Antimicrobial resistance in clinical bacterial isolates from horses in the UK

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Abstract

Background: Surveillance of antimicrobial resistance (AMR) in horses is important to aid empirical treatment decisions and highlight emerging AMR threats.

Objective: To describe the AMR patterns of common groups of bacteria from clinical submissions from horses in the UK during 2018, and to determine how this varies by sample site and type of submitting veterinary practice.

Study design: Prospective observational study.

Methods: All data on bacterial culture and subsequent antimicrobial susceptibility testing (AST) collected in 2018 from six large equine diagnostic laboratories were included. Resistance patterns were analysed including resistance to 1 or 2 antimicrobial classes, multidrug resistance (MDR), extensively drug resistant (XDR), resistance to highest priority critically important antimicrobials and isolates where there was no readily available treatment for adult horses in the UK. Submitting practices were classified according to whether they treated referral cases or not (first opinion). Comparisons between proportions and resistance for each bacterial group and sample site was performed using Chi-squared (or Fisher’s exact test).

Results: A total of 6,018 bacterial isolates from 4,038 diagnostic submissions were included from respiratory (n = 1,555), urogenital (n = 1,010), skin/hair/wound/abscess (n = 753), surgical site infection (SSI) /catheter-related-infection (CRI) /orthopaedic infections (n = 347) and unknown/’other’ submissions (n = 373). There were 2,711 Gram-negative isolates and 3,307 Gram-positive isolates. Prevalence of MDR for Escherichia coli was 31.7%, Staphylococcus spp. 25.3% and >25% for the majority of bacterial isolates from SSI/CRI/orthopaedic submissions. For Enterococcus spp. there was no readily available treatment for adult horses in the UK. MDR was significantly higher from referral hospital than first opinion submissions for the majority of pathogens (except Actinobacillus spp. and Pasteurella spp. and β-haemolytic Streptococcus spp.).

Main limitations: Since culture and susceptibility results are not systematic analyses based on harmonised methods, selection bias could impact the findings.

Conclusions: Ongoing surveillance is essential to understand emerging patterns of resistance. MDR is high in SSI/CRI/orthopaedic infections, which is important for...
Antimicrobial resistance (AMR) is a global problem with implications for both human and equine health. AMR in horses poses a threat not only to the individual horse but also to the owners and caregivers as well as to the environment from faecal and urine excretion of antimicrobials and their metabolites. This problem is more concerning since transmission of multidrug-resistant (MDR, isolates with acquired non-susceptibility to at least one antimicrobial in three or more antimicrobial classes) pathogenic strains from animals to humans has also been reported. There are also few antimicrobials available for use in UK horses due to a limited number of drugs being authorised for use in this species, cost implications and safety concerns due to hindgut fermentation. Certain antimicrobial classes, such as macrolides, which are commonly used in humans and other veterinary species, are rarely used in horses over 12 months of age (although macrolides are used in foals in the treatment of Rhodococcus pneumonia). Similarly, lincosamides are never used in horses due to risk of severe and potentially fatal colitis. Some antimicrobials such as doxycycline and enrofloxacin, which are considered safe for use in horses but are not authorised for equine use in the UK, are frequently prescribed under the cascade for treating equine infections. Other antimicrobials authorised for use in other veterinary species are rarely used in adult horses due to cost (e.g. amoxicillin), even though they are considered safe to use in adult horses.

Surveillance of AMR in clinical isolates is important in order to monitor and detect emerging resistance patterns, which may be a threat to horse or human health. In addition, surveillance data can be used to guide policies on antimicrobial use and local geographical empirical therapy. Antimicrobial stewardship and appropriate antimicrobial prescribing practices are also important to ensure antimicrobials remain effective, especially with limited treatment options in the horse. Intrinsic resistance (IR), the innate ability of wild-type bacterial species to resist activity of a particular antimicrobial, is particularly high in some bacterial species that are commonly isolated in horses, eg Enterococcus spp. and Pseudomonas spp., which further limits treatment options and may be compounded by acquired resistance also present in such bacteria.

Previous reports in horses have mostly focused on susceptibility patterns of particular bacteria or from a particular sample site or age group, or used results from a single hospital or laboratory. Recent publications from France have reported on susceptibility patterns from a variety of bacteria from clinical submissions from 2012 to 2016 and identified increasing resistance to trimethoprim-sulfamethoxazole in Streptococcus spp. and E. coli. Another report from France identified a decrease in MDR in E. coli and Staphylococcus aureus clinical isolates from 2006 to 2016, however, prevalence of MDR still remained above 18% and 22.5% for S. aureus and E. coli respectively. The Defra AHT BEVA Equine Quarterly Disease Surveillance Report provides information on the prevalence of bacteria such as Streptococcus equi subspecies equi (S. equi), methicillin resistant Staphylococcus aureus (MRSA) and several other bacteria; however, it does not report on antimicrobial susceptibility of these organisms. While there are several studies reporting on carriage of AMR in bacterial isolates in horses, to the authors’ knowledge, there is currently a lack of data on antimicrobial susceptibility patterns in bacterial isolates from equine clinical submissions globally.

In the UK, a variety of different types of independent diagnostic laboratories operate; these include those based within large private equine hospitals, university-based laboratories, large commercial veterinary laboratories that predominantly process small animal submissions with fewer equine submissions as well as small in-house laboratories with mainly internal submissions. Currently, there are no standardised veterinary laboratory methods in the UK, although most laboratories use Clinical and Laboratory Standards Institute (CLSI) standards for performing antimicrobial susceptibility testing (AST) and for interpretation of clinical breakpoints. Culture and susceptibility data are crucial for informing treatment decisions and determining emerging AMR threats. Therefore, the aim of the study was to describe the prevalence of bacteria most commonly isolated from clinical specimens and patterns of AMR among bacterial isolates from equine clinical samples submitted to diagnostic laboratories in the UK over a 12-month period in 2018. We hypothesised that there would be increased MDR from submissions from referral practices compared with first opinion practices, as referral caseloads are more likely to have already been administered first-line antimicrobial treatment, with subsequent referral only following treatment failure.

2 | MATERIALS AND METHODS

2.1 | Data collection

Bacterial culture and subsequent AST data from bacterial isolates were obtained from clinical submissions during the calendar year.
2018, from six equine diagnostic laboratories across England, including commercial, practice-based and University-based laboratories. Microorganisms isolated from positive cultures were identified using commercial biochemical tests including API kits (Biomérieux) and GNID and GPID Sensititre Identification plates (TREK Diagnostic Systems) at four of the laboratories, while two used the Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) platform for bacterial species identification (Bruker Daltonics).

AST was performed using minimum inhibitory concentration (MIC) at two laboratories while the remaining four used Kirby-Bauer disc diffusion testing. All laboratories used CLSI methods and used CLSI breakpoints where available for horses. When no breakpoints were available for horses, other veterinary breakpoints were used, followed by human breakpoints (CLSI or EUCAST) if no other veterinary breakpoints were available.15 The individual breakpoints used by each laboratory for the most common bacteria in this study are listed in Table S1 and although many breakpoints were identical (eg benzyl-penicillin for β-haemolytic Streptococcus spp., Pasteurella spp. and Actinobacillus spp., oxytetracycline and doxycycline for Enterobacteriaceae and folate pathway inhibitors for Acinetobacter spp.), some differed between laboratories (eg oxytetracycline and doxycycline for bacteria other than Enterobacteriaceae). No laboratories used urine-specific breakpoints.

Breakpoints were displayed to reflect whether MIC or Kirby-Bauer disc diffusion testing was performed. Laboratories also used different antimicrobial susceptibility panels (Table S2). From all laboratories, a range of information was provided including a unique submission identification code: first part of the postcode of the submitting veterinary practice address; date the results report was produced; the type or site of infection; number of isolates submitted; sample number; and the results for each antimicrobial class. Pathway inhibitors used included folate pathway inhibitors (trimethoprim-sulfamethoxazole), fluoroquinolones (enrofloxacin) or phenicols (chloramphenicol). Polymyxin B, although tested for using Kirby-Bauer disc diffusion methods by two laboratories was not included due to inaccuracy of this method; testing using MIC by microbroth dilution is advocated.12

### 2.2 | Data analysis

Sample sites were categorised into five different categories as follows: (a) respiratory tract/guttural pouch, (b) urogenital, (c) skin/hair/wound/abscess, (iv) surgical site infection (SSI)/catheter-related infection (CRI)/orthopaedic infections and (v) other or absent. Orthopaedic infections included positive synovial cultures, septic tendinitis and osteomyelitis submissions and were grouped with SSI and CRI as these infections are difficult to treat and often require surgery and hospitalisation. Unknown submissions included those where no site was reported (n = 520 isolates from 447 submissions), while ‘others’ were those present in less than 100 isolates and included the following sites: faecal (n = 25), peritoneal fluid (n = 33), liver (n = 11), dental (n = 4), gastric (n = 7) and rectal (n = 5) submissions.

Bacterial species were separated based on their IR and genetic similarity into the following groups: for Gram-negative bacteria E. coli; Actinobacillus spp. & Pasteurella spp.; Citrobacter spp., Enterobacter spp., Klebsiella spp., Serratia spp., & Pantoea spp.; Pseudomonas spp.; Acinetobacter spp.; Proteus spp., Morganella spp., and Providencia spp.; for Gram-positive bacteria β-haemolytic Streptococcus spp.; α-haemolytic Streptococcus spp.; Staphylococcus spp.; Enterococcus spp.; Corynebacterium spp. & Bacillus spp. Although there are CLSI MIC breakpoints for amikacin only for β-haemolytic Streptococcus spp., EUCAST expert rules consider all Streptococcus spp. IR to all aminoglycosides16 due to increasing levels of resistance hence this classification was used for the methods for this project. Confidence intervals (95% CIs) for the proportions resistant were calculated using the Wilson Score intervals.21 ‘Broadly susceptible isolates’ were those which were susceptible to all classes of antimicrobials tested (IR excluded) and described in Table 1; ‘Resistant to 1 or 2 classes’ were those resistant to one or two antimicrobials from different classes; MDR was defined as isolates with acquired non-susceptibility to at least one antimicrobial in three or more antimicrobial classes. ‘XDR isolates’ were those which were resistant to all classes of antimicrobials considered.8 Isolates with ‘no readily available treatment for adult horses in the UK’ included those that were not susceptible to any of the following antimicrobials; penicillin (penicillin G), 3rd-generation cephalosporins (3GC; cefotiofur), aminoglycosides (gentamicin/amikacin), tetracyclines (oxytetracycline/doxycycline), folate pathway inhibitors and tetracyclines. Royal College of Veterinary Surgeons (RCVS) Veterinary Practice Directory (VPD) and the National Statistics Postcode Look-up (NSPL) were used to determine if the submitting practice was a practice that accepted referral cases. Submissions from practices that accepted referral cases and with an ambulatory branch also were categorised as referral.
### TABLE 1  List of antimicrobial classes and agent used to define multidrug resistance (MDR) for common bacterial isolates in horses (modified from resources in literature such as Magiorakos et al. (2012), EUCAST 3.1 and CLSI VET08 ED4:2018) and Giguère et al. (2013)

| Bacterial genus or species | Antimicrobial class | Antimicrobial agent |
|---------------------------|--------------------|--------------------|
| Gram-negative             | **Escherichia coli** | **Amino-penicillins** | Ampicillin  |
|                           |                    |                    | Amoxicillin |
|                           | Beta-lactamase inhibitor combinations | **Amoxicillin-clavulanic acid** |
|                           |                    |                    | **Ticarcillin-clavulanic acid** |
|                           |                    |                    | **Piperacillin-tazobactam** |
|                           | 3rd and 4th GCs    | Cefotaxime          |
|                           |                    | Ceftazidime         |
|                           |                    | Cefpodoxime         |
|                           |                    | Ceftiofur           |
|                           |                    | Cefquinome          |
|                           | Aminoglycosides    | Gentamicin          |
|                           |                    | Amikacin            |
|                           |                    | Neomycin            |
|                           |                    | Framycetin          |
|                           |                    | Tobramycin          |
|                           | Tetracyclines      | Oxytetracycline     |
|                           |                    | Doxycycline         |
|                           | Folate pathway inhibitors | Trimethoprim sulphadiazine |
|                           |                    | Trimethoprim-sulfamethoxazole |
|                           | Fluoroquinolones   | Enrofloxacin        |
|                           |                    | Ciprofloxacin       |
|                           |                    | Marbofloxacin       |
|                           | Phenicols          | Chloramphenicol     |

