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Antibiotic Susceptibility Patterns of Bacterial Isolates from Equine Clinical Submissions in Texas and Oklahoma, 2011–2024

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Abstract

This study characterized the epidemiology and antimicrobial resistance patterns of bacterial isolates recovered from equine clinical submissions in Texas and Oklahoma between 2011 and 2024. A retrospective observational analysis was conducted using bacterial culture and antimicrobial susceptibility testing records from equine samples. Data was cleaned and standardized prior to analysis. The distribution of bacterial organisms, anatomical sites of infection, antimicrobial susceptibility across major drug classes, and prevalence of multidrug resistance (MDR) were evaluated, with MDR defined as resistance to three or more antimicrobial classes. A total of 2,812 bacterial isolates were included. The most frequently identified organisms were *Streptococcus equi* subsp. *zooepidemicus*, *Escherichia coli*, *Staphylococcus* spp., *Pseudomonas* spp., and *Klebsiella* spp. Most isolates originated from reproductive, integumentary/wound, and respiratory sites. Antimicrobial susceptibility varied across organisms and drug classes, with generally high susceptibility to fluoroquinolones and aminoglycosides, and greater variability observed for β-lactams and tetracyclines. Multidrug resistance was identified in 19.0% of isolates and was more common among Gram-negative organisms. These findings highlight the diversity of antimicrobial susceptibility patterns among equine bacterial pathogens and underscore the importance of routine culture and susceptibility testing to guide therapy and support antimicrobial stewardship in equine practice.

Introduction

Antimicrobial resistance (AMR), particularly bacterial AMR, is a growing global health threat that reduces the effectiveness of infection treatment (Ho et al., 2025). In veterinary medicine, antimicrobial use for prevention and treatment contributes to the emergence of resistant microorganisms, challenging stewardship efforts within the One Health framework (Ekakoro and Okafor, 2019).

In horses, antimicrobials are commonly used to treat bacterial infections across multiple body systems (Haggett and Wilson, 2008). However, rising antimicrobial resistance (AMR), including multidrug-resistant (MDR) bacteria, poses significant risks to both animal and human health, as horses can act as reservoirs for zoonotic pathogens (Kabir et al., 2024). Inappropriate antimicrobial use—often driven by factors such as client pressure, fear of clinical deterioration, and perceived low risk—further accelerates resistance development (Hardefeldt et al., 2018; 2021). This highlights the need for improved stewardship within the equine industry.

Equine bacterial infections are caused by diverse Gram-positive and Gram-negative organisms, including *Streptococcus equi* subsp. *zooepidemicus*, *Staphylococcus* spp., *Escherichia coli*, and *Pseudomonas* spp. (Nielsen et al., 2021), affecting multiple body systems. Antimicrobial susceptibility testing (AST) guides evidence-based therapy and supports stewardship in clinical practice. However, comprehensive epidemiological assessments of equine AMR remain limited, with most studies focused on specific diseases, locations, or short timeframes. As a result, long-term trends in bacterial distribution,

antimicrobial susceptibility, and multidrug resistance—and their relationship with anatomical infection sites—are insufficiently understood.

Therefore, this study aimed to evaluate and characterize the epidemiology and antimicrobial resistance (AMR) patterns of bacterial isolates from equine clinical samples submitted to commercial laboratories in Texas and Oklahoma from 2011 to 2024. Specifically, it describes the distribution of bacterial organisms, assesses their association with infection sites, evaluates antimicrobial susceptibility across major drug classes, and quantifies the prevalence and distribution of multidrug-resistant isolates.

Methodology

This study used data from equine clinical samples submitted by veterinarians for routine bacterial culture and antimicrobial susceptibility testing (AST) to commercial diagnostic laboratories in Texas and Oklahoma between 2011 and 2021. Records included bacterial identification, submission year, anatomical site, and AST results with corresponding antimicrobial agents.

