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Original article

Antimicrobial resistance in bacteria isolated from diseased horses in Poland, 2010-2022

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Abstract

The rise of antibiotic resistance is a global health crisis affecting human and veterinary medicine, highlighted within the One Health framework. Horses, classified as both meat and companion animals, play a crucial role in facilitating resistant bacteria spread to humans. Despite increased awareness and reduced antibiotic use in livestock, data on equine antibiotic use and resistance remain limited. This retrospective study examines antimicrobial resistance in 978 bacterial isolates from horses in Poland over 12 years (2010-2022), utilizing data from the Microbiological Diagnostic Laboratory at the Warsaw University of Life Sciences. The most common isolates were *Streptococcus* spp. (45.60%) and *Staphylococcus* spp. (19.22%). Clinical isolates resistance pattern observed in this study, particularly to gentamicin, tetracyclines, enrofloxacin, third-generation cephalosporins, and chloramphenicol, strongly suggests the overuse and misuse of these antibiotics, a trend that urgently needs to be addressed to preserve their efficacy. The detection of high-resistance *Rhodococcus equi* isolates resistant to erythromycin, rifampicin, and doxycycline may pose challenges for foal rhodococcosis treatment soon, especially considering the lack of alternative treatment.

The outcomes of this study show the urgent and critical need to collect and analyze local data for improved antimicrobial stewardship. They also emphasize the importance of continuous monitoring of resistance patterns in equine pathogens, as this is crucial for developing effective treatment strategies and staying ahead of potential threats.

Keywords: antibiotic, clinical isolates, epidemiology, equine, One Health, susceptibility

Introduction

Antimicrobial resistance (AMR) is a global health problem affecting human and veterinary medicine. It is recognized as a critical concern within One Health initiatives. Responsible antibiotic use is essential to prevent bacterial resistance, especially for antibiotics used in treating both humans and animals, highlighting our shared responsibility (Slater 2015, White et al. 2019, EFSA Panel on Animal Health and Welfare (AHAW) et al. 2022, Gehring et al. 2023, Tyrnenopoulou et al. 2023, WHO 2024).

Horses serve diverse societal roles, from farm/meat, race, and sport to companionship and biodiversity efforts. Their close interaction with humans facilitates the exchange of resistant strains, highlighting their pivotal role in the One Health framework, which aims to maintain the health of people, animals, and ecosystems (Slater 2015, Groves et al. 2016, Waqar et al. 2019).

Antimicrobial use leads to resistance and increased European efforts to improve veterinary antibiotic prescription rules. Prudent antibiotic use is essential for preserving their effectiveness and protecting public health. In the EU, most critical antibiotics, vital for severe human infections, are not authorized for veterinary use (EFSA Panel on Animal Health and Welfare (AHAW) et al. 2022). Data from antimicrobial usage monitoring programs for livestock animals in European countries indicate a decreasing trend in the sales of all antibiotic-active substances for food-producing animals over the last decade. However, in Poland, between 2010 and 2022, antimicrobials sales nearly doubled, highlighting an extraordinary trend in antimicrobial usage (European Medicines Agency 2022).

Although AMR within food animal production has garnered substantial attention, the extent of AMR in horses and companion animals has been underestimated. Data on equine medicine and antimicrobial use are limited, with most official reports relying on general veterinary medicinal product registers that do not specify individual animals. This makes it challenging to attribute drug usage to specific species, and off-label use of human medications in horses often goes unreported (Schnepf et al. 2020, Prouillac 2021, Redpath et al. 2021, Bollig et al. 2022, Jacobsen et al. 2023, Tallon et al. 2024). Recent studies indicate that AMR in horses is a growing concern, as resistant bacteria can transfer resistance to human-associated strains, complicating infection control (Maddox et al. 2015, Slater 2015, Alvarez-Narvaez et al. 2020, Leonard 2020, Steinman et al. 2020, Tyrnenopoulou et al. 2023).

In the EU, horses are considered meat animals, influencing antibiotic regulations. While most critical antibiotics for human health are banned in food-producing animals, they may be used in non-food horses, such as sports or racehorses, under certain conditions. Equine vets must balance individual horse health with the broader implications of antimicrobial use, as highly resistant bacteria increase the risks of morbidity, mortality, and treatment costs (Bowen et al. 2015, Leonard 2020, Steinman et al. 2020, Arafa et al. 2021, Knych et al. 2021).