**Intrinsic resistance:** benzyl-penicillins and macrolides

| Pasteurella spp. & , Actinobacillus spp. | Penicillins | Benzyl-penicillins | Penicillin G |
|------------------------------------------|------------|--------------------|--------------|
|                                          | Amino-penicillins | Ampicillin  |
|                                          |                    | Amoxicillin |
|                                          | Beta-lactamase inhibitor combinations | **Amoxicillin-clavulanic acid** |
|                                          |                    | **Ticarcillin-clavulanic acid** |
|                                          |                    | **Piperacillin-tazobactam** |
|                                          | 3rd and 4th GCs    | Cefotaxime          |
|                                          |                    | Ceftazidime         |
|                                          |                    | Cefpodoxime         |
|                                          |                    | Ceftiofur           |
|                                          |                    | Cefquinome          |
|                                          | Aminoglycosides    | Gentamicin          |
|                                          |                    | Amikacin            |
|                                          |                    | Neomycin            |
|                                          |                    | Framycetin          |
|                                          |                    | Tobramycin          |
|                                          | Tetracyclines      | Oxytetracycline     |
|                                          |                    | Doxycycline         |
|                                          | Folate pathway inhibitors | Trimethoprim sulphadiazine |
|                                          |                    | Trimethoprim-sulfamethoxazole |
|                                          | Fluoroquinolones   | Enrofloxacin        |
|                                          |                    | Ciprofloxacin       |
|                                          |                    | Marbofloxacin       |
|                                          | Macrolides         | Erythromycin        |
|                                          |                    | Clarithromycin      |
|                                          |                    | Azithromycin        |
|                                          | Phenicols          | Chloramphenicol     |

**Intrinsic resistance:** Pasteurella spp. & Actinobacillus spp. are considered IR to 1 & 2nd GC, and Actinobacillus spp. are considered IR to benzyl-penicillins.

(Continues)
| Bacterial genus or species | Antimicrobial class | Antimicrobial agent |
|---------------------------|---------------------|---------------------|
| Citrobacter spp., Enterobacter spp., Klebsiella spp., Serratia spp., & Pantoea spp. | Extended-spectrum β-lactamase inhibitor combinations | Ticarcillin-clavulanic acid, Piperacillin-tazobactam |
| | 3rd and 4th GCs | Cefotaxime, Ceftazidime, Cefpodoxime, Ceftriaxone, Cefquinome |
| | Aminoglycosides | Gentamicin, Amikacin, Neomycin, Framycetin, Tobramycin |
| | Tetracyclines | Oxytetracycline, Doxycycline |
| | Folate pathway inhibitors | Trimethoprim sulphadiazine, Trimethoprim-sulfamethoxazole |
| | Fluoroquinolones | Enrofloxacin, Ciprofloxacin, Marbofloxacin |
| | Phenicols | Chloramphenicol |
| **Intrinsic resistance:** | benzyl and amino penicillins, 1 & 2nd GCs and macrolides |
| Pseudomonas spp. | Extended-spectrum β-lactamase inhibitor combinations | Ticarcillin-clavulanic acid, Piperacillin-tazobactam |
| | 3rd and 4th GCs\(^a\) | Ceftazidime, Cefquinome |
| | Aminoglycosides | Gentamicin, Amikacin, Neomycin, Framycetin, Tobramycin |
| | Fluoroquinolones | Enrofloxacin, Ciprofloxacin, Marbofloxacin |
| **Intrinsic resistance:** | benzyl and amino penicillins, 1 & 2nd GCs, tetracyclines, folate pathway inhibitors, phenicols and macrolides. \(^a\)Ceftazidime/Cefquinome only |

**Acinetobacter spp.**

| | Extended-spectrum β-lactamase inhibitor combinations | Ticarcillin-clavulanic acid, Piperacillin-tazobactam |
| | 3rd and 4th GCs\(^b\) | Cefotaxime, Ceftazidime, Cefquinome |
| | Aminoglycosides | Gentamicin, Amikacin, Neomycin, Framycetin, Tobramycin |
| | Folate pathway inhibitors | Trimethoprim sulphadiazine, Trimethoprim-sulfamethoxazole |
| | Fluoroquinolones | Enrofloxacin, Ciprofloxacin, Marbofloxacin |
| **Intrinsic resistance:** | benzyl and amino penicillins, 1 & 2nd GCs, tetracyclines, phenicols and macrolides. \(^b\)Cefotaxime/Ceftazidime/Cefquinome only |

(Continues)
| Bacterial genus or species | Antimicrobial class | Antimicrobial agent |
|---------------------------|--------------------|--------------------|
| *Proteus* spp., *Morganella* spp., & *Providencia* spp., | Extended-spectrum β-lactamase inhibitor combinations | Ticarcillin-clavulanic acid, Piperacillin-tazobactam |
| 3rd and 4th GCs | Cefotaxime, Ceftazidime, Cefpodoxime, Cefiofur, Cefquinome |
| Aminoglycosides | Gentamicin<sup>c</sup>, Amikacin, Neomycin, Framycetin, Tobramycin |
| Folate pathway inhibitors | Trimethoprim-sulphadiazine, Trimethoprim-sulfamethoxazole |
| Phenicols | Chloramphenicol |
| **Intrinsic resistance**: benzyl and amino penicillins, 1&2nd GCs, tetracyclines and macrolides. |  |
| Gram-positive | Staphylococcus spp. (coagulase positive and negative) | Anti-staphylococcal β-lactam | Oxacillin<sup>d</sup>, Cefoxitin<sup>d</sup> |
| | | Aminoglycosides | Gentamicin, Amikacin, Neomycin, Framycetin, Tobramycin |
| | Tetracyclines | Oxytetracycline, Doxycycline |
| | Folate pathway inhibitors | Trimethoprim-sulphadiazine, Trimethoprim-sulfamethoxazole |
| | Fluoroquinolones | Enrofloxacin, Ciprofloxacin, Marbofloxacin |
| | Macrolides | Erythromycin, Clarithromycin, Azithromycin |
| | Phenicols | Chloramphenicol |
| | Fusidanes | Fusidic acid |
| | Ansamycins | Rifampicin |
| **Intrinsic resistance**: benzyl and amino penicillins and all cephalosporins. |  |
| | (no treatment option horses) |  |
| Beta-haemolytic | Penicillins | Benzyl-penicillins | Penicillin G |
| *Streptococcus* spp. | Amino-penicillins | Ampicillin, Amoxicillin |
| Beta-lactamase inhibitor combinations | Amoxicillin-clavulanic acid, Ticarcillin-clavulanic acid, Piperacillin-tazobactam |
| 3rd and 4th GC | Cefotaxime, Ceftazidime, Cefpodoxime, Cefiofur, Cefquinome |
| Tetracycline | Doxycycline, Oxytetracycline |

<sup>c</sup>Gentamicin excluded for *Providencia* spp.
<sup>d</sup>Diagnostic purpose (no treatment option horses)
| Bacterial genus or species          | Antimicrobial class | Antimicrobial agent                  |
|-----------------------------------|---------------------|--------------------------------------|
| Folate pathway inhibitors         | Trimethoprim sulphadiazine |
|                                   | Trimethoprim- sulfamethoxazole |
| Fluoroquinolones                  | Enrofloxacin         |
|                                   | Ciprofloxacin        |
|                                   | Marbofloxacin        |
| Macrolides                        | Erythromycin         |
|                                   | Clarithromycin       |
|                                   | Azithromycin         |
| Phenicols                         | Chloramphenicol      |

**Intrinsic resistance:** aminoglycosides

| Inh-aemolytic Strep. Spp          | 3rd and 4th GCs | Ceftiofur |
|----------------------------------|-----------------|-----------|
|                                  |                 | Cefquinome|
| Macrolides (only in combination) | Erythromycin    |
|                                  | Clarithromycin  |
|                                  | Azithromycin    |
| Fluoroquinolones                 | Enrofloxacin    |
|                                  | Ciprofloxacin   |
|                                  | Marbofloxacin   |

**Intrinsic resistance:** benzyl and amino penicillins, beta-lactamase inhibitor combinations, 1&2nd GCs, aminoglycosides, tetracyclines, folate pathway inhibitors, macrolides and phenicols.

| Enterococcus spp.                | Amino/Ureido- Penicillins | Ampicillin |
|---------------------------------|----------------------------|------------|
|                                  |                            | Amoxicillin|
|                                  |                            | Ticarcillin|
| Tetracyclines                    | Doxycycline                |
|                                  | Oxytetracycline            |
| Fluoroquinolones                 | Enrofloxacin               |
|                                  | Ciprofloxacin              |
|                                  | Marbofloxacin              |

**Intrinsic resistance:** benzyl penicillin, beta-lactamase inhibitor combinations, all cephalosporins, aminoglycosides, folate pathway inhibitors, macrolides and phenicols.

| Corynebacterium spp. Bacillus spp | Penicillin | Benzyl-penicillins | Penicillin G |
|-----------------------------------|------------|-------------------|-------------|
|                                   |            | Amino-penicillins |             |
| Beta-lactamase inhibitor combinations |            | Amoxicillin-clavulanic acid |
|                                   |            | Ticarcillin-clavulanic acid |
|                                   |            | Piperacillin-tazobactam |
| 3rd and 4th GCs                   |            | Cefotaxime         |
|                                   |            | Ceftzidime         |
|                                   |            | Cefpodoxime        |
|                                   |            | Ceftiofur          |
|                                   |            | Cefquinome         |
| Aminoglycosides                   |            | Gentamicin         |
|                                   |            | Amikacin           |
|                                   |            | Neomycin           |
|                                   |            | Framycetin         |
|                                   |            | Tobramycin         |
| Tetracyclines                     |            | Oxytetracycline    |
|                                   |            | Doxycycline        |

(Continues)
Additionally, although polymyxin B may be used in horses as part of treatment of systemic inflammatory response syndrome (SIRS), it is used at an anti-endotoxin dose rate and not at an antimicrobial dose rate. The recommended dose for polymyxin B in the treatment of SIRS in horses is 6,000 iu/kg IV every 8 to 12 hours, although the dose range varies between 5,000 and 10,000 iu/kg. The antimicrobial dose is higher (20,000 iu/kg), although neurotoxic and nephrotoxic effects have been seen at this dose hence should not be used in horses. The human antimicrobial polymyxin B dose is 30,000 iu/kg/day. Comparisons between proportions for sample site, referral or first opinion practice and resistance for each bacterial group was performed using Chi-squared (or Fisher’s exact test, [f] when sample size in any category was <5). A p-value of <0.05 was considered statistically significant. A bivariate choropleth map was constructed displaying geographical variation in the proportion of MDR isolates across all isolates and for those bacteria that were present in sufficient numbers for analysis.

