (Constable et al. 2017). In the aspirate sampled, *E. coli* was the most prevalent enterobacterium isolated, followed by *Enterobacter cloacae*, *Enterobacter aerogenes*, and *Salmonella* spp. These pathogens are responsible for enteric and extra-enteric infections in livestock and have various virulence factors (Quinn et al. 2011). As noted previously (Constable et al. 2017), the high occurrence of enterobacteria in LRD observed in the present study is likely due to systemic dissemination of these pathogens, mainly secondary to enteric infections. *Escherichia coli* was identified as the causative agent in five horses with varying degrees of pneumonia in northeastern Brazil, all of which recovered following treatment (Melo & Ferreira 2022). Additionally, as previously described (DebRoy et al. 2008), *E. coli* was detected in a fatal case of bronchopneumonia in this study.

Rhodococcus equi is an intracellular Gram-positive opportunistic pathogen that causes life-threatening pyogranulomatous pneumonia and ulcerative colitis in young foals (Giguère & Prescott 1997), while adult horses rarely show clinical rhodococcosis. Additionally, *R. equi*-induced infections in humans have increased globally, posing an emergent issue for both immunocompromised and immunocompetent individuals, particularly those with HIV/AIDS (Vázquez-Boland et al. 2013). In this study, *R. equi* was recovered mainly in mono-infections, exclusively in foals, consistent with other studies showing it as a major cause of bacterial pneumonia in foals up to six months of age (Giguère et al. 2004, Ribeiro et al. 2005, Vázquez-Boland et al. 2013). Interestingly, only one foal died from *R. equi*-induced infection despite the generally poor prognosis of rhodococcosis in young animals.

The first-choice therapy for rhodococcosis involves combining macrolides and rifamycin drugs, such as erythromycin and rifampicin (Giguère & Prescott 1997). However, erythromycin requires multiple daily doses, has variable absorption in foals,

Table 2. *In vitro* antimicrobial susceptibility of bacteria from transtracheal aspirate samples of 40 horses with lower respiratory diseases