Antimicrobial stewardship programs, practice policies, guidelines, and toolkits exist in some countries to promote the responsible treatment of horses. Those guidelines are based on fundamental principles, including data from the literature or implemented local surveillance systems (infection likelihood, bacterial identification, resistance data), and have proven effective (Bowen et al. 2015, Weese 2015, Wilson et al. 2021, Wilson et al. 2023). Limited data from specific countries where those initiatives have been implemented indicate a similar trend in equine medicine with an apparent overall decrease in antimicrobial usage (Schnepf et al. 2020, Tallon et al. 2024). This decline is noteworthy even in the context of high-priority critical antimicrobial utilization within equine practice.

However, large-scale and long-term data on antibiotic use and AMR in equine medicine remain scarce. Furthermore, pathogen types and resistance profiles vary significantly across regions. Thus, access to up-to-date data regarding antibiotic use, local pathogens, and their AMR profiles is essential for making right decisions about empirical antimicrobial therapy in horses (Weese 2015, Alvarez-Narvaez et al. 2020, Knych et al. 2021, Wilson et al. 2021, Wilson et al. 2023).

To date, limited work has been undertaken to evaluate risk factors and the epidemiology of AMR in bacteria from horses in Poland (Wolny-Koladka et al. 2018, Kalinowski et al. 2020). This study aimed to provide valuable insights into the occurrence of equine bacterial pathogens and AMR in equine medicine in Poland.

Materials and Methods

The Microbiological Diagnostic Laboratory of the Institute of Veterinary Medicine of the Warsaw University of Life Sciences database was retrospectively analyzed for susceptibility testing results from bacterial pathogens cultured from horses from January 2010 to August 2022. Considering the diversity of the collected material, the authors decided to divide it into ten categories: respiratory airways (guttural pouch washes, tracheobronchial aspirates, bronchoalveolar lavages), cerebrospinal fluid (CSF), abscesses (different locations), nasal swabs, cutaneous (skin scrapes and wound swabs), tissues, sinuses, feces, synovial fluid, and genital.

The samples were investigated using standard bacteriological methods, mainly by bacterial growth on aerobic culture. When ordered, procedures for anaerobic bacteria were used. Gram staining, catalase, oxidase, oxidation-fermentation, agglutination, and CAMP tests were used. The API biochemical test (bioMerieux) was used to identify the species level.

Using the disk diffusion method, AMR was tested according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations (EUCAST 2024). Based on zone diameters, isolates were deemed susceptible, intermediate, or resistant. Intermediate susceptibility was classified as resistant.

Ethical review and approval were waived for this study due to Directive 2010/63/EU and the Act of the Polish Parliament of 15 January 2015 on the protection of animals used for scientific purposes (Journal of Laws 2015, item 266).

Results

The most numerous samples were nasal swabs (45.60%), tissues (12.26%), respiratory samples (12.14%), genital (12.14%), and abscesses in different locations (9.52%) (Table 1).

Between 2010 and 2022, 1680 clinical samples were investigated, and 978 clinically significant bacteria isolates were collected. The most commonly detected were *Streptococcus spp*., accounting for 45.60% of the isolates, followed by *Staphylococcus* spp., at 19.22% (Table 2). No pathogenic bacteria growth (not proven to have any clinical importance in horses) was recorded in 496 samples. The microbiological examination outcome was described as “saprophytic flora” or “no pathological bacteria growth.”

The available data allowed for summarizing the results for several antimicrobials, including amoxicillin (AMO), amoxicillin-clavulanate (AMC), benzylpenicillin (BEN), cefepime (FEP), cefoperazone (CFP), cefpodoxime (CPO), ceftiofur (CFT), cephalothin (CEF), chloramphenicol (CHL), clindamycin (CLI), colistin (CST), doxycycline (DOX), enrofloxacin (ENR), erythromycin (ERY), florfenicol (FLO), gentamicin (GEN), penicillin (PEN), rifampin (RIF), tetracycline (TET), trimethoprim-sulfamethoxazole (SXT). AMR for investigated isolates is shown in Fig. 1.

Discussion

According to the best authors’ knowledge, this is the first report describing the analysis of AMR of equine pathogens isolated from equine clinical samples in Poland. This retrospective study is subject to several typical limitations inherent to projects of this nature. Firstly, the available data did not permit the identification of the disease status of the animals. Additionally, there was variability in the number and types of clinical sample submissions throughout the study period. Most samples were provided by a limited number of equine practitioners primarily operating in the Warsaw region or sourced from equine farms collaborating with the authors. It may be assumed that most samples came from horses used as companion animals. Furthermore, certain sample types and bacterial isolations, such as abscesses and *Rhodococcus equi*, may be overrepresented due to specific research projects focusing on these pathogens. Therefore, it is essential to acknowledge that the samples investigated in this study may not directly reflect the prevalence of bacterial diseases and AMR among the horse population in Poland.

