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Antimicrobial resistance in clinical bacterial isolates from horses in the UK

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Summary

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 6018 bacterial isolates from 4038 diagnostic submissions were included from respiratory (n=1555), urogenital (n=1010), 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 2711 Gram-negative isolates and 3307 Gram-positive isolates. Prevalence of MDR for *E. 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 in 30.2% of positive submissions. 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 hospital biosecurity and guiding treatment decisions. Harmonisation of diagnostic procedures and interpretation of results amongst veterinary laboratories will improve AMR surveillance and data comparison amongst laboratories.

Introduction

Antimicrobial resistance (AMR) is a global problem with implications for both human and equine health [1]. 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 [2]. 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 [3]. 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 [4,5]. 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 [6]. 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 [6].

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 [7], is particularly high in some bacterial species that are commonly isolated in horses e.g. *Enterococcus* spp. and *Pseudomonas* spp. [8], 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 [9] or from a particular sample site [10] or age group [11], or used results from a single hospital or laboratory [9]. 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* [12]. 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 [13]. The Defra AHT BEVA Equine Quarterly Disease Surveillance Report [14] 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. Whilst 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 [15]. Culture and susceptibility data is 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 amongst bacterial isolates from equine clinical samples submitted to diagnostic laboratories in the UK over a twelve-month period in 2018. We hypothesised that there would be increased MDR from submissions from referral practices compared to 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.

Materials and methods

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 (Biomerieux, France) and GNID and GPID Sensititre Identification plates (TREK Diagnostic Systems, West Sussex, UK) 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, Germany). 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 lab for the most common bacteria in this study are listed in Table S1 and although many breakpoints were identical (e.g. 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 (e.g. 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 anatomical location of the submitted clinical specimen; the bacterial culture and AST results for each bacterial species isolated from clinical specimens.

Due to laboratories using different antimicrobial panels, antimicrobials were grouped by class. IR was not included when determining the susceptibility of isolates. Bacteria giving intermediate results i.e. not fully susceptible were categorised as resistant [8]. Table 1 shows the classification of IR by bacterial species and was developed by the authors using available relevant recent literature [8,15–18], whilst taking into account equine pharmacokinetic and pharmacodynamic interactions and available antimicrobials for horses, including those not authorised for horses but prescribed under the cascade. Antimicrobial prescriptions under the cascade is a unique UK and Irish process [19] and antimicrobials commonly used for both authorised and non-authorised use in horses and their Committee for Medicinal Products for Veterinary Use (CVMP) category is shown in Table S3. In the latest documentation by the CVMP [20], antimicrobials readily available in the UK for horses include Category A ("Avoid") - rifampicin; Category B ("Restrict") – 3rd and 4th generation cephalosporins (3/4GC) and fluoroquinolones; Category C ("Caution") – aminoglycosides; Category D ("Prudence") – metronidazole, benzylpenicillins, folate pathway inhibitors and tetracyclines. Royal College of Veterinary Surgeons (RCVS) Veterinary Practice Directory (VPD) and the National Statistics Postcode Look-up (NSPL) was 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.

Data analysis

Sample sites were categorised into five different categories as follows: 1. respiratory tract/guttural pouch, 2. urogenital, 3. skin/hair/wound/abscess, 4. surgical site infection (SSI)/catheter related infection (CRI)/orthopaedic infections, 5. 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), whilst '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 considers all *Streptococcus* spp. IR to all aminoglycosides [16] 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; ceftiofur), aminoglycosides (gentamicin/amikacin), tetracyclines (oxytetracycline/doxycycline), folate pathways 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 [22]. 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-endotoxic 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 -10,000 iu/kg [23–26]. The antimicrobial dose is higher (20,000 iu/kg), although neurotoxic and nephrotoxic effects have been seen at this dose [27,28] hence should not be used in horses. The human antimicrobial polymyxin B dose is 30,000 iu/kg/day [29]. 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) [21]. A p-value of <0.05 was considered statistically significant. A bi-variate 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.