3 | RESULTS

AST data were available from 6,018 bacterial isolates obtained from 4,038 clinical submissions during 2018 and included 1555 respiratory, 1,010 urogenital, 753 skin/hair/wound/abscess, 347 SSI/CRI/orthopaedic infections and 373 unknown or ‘other’ submissions. A single pure bacterial isolate was recovered from 63.6% (2553/4038) of submissions, while the remaining submissions revealed polymicrobial cultures ranging from 2 to 7 isolates. Of the remaining 1,485 submissions there were 1,093, 319, 66, 3, 1 and 3 submissions with 2, 3, 4, 5, 6 and 7 isolates respectively. The 6,018 bacterial isolates included 2,711 Gram-negative isolates and 3,307 Gram-positive isolates. Only isolates belonging to the major bacterial groups identified in Table 1 were included (n = 5,698) for AMR and MDR calculations, omitting 212 and 108 other Gram-negative and Gram-positive bacterial isolates respectively (breakdown shown in Table S4).

The submissions came from 208 veterinary practices distributed across the UK (shown in Figure 1). The most common Gram-positive bacterial isolate was β-haemolytic Streptococcus spp. (45.9%) followed by Staphylococcus spp. (28.6%; in this group 56.8% were S. aureus, see Table S4 for further information) and β-haemolytic Streptococcus spp. (11.0%). In the β-haemolytic Streptococcus spp., the majority were unspecified species (54.4%) followed by Streptococcus equi subspecies zooepidemicus (S. zooepidemicus) (34.2%), Streptococcus dysgalactiae subsp. equisimilis (8.4%) and S. equi (3.0%). E. coli (38.3%) represented the most common Gram-negative isolates followed by Actinobacillus spp. & Pasteurella spp. (22.8%) and Citrobacter spp., Enterobacter spp., Klebsiella spp., Serratia spp., & Pantoea spp. (16.9%). The full breakdown of bacterial isolates is shown in Table S4. The most common bacterial isolates from respiratory submissions included β-haemolytic Streptococcus spp. (31.1%) and Actinobacillus spp. & Pasteurella spp. (21.6%), while the most common urogenital pathogens included E. coli (31.9%) and β-haemolytic Streptococcus spp. (29.5%). The most common bacterial isolates from skin/hair/wound/abscess submissions included Staphylococcus spp. (32.2%) and β-haemolytic Streptococcus spp. (20.0%), while SSI/CRI/orthopaedic infections, also most commonly included Staphylococcus spp. (28.1%) but also E. coli (18.8%) and Enterococcus spp. (12.2%). The breakdown of AMR in bacterial isolates according to sample site is shown in Table 2.

3.1 | Antimicrobial resistance

The proportion of resistance of bacterial isolates is shown in Tables 2 and 3. Resistance to 1 or 2 antimicrobial classes was most common in Enterococcus spp. (66.5%), Acinetobacter spp. (63.1%) and β-haemolytic Streptococcus spp. (45.2%). In Gram-negative bacterial isolates, the proportion of resistance was highest to β-lactams (83.8%), followed by β-lactam/β-lactamase inhibitors (79.0%) and β-lactam/β-lactamase inhibitors plus clavulanic acid (73.5%). The proportion of resistance was lowest to trimethoprim-sulfamethoxazole (24.5%), followed by trimethoprim-sulfadiazine (33.9%) and chloramphenicol (39.4%).
isolates there was high tetracycline and folate pathway inhibitor resistance in E. coli (48.0% and 44.3%, respectively) and Citrobacter spp., Enterobacter spp., Klebsiella spp., Serratia spp. and Pantoena spp. (42.8% and 35.1%, respectively); high folate pathway inhibitor resistance in Acinetobacter spp. (70.2%) and Proteus spp., Morganella spp. & Providencia spp. (57.5%); and high macrolide resistance in Actinobacillus spp. & Pasteurella spp. (82.7%). Resistance to 3/4GC in E. coli and Citrobacter spp., Enterobacter spp., Klebsiella spp., Serratia spp. and Pantoena spp. was 14.0% and 27.6% respectively. Prevalence of fluoroquinolone resistance was >20% for Pseudomonas spp., Proteus spp., Morganella spp. and Providencia spp. And >10% for E. coli, Citrobacter spp., Enterobacter spp., Klebsiella spp., Serratia spp. and Pantoena spp. and Acinetobacter spp.

In Gram-positive isolates there was a very high prevalence of tetracycline resistance in Enterococcus spp. (49.6%) and >30% for Staphylococcus spp. and β-haemolytic Streptococcus spp. Fluoroquinolone resistance was also high in Enterococcus spp. (50.7%) but lower in β-haemolytic Streptococcus spp. (27.9%) and <15% for other relevant Gram-positive isolates. The prevalence of oxacillin or cefoxitin resistance in Staphylococcus spp. isolates was 15.9%, however, only 34.3% of isolates (315 of 916 isolates) were tested against either of these antimicrobials. In S. aureus, the prevalence of oxacillin or cefoxitin resistance was 12.1% (30 of 247 isolates).

3.2 | Multidrug and extensively drug-resistant isolates

MDR was high in Corynebacterium spp. & Bacillus spp. (50.8%), E. coli (31.7%), Citrobacter spp., Enterobacter spp., Klebsiella spp., Serratia spp., & Pantoena spp. (25.3%) and Staphylococcus spp. (25.3%). Isolates with no readily available treatment for adult horses in the UK were highest in Enterococcus spp. (30.2%) followed by Acinetobacter spp. (9.2%), while in all other bacterial isolates this category accounted for less than 6.4% of isolates. The most broadly susceptible isolates included α-haemolytic Streptococcus spp. (92.1%), Pseudomonas spp. (60.1%), Actinobacillus spp. & Pasteurella spp. (51.7%). Proportion of broadly susceptible, resistant to 1 or 2 classes, MDR and XDR is shown in Table 3.

3.3 | Resistance by sampling site

The most frequent source of bacterial isolates included respiratory (n = 2,334), urogenital (n = 1,286), skin/hair/wound/abscess (n = 1,230), SSI/CRI and orthopaedic infections (n = 549). The proportion of bacterial isolates with resistance and MDR by species and sample site is shown in Table 2. Proportions of resistance varied significantly by sample sites with SSI/CRI and orthopaedic infections having high prevalence of MDR and resistance to most antimicrobials tested for many in many of the bacterial species including E. coli, Citrobacter spp., Enterobacter spp., Klebsiella spp., Serratia spp., Pantoena spp. & Acinetobacter spp. and Staphylococcus spp. MDR was also prevalent in samples from unknown and other sites in Actinobacillus spp. & Pasteurella spp., Proteus spp., Morganella spp., Providencia spp. and Staphylococcus spp. Of concern, resistance to 3/4GC was >20% in E. coli isolates and >35% in Acinetobacter spp. from SSI/CRI/orthopaedic infections and unknown/other. Resistance to 3/4GC was >40% in Citrobacter spp., Enterobacter spp., Klebsiella spp., Serratia spp., Pantoena spp. from respiratory, SSI/CRI/orthopaedic infections and unknown/other. Fluoroquinolone resistance was >45% in SSIs for Citrobacter spp., Enterobacter spp., Klebsiella spp., Serratia spp., Pantoena spp. and Pseudomonas spp. and >50% for β-haemolytic Streptococcus spp. from SSI/CRI/orthopaedic infections and unknown/other and >75% in Enterococcus spp. from SSI/CRI/orthopaedic infections and unknown/other.

3.4 | Submitting practice demographics

From the 4,038 original submissions, there were 3,926 where the submitting practice details included a UK veterinary practice postcode, of which 2008 were referral submissions and 1918 first opinion submissions. Submissions were excluded (n = 112) either due to submitting practice details not being recorded (n = 6), or submissions were from outside of the UK (n = 106). There were significantly more respiratory and SSI/CRI and orthopaedic submissions from practices with referral.
TABLE 2  Proportion of resistance (in %) of 5,698 bacteria isolated from clinical infections in horses classified by sample site