The analyzed database system underwent several changes over the 12 years (from 2010 to 2022). As a result, there was a lack of precise and consistent information regarding the investigated samples and the origin of bacterial isolates. At times, all sample types and isolates obtained from a single animal were assigned the same identification number, and the source of bacterial isolation was not recorded. Conversely, on other occasions, each sample from the same animal received a distinct identification number, and individual isolates were coded separately with different identification numbers. Moreover, neither the number of 1680 samples nor 978 clinically significant isolates reflects the number of animals tested. Various sample types or the same material collected at intervals were investigated from a single animal. Only a single isolate was routinely tested for clinically significant bacteria isolation. In some cases, different pathogenic bacteria were isolated from a single case.

From 2010 to 2022, bacteria isolates were assessed for their susceptibility to various panels of antibiotics, reflecting the antimicrobials utilized. Due to this variability in antimicrobial usage, comparing the resistance of frequently isolated bacteria to commonly used antimicrobials across different periods was not feasible. Although lincosamides are typically not used in horses due to severe adverse effects in the gastrointestinal tract, clindamycin was still included in susceptibility evaluation.

It should be noted that a false negative result could have been obtained in some cases. Inappropriate material collection, storage, or transport (especially nose swabs) might been a reason. Antibiotic usage can not be excluded because clinical data, including previous antimicrobial treatment, were often scarce or unavailable. Bacterial growth might also have been classified

Table 1. Number of investigated sample types and bacteria species isolated from each material type from diseased horses in Poland over the 2010-2022 period.

Type of sample	Number of samples	% of samples	Identified microorganism	% of each origin isolates
Nasal swabs	766	45.60	*Streptococcus* β-hem.	66.59
			Staphylococcus aureus	19.69
			Pseudomonas aeruginosa	9.59
			Klebsiella ssp.	1.55
			Staphylococcus aureus MRSA	1.03
			Staphylococcus delphini B	1.03
			Staphylococcus intermedius	0.52
Tissues	206	12.26	*Streptococcus* β-hem.	31.65
			Klebsiella ssp.	22.30
			Escherichia coli	15.83
			Pseudomonas aeruginosa	12.23
			Rhodococcus equi	10.08
			Enterococcus ssp.	5.75
			Clostridium perfringens	0.73
			Pasteurella spp.	1.44
Respiratory airways	204	12.14	*Pseudomonas aeruginosa*	52.38
			Streptococcus β-hem.	31.75
			Rhodococcus equi	4.86
			Escherichia coli	4.70
			Staphylococcus aureus	4.70
			Staphylococcus intermedius	1.62
Genital	160	9.52	*Streptococcus ssp.*	52.24
			Escherichia coli	23.88
			Corynebacterium spp.	20.89
			Klebsiella pneumoniae	2.98
Abscesses	146	8.69	*Streptococcus* β-hem.	28.21
			Rhodococcus equi	9.83
			Klebsiella spp.	5.13
			Staphylococcus aureus	4.70
			Pseudomonas aeruginosa	1.28
			Clostridium ssp.	0.85
Skin scrapes and wound swabs	105	6.25	*Streptococcus* β-hem.	46.15
			Staphylococcus aureus	36.54
			Pseudomonas aeruginosa	9.61
			Corynebacterium spp.	5.77
			Staphylococcus aureus MRSA	1.92
Feces	40	2.38	*Streptococcus* β-hem.	63.64
			Staphylococcus aureus	18.18
			Clostridium perfringens	9.09
			Klebsiella spp.	9.09
Synovial fluid	29	1.73	*Rhodococcus equi*	42.85
			Staphylococcus spp.	34.50
			Streptococcus spp.	34.50
			Klebsiella spp.	33.33
			Escherichia coli	9.53
			Enterococcus ssp.	4.76
Sinuses	16	0.95	*Streptococcus* β-hem.	46.15
			Klebsiella spp.	30.77
			Acinetobacter spp.	15.38
			Pseudomonas aeruginosa	7.69
Cerebrospinal fluid (CSF)	8	0.48	*Klebsiella* spp.	50
			Streptococcus β-hem.	50
Total	**1680**	**100.00**		

Table 2. Bacterial species isolated from diseased horses in Poland over the 2010-2022 period.