Agents	AMP		AZM		CEF		ENR		GEN		PEN G		RIF		TET		SXT	
	R ^a	S ^b	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
<i>α</i> -haemolytic <i>Streptococcus</i>	50 ^c (1/2)	50 (1/2)	50 (1/2)	50 (1/2)	50 (2/4)	50 (2/4)	0 (0/2)	100 (2/2)	100 (1/1)	0 (0/1)	33 (2/6)	66 (4/6)	0 (0/1)	100 (1/1)	100 (1/1)	0 (0/1)	50 (1/2)	50 (1/2)
<i>A. lignieresii</i>	\ ^d	\	0 (0/1)	100 (1/1)	0 (0/1)	100 (1/1)	100 (1/1)	0 (0/1)	0 (0/1)	100 (1/1)	\	\	\	\	0 (0/1)	100 (1/1)	100 (1/1)	0 (0/1)
<i>A. faecalis</i>	\	\	\	\	100 (1/1)	0 (0/1)	0 (0/1)	100 (1/1)	0 (0/1)	100 (1/1)	\	\	\	\	\	\	0 (0/1)	100 (1/1)
<i>E. aerogenes</i>	\	\	\	\	100 (2/2)	0 (0/2)	100 (1/1)	0 (0/1)	0 (0/2)	100 (2/2)	\	\	\	\	\	\	0 (0/1)	100 (1/1)
<i>E. cloacae</i>	\	\	100 (1/1)	0 (0/0)	0 (0/1)	100 (1/1)	0 (0/1)	100 (1/1)	0 (0/1)	100 (1/1)	\	\	\	\	\	\	\	\
<i>E. coli</i>	0 (0/2)	100 (2/2)	0 (0/1)	100 (1/1)	66 (4/6)	33 (2/6)	0 (0/10)	100 (10/10)	50 (3/6)	50 (3/6)	100 (1/1)	0 (0/1)	50 (1/2)	50 (1/2)	33 (1/3)	66 (2/3)	33 (1/3)	66 (2/3)
<i>E. tarda</i>	\	\	\	\	\	\	0 (0/1)	100 (1/1)	100 (1/1)	0 (0/1)	\	\	100 (1/1)	0 (0/1)	\	\	\	\
<i>K. pneumoniae</i>	100 (2/2)	0 (0/2)	0 (0/1)	100 (1/1)	100 (1/1)	0 (0/1)	100 (1/1)	0 (0/1)	0 (0/1)	100 (1/1)	\	\	100 (1/1)	0 (0/1)	0 (0/1)	100 (1/1)	0 (0/1)	100 (1/1)
<i>M. haemolytica</i>	0 (0/1)	100 (1/1)	0 (0/1)	100 (1/1)	0 (0/1)	100 (1/1)	50 (1/2)	50 (1/2)	0 (0/1)	100 (1/1)	0 (0/1)	100 (1/1)	100 (2/2)	0 (0/2)	\	\	0 (0/1)	100 (1/1)
<i>Nocardia</i> sp.	0 (0/1)	100 (1/1)	\	\	0 (0/1)	100 (1/1)	0 (0/1)	100 (1/1)	0 (0/1)	100 (1/1)	0 (0/1)	100 (1/1)	0 (0/1)	100 (1/1)	0 (0/1)	100 (1/1)	100 (1/1)	0 (0/1)
<i>P. multocida</i>	\	\	0 (0/3)	100 (3/3)	0 (0/3)	100 (3/3)	50 (2/4)	50 (2/4)	0 (0/2)	100 (2/2)	0 (0/1)	100 (1/1)	\	\	\	\	\	\
<i>Salmonella</i> spp.	\	\	\	\	100 (1/1)	0 (0/1)	0 (0/1)	100 (1/1)	0 (0/1)	100 (1/1)	\	\	\	\	\	\	0 (0/1)	100 (1/1)
<i>Staphylococcus</i> sp.	50 (1/2)	50 (1/2)	0 (0/2)	100 (2/2)	0 (0/2)	100 (2/2)	0 (0/1)	100 (1/1)	0 (0/1)	100 (1/1)	\	\	50 (2/4)	50 (2/4)	\	\	100 (2/2)	0 (0/2)
<i>S. equi</i>	66 (4/6)	33 (2/6)	0 (0/3)	100 (3/3)	10 (1/10)	90 (9/10)	90 (9/10)	1 (1/10)	75 (3/4)	25 (1/4)	80 (4/5)	20 (1/5)	50 (1/2)	50 (1/2)	33 (1/3)	66 (2/3)	50 (1/2)	50 (1/2)
<i>R. equi</i>	\	\	0 (0/7)	100 (7/7)	11 (1/9)	89 (8/9)	0 (0/4)	100 (4/4)	0 (0/3)	100 (3/3)	100 (3/3)	0 (0/3)	0 (0/9)	100 (9/9)	\	\	0 (0/4)	100 (4/4)
TOTAL (%)	50 (8/16)	50 (8/16)	8 (2/25)	92 (23/25)	28 (13/46)	72 (33/46)	38 (17/45)	62 (28/45)	28 (8/29)	72 (21/29)	5 (10/19)	47 (9/19)	35 (8/23)	65 (15/23)	30 (3/10)	70 (7/10)	35 (7/20)	65 (13/20)

AMP = ampicillin, AZM = azithromycin, CEF = ceftiofur, ENR = enrofloxacin, GEN = gentamicin, PEN G = penicillin G, RIF = rifampicin, TET = tetracycline, SXT = sulfamethoxazole/trimethoprim, *A. lignieresii* = *Actinobacillus lignieresii*, *A. faecalis* = *Alcaligenes faecalis*, *E. aerogenes* = *Enterobacter aerogenes*, *E. cloacae* = *Enterobacter cloacae*, *E. coli* = *Escherichia coli*, *E. tarda* = *Edwardsiella tarda*, *K. pneumoniae* = *Klebsiella pneumoniae*, *M. haemolytica* = *Mannheimia haemolytica*, *P. multocida* = *Pasteurella multocida*, *S. equi* = *Streptococcus equi*, *R. equi* = *Rhodococcus equi*; ^a resistant, ^b sensitive, ^c % *in vitro* antimicrobial susceptibility, ^d not applicable.

and may cause adverse effects. Furthermore, azithromycin and clarithromycin have shown better results as alternatives to erythromycin (Giguère et al. 2004). In this study, *R. equi* isolates were not resistant to azithromycin or rifampicin. The successful treatment of *R. equi* in the sampled horses is likely due to early definitive diagnosis through microbiological culture of transtracheal aspirates and treatment based on the *in vitro* sensitivity patterns of the isolates (Vázquez-Boland et al. 2013, Constable et al. 2017).