| Pathogen (n)                      | Antimicrobial                              | Total number of isolates tested | Proportion of resistance (% and 95%CI) | Respiratory (2,187) | Urogenital (1,227) | Skin/Wound (1,163) | SSI/CRI/Orthopaedic (526) | Unknown & Other (595) | P value |
|-----------------------------------|--------------------------------------------|---------------------------------|---------------------------------------|---------------------|--------------------|--------------------|--------------------------|------------------------|---------|
| Gram-negative bacteria            |                                            |                                 |                                       |                     |                    |                    |                          |                        |         |
| Escherichia coli (958)            | Total                                      | 958                             |                                       | 8.4 (183)           | 31.9 (391)         | 13.1 (152)         | 18.8 (99)                | 22.4 (133)             | <0.001  |
|                                   | Aminopenicillins                           | 627                             | 35.4 (31.8-39.2)                      | 39.0 (141)          | 27.3 (300)         | 44.9 (91)          | 64.4 (48)                | 29.8 (47)              |         |
|                                   | β-lactamase inhibitor combinations         | 402                             | 8.7 (6.3-11.9)                        | 7.0 (158)           | 12.2 (41)          | 9.6 (104)          | 12.2 (49)                | 6.0 (50)               | 0.5     |
|                                   | 3/4th GCs                                  | 955                             | 14.0 (12.0-16.4)                      | 11.5 (183)          | 9.0 (390)          | 14.6 (151)         | 23.5 (98)                | 24.8 (133)             | <0.001  |
|                                   | Aminoglycosides                            | 955                             | 23.4 (20.8-26.1)                      | 18.0 (183)          | 18.0 (389)         | 25.0 (152)         | 43.9 (98)                | 29.3 (133)             | <0.001  |
|                                   | Tetracyclines                              | 954                             | 48.0 (44.9-51.2)                      | 42.1 (183)          | 37.1 (388)         | 55.3 (152)         | 60.2 (98)                | 70.7 (133)             | <0.001  |
|                                   | Folate pathway inhibitors                  | 945                             | 44.3 (41.2-47.5)                      | 37.0 (181)          | 38.1 (381)         | 53.3 (152)         | 60.2 (98)                | 50.4 (133)             | <0.001  |
|                                   | Fluoroquinolones                           | 955                             | 10.7 (8.9-12.8)                       | 9.3 (183)           | 5.9 (389)          | 17.1 (152)         | 21.4 (98)                | 11.3 (133)             | <0.001  |
|                                   | Phenics                                    | 954                             | 26.5 (20.9-32.9)                      | 28.0 (25)           | 11.8 (34)          | 24.4 (41)          | 28.0 (25)                | 32.9 (79)              | <0.001  |
|                                   | MDR                                        | 958                             | 31.7 (28.9-34.8)                      | 30.6 (183)          | 21.5 (391)         | 37.5 (152)         | 50.5 (99)                | 42.9 (133)             | <0.001  |
| Actinobacillus spp. & Pasteurella spp. (571) | Total                                      | 571                             |                                       | 21.6 (472)          | 1.3 (16)           | 3.7 (43)           | 3.6 (19)                 | 3.5 (21)               | <0.001  |
|                                   | Aminopenicillins                           | 493                             | 16.0 (13.1-19.5)                      | 15.3 (425)          | 36.4 (11)          | 17.1 (35)          | 10.0 (10)                | 27.3 (11)              | <0.001  |
|                                   | β-lactamase inhibitor combinations         | 462                             | 0.6 (0.2-1.9)                         | 0.2 (408)           | 25.0 (4)           | 3.3 (30)           | 0.0 (9)                  | 0.0 (11)               | <0.001  |
|                                   | 3/4th GCs                                  | 570                             | 2.5 (1.5-4.1)                         | 2.5 (471)           | 6.3 (16)           | 2.3 (43)           | 0.0 (19)                 | 0.0 (21)               | 0.02    |
|                                   | Aminoglycosides                            | 571                             | 32.2 (28.5-36.2)                      | 29.4 (472)          | 37.5 (16)          | 34.9 (43)          | 63.2 (19)                | 57.1 (21)              | <0.001  |
|                                   | Tetracyclines                              | 571                             | 5.8 (4.1-8.0)                         | 4.9 (472)           | 6.3 (16)           | 7.0 (43)           | 15.8 (19)                | 14.3 (21)              | 0.03    |
|                                   | Folate pathway inhibitors                  | 571                             | 15.9 (13.2-19.2)                      | 15.3 (472)          | 18.8 (16)          | 14.0 (43)          | 26.3 (19)                | 23.8 (21)              | 0.1     |
|                                   | Fluoroquinolones                           | 571                             | 3.7 (2.4-5.6)                         | 3.2 (472)           | 0.0 (11)           | 4.7 (43)           | 15.8 (19)                | 4.8 (21)               | <0.001  |
|                                   | Macrolides                                 | 104                             | 82.7 (74.3-88.8)                      | 85.3 (68)           | 88.9 (9)           | 75.0 (8)           | 60.0 (10)                | 88.9 (9)               | <0.001  |
|                                   | Phenics                                    | 93                              | 5.4 (2.3-12.0)                        | 6.7 (60)            | 0.0 (5)            | 0.0 (13)           | 0.0 (6)                  | 11.1 (9)               | <0.001  |
|                                   | MDR                                        | 571                             | 9.3 (7.2-11.9)                        | 7.8 (472)           | 18.8 (16)          | 9.3 (43)           | 15.8 (19)                | 28.6 (21)              | <0.001  |

(Continues)
## TABLE 2 (Continued)

| Pathogen (n) | Antimicrobial | Total number of isolates tested | Proportion of resistance (% and 95% CI) | Respiratory (2,187) | Urogenital (1,227) | Skin/Wound (1,163) | SSI/CRI/Orthopaedic (526) | Unknown & Other (595) | P value |
|--------------|---------------|---------------------------------|----------------------------------------|--------------------|-------------------|--------------------|--------------------------|------------------------|---------|
| Citrobacter spp., Enterobacter spp., Klebsiella spp., Serratia spp. & Pantoea spp. (423) | **Total** | 423 | 7.2 (158) | 9.9 (121) | 5.5 (64) | 8.7 (46) | 5.9 (34) | >0.9 (f) |
| | Extended spectrum penicillins/β-lactamase inhibitors | 16 | 0 (0.0-19.4) | 0.0 (6) | 0 (4) | 0 (2) | 0 (5) | 0 (4) |
| | **3/4th GCs** | 420 | 27.6 (23.6-32.1) | 13.9 (158) | 26.4 (121) | 27.0 (63) | 56.8 (44) | 58.8 (34) | <0.001 |
| | Aminoglycosides | 423 | 25.3 (21.4-29.7) | 15.8 (158) | 18.2 (121) | 21.9 (64) | 73.9 (46) | 35.3 (34) | <0.001 |
| | Tetracyclines | 423 | 42.8 (38.2-47.6) | 28.5 (158) | 38.8 (121) | 50.0 (64) | 78.3 (46) | 61.8 (34) | <0.001 |
| | Folate pathway inhibitors | 416 | 35.1 (30.7-39.8) | 21.7 (157) | 33.3 (117) | 38.1 (63) | 75.6 (45) | 44.1 (34) | <0.001 |
| | Fluoroquinolones | 423 | 12.8 (9.9-16.3) | 5.7 (158) | 9.9 (121) | 9.4 (64) | 47.8 (46) | 14.7 (34) | <0.001 |
| | Phenicol | 101 | 23.8 (16.5-32.9) | 34.6 (26) | 0 (21) | 22.7 (22) | 28.6 (7) | 32.0 (25) | <0.001 (f) |
| | MDR | 423 | 25.3 (21.4-29.7) | 13.3 (158) | 16.5 (121) | 25.0 (64) | 76.1 (46) | 44.1 (34) | <0.001 |
| Pseudomonas spp. (286) | **Total** | 286 | 7.0 (152) | 5.6 (69) | 3.3 (38) | 2.3 (12) | 2.5 (15) | <0.001 (f) |
| | Extended spectrum penicillins/β-lactamase inhibitors | 13 | 7.7 (1.4-33.3) | 14.3 (7) | 0 (0) | 0 (0) | 0 (6) | 0 (0) | <0.001 (f) |
| | **3/4th GCs** | 180 | 11.1 (7.3-16.5) | 12.8 (133) | 0 (3) | 0 (26) | 0 (10) | 37.5 (8) | <0.001 (f) |
| | Aminoglycosides | 286 | 19.9 (15.7-24.9) | 21.7 (152) | 23.2 (69) | 5.3 (38) | 33.3 (12) | 13.1 (15) | <0.001 (f) |
| | Fluoroquinolones | 285 | 23.5 (19.0-28.8) | 17.8 (152) | 23.5 (68) | 28.9 (38) | 41.7 (12) | 53.3 (15) | <0.001 |
| | MDR | 286 | 0.7 (0.2-2.5) | 1.3 (152) | 0 (69) | 0 (38) | 0 (12) | 0 (15) | >0.9 (f) |
| Acinetobacter spp. (141) | **Total** | 141 | 1.1 (24) | 2.6 (32) | 4.4 (51) | 3.6 (19) | 2.5 (15) | <0.001 (f) |
| | Extended spectrum penicillins/β-lactamase inhibitors | 6 | 0 (0.0-39.0) | 0 (1) | 0 (0) | 0 (1) | 0 (4) | 0 (1) | >0.9 (f) |
| | **3/4th GCs** | 118 | 23.7 (17.0-32.2) | 20.8 (24) | 13.3 (15) | 18.4 (49) | 41.2 (17) | 38.5 (13) | <0.001 (f) |
| | Aminoglycosides | 141 | 19.2 (13.5-26.4) | 8.3 (24) | 6.3 (32) | 17.6 (51) | 57.9 (19) | 20.0 (15) | <0.001 (f) |
| | Folate pathway inhibitors | 141 | 70.2 (62.2-77.1) | 62.5 (24) | 71.9 (32) | 68.6 (51) | 73.7 (19) | 80.0 (15) | 0.09 |
| | Fluoroquinolones | 139 | 15.8 (10.7-22.8) | 8.3 (24) | 9.4 (32) | 15.7 (51) | 29.4 (17) | 26.7 (15) | <0.001 (f) |
| | MDR | 141 | 13.5 (8.8-20.1) | 12.5 (24) | 0.0 (32) | 11.8 (51) | 36.8 (19) | 20.0 (15) | <0.001 (f) |

(Continues)
### TABLE 2 (Continued)

| Pathogen (n) | Antimicrobial | Total number of isolates tested | Proportion of resistance (% and 95%CI) | Respiratory (2,187) | Urogenital (1,227) | Skin/Wound (1,163) | SSI/CRI/Orthopaedic (526) | Unknown & Other (595) | P value |
|--------------|---------------|---------------------------------|----------------------------------------|---------------------|--------------------|--------------------|--------------------------|----------------------|---------|
| *Proteus spp.*, *Morganella spp.*, & *Providencia spp.* (120) | Total | 120 | 0.1 (3) | 1.0 (12) | 4.5 (52) | 4.0 (21) | 5.4 (32) | >0.9 (f) |
| | Extended spectrum penicillins/β-lactamase inhibitors | 7 | 0 (0-35.4) | 0 (2) | 0 (3) | 0 (2) | 0 (0) | 0 (0) | <0.001 (f) |
| | 3/4th GCs | 120 | 19.2 (13.1-27.1) | 0 (3) | 8.3 (12) | 7.7 (52) | 19.0 (21) | 43.8 (32) | <0.001 (f) |
| | Aminoglycosides | 120 | 32.5 (24.8-41.3) | 0 (3) | 33.3 (4) | 23.1 (52) | 33.3 (21) | 50.0 (32) | <0.001 (f) |
| | Folate pathway inhibitors | 120 | 57.5 (48.6-66.0) | 33.3 (3) | 83.3 (12) | 42.3 (52) | 76.2 (21) | 62.5 (32) | <0.001 (f) |
| | Fluoroquinolones | 120 | 25.0 (18.1-33.4) | 0 (3) | 16.7 (12) | 23.1 (52) | 28.6 (21) | 31.3 (32) | <0.001 (f) |
| | Phenicois | 53 | 34 (22.7-47.4) | 0 (0) | 0 (2) | 30.8 (13) | 45.5 (11) | 30.0 (30) | <0.001 (f) |
| | MDR | 120 | 26.7 (19.6-35.2) | 0 (3) | 16.7 (12) | 15.4 (52) | 33.3 (21) | 46.9 (32) | <0.001 (f) |
| Gram-positive bacteria | 3,199 | | 54.6 (1.195) | 47.8 (586) | 65.6 (763) | 58.9 (310) | 58.0 (345) | 0.9 (f) |
| Beta haemolytic *Streptococcus spp.* (1,467) | Total | 1,467 | 31.3 (685) | 29.5 (362) | 20.0 (233) | 10.3 (54) | 22.0 (131) | 0.9 (f) |
| | Penicillin | 1,466 | 2.5 (1.8-3.4) | 3.1 (684) | 0.8 (362) | 3.0 (233) | 1.9 (54) | 3.0 (133) | 0.9 (f) |
| | β-lactamase inhibitor combinations | 756 | 0.7 (0.3-1.5) | 0.9 (533) | 0.0 (30) | 0.0 (145) | 0.0 (26) | 0.0 (22) | >0.9 (f) |
| | 3/4th GCs | 1,467 | 1.7 (1.2-2.5) | 1.8 (685) | 0.8 (362) | 2.6 (233) | 1.9 (54) | 2.3 (133) | >0.9 (f) |
| | Tetracycline | 1,460 | 33.8 (31.5-36.3) | 37.8 (685) | 19.4 (355) | 33.1 (233) | 59.3 (54) | 42.9 (133) | <0.001 |
| | Folate pathway inhibitors | 1,465 | 15.0 (12.2-16.9) | 15.7 (683) | 15.8 (362) | 10.7 (233) | 20.4 (54) | 14.3 (133) | 0.5 |
| | Fluoroquinolones | 1,467 | 27.9 (25.7-30.2) | 25.0 (685) | 11.9 (362) | 33.9 (233) | 50.0 (54) | 66.9 (133) | <0.001 |
| | Macrolides | 599 | 15.4 (12.7-18.5) | 12.8 (258) | 20.3 (64) | 11.1 (126) | 26.7 (30) | 19.8 (121) | <0.001 |
| | Phenicois | 393 | 13.7 (10.7-17.5) | 13.0 (146) | 14.0 (43) | 16.5 (79) | 13.3 (15) | 12.7 (110) | >0.9 (f) |
| | MDR | 1,467 | 8.3 (7.0-9.8) | 7.5 (685) | 3.9 (362) | 6.4 (233) | 18.5 (54) | 21.8 (133) | <0.001 |