Identified microorganism	Total number of isolates	% of isolates
Streptococcus spp.	446	45.60
Staphylococcus spp.	188	19.22
Escherichia coli	72	7.36
Pseudomonas spp.	72	7.36
Klebsiella spp.	58	5.93
Rhodococcus equi	49	5.01
Enterococcus spp.	33	3.37
Proteus spp.	32	3.27
Corynebacterium spp.	28	2.86
Total	978	100.00

as saprophytic flora by a human error that disrupted data analysis.

Many of the isolates in the database were identified only at the genus level rather than the species level. Although further strain identification was occasionally conducted, this data was often not recorded in the database. Due to these inconsistencies, the available resources only permit partial data analysis. Taking all of the above, the results presented in this study should be interpreted with caution. Despite these limitations, the data provide crucial information about the occurrence of antibiotic resistance between isolates from clinical cases.

Streptococcus spp. was the primarily detected pathogen (45.60%) across various sample types, except for respiratory samples and synovial and cerebrospinal fluids. Nasal swabs and feces were the most common sources of Streptococcus spp. isolates, comprising 66.59% and 63.64%, respectively, followed by genital and sinus samples at 52.38% and 46.15%, respectively.

While strain identification data were only partially available in the database, among the 446 *Streptococcus* spp. isolates, 412 (92.38%) were identified as beta-hemolytic strains, 8 (1.79%) as alpha-hemolytic strains, and 26 (5.86%) as *Streptococcus equi* subsp. *equi*. The bacteria occurrence findings are consistent with previous studies, highlighting *Streptococcus* spp. as a prevalent cause of bacterial infections in horses. Beta-hemolytic Streptococci, particularly *Streptococcus equi subsp. zooepidemicus*, sometimes also *Streptococcus dysgalactiae* subsp. *equisimilis* are frequently isolated from clinical cases, especially genital and respiratory samples (Awosile et al. 2018, Bourely et al. 2020, Rathbone et al. 2023). Strangles caused by *Streptococcus equi* subsp. *equi* is among the most widespread diseases affecting horses globally (Mitchell et al. 2021, Vercruysse et al. 2022). Therefore, the actual number of *Streptococcus equi* subsp. *equi* among isolates may be higher, particularly considering that 111 out of 446 beta-hemolytic Streptococci were obtained from guttural pouch washes, sinuses, and abscesses – locations commonly associated with strangles infection.

Despite their significance as equine pathogens, little is known about the resistance patterns of *Streptococcus* spp. in horses. However, recent findings have indicated that streptococcal resistance can increase sharply within a short timeframe (Arafa et al. 2021, Lord et al. 2022, Malaluang et al. 2022, Nocera et al. 2022). *Streptococcus* spp. has been considered susceptible to penicillin, and this study reaffirmed their susceptibility to penicillin among the investigated isolates. There was little discernible difference in susceptibility between penicillin and other beta-lactam antimicrobials (Fonseca et al. 2020). Over 90% of the isolates remained susceptible to amoxicillin, amoxicillin/clavulanate, and third-generation cephalosporins. Higher resistance rates were observed for phenicols, particularly chloramphenicol, and tetracyclines. Additionally, over 90% of the isolates were resistant to clindamycin, gentamicin, and enrofloxacin. This contrasts with the majority of previous studies, where gentamicin and enrofloxacin were found to be effective against streptococcal isolates. For instance, the overall susceptibility of *Streptococcus* spp. to gentamicin was reported to be close to 90% (Van den Eede et al. 2013, Toombs-Ruane et al. 2015, Bourely et al. 2020, Chalder et al. 2020, Arafa et al. 2021, Yuen et al. 2021, Anwaar et al. 2023). High levels of resistance may be attributed to methodological biases in susceptibility testing. Streptococci are inherently resistant to low concentrations of aminoglycosides, and accurate susceptibility evaluation requires high-concentration discs (Schoster et al. 2021). Unfortunately, the documentation analyzed in this study did not specify the type of disc used for susceptibility testing. However, an alternative explanation is more plausible. The aminoglycoside antibiotics are commonly used in equine practice (Redpath et al. 2021). While gentamicin may not be the ideal first-choice drug, it is frequently used in horses in Poland. Consequently, the overuse of this drug may contribute to high resistance.