Data were cleaned and standardized in Microsoft Excel by removing duplicates and harmonizing anatomical sites into major system-based categories, including reproductive, integument/wound, respiratory, musculoskeletal/neurologic, gastrointestinal, urinary, ocular, otic, mammary, blood, and other/unknown. This classification enabled consistent evaluation of pathogen distribution and analysis of associations between bacterial species and infection sites.

AST results, originally reported as susceptible (S), intermediate (I), or resistant (R), were converted into a binary format for analysis: susceptible (1) and resistant (0), with intermediate results excluded. Only isolates tested against a given antimicrobial agent were included in susceptibility calculations.

To evaluate susceptibility at a broader pharmacological level, antimicrobial agents were grouped into major classes, including aminoglycosides, β-lactams, fluoroquinolones, tetracyclines, macrolides, amphenicols, rifamycins, and lincosamides. This grouping enabled comparison of resistance patterns across related drugs and improved interpretation for antimicrobial stewardship. Organism–class combinations with fewer than 30 tested isolates were excluded to ensure robust estimates.

Multidrug resistance (MDR) was defined as resistance to three or more antimicrobial classes. Susceptibility data were restructured to determine, for each isolate, the number of classes tested and the number to which resistance was observed. Only isolates tested against at least three classes were included, and those resistant to three or more classes were classified as MDR.

Following preliminary cleaning, the dataset was imported into R (version 4.5.2) for data processing and statistical analysis using appropriate packages. After cleaning, classification, and standardization, a total of 2,812 bacterial isolates were retained for analysis.

Statistical Analysis

Descriptive statistics were used to summarize bacterial distribution, anatomical sites, antimicrobial susceptibility patterns, and MDR prevalence, with frequencies and percentages reported for categorical variables. Associations between bacterial species and infection sites were assessed using Pearson's chi-square test, with Cramér's V used to measure effect size. Differences in MDR prevalence between Gram-positive and Gram-negative organisms were also evaluated using chi-square tests. Temporal trends in MDR were analyzed using logistic regression, with MDR status as the outcome and year as the predictor. Statistical significance was set at $p < 0.05$.

Results

Isolate Submissions and Temporal Distribution

A total of 2,812 bacterial isolates were analyzed. Submissions were low between 2011 and 2017, followed by a marked increase from 2018, peaking in 2021. Although submissions declined thereafter, they remained higher than in earlier years. The temporal distribution is presented in Supplementary Table 1 and illustrated in Fig. 1.

Distribution of Bacterial Organisms

A diverse range of bacterial organisms was identified, with *Streptococcus equi* subsp. *zooepidemicus* being the most common (19.2%), followed by *Escherichia coli* (14.8%), *Staphylococcus* spp. (7.6%), *Pseudomonas* spp. (6.2%), and *Klebsiella* spp. (5.8%). Other frequently detected organisms included *Acinetobacter* spp., *Streptococcus* spp., *Staphylococcus aureus*, *Enterobacter* spp., and *Enterococcus* spp. Their distribution is presented in Fig. 2.

Temporal Trends in Bacterial Organisms

Temporal patterns of bacterial isolates from equine clinical submissions (2011–2024) showed consistent detection of key species, including *Streptococcus equi subsp. zooepidemicus, Escherichia coli, Staphylococcus spp., Pseudomonas spp., and Klebsiella spp.* Although their relative abundances varied annually, the overall composition of dominant species remained stable. Detailed organism-specific trends are presented in Supplementary Figures S1–S6.

Anatomical Site Distribution of Isolates

Bacterial isolates were recovered from multiple anatomical sites, with the reproductive system accounting for the highest proportion (29.9%), followed by integumentary/wound (23.8%) and respiratory samples (20.1%). Other categories included unknown (19.0%) and smaller proportions from musculoskeletal/neurologic, ocular, urinary, gastrointestinal, ear, blood, and mammary sites. Table 1 summarizes this distribution, while Fig. 3 illustrates bacteria–site associations. A significant association

was observed ($\chi^2 = 1606$, df = 270, $p < 0.001$), with a small-to-moderate effect size (Cramér's V = 0.239), indicating species-specific site preferences.