(Continues)
| Pathogen (n) | Antimicrobial | Total number of isolates tested | Proportion of resistance (% and 95%CI) | Respiratory (2,187) | Urogenital (1,227) | Skin/Wound (1,163) | SSI/CRI/Orthopaedic (526) | Unknown & Other (595) | P value |
|-------------|---------------|---------------------------------|----------------------------------------|---------------------|-------------------|--------------------|--------------------------|------------------------|----------|
| *Staphylococcus* spp. (916) | Total         | 916                             | 7.1 (155) 8.8 (108) 32.2 (374) 28.1 (148) 22.0 (131) | 10.7 (28) 28.6 (14) 8.2 (98) 38.6 (70) 7.6 (105) | <0.001 (f) |
|             | Oxacillin/Cefoxitin | 315                             | 15.9 (12.3-20.3)% | 10.7 (28) 28.6 (14) 8.2 (98) 38.6 (70) 7.6 (105) | <0.001 (f) |
|             | Aminoglycosides   | 894                             | 24.9 (22.2-27.9) | 11.0 (154) 22.4 (107) 18.4 (370) 51.5 (132) 35.1 (131) | <0.001 |
|             | Tetracyclines     | 894                             | 35.6 (32.5-38.8) | 26.0 (154) 34.6 (107) 27.0 (370) 65.2 (132) 42.0 (131) | <0.001 |
|             | Folate pathway inhibitors | 894                         | 25.8 (23.1-28.8) | 15.6 (154) 22.4 (107) 19.5 (370) 47.0 (132) 37.4 (131) | <0.001 |
|             | Fluoroquinolones  | 893                             | 13.1 (11.3-15.7) | 6.5 (154) 8.3 (108) 8.4 (370) 22.9 (131) 30.0 (130) | <0.001 |
|             | Macrolides        | 407                             | 34.6 (30.2-39.4) | 18.6 (59) 29.0 (31) 25.8 (120) 32.6 (86) 55.9 (111) | <0.001 |
|             | Phenicols         | 259                             | 6.2 (3.8-9.8)    | 7.4 (27) 27.3 (11) 5.4 (93) 7.4 (27) 4.0 (101) | <0.001 (f) |
|             | Fusidanes         | 736                             | 15.6 (13.2-18.4) | 17.3 (139) 10.9 (46) 11.9 (337) 12.6 (87) 27.6 (127) | <0.001 |
|             | Ansamycins (Rifampicin) | 724                        | 6.5 (4.9-8.5)    | 4.5 (112) 7.0 (43) 4.1 (321) 10.7 (122) 10.3 (126) | 0.2 (f) |
|             | MDR               | 916                             | 25.3 (22.6-28.2) | 14.2 (155) 17.6 (108) 16.6 (374) 44.6 (148) 48.1 (131) | <0.001 |
| Alpha haemolytic *Streptococcus* spp. (353) | Total         | 353                             | 12.7 (277) 1.8 (22) 2.4 (28) 3.8 (20) 1.0 (6) | 0.0 (6) | <0.001 (f) |
|             | 3/4th GCs         | 353                             | 0.85 (0.3-2.5)   | 0.36 (277) 0.0 (22) 3.6 (28) 5.0 (20) 0.0 (6) | <0.001 (f) |
|             | Fluoroquinolones  | 352                             | 7.1 (4.9-10.3)   | 4.7 (276) 13.6 (22) 0.0 (28) 35.0 (20) 33.3 (6) | <0.001 (f) |
|             | Macrolides        | 29                              | 10.3 (3.6-26.4)  | 12.5 (8) 25.0 (4) 0.0 (1) 7.7 (13) 0.0 (3) | <0.001 (f) |
|             | MDR               | 353                             | 0 (0.0-1.1)      | 0.0 (277) 0.0 (22) 0.0 (28) 0.0 (20) 0.0 (6) | >0.9 (f) |
| *Enterococcus* spp. (278) | Total         | 278                             | 2.7 (58) 5.1 (63) 4.0 (46) 12.2 (64) 7.9 (47) | 10.2 (34) 2.8 (36) 12.0 (25) 27.3 (33) 0.0 (9) | <0.001 (f) |
|             | Aminopenicillins  | 137                             | 10.2 (6.2-16.4)  | 2.9 (34) 2.8 (36) 12.0 (25) 27.3 (33) 0.0 (9) | <0.001 (f) |
|             | Tetracyclines     | 276                             | 49.6 (43.8-55.5) | 22.4 (58) 48.4 (62) 54.3 (46) 77.8 (63) 42.6 (47) | <0.001 |
|             | Fluoroquinolones  | 276                             | 50.7 (44.9-56.7) | 13.8 (58) 41.3 (63) 43.5 (46) 79.0 (62) 78.7 (47) | <0.001 |
|             | MDR               | 278                             | 0 (0.0-1.4)      | 0.0 (58) 0.0 (63) 0.0 (46) 0.0 (64) 0.0 (47) | >0.9 (f) | (Continues)
| Pathogen (n)                        | Antimicrobial                      | Total number of isolates tested | Proportion of resistance (% and 95% CI) | Proportion of resistant isolates by sample site, % (total tested) |
|------------------------------------|-----------------------------------|---------------------------------|----------------------------------------|------------------------------------------------------------------|
| **Corynebacterium spp. & Bacillus spp. (185)** | Total                             | 185                             | 0.9 (20)                               | Respiratory (2,187) 0.2 (31) Urogenital (1,227) 7.1 (82) Skin/Wound (1,163) 4.6 (24) SSI/CRI/Orthopaedic (526) 4.7 (28) | P value |
|                                   | Penicillins                        | 185                             | 70.3 (63.3-76.4)                       | 60.0 (20) 58.1 (31) 72.0 (82) 70.8 (24) 85.7 (28)               | <0.001 |
|                                   | β-lactamase inhibitor combinations | 85                              | 27.1 (18.8-37.3)                      | 25.0 (12) 50.0 (6) 30.4 (46) 7.7 (13) 25.0 (8)                | <0.001 |
|                                   | 3/4th GCs                          | 184                             | 52.2 (45.0-59.3)                      | 55.0 (20) 56.7 (30) 53.7 (82) 33.3 (24) 57.1 (28)            | <0.001 |
|                                   | Aminoglycosides                    | 185                             | 17.8 (13.0-24.0)                      | 10.0 (20) 9.7 (31) 17.1 (82) 16.7 (24) 35.7 (28)              | <0.001 |
|                                   | Tetracyclines                      | 185                             | 21.6 (16.3-28.1)                      | 20.0 (20) 9.7 (31) 17.1 (82) 16.7 (24) 35.7 (28)             | <0.001 |
|                                   | Folate pathway inhibitors          | 185                             | 41.6 (34.8-48.8)                      | 35.0 (20) 41.9 (31) 45.1 (82) 45.8 (24) 32.1 (28)            | 0.1 |
|                                   | Fluoroquinolones                   | 185                             | 12.4 (8.4-18.0)                       | 5.0 (20) 9.7 (31) 7.3 (82) 25.0 (24) 25.0 (28)               | <0.001 |
|                                   | Macrolides                         | 76                              | 60.5 (49.3-70.8)                      | 75.0 (4) 70.0 (10) 50.0 (32) 60.0 (10) 70.0 (20)             | <0.001 |
|                                   | Phenicols                          | 89                              | 36.0 (26.8-46.3)                      | 12.5 (8) 22.2 (9) 40.5 (42) 40.0 (10) 40.0 (20)             | <0.001 |
|                                   | MDR                                | 185                             | 50.8 (43.7-57.9)                      | 45.0 (20) 45.2 (31) 51.2 (82) 41.7 (24) 67.9 (28)             | <0.001 |

Note: P value is provided for comparisons between proportions using Chi-squared (or Fisher’s exact test (f) when sample size in any category was <5).

Abbreviation: GC, generation cephalosporin.

^{a} Penicillin and Aminopenicillin combined for Pasteurella spp.

^{b} Cefazidime/Cefquinome only.

^{c} Ceftoxime/Cefazidime/Cefquinome only.

^{d} Gentamicin excluded for Providencia spp. Bacterial isolates where there was <100 in a genus were not included (n = 320) from the original 6,018. Unknown included those submissions where no site was reported (n = 520) while ‘others’ were those present in low numbers (n = 99) and included sample sites such as faecal, peritoneal fluid, liver, dental, gastric and rectal submissions. Full breakdown of bacterial isolates is shown in Table S4.