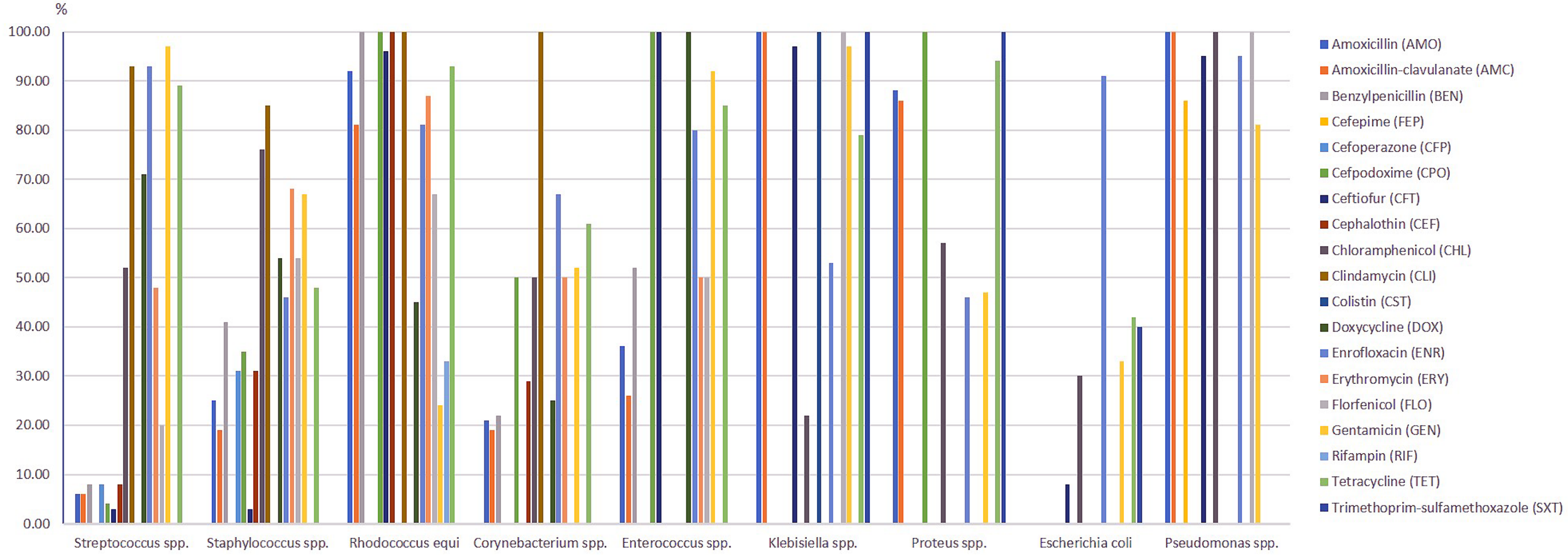

Fig. 1. Percentage of resistant isolates of bacteria isolated from diseased horses in Poland over the 2010-2022 period.

Coagulase-positive *Staphylococcus* spp. are part of the normal skin flora and were commonly recovered from abscesses, joints, wounds, surgical sites, skin, and samples from the genital and respiratory tracts. Methicillin-resistant *Staphylococcus aureus* (MRSA) represents a global threat that has become a major clinical challenge. *Staphylococcus* spp. emerged as the second most frequently isolated bacteria throughout the study period, consistent with findings from other studies utilizing retrospective data from diagnostic laboratories (Tirosh-Levy et al. 2015, Guérin et al. 2017, Oguttu et al. 2017, Adams et al. 2018, Albert et al. 2019, Chalder et al. 2020, Roudaud et al. 2020, Yuen et al. 2021, EFSA Panel on Animal Health and Welfare (AHAW) et al. 2022, Malaluang et al. 2022, Vercruysse et al. 2022, Anwaar et al. 2023, Nwobi et al. 2023, Rasheed et al. 2023).

In our study, *Staphylococcus* spp. were primarily detected in skin and wound samples, synovial fluid, nasal swabs, and fecal samples, with fewer isolates obtained from respiratory tract and abscess samples. Of the 188 isolates, the majority (64%) were identified as *Staphylococcus aureus*, including MRSA (3%). *Staph. intermedius* (3%), *Staph. delphini* accounted for 2% of isolates each.

Ceftiofur demonstrated the highest susceptibility rate (97%) against investigated *Staph. aureus* isolates and was the most effective antimicrobial overall. Beta-lactams, in general, exhibited the highest efficacy. Higher resistance levels were observed for tetracyclines, gentamicin, and erythromycin. The most significant resistance was detected against chloramphenicol and clindamycin.