Table 1
Distribution of equine bacterial isolates by anatomical site (n = 2,812)

Anatomical system	**No. (%)**
Reproductive	840 (29.9)
Integument/wound	668 (23.8)
Respiratory	565 (20.1)
Other/unknown	535 (19.0)
Musculoskeletal/neurologic	66 (2.3)
Eye	56 (2.0)
Urinary	31 (1.1)
Gastrointestinal	27 (1.0)
Ear	18 (0.6)
Blood	4 (0.1)
Mammary	2 (0.1)
Total	**2,812 (100)**

AST Patterns

Antimicrobial susceptibility patterns varied across bacterial species and drug classes. Overall, fluoroquinolones and aminoglycosides showed consistently high susceptibility, whereas β-lactams and tetracyclines were more variable. Among Gram-negative organisms (*Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp.), resistance to β-lactams was generally lower, and susceptibility to fluoroquinolones remained high. In Gram-positive bacteria, *Streptococcus dysgalactiae* and *Streptococcus equi* subsp. *zooepidemicus* were highly susceptible to β-lactams, while *Staphylococcus* spp. showed more variable patterns.

Susceptibility was categorized as high (≥ 90%), moderate (70–89%), or low (< 70%). Supplementary Tables S2 and S3 summarize organism distribution and susceptibility proportions, respectively. Class-level patterns are shown in supplementary Figs. 14 (Gram-positive) and 15 (Gram-negative). Drug-level heatmaps further highlight variability within classes (Supplementary Figures S7–S12).

MDR Prevalence and temporal trend

MDR analysis included isolates tested against at least three antimicrobial classes. Of 2,688 eligible isolates, 512 (19.0%) were classified as MDR, with prevalence varying by species. The highest MDR proportions were observed in *Citrobacter*, *Klebsiella*, and *Enterobacter* spp., while it was relatively low in streptococci (Supplementary Table S5). MDR was more common in Gram-negative (23.3%) than Gram-positive (14.9%) isolates, a statistically significant difference ($\chi^2 = 30.24$, df = 1, $p = 3.81 \times 10^{-8}$). Annual MDR prevalence fluctuated over time without a clear trend (Supplementary Figure S13).

Discussion

This study provides a longitudinal assessment of the epidemiology and AMR patterns of equine bacterial isolates in Texas and Oklahoma (2011–2024). Among 2,812 isolates, the most common species were *Streptococcus equi* subsp. *zooepidemicus*, *Escherichia coli*, *Staphylococcus* spp., *Pseudomonas* spp., and *Klebsiella* spp., with most samples originating from reproductive, integumentary/wound, and respiratory sites. Susceptibility varied across organisms and drug classes, with generally high susceptibility to fluoroquinolones and aminoglycosides, and more variable responses to β-lactams and tetracyclines. MDR was identified in about 20% of isolates and was significantly more common in Gram-negative bacteria.

The high prevalence of *Streptococcus equi* subsp. *zooepidemicus* in this study is consistent with previous reports identifying it as a common equine pathogen, particularly in respiratory and reproductive infections (Nocera et al., 2021). As an opportunistic commensal of the respiratory and genital mucosa, it is frequently associated with conditions such as endometritis and respiratory disease (Mohamed et al., 2025). Similar patterns have been reported in large-scale surveillance studies, where group C streptococci, especially *S. zooepidemicus*, are among the most prevalent isolates (Léon et al., 2020).

Gram-negative bacteria, including *Escherichia coli*, *Pseudomonas* spp., and *Klebsiella* spp., were consistently identified across the study period and are commonly associated with wound, respiratory, and uterine infections, particularly in hospitalized or immunocompromised horses (Hallowell et al., 2024; Pottier et al., 2022). Previous studies also highlight *E. coli* as a major Gram-negative isolate in equine samples (Albihn et al., 2003).