^{e} For S. aureus, prevalence of oxacillin/cefoxitin resistance was 12.1% (30 of 247 isolates tested).
| Bacteria (total number of isolates) | Susceptibility patterns of isolates | Number of isolates | Proportion (%) [95% CI] |
|-----------------------------------|-----------------------------------|-------------------|------------------------|
| **Gram-negative bacteria**         |                                   |                   |                        |
| *Escherichia coli* (958)          | Broadly susceptible               | 342               | 35.7 (32.7-38.8)       |
|                                    | Resistant to 1 or 2 classes       | 312               | 32.6 (29.7-35.6)       |
|                                    | MDR                               | 304               | 31.7 (28.9-34.8)       |
|                                    | XDR                               | 23                | 2.4 (1.6-3.6)          |
|                                    | No readily available treatment for adult horses in the UK | 31 | 3.2 (2.3-4.6) |
| *Actinobacillus* spp. *Pasteurella* spp. (571) | Broadly susceptible | 295 | 51.7 (47.6-55.7) | |
|                                    | Resistant to 1 or 2 classes       | 223               | 39.1 (35.1-43.1)       |
|                                    | MDR                               | 53                | 9.3 (7.2-11.9)         |
|                                    | XDR                               | 0                 | 0.0 (0.0-0.6)          |
|                                    | No readily available treatment for adult horses in the UK | 0 | 0.0 (0.0-0.6) |
| *Citrobacter* spp., *Enterobacter* spp., *Klebsiella* spp., *Serratia* spp., & *Pantoea* spp. (423) | Broadly susceptible | 174 | 41.1 (36.6-45.9) | |
|                                    | Resistant to 1 or 2 classes       | 142               | 33.6 (29.3-38.2)       |
|                                    | MDR                               | 107               | 25.3 (21.4-29.7)       |
|                                    | XDR                               | 6                 | 1.4 (0.7-3.1)          |
|                                    | No readily available treatment for adult horses in the UK | 26 | 6.1 (4.2-8.9) |
| *Pseudomonas* spp. (286)           | Broadly susceptible               | 172               | 60.1 (54.4-65.6)       |
|                                    | Resistant to 1 or 2 classes       | 112               | 39.2 (33.7-44.9)       |
|                                    | MDR                               | 2                 | 0.7 (0.2-2.5)          |
|                                    | XDR                               | 0                 | 0.0 (0.0-1.3)          |
|                                    | No readily available treatment for adult horses in the UK | 18 | 6.3 (4.0-9.7) |
| *Acinetobacter* spp. (141)        | Broadly susceptible               | 33                | 23.4 (17.2-31.0)       |
|                                    | Resistant to 1 or 2 classes       | 89                | 63.1 (54.9-70.6)       |
|                                    | MDR                               | 19                | 13.5 (8.8-20.1)        |
|                                    | XDR                               | 6                 | 4.3 (2.0-9.0)          |
|                                    | No readily available treatment for adult horses in the UK | 13 | 9.2 (5.5-15.1) |
| *Proteus* spp., *Morganella* spp., & *Providencia* spp. (120) | Broadly susceptible | 36 | 30.0 (22.5-38.7) | |
|                                    | Resistant to 1 or 2 classes       | 52                | 43.3 (34.8-52.3)       |
|                                    | MDR                               | 32                | 26.7 (19.6-35.2)       |
|                                    | XDR                               | 3                 | 2.5 (0.9-7.1)          |
|                                    | No readily available treatment for adult horses in the UK | 3 | 2.5 (0.9-7.1) |
| **Gram-positive bacteria**         |                                   |                   |                        |
| *β-haemolytic Streptococcus* spp. (1,467) | Broadly susceptible | 683 | 46.6 (44.0-49.1) | |
|                                    | Resistant to 1 or 2 classes       | 663               | 45.2 (42.7-47.8)       |
|                                    | MDR                               | 121               | 8.3 (7.0-9.8)          |
|                                    | XDR                               | 1                 | 0.1 (0.0-0.4)          |
|                                    | No readily available treatment for adult horses in the UK | 1 | 0.1 (0.0-0.4) |
| *Staphylococcus* spp. (916)        | Broadly susceptible               | 427               | 46.6 (43.4-49.9)       |
|                                    | Resistant to 1 or 2 classes       | 257               | 28.1 (25.3-31.1)       |
|                                    | MDR                               | 232               | 25.3 (22.6-28.2)       |
|                                    | XDR                               | 2                 | 0.2 (0.0-0.8)          |
|                                    | No readily available treatment for adult horses in the UK | 4 | 0.4 (0.2-1.1) |

(Continues)
TABLE 3 (Continued)

| Bacteria (total number of isolates) | Susceptibility patterns of isolates | Number of isolates | Proportion (% [95% CI]) |
|-------------------------------------|------------------------------------|-------------------|-------------------------|
| α-haemolytic Streptococcus spp. (353) | Broadly susceptible                  | 325               | 92.1 (88.8-94.5)         |
|                                    | Resistant to 1 or 2 classes          | 28                | 7.9 (5.5-11.2)           |
|                                    | MDR/XDR (≠ all classes)              | 0                 | 0.0 (0.0-1.1)            |
|                                    | No readily available treatment for adult horses in the UK | 1                 | 0.3 (0.0-1.6)            |
| Enterococcus spp. (278)            | Broadly susceptible                  | 84                | 30.2 (25.1-35.9)         |
|                                    | Resistant to 1 or 2 classes          | 185               | 66.5 (60.8-71.8)         |
|                                    | MDR / XDR (≠ all classes)            | 9                 | 3.2 (1.7-6.0)            |
|                                    | No readily available treatment for adult horses in the UK | 84               | 30.2 (25.1-35.9)         |
| Bacillus spp. & Corynebacterium spp. (185) | Broadly susceptible                  | 26                | 14.1 (9.8-19.8)          |
|                                    | Resistant to 1 or 2 classes          | 65                | 35.1 (28.6-42.3)         |
|                                    | MDR                                 | 94                | 50.8 (43.7-57.9)         |
|                                    | XDR                                 | 0                 | 0.0 (0.0-2.0)            |
|                                    | No readily available treatment for adult horses in the UK | 1                 | 0.5 (0.1-3.0)            |

Note: Multidrug-resistant (MDR) isolates were those with acquired non-susceptibility to at least one antimicrobial in three or more different antimicrobial classes. Extensively drug-resistant (XDR) isolates were those, which were resistant to all classes of antimicrobials tested. ‘No readily available treatment for adult horses in the UK’ included those isolates, which were resistant to commonly used (authorised or non-authorised) antimicrobials available for adult horses in the UK. All calculations are based on antimicrobials considered in Table 1 and excludes intrinsic resistance.

4 | DISCUSSION

This is the largest study investigating bacterial isolates and their resistance patterns from equine clinical submissions to multiple laboratories in the UK and provides important information on AMR in common equine pathogens. The current study identified potential geographical differences in MDR for the most common bacterial isolates as well as significantly different prevalence of resistance in bacterial isolates from different sample sites and from referral practices compared with first opinion practices. These variables are unlikely to be independent; for example, there was increased MDR in SSI/CRI and orthopaedic isolates, however, the majority of these were from referral practices (80.3%) where horses may be more likely to have received previous antimicrobials, having undergone surgery, have co-morbidities such as systemic inflammatory response syndrome (SIRS) after colic surgery or be/have been hospitalised (although the exact proportion hospitalised is unknown). In isolates from SSI/CRI and orthopaedic infections in the major categories (listed in Table 1) from referral practices, 37.7% (160/424) were MDR. Previous studies have reported increased AMR and MDR in clinical isolates from hospitalised compared with non-hospitalised horses. Similar to our study, previous equine studies have also reported lower prevalence of AMR in bacteria from respiratory and urogenital submission compared with wounds. Human and companion animal studies have also identified high MDR in hospital-acquired infections due to a variety of factors such as previous antimicrobials, co-morbidities, duration of hospitalisation and severity of disease. Gram-negative MDR bacteria have been associated with increased mortality in horses with synovial caseloads (p < 0.001), while urogenital, skin/hair/wound/abscess and unknown/other submissions were higher from first opinion practices (p < 0.001) (Table 4). From the 5,861 isolates which belonged to the major bacterial groups with AST results presented in Table 2, postcode data were available for 5,564 isolates. This included 2,422 Gram-negative and 3,142 Gram-positive isolates with 2,820 isolates from referral and 2,744 isolates from first opinion practices. The proportions of MDR in bacterial isolates were significantly different in referral hospitals compared with first opinion practices (Table 5). MDR was significantly higher in submissions from referral hospitals in E. coli (p < 0.001), Citrobacter spp., Enterobacter spp., Klebsiella spp., Serratia spp., & Pantoea spp. (p < 0.001), Acinetobacter spp. (p < 0.001), Staphylococcus spp. (p < 0.001) and Enterococcus spp.(p = 0.01). MDR was significantly higher in submissions from first opinion practices in Actinobacillus spp. & Pasteurella spp. (p < 0.001) and β-haemolytic Streptococcus spp. (p < 0.001). The majority of S. equi were from first opinion submissions (77.3%), while only 18.1% were from referral practices. The majority of S. zooepidemicus were also from first opinion submissions (64.9%) while 33.7% were from referral practices. In unspecified β-haemolytic Streptococcus, which made up 54.4% of all β-haemolytic Streptococcus spp. 72.2% were from referral practices. Where data were available regarding postcode (n = 5,861), a bivariate choropleth map displaying the proportion of MDR isolates (and standard error) for each UK constituent postcode area identified variations in MDR across the UK (shown in Figure 2) across all isolates and for those bacteria which were present in large enough numbers for analysis (E. coli, β-haemolytic Streptococcus spp. and Staphylococcus spp.). Although descriptive, this revealed some postcode areas with relatively higher resistance prevalence and low standard errors.
sepsis (orthopaedic infection). However, depending on the severity and site of the infection, MDR bacteria particularly from SSI do not always require systemic antimicrobials as many are superficial infections, which are often self-limiting. The current human guidelines for SSIs recommend local treatment consisting of topical antimicrobials in conjunction with debridement and specialist wound dressings, as well as regular bandage changes and close monitoring of the progress of the infection.

Knowledge of these MDR bacteria is important in order to implement targeted biosecurity measures such as increasing hand hygiene when handling surgical patients, high-level cleaning of stables between patients and sampling the stable environment after cleaning and before admitting the next patient in the same stable in order to prevent spread of MDR bacteria in the hospital. Ideally, patients with MRSA or ESBL-producing bacteria should be placed in isolation to prevent spread to other horses in the hospital. By

### Table 4: Proportion of clinical submissions (n = 3,926) from different sample sites (in % with 95% CI) from clinical infections in horses at referral and first opinion equine practices in the UK in 2018

| Sample site (n)                              | Referral hospital (n = 2008) | First opinion practice (n = 1918) |
|---------------------------------------------|-----------------------------|----------------------------------|
|                                             | Total number of submissions | Proportion of isolates (% and 95%CI) | Total number of submissions | Proportion of isolates (% and 95%CI) | P value |
| Respiratory tract (1505)                    | 885                         | 58.8 (56.3-61.3)                 | 620                         | 41.2 (38.7-43.7)                  | <0.001  |
| Urogenital (990)                            | 406                         | 41.0 (38.0-44.1)                 | 584                         | 59.0 (55.9-62.0)                  | <0.001  |
| Skin/Hair/Wound/Abscess (723)               | 293                         | 40.5 (37.0-44.2)                 | 430                         | 59.5 (55.9-63.0)                  | <0.001  |
| SSI/CRI/Orthopaedic Infection (342)         | 283                         | 82.8 (78.4-86.4)                 | 59                          | 17.3 (13.6-21.6)                  | <0.001  |
| Unknown and other (366)                     | 141                         | 38.5 (33.7-43.6)                 | 225                         | 61.5 (56.4-66.3)                  | <0.001  |

Note: P-value is provided for comparisons between the proportions of submissions from different practices using Chi squared. Clinical submissions without information regarding referral status of submitting practice including submissions from abroad were excluded from analysis (n = 112). SSI-surgical site infection, CRI-catheter-related infection.