Escherichia coli, naturally occurring in the digestive tracts of animals, can be implicated in various conditions such as gastrointestinal disorders, urinary tract infections, and septicemia (Awosile et al. 2018, Dziubinski et al. 2020, Bookbinder et al. 2023). Diarrheagenic *Escherichia coli* (DEC) is classified as a particular concern in both veterinary and human medicine. Given the close interaction between horses and humans, transmitting virulent *E. coli* is considered a public health issue. A growing trend in resistance among *E. coli* isolates, including multi-drug-resistant (MDR) strains, is observed. Among the common genera and species, *E. coli* is frequently encountered in horses, although our findings differ from those of previous studies. In this study, *E. coli* isolates comprised 7.36% of the total bacteria isolates and were primarily obtained from genital, tissue, synovial fluid, and respiratory samples. *E. coli* resistance is commonly encountered, with β-lactam resistance representing a particular concern (Fonseca et al. 2020, Malaluang et al. 2022, Malaluang et al. 2023). Interestingly, no resistance to amoxicillin/clavulanate and amoxicillin was observed over the study period, and susceptibility to ceftiofur exceeded 90%. Resistance rates between 30% and 42% were observed for chloramphenicol, gentamicin, sulfamethoxazole, and tetracycline. Over 90% of the *E. coli* strains were resistant to enrofloxacin. This is particularly worrisome as enrofloxacin is an antimicrobial highly relevant to human and equine medicine but not surprising.

The development of *E. coli* resistance against enrofloxacin has been documented in human and equine populations, and the growing number of equine isolates with resistance to enrofloxacin has been observed in the last decades (Sanchez et al. 2008, Malo et al. 2016).

Pseudomonas aeruginosa is a major opportunistic pathogen in human medicine, capable of forming biofilms and commonly encountered multi-drug resistance. It can develop resistance rapidly and is intrinsically resistant to many antimicrobials, such as beta-lactams (Fonseca et al. 2020, Ammazzalorso et al. 2024). AMR data in horses are limited, making our study a significant contribution to understanding *Pseudomonas* spp. resistance in equine populations (Maddox et al. 2015, Chipangura et al. 2017, Duchesne et al. 2019, Bourely et al. 2020, Fonseca et al. 2020, Leon et al. 2020, Vercruysse et al. 2022). Bacterium in horses is often associated with endometritis in mares. However, no isolates were obtained from genital samples in our study. *Pseudomonas aeruginosa* constituted 7.36% of the total bacterial isolates, primarily being isolated from the respiratory tract, with the highest proportions from respiratory airways (52.38%), followed by nasal swabs (9.59%) and sinuses (7.69%). Additionally, isolates were obtained from tissue samples (12.23%), skin (9.61%), and abscesses (1.28%). All 72 isolates in our study showed high resistance levels. Previous research noted the most substantial resistance in *Pseudomonas* spp. to gentamicin and enrofloxacin, with resistance proportions ranging from 30% to 50% and showing a decreasing trend. Extremely high resistance rates were observed in our study, ranging from 81% to 86% for gentamicin and chloramphenicol, 95% for enrofloxacin, and 100% for other investigated antimicrobials. These findings differ from those reported in the literature, where these antimicrobial agents remained active against *Pseudomonas aeruginosa* (Ammazzalorso et al. 2024).

Klebsiella pneumoniae is commonly found in the normal urogenital and intestinal flora of humans and animals. The World Health Organization recognizes multidrug-resistant *Klebsiella pneumoniae* strains as a global concern (WHO 2024). While it ranks as the third most common cause of healthcare-associated infections in humans, it is also frequently isolated in horses, where it can lead to conditions such as metritis, infertility, pneumonia, and sepsis (Estell et al. 2016, Duchesne et al. 2019, Bourely et al. 2020, Leon et al. 2020, Ribeiro et al. 2022, Gravey et al. 2024). In our study, 58 *Klebsiella pneumoniae* isolates accounted for 5.93% of the total bacterial isolates. These isolates were often obtained from cerebrospinal fluid, synovial fluid, and tissues, with individual isolates retrieved from genital samples. Data on the resistance of *Klebsiella pneumoniae* to commonly used antimicrobials in equine medicine differ. Some studies suggest the susceptibility of isolates from mare uterine infections to widely used antimicrobials. In contrast, others report a significant prevalence of multidrug-resistant strains, including hypervirulent strains (Estell et al. 2016, Chipangura et al. 2017, Awosile et al. 2018, Duchesne et al. 2019, Leon et al. 2020, Ribeiro et al. 2022, Bookbinder et al. 2023, Rathbone et al. 2023, Tyrnenopoulou et al. 2023, Gravey et al. 2024). In our study, all investigated strains were susceptible to cefepime, and the majority (78%) exhibited susceptibility to chloramphenicol. However, most isolates showed resistance to other antimicrobials. While significant resistance to aminoglycosides and beta-lactams has been reported in previous studies, the extraordinarily high resistance levels observed in our study, such as 97% resistance to gentamicin and 100% to amoxicillin, amoxicillin/clavulanate and other antimicrobials are notable.