The association between bacterial species and anatomical sites supports pathogen tropism in equine infections. Streptococci were primarily linked to respiratory and reproductive sites, while Gram-negative organisms were more frequently associated with wounds and reproductive infections, consistent with earlier reports (Adams *et al*., 2014).

AST results showed marked variability across bacterial species and antimicrobial classes. Fluoroquinolones and aminoglycosides demonstrated consistently high susceptibility, whereas β-lactams and tetracyclines were more variable. These findings align with previous studies reporting strong efficacy of fluoroquinolones and aminoglycosides against Gram-negative equine pathogens (Isgren et al., 2025). Reduced β-lactam susceptibility among Enterobacterales reflects known trends, likely driven by antimicrobial pressure and the emergence of ESBL-producing strains (Steinman and

Navon-Venezia, 2020). Similarly, lower tetracycline susceptibility is consistent with documented increases in resistance, particularly in *E. coli* (Moura et al., 2013).

In contrast, Gram-positive streptococci, including *S. dysgalactiae* and *S. equi* subsp. *zooepidemicus*, remained highly susceptible to β-lactams, supporting their continued use as first-line treatments for equine streptococcal infections (Veiga et al., 2024).

MDR was identified in ~ 19% of isolates and was significantly more common in Gram-negative bacteria, consistent with their ability to acquire resistance genes via mobile genetic elements (Kumavath et al., 2025). Previous studies also report increasing MDR *E. coli* and other Gram-negative infections in horses (De Lagarde et al., 2019), with evidence that horses can carry resistant organisms such as ESBL-producing *E. coli* and MRSA (Maddox et al., 2012). This highlights the risk of escalating resistance, particularly with inappropriate antimicrobial use.

These findings emphasize the importance of routine culture and AST to guide therapy and support antimicrobial stewardship. However, limitations include potential selection bias from diagnostic submissions, variability in AST panels, and lack of data on prior treatment or clinical outcomes, which may influence resistance patterns.

Conclusion

In conclusion, a limited number of pathogens, including *Streptococcus equi* subsp. *zooepidemicus*, *Escherichia coli*, *Staphylococcus* spp., and other Gram-negative organisms, account for most equine bacterial infections. Susceptibility varied across organisms and drug classes, with MDR more common in Gram-negative bacteria. These findings highlight the need for routine AST, improved antimicrobial stewardship, and continued surveillance, alongside future studies incorporating genetic data, antimicrobial use, and clinical outcomes.

Declarations

Funding

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Daniel Braxton Stone contributed to study conceptualization and data acquisition. Ibrahim Idris conducted data analysis, interpreted the results, and drafted the manuscript. Godwin Ohemu, Nancy Zimmerman, Marcelo Schmidt, and Tianxi Ji contributed to data management and manuscript revision. Babafela Awosile contributed to study design, supervision, data interpretation, and critical revision. All authors approved the final manuscript.

Data Availability

The datasets analysed during the current study are not publicly available due to institutional and data-use restrictions but are available from the corresponding author on reasonable requests.

Ethics Approval

This study was a retrospective observational analysis of laboratory data and did not involve direct interaction with animals. No ethical approval was required.

Consent to Participate

Not applicable.

Consent to Publish

Not applicable.