Asthma may be associated with exposure to dust or other aerosols and is characterized by suppurative nasal discharge due to neutrophilic chemotaxis and increased respiratory tract hyperresponsiveness (Constable et al. 2017, Bond et al. 2017). *Streptococcus zooepidemicus*, *Streptococcus pneumoniae*, *Actinobacillus* spp., and *Mycoplasma equirhinis* have been isolated in asthma cases in horses (Bond et al. 2017). Conversely, in our study, *Nocardia* spp. and α -hemolytic streptococci were found in horses with clinical asthma. Therefore, bacterial agents can act as opportunists in cases of asthma in horses.

Pathogens recovered from 40 LRD cases, including *E. coli*, *R. equi*, *S. equi*, *Klebsiella pneumoniae*, *Pasteurella multocida*, and *Salmonella* spp., exhibit complex virulence mechanisms (Ribeiro et al. 2005, Quinn et al. 2011, Vázquez-Boland et al. 2013, Constable et al. 2017). However, the multifaceted virulence factors of these isolates were not investigated, which limits the current study.

β -lactams (penicillin, cephalosporins), aminoglycosides, and fluoroquinolones are first-choice antimicrobials for treating respiratory infections in horses (Racklyeft & Love 2000, Reuss & Giguère 2015). In the present study, azithromycin, ceftiofur, and gentamicin demonstrated *in vitro* efficacy of over 70% to isolates, suggesting these agents as effective alternatives for treating lower respiratory infections in horses in Brazil. However, higher resistance rates of isolates were observed for penicillin G and ampicillin. Discrepancies between the general efficacy of antimicrobials and their effectiveness against individual pathogens suggest that therapy should ideally be guided by *in vitro* sensitivity patterns of isolates (Ensink et al. 1993, Giguère et al. 2010).

The high resistance of isolates to penicillin and ampicillin may be attributed to their use for over four decades in the systemic treatment of equine infections (Giguère & Prescott 1997), including in Brazil (Melo et al. 2021, Melo & Ferreira 2022), where misuse could contribute to selective pressure for multidrug-resistant bacteria (Giguère et al. 2010). Additionally, 36% of bacteria recovered from sampled horses were multidrug-resistant. Responsible use of antimicrobials in livestock is crucial within the One Health framework, as these drugs need to be preserved for human therapy (Ribeiro et al. 2015).

Bronchopneumonia was the most common disease in horses with lower respiratory infections, with 91% of these horses being discharged. Other conditions, such as pleuropneumonia, asthma, and interstitial pneumonia, were less frequent but were cured in all cases. Despite the high pathogenicity of the isolated agents, these findings emphasize the importance of clinical examination, early diagnosis, and therapy based on *in vitro* sensitivity to improve cure rates.

Although the Veterinary Hospital of FMVZ-Unesp, Botucatu, serves animals from various regions of São Paulo state and neighboring states, studies that compile the findings of

isolation and antimicrobial resistance patterns, as well as the clinical findings of LRD in horses from various veterinary hospitals or training centers across different Brazilian states, would significantly contribute to advancing knowledge on this subject in Brazil.

CONCLUSION

We observed substantial bacterial diversity in lower respiratory infections in horses, with streptococci, enterobacteria, and *Rhodococcus equi* prevalent in mono- and coinfections. Multidrug-resistant isolates, notably penicillin and ampicillin, were remarkable. These results highlight the need to investigate bacterial profiles and antimicrobial susceptibility in equine pulmonary infections to improve treatment and prevention.

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Data availability statement.- The data presented are available in the clinical records of animals treated at the Large Animal Clinic of the Veterinary Hospital, "Faculdade de Medicina Veterinária e Zootecnia", "Universidade Estadual Paulista 'Júlio de Mesquita Filho'" (FMVZ-Unesp), Botucatu campus.

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