Rhodococcus equi is widespread in the environment and has emerged as an essential challenge in the global equine breeding industry; a soil saprophyte that is part of the gastrointestinal flora of grazing animals poses a significant threat in equine medicine as the causative agent of foal rhodococcosis, a highly fatal disease primarily characterized by pyogranulomatous bronchopneumonia (Rakowska et al. 2020, Bordin et al. 2022, Takai et al. 2023). Additionally, cases of infection in other species, including humans, have been reported, highlighting *R. equi* potential as an emerging zoonotic pathogen (Witkowski et al. 2015, Witkowski et al. 2016, Zychska et al. 2021, EFSA Panel on Animal Health and Welfare (AHAW) et al. 2022). In our study, *R. equi* was detected in synovial fluid, abscesses, tissues, and respiratory tract samples, typical sites for lesions associated with rhodococcosis in infected foals (Rakowska et al. 2022). The availability of effective and safe alternative treatment methods is limited to the combination of macrolides and rifampicin. It has been the standard therapy for foals with *R. equi* pneumonia for decades, increasing antimicrobial resistance to these drugs. Only one alternative antibiotic turned out to be partially effective, a doxycycline. Increasing resistance of *R. equi* to macrolides and rifampicin is particularly concerning (Alvarez-Narvaez et al. 2020, Higgins et al. 2023). These drugs are widely used in human medicine to treat tuberculosis, with macrolides designated as medically essential drugs by the World Health Organization (WHO 2024). Furthermore, recent reports highlighted the alarming prevalence of resistant isolates in the environment, with concerns growing over the emergence of multidrug-resistant *R. equi* (MDR-RE) (Huber et al. 2019, Erol et al. 2020, Kalinowski et al. 2020, Alvarez-Narvaez, Giguere, et al. 2021, Alvarez-Narvaez, Huber, et al. 2021, Bordin et al. 2022, Lord et al. 2022, Zuniga et al. 2023, Higgins et al. 2024, Vazquez-Boland et al. 2024). In our study, a high proportion (87%) of the 49 investigated strains exhibited resistance to erythromycin, while 33% showed resistance to rifampicin. Because of the severe adverse effects caused by erythromycin, it is not used in equine practice, and most foals receive clarithromycin or azithromycin. In previous studies, reported resistance to those drugs was linked to erythromycin. This poses challenges for treatment, especially considering the lack of alternatives available. Doxycycline is recommended in monotherapy or combined with azithromycin in case of rifampin-resistant strain infection. Detected high resistance (45%) significantly affects this option. Extremely high in vitro resistance to other antimicrobials is less significant. Because those drugs lack clinical efficacy *in vivo* in *R. equi* infection cases, they are not used in therapy.

Epidemiological studies regarding enterococci in horses are lacking. *Enterococci*, traditionally regarded as commensal bacteria in human and animal medicine, have recently emerged as significant nosocomial pathogens, mainly due to antimicrobial resistance. For example, the prevalence of enterococci in foal sepsis has increased over recent decades (Bowen et al. 2015, Kim et al. 2016, Willis et al. 2019, Alvarez-Narvaez et al. 2020, Knych et al. 2021, Williams et al. 2022). It is in line with the data analyzed in this study. *Enterococcus* spp. isolates were predominantly obtained from tissue and synovial fluid samples, constituting 3.37% of isolated bacteria over the study period. All enterococci are intrinsically resistant to cephalosporins and aminoglycosides. They are now recognized as potential reservoirs of resistance genes, capable of transmitting them to strains found in the environment, animals, and humans (Billington et al. 2014, Redpath et al. 2021). The observed resistance to most tested antimicrobials was notably high. All isolates exhibited resistance to ceftiofur, cefpodoxime, and doxycycline. However, amoxicillin and amoxicillin//clavulanate demonstrated relatively higher effectiveness, with susceptibility rates among isolates at 64% and 74%, respectively. These findings underscore the importance of vigilant antimicrobial stewardship practices to mitigate the spread of resistant enterococci strains in veterinary and human healthcare settings (Toombs-Ruane et al. 2015, Chipangura et al. 2017, Yuen et al. 2021, Isgren et al. 2022, Malaluang et al. 2023).