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Figures

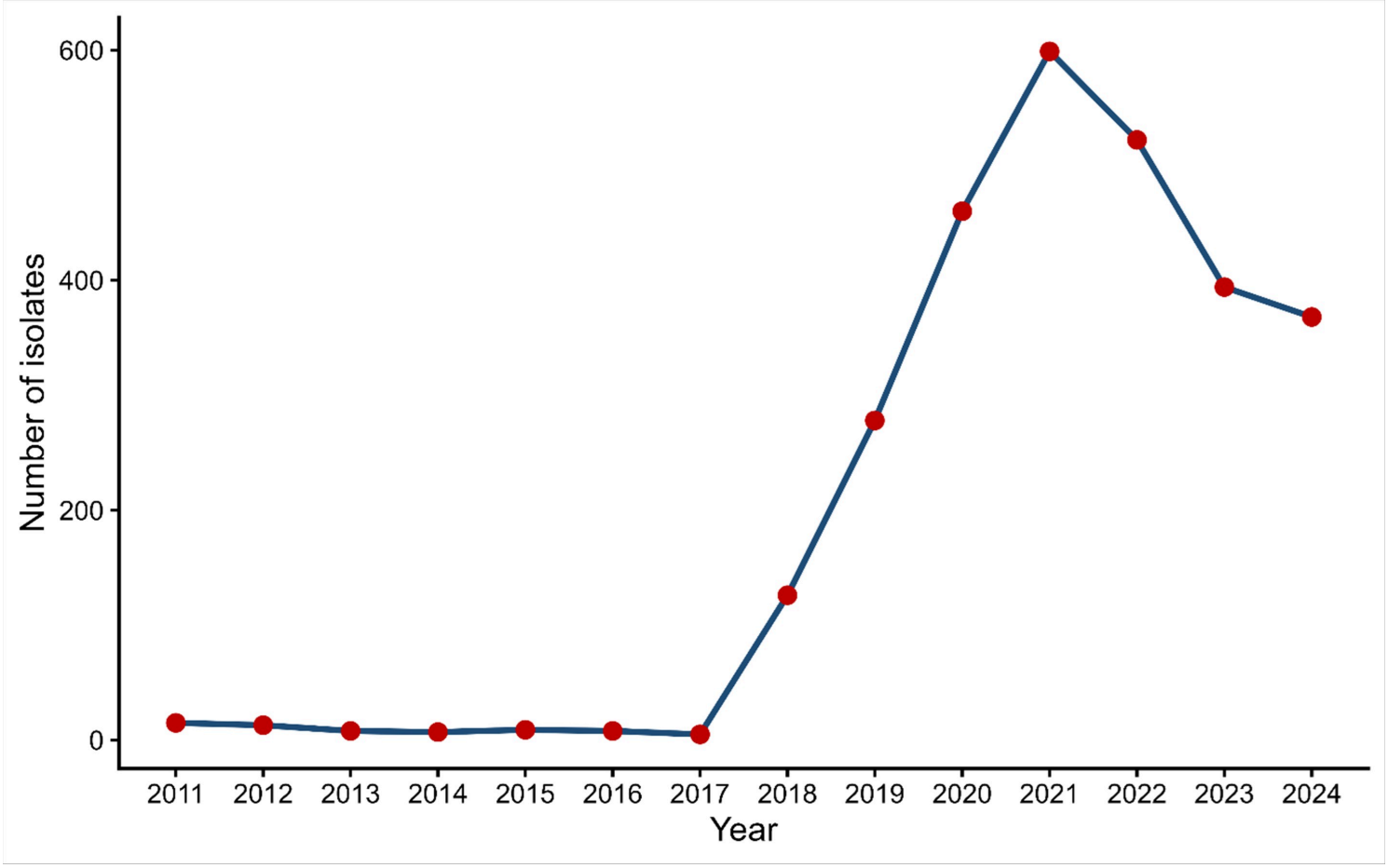


Figure 1

Annual distribution of equine bacterial isolates submitted for culture and AST in Texas and Oklahoma, 2011–2024 (n = 2,812).