### Table 5: Proportion of multidrug resistance (MDR) (in % with 95% CI) in bacteria isolated from clinical infections in horses at referral and first opinion equine practices in the UK in 2018 based on 5,564 isolates with UK postcode data in the major bacterial genera included in this study. P-value is provided for comparisons between proportions using Chi squared (or Fisher’s exact test (f) when sample size in any category was <5)

| Pathogen (n)                        | Referral hospital (n = 2,820) | First opinion practice (n = 2,744) |
|-------------------------------------|-----------------------------|----------------------------------|
|                                     | Total number of isolates    | Proportion of MDR (% and 95%CI)  | Total number of isolates    | Proportion of MDR (% and 95%CI)  | P value |
| Gram-negative bacteria (n = 2,422)  |                             |                                 |                             |                                 |         |
| *Escherichia coli* (926)            | 387                         | 36.7 (32.0-41.6)                 | 539                         | 27.1 (23.5-31.0)                 | <0.001  |
| *Actinobacillus* spp. & *Pasteurella* spp. (569) | 425                         | 6.4 (4.4-9.1)                    | 144                         | 18.1 (12.6-25.1)                 | <0.001  |
| *Citrobacter* spp., *Enterobacter* spp., *Klebsiella* spp., *Serratia* spp., & *Pantoea* spp. (406) | 142                         | 35.2 (27.8-43.4)                 | 264                         | 20.8 (16.4-26.2)                 | <0.001  |
| *Pseudomonas* spp. (267)<sup>a</sup> | 121                         | 0 (0-3.1)                       | 146                         | 1.4 (0.4-4.9)                    | 0.5 (f)  |
| *Acinetobacter* spp. (135)          | 44                          | 27.3 (16.4-41.9)                 | 91                          | 6.6 (3.1-13.7)                   | <0.001  |
| *Proteus* spp., *Morganella* spp., & *Providencia* spp. (119) | 58                          | 34.5 (23.6-47.3)                 | 61                          | 19.7 (11.6-31.3)                 | 0.1      |
| Gram-positive bacteria (n = 3,142)   |                             |                                 |                             |                                 |         |
| *Beta haemolytic Streptococcus* spp. (1,455) | 789                         | 5.1 (3.7-6.8)                    | 666                         | 11.7 (9.5-14.4)                  | <0.001  |
| *Staphylococcus* spp. (888)         | 405                         | 34.8 (30.3-39.6)                 | 483                         | 18.4 (15.2-22.1)                 | <0.001  |
| *Alpha haemolytic Streptococcus* spp. (351)<sup>a</sup> | 273                         | 0.0 (0.0-1.4)                    | 78                          | 0.0 (0.0-4.7)                    | >0.9 (f) |
| *Enterococcus* spp. (271)<sup>a</sup> | 127                         | 6.3 (3.2-11.9)                   | 144                         | 0.7 (0.1-3.8)                    | 0.01 (f) |
| *Bacillus* spp. & *Corynebacterium* spp. (177) | 49                          | 44.9 (31.9-58.7)                 | 128                         | 50.8 (42.2-59.3)                 | 0.6      |

<sup>a</sup>There are several bacterial isolates with high levels of IR leaving limited treatment options available in adult horses (eg *Enterococcus* spp., *Pseudomonas* spp. and *α*-haemolytic *Streptococcus* spp.) Thus, using a classification of MDR of resistance to three or more classes results often results in artificially low MDR estimates despite there being limited treatment options for adult horses, hence MDR calculations in bacterial isolates with high IR should be interpreted in light of IR.
monitoring bacteria in SSI/CRI and orthopaedic infections, hospitals are also better able to identify breaches in biosecurity if multiple patients develop infections with the same bacteria and AST phenotype. Surveillance data are also important from a public health aspect to monitor emerging zoonotic bacteria in companion animals and horses such as toxigenic Corynebacterium ulcerans, Clostridium difficile, Leptospira spp. or Staphylococcus spp. In addition to submissions with missing postcodes, 8.6% (520/6018) of isolates had the sample site information missing, which is similar to human and other veterinary studies where information was commonly missing from diagnostic submission forms. Isolates from unknown sample sites often also had high prevalence of MDR, however, this is of limited value without knowing the source of the samples. We elected to include “unknown” site for completeness of reporting, and to highlight the importance of encouraging submitting veterinarians to provide more complete information on diagnostic submissions for improved laboratory reporting and surveillance. Knowledge of the sample site is also valuable information for the microbiology laboratory to allow adherence to appropriate culture protocols according to the sample site and also for the clinical microbiologist when interpreting the results and deciding whether the presence of certain bacterial isolates is likely clinically significant or due to contamination. Unless present as a pure growth from a normally sterile site (such as urine obtained via cystocentesis), it is difficult to distinguish between simple bacterial presence and true infection. Many bacterial isolates in equine infections are opportunistic pathogens that may colonise body sites together with other commensal bacteria and when the conditions are optimal can cause infections. For example, MDR Acinetobacter baumannii has been reported in vascular catheters in horses, but only in 42.9% of cases was there evidence of local infection. Immunocompromised patients, in particular, are at risk of infections caused by diverse bacteria, including opportunistic pathogens and anatomical differences, between different sexes and age groups may also predispose to infection. Furthermore, administration of antimicrobials exerts selective pressure on commensal bacterial populations within a host, which can select for opportunistic pathogens, for example S. aureus on mucosal surfaces of carriers following cephalosporin exposure will undergo collateral selective pressure, conferring advantage to resistant subpopulations, including MRSA.

This study identified increased MDR in submissions from referral practices compared with first opinion practices in common opportunistic pathogens, such as E. coli, Citrobacter spp., Enterobacter spp., Klebsiella spp., Serratia spp., and Pantoea spp., Acinetobacter spp., Staphylococcus spp. and Enterococcus spp. Interestingly, there was increased MDR in submissions from first opinion practices for Actinobacillus spp. and Pasteurella spp. and β-haemolytic Streptococcus spp. These are common respiratory and mucosal pathogens, but surprisingly there were significantly more respiratory submissions from practices with referral caseloads, which is different to a previous study where 64% of β-haemolytic Streptococcus submissions were from non-hospitalised horses. In the current study, the majority of S. equi (77.3%) and S. zooepidemicus (64.9%) were from first opinion submission while the majority of unspecified β-haemolytic Streptococcus spp. (72.2%) were from referral submissions, hence it is difficult to comment further on the pathogenicity of these isolates. However, as submissions from practices with both referral and first opinion caseloads were categorised as referral, it was not possible to distinguish further between submissions and it is possible there was some misclassification. Some of these referral respiratory

**FIGURE 2** Quintile bivariate postcode map displaying the proportion of multidrug-resistant (MDR) equine bacterial isolates that were submitted by veterinary practice sites in the UK. Only bacterial isolates present in sufficient numbers for analysis were included showing (A) overall, (B) β-haemolytic Streptococcus spp., (C) E. coli and (D) Staphylococcus spp. Proportions are displayed against standard error to provide a measure in relative confidence in findings depending on data volume provided within each postcode area.
submissions are likely to originate from some large equine practices that have both hospital and ambulatory branches and may undertake more poor performance/subclinical respiratory disease screening in sport and racehorses rather than sampling horses with overt clinical disease which may have biased these results. In these horses, S. zoopneumoniae, for example, is viewed as performance limiting, which may well be associated with tracheal mucus and inflammatory airway disease and is likely to be treated with antimicrobials.46 As it was not possible to distinguish between upper and lower respiratory tract submissions, it is not possible to explore this further.

It is important to highlight that despite there being higher prevalence of MDR in β-haemolytic Streptococcus spp. in first opinion submissions than referral submissions, overall MDR in β-haemolytic Streptococcus spp. was only 8.3% and importantly 97.5% of isolates were susceptible to penicillin, which is the current first-line treatment for equine respiratory infections listed in the BEVA Protect ME toolkit.49 As described in the methods in this study, all Streptococcus spp. were considered intrinsically resistant to aminoglycosides according to EUCAST Expert Rules.50 Increased doses may overcome this low-level of intrinsic resistance, although this may not be practical or safe in equine practice. Interestingly, in recent reports from clinical isolates from horses in France gentamicin resistance were low (1.2%) in all Streptococcus spp. (75.1% of all streptococci in that study were S. zoopneumoniae), although that study used a high concentration of gentamicin (500µg) for AST. Similarly, in respiratory submissions in horses from New Zealand, gentamicin resistance was low in all Streptococcus spp. (7.4%) despite that study using a lower standard concentration of gentamicin (10µg) for AST.51

Therefore, for these bacterial isolates MDR is often artificially low (<4% in this study) despite there often being no readily available treatment options in adult horses in the UK (30.2% for Enterococcus spp.). It is important to recognise that bacterial isolates with high IR should not be overlooked due to their low MDR as they pose a therapeutic challenge when involved in infection.52 These bacteria, as well as posing a risk for the individual horse, are also of zoonotic concern as they have also been reported in humans. A genotypically identical strain of Pseudomonas spp. from a water source has been reported as a cause of an outbreak of equine endometritis in Australia,53 from a variety of equine samples in Ireland,54 from companion animals55 and from human cystic fibrosis patients.56 Enterococcus spp. are common pathogens in hospital-acquired infections in humans,57 equine synovial infections58 and companion animals59 and have been associated with increased mortality in foals.60 However, they are often present in human and animal gut flora,61 on skin62 and urogenital mucosa63 and therefore are often present in clinical specimens as contaminants.64,65 It is important that their clinical significance is thoroughly evaluated, and susceptibility testing is issued only when their clinical significance is established. Future studies should investigate the relevance of Enterococcus spp. by including cytological evidence of association with infection. Alpha-haemolytic Streptococcus spp., in particular S. pneumoniae, are also troublesome to treat and are a common cause of human septicaemia66 and have been reported in bacteraemia and pneumonia in a neonatal foal67 and companion animals.68 These bacteria form an exceptional clinical challenge in human and veterinary medicine, as the isolates are frequently MDR and have susceptibility patterns that are difficult to predict.60,71,72

Our study identified higher MDR compared with a recent study of clinical isolates in France, where the highest MDR was 22.5% (S. aureus). MDR in Staphylococcus spp. in our study was slightly higher (25.3%) with high MDR in other common opportunistic pathogens such as E. coli (31.7%) and Citrobacter spp., Enterobacter spp.,
Klebsiella spp., Serratia spp. and Pantoea spp. (25.3%). This is similar to other studies in the UK where MDR in clinical E. coli from horses was 39.9%.5 MRSA and EBSL-producing Enterobacteriaceae are common bacterial isolates in nosocomial infections and of much clinical interest. It was not possible to report the exact prevalence of these organisms in this study due to no confirmatory genotyping or phenotypic testing being performed in the majority of laboratories. The prevalence of oxacillin- or cefoxitin-resistant S. aureus isolates was 6.1% which is lower than the prevalence of cefoxitin-resistant S. aureus from horses in France (23%). The highest prevalence of oxacillin- or cefoxitin-resistant S. aureus in the current study was from SSI/CRI/orthopaedic (21.6%, 16/74) and urogenital (6.6%, 3/44) and skin/hair/wound/abscess (2.3%, 5/214). This included isolates from one laboratory that used PCR assay to confirm presence of meca gene73 and this gene was identified in 26.3% (5/19) of oxacillin- or cefoxitin-resistant S. aureus in this laboratory. Although MRSA screening was based on oxacillin or cefoxitin testing which could result in an overestimation of the real proportion of MRSA, our results indicate that oxacillin- or cefoxitin-resistant S. aureus are less prevalent in UK than French submissions. Most laboratories did not perform phenotypic testing to detect ESBL producers in 3/4GC-resistant Gram-negative isolates, hence the prevalence of ESBL producers cannot be reported. However, resistance to 3/4GC in the current study was 14.0% of E. coli and 27.6% in Citrobacter spp., Enterobacter spp., Klebsiella spp., Serratia spp. & Pantoea spp., which is higher than in E. coli (7.6%) and Klebsiella spp. (5.2%) in clinical isolates from France,12 but similar to a previous UK study where 3GC resistance in E. coli was 14.2%.9 There was a lower prevalence of cefotiofur resistance in E. coli from respiratory submissions in horses in the UK (0%-2.9%) than the current study, while in Pseudomonas spp. 3GC resistance was higher (over 64.6%) compared with the current study (11.1%).53