The analyzed database contained antibiotic resistance evaluation of 32 *Proteus* spp. isolates recognized as clinically significant bacteria but without additional information on the sample type. Literature on *Proteus* spp. antimicrobial resistance patterns among horse isolates are limited. However, studies on small animals suggest that clinical isolates are commonly derived from skin and genital samples, with associated resistance percentages reported (Vercelli et al. 2021, Yuen et al. 2021, Isgren et al. 2022). In our investigation, all examined isolates demonstrated susceptibility to penicillin and florfenicol. Conversely, the highest resistance rates were observed against sulfamethoxazole, cefpodoxime (100%), and tetracycline (94%), findings consistent with prior reports (Robinson et al. 2016, Chipangura et al. 2017, Yuen et al. 2021, Vercruysse et al. 2022). These results emphasize the importance of continued surveillance and appropriate antibiotic stewardship practices to manage antimicrobial resistance in *Proteus* spp – infections among horses.

Corynebacterium pseudotuberculosis biovar Equi is an equine pathogen responsible for various clinical manifestations, such as external and internal abscesses and ulcerative lymphangitis (Britz et al. 2014). However, data on antibiotic resistance in this bacterium are limited. Evaluation of equine isolates of *C. pseudotuberculosis* has indicated that commonly used antimicrobials remain effective against the bacterium (Britz et al. 2014, Barba et al. 2015, Rhodes et al. 2015, Isgren et al. 2022). In our study, all 28 isolates were derived from genital and cutaneous samples. Notably, all isolates demonstrated susceptibility to florfenicol, while resistance to clindamycin was universal. Resistance to other antimicrobials varied between 19% and 67% among the isolates. These findings highlight the importance of continued surveillance of antimicrobial resistance patterns in *C. pseudotuberculosis* biovar Equi to guide effective treatment strategies for horse infections.

Our results describe the local situation based on a limited number of isolates, but despite several limitations, they provide crucial information on antimicrobial resistance in horses in Poland. Moreover, such data shed light on the evolving landscape of antimicrobial resistance within local equine populations but with a significant impact. Familiarity with local antimicrobial susceptibility patterns data is crucial and enables informed and prudent decision-making in the early stages of treatment.

Most available resources give data on local or regional importance. The main pathogenic bacteria types and resistance profiles are highly variable and depend on local conditions. It isn't easy to compare the data obtained in this study with those of other studies (Toombs-Ruane et al. 2015, Cummings et al. 2016, Toombs-Ruane et al. 2016, Chipangura et al. 2017, Awosile et al. 2018, Duchesne et al. 2019, Bourely et al. 2020, Chalder et al. 2020, Fonseca et al. 2020, Leonard 2020, Mustikka et al. 2020, Sukmawinata et al. 2020, Yuen et al. 2021, Isgren et al. 2022, Bookbinder et al. 2023, Rathbone et al. 2023).

The differences between the studies may be due to differences in study design or the geographical distribution of different bacteria clones. Still, they also suggest differences in antimicrobial use between countries. Low regulations, implemented or unsustainable stewardship, drug availability, or individual preferences of equine practitioners all impact this (Schnepf et al. 2020, Knych et al. 2021, Prouillac 2021, Redpath et al. 2021, Jacobsen et al. 2023, Tallon et al. 2024). Thus, monitoring antibiotic usage is an integral part of tackling antimicrobial resistance because antimicrobial resistance depends on the amount and type of antibiotics used.

Our study described significant and worrisomely high or very high resistance levels, which should be highlighted. Antibiotic resistance of horse pathogens might seriously threaten veterinary medicine and public health, which is essential to the One Health initiative. The high resistance to gentamicin, tetracyclines, enrofloxacin, and third-generation cephalosporins in Poland likely results from overuse. The surprising resistance to chloramphenicol, which is banned in EU food animals but used in Poland, especially for local treatment of skin infections in horses with companion animal status.

There is an urgent need to create a surveillance system for equine pathogens in Poland and develop veterinary antimicrobial stewardship to encourage proper antibiotic use in horses.

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