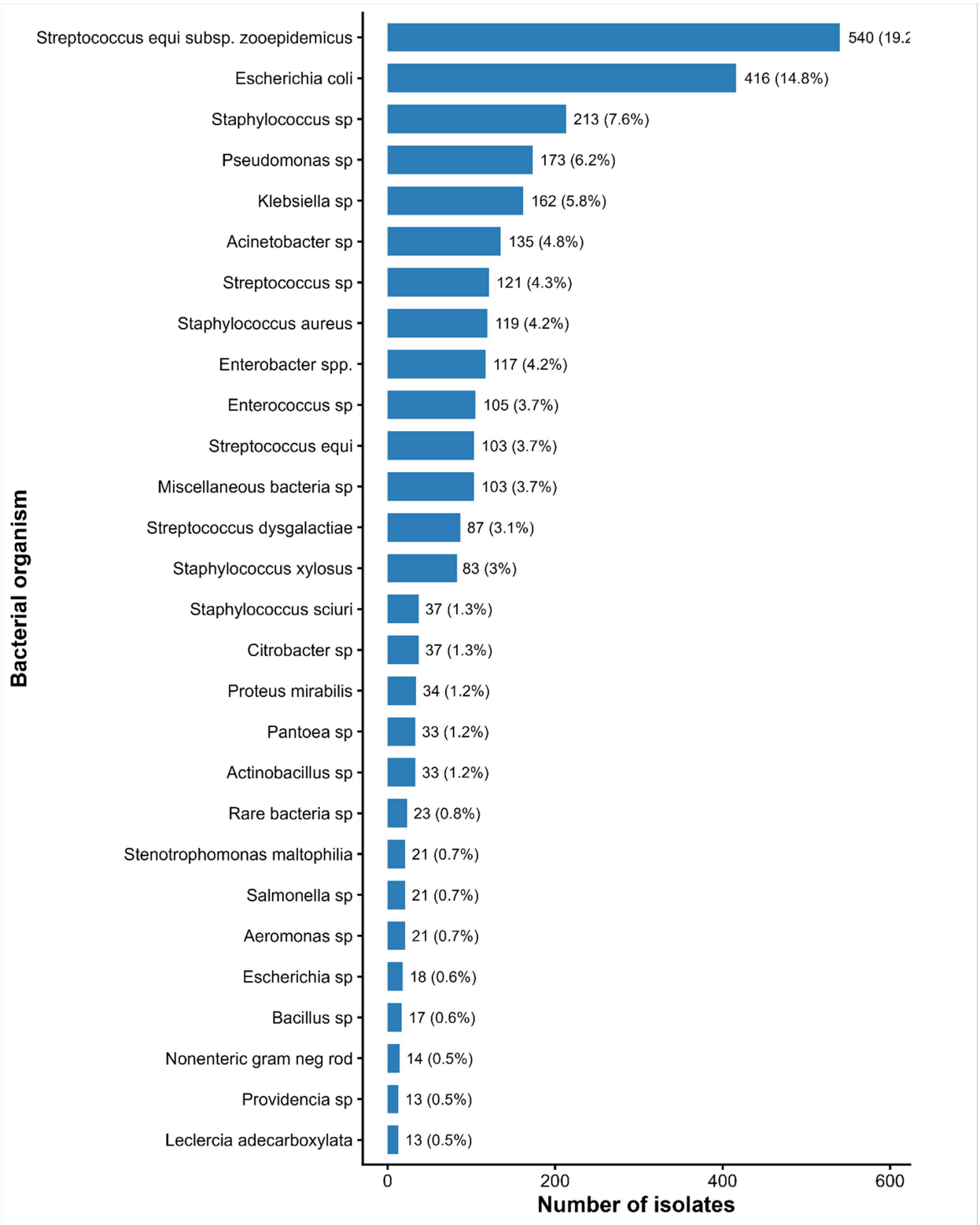


Figure 2

Distribution of bacterial organisms isolated from equine clinical submissions (n = 2,812). Bars represent the number of isolates for each bacterial organism, with percentages shown in parentheses.