The current study has some inherent limitations. The results were generated from different laboratories using slightly different antimicrobial panels and different technical equipment and staff. Since interpretative criteria of disc diffusion data are set, so there is optimal correlation with MIC from microbroth dilution, for most bacterial species from both human and veterinary samples, one method of susceptibility testing is not considered superior to the other24-78 for the majority of antimicrobials against bacterial species such as Salmonella spp., Enterobacteriaceae, A. baumannii.77-80 For some bacterial species there are discrepancies between the methods in particular for methicillin-resistant Staphylococcus pseudintermedius (MRSP) and for some antimicrobials when testing against Pseudomonas spp. and Corynebacterium spp.78,82,83 For polymyxin B and colistin disc diffusion methods are not recommended as these do not diffuse well in agar84 and microbroth dilutions are also recommended for S. pneumoniae (α-haemolytic Streptococcus spp.) to penicillins and some cephalosporins due to better accuracy.85,86 Larger and more modern laboratories are commonly using automated microbroth dilution methods due to its versatility and ability to determine the MIC likely to achieve effective antimicrobial plasma concentration. This means that if the MIC indicates that an isolate is susceptible but at the higher end of the range, near the epidemiological cut-off value (ECOFF), it may require a higher dose to achieve therapeutic concentrations.87 Although there are also inaccuracies in MIC, such that the accepted MIC ranges of quality control strains, often span over two to three dilutions and even four dilutions in some cases.88 Smaller laboratories often use Kirby-Bauer disc diffusion methods due to lower costs and no requirement for extensive equipment. Furthermore, another limitation was that a pooled approach to reporting was used by combining some bacterial species based on their similarities in intrinsic resistance patterns. This is similar to human studies8 and was done in order to avoid having several smaller groups making conclusions and presentation of results difficult, but the authors acknowledged that this does somewhat limit the application of these pooled results.

The current study reports on the presence of different bacterial isolates from clinical submissions and all isolates with reported susceptibility were included in this study. However, some bacteria may be contaminants such as Bacillus spp. and Enterococcus spp., and Pseudomonas spp. in some submissions. Although in the majority of submissions only a single bacterial isolate was reported, it is difficult to establish the main pathogenic organism in polymicrobial cultures,51 which is another limitation of the current study and a general challenge of diagnostic microbiology. However, in this study, we identified less polymicrobial cultures (36.4%) than previously reported in equine respiratory submissions where 58.2%-76.4% of cultures yielded polymicrobial growth.53 Performing AST on multiple isolates from the same specimen without consideration of clinical relevance does not promote prudent antimicrobial prescribing practices.89 Bacterial isolates with intermediate susceptibility were considered resistant, as treatment with an antimicrobial with intermediate susceptibility would likely not be recommended in most situations in a clinical infection and is also consistent with reporting in other EU and UK surveillance projects.90,91 In circumstances where there are no other treatment options, antimicrobial therapy may be guided by MIC to safely determine the antimicrobial dose for an antimicrobial agent with intermediate susceptibility. As certain antimicrobials are excreted in urine, such as penicillin and folate pathway inhibitors, higher concentration can be achieved in urine. There are a small number of breakpoints specific to urinary tract infections (UTI) for this reason (eg for amoxicillin in Enterobacteriaceae in dogs), but it is not relevant to include these breakpoints as they were not used by any of the laboratories in this study. Classifying intermediate susceptibility as resistant is likely to have overestimated resistance outcomes, including MDR. Furthermore, intermediate susceptibility may incorrectly reflect the outcome in topical use in cases involving the eye/skin/wounds where resistance was relatively high in this study. Care is also warranted over overestimation of susceptibility for treatment of infections confined in the central nervous system, or systemic use of antimicrobials for treatment of infections in the eye, where some antimicrobials may penetrate poorly. Although these were not common sites reported in this study. It was also not possible to assess the way samples were collected and, for example, obtaining a respiratory sample via a nasal swab has higher potential
for contamination compared with obtaining a transtracheal wash (TTW) or bronchoalveolar lavage (BAL) sample.

Another limitation is the inherent selection bias associated with clinical submissions, as infections, which are not responding to treatment, are more likely to be submitted and similarly, infections which are responding to treatment are often not sampled, particularly in non-hospitalised horses. This is a limitation common to clinical diagnostic microbiology data, which is unavoidable. However, these sources of data are a valuable part of AMR surveillance in humans\(^{90,92}\) and other veterinary animal species\(^{91,93}\) and can help to identify new and emerging patterns of resistance, particularly because treatment failure is a frequent reason for submission of samples. Furthermore, there are likely to be differences in prudence in sampling between different practices and veterinary surgeons. The use of different AST methods and different clinical breakpoints is considered a major limitation but is a problem common to other multilaboratory studies\(^{93,94}\) and in well-established reports of resistance on bacteria from human invasive infections.\(^{90}\) This limitation was unavoidable and also complicates comparison of resistance among current and future surveillance studies. Harmonisation of methods and interpretative criteria in veterinary medicine should be a priority and would allow future comparisons over time in resistance frequencies. There are national and international systems for monitoring and reporting AMR in food-producing animals, such as the National Antimicrobial Resistance Monitoring System in the USA and the harmonised monitoring of AMR conducted in the EU. However, systematic surveillance systems for AMR in veterinary clinical samples are frequently lacking, and surveillance of this kind is not currently carried out for AMR in horses. Even systems such as the RESAPATH network in France,\(^{95}\) which is a national passive surveillance system that includes equine samples, have the inherent biases associated with voluntary submission of results by laboratories and selection of cases for sampling by practising vets.\(^{12}\) The European Antimicrobial Resistance Surveillance Network (EARS-Net) for monitoring AMR in organisms associated with human diseases is based on routine clinical antimicrobial susceptibility data that are reported to the European Centre for Disease Prevention and Control by EU countries and the UK.\(^{90}\) The data originate from national AMR surveillance initiatives and laboratory networks. Furthermore, the veterinary medicines directorate (VMD) collates data from laboratories on AMR in bacteria in samples from animals in the annual Veterinary Antimicrobial Resistance and Sales Surveillance (VARSS) report.\(^{91}\) This is managed through two programmes: EU Harmonised Monitoring and a clinical surveillance programme, which relies on voluntary submission of samples by farmers and veterinary surgeons, although this has limited data from equine and companion animals. Current efforts include developing a system for diagnostic surveillance of AMR in veterinary medicine, European Antimicrobial Resistance Veterinary Surveillance Network (EARS-VET),\(^{96}\) which eventually may include equine data. The role of the veterinary committee on AST, VetCAST\(^{97}\) and ENOVAT (European Network for Optimization of Veterinary Antimicrobial Treatment)\(^{98}\) may be crucial in this harmonisation process. However, veterinary laboratories must adopt the same laboratory standards in order to achieve this.\(^{99}\) There are many barriers to implementation of harmonised methods including cost and availability of equipment, skills and training of the laboratory staff, and the time-consuming nature of updating the latest breakpoints while running a commercial service. As there is no governing body which veterinary laboratories have to subscribe to that regulates or audits methods and results, laboratories are able to use their own in-house methods. Despite these limitations, the results from this study provide relevant and updated information on the current AMR situation in clinical bacterial isolates from horses in the UK.

Apart from establishing if practices were referral or first opinion, it was not possible to determine further practice characteristics such as case load. Descriptive spatial analysis suggested there may be geographical differences in levels of resistance prevalence, as has been observed in humans.\(^{100,101}\) However, in the current study, data were based on the submitting practice postcode, rather than horse or owner location, and it is therefore not accurate to compare geographical differences. Furthermore, the submissions were from a limited number of diagnostic laboratories, hence the results from this study are not representative of all infections encountered in horses in the UK. Further research and surveillance are needed to enable practitioners to use local resistance trends to guide prescribing. The study did identify that current guidelines regarding first-line antimicrobials are relevant, such as recommendation for trimethoprim-sulphadiazine for first-line treatment for most urogenital conditions\(^{49}\) [where the most common bacterial isolates were E. coli (31.9%) and β-haemolytic Streptococcus spp. (29.5%)], unless the infection is due to Proteus spp., Morganella spp. and Providencia spp. (83.3% resistance) or Acinetobacter spp. (62.5% resistance), or any of the bacteria which are IR to such as Pseudomonas spp., α-haemolytic Streptococcus spp. or Enterococcus spp. Although these bacterial isolates combined accounted for only 16.1% (198/1227) of bacterial isolates from urogenital submissions in the current study, it does highlight the need for culture and susceptibility testing in infections, which are not responding to first-line treatment.

### 5 | CONCLUSION

This study provides important information about patterns of AMR in major equine pathogens in the UK. Our results are useful for veterinarians to guide their initial empirical treatment. Our results also emphasise the importance of antimicrobial stewardship and judicious use of antimicrobials especially in horses undergoing surgery as SSI/CRI and orthopaedic infections had increased levels of MDR. It also highlights the need for concerted efforts for harmonisation and standardisation of culture and susceptibility methods at least at national level to support AMR surveillance. Furthermore, resistance patterns were different in referral and first opinion submission, which is vital information for risk assessment and implementation of biosecurity measures. This study only provides information on equine isolates submitted during 2018 and ongoing surveillance is
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CONFLICT OF INTERESTS

No competing interests have been declared.

AUTHOR CONTRIBUTIONS

This project was executed by C. Isgren with assistance from N. Williams, D. Timofte, O. Fletcher, R. Newton, T. Maddox, P. Clegg and G. Pinchbeck who also contributed to the study conception and design. G. Pinchbeck assisted with the statistical analysis. C. Isgren wrote the article, and all authors revised the manuscript and approved the final version for submission.

ETHICAL ANIMAL RESEARCH

Ethical approval for the study was granted by the University of Liverpool Veterinary Research Ethics Committee (VREC544). Data were collected confidentially, and all laboratories provided written consent to participate in the study.

INFORMED CONSENT

Explicit owner informed consent for inclusion of samples from animals in this study was not sought but owners were given the option to opt out of research. Data from laboratory submissions were excluded where the option to exclude data from future research had been selected.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/evj.13437.

DATA ACCESSIBILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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**SUPPORTING INFORMATION**
Additional supporting information may be found online in the Supporting Information section.

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