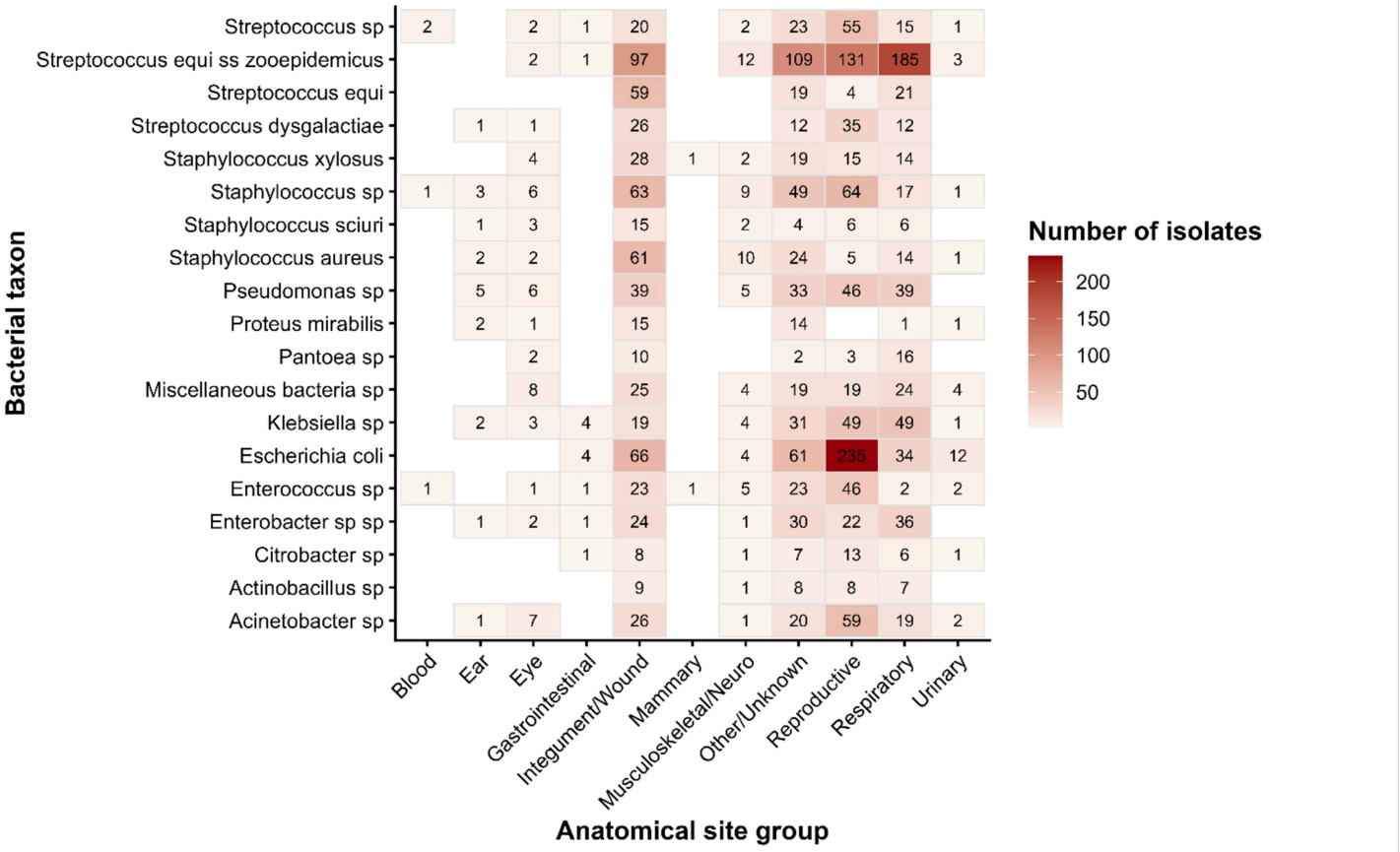


Figure 3

Heatmap showing the distribution of equine bacterial isolates across anatomical site categories. Rows represent bacterial organisms and columns represent anatomical systems. Color intensity corresponds to the number of isolates recovered from each anatomical site for each bacterial species.

Supplementary Files

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- SupplementaryMaterials.docx