Results

AST data were available from 6018 bacterial isolates obtained from 4038 clinical submissions during 2018 and included 1555 respiratory, 1010 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 2-7 isolates. Out of the remaining 1485 submissions there were 1093, 319, 66, 3, 1 and 3 submissions with 2, 3, 4, 5, 6 and 7 isolates, respectively. The 6018 bacterial isolates included 2711 Gram-negative isolates and 3307 Gram-positive isolates. Only isolates belonging to the major bacterial groups identified in Table 1 were included (n=5698) 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

α-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.

Antimicrobial resistance

The proportion of resistance of bacterial isolates is shown in Table 2 and Table 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 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 *Pantoea* spp. (42.8% and 35.1%, respectively); high folate pathway inhibitor resistance in *Acinetobacter* spp. (70.2%) and *Proteu*s 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 *Pantoea* 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 *Pantoea* spp. and *Acinetobacte*r 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).

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., & *Pantoea* 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.

Resistance by sampling site

The most frequent source of bacterial isolates included respiratory (n=2334), urogenital (n=1286), skin/hair/wound/abscess (n=1230), 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., & *Pantoea and 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., & *Pantoea* 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., & *Pantoea* 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.

Submitting practice demographics

From the 4038 original submissions, there were 3926 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 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 5861 isolates which belonged to the major bacterial groups with AST results presented in Table 2, postcode data was available for 5564 isolates. This included 2422 Gram-negative and 3142 Gram-positive isolates with 2820 isolates from referral and 2744 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=5861), a bi-variate 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.) Though descriptive, this revealed some postcode areas with relatively higher resistance prevalence and low standard errors.

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 to 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 [9]. Similar to our study, previous equine studies have also reported lower prevalence of AMR in bacteria from respiratory and urogenital submission compared to wounds [30]. Human [31] and companion animal [32] 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 sepsis (orthopaedic infection) [33]. 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 [34], as well as regular bandage changes and close monitoring 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 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 is also important from a public health aspect to monitor emerging zoonotic bacteria in companion animals and horses such as toxigenic *Corynebacterium ulcerans* [35]*, Clostridium difficile*, *Leptospira* spp. or *Staphylococcus* spp. [36]. In addition to submissions with missing postcodes, 8.6% (520/6018) of isolates had the sample site information missing, which is similar to human [37,38] and other veterinary studies [39] 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 [40]. 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 [41]. Many bacterial isolates in equine infections are opportunistic pathogens that may colonise body sites together with other commensal bacteria [42] 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 [43]. Immunocompromised patients in particular, are at risk of infections caused by diverse bacteria, including opportunistic pathogens [44] and anatomical differences between different sexes and age groups may also predispose to infection [45]. 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 [46].

This study identified increased MDR in submissions from referral practices compared to 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 [47], 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 [9]. 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 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. zooepidemicus,* 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 [48]. 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 [16]. 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 was low (1.2%) in all *Streptococcus* spp. (75.1% of all streptococci in that study were *S. zooepidemicus*), 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 [10] than the French study. In contrast, a previous UK study identified high prevalence of resistance to gentamicin (ranging from 50-74%) in β-haemolytic *Streptococcus* spp. with increasing resistance in *S equi*, *Streptococcus equisimilis* and unidentified β-haemolytic *Streptococcus* spp. from 2004-2012 [9] using a standard concentration of gentamicin (10µg). However, these results need to be interpreted with some caution, as older CLSI interpretation guidelines were used [50–52]. Similarly, in β-haemolytic *Streptococcus* spp. from respiratory submissions in UK horses, gentamicin and streptomycin resistance were 100%, which adds further evidence that all *Streptococcus* spp. should be considered resistant to aminoglycosides [53]. Other differences include high enrofloxacin (68.4%) and tetracycline (60.1%) resistance in French *Streptococcus* spp. [12], while in the current study fluoroquinolone (27.9%) and tetracycline (33.8%) resistance was much lower in β-haemolytic *Streptococcus* spp. Similar to the French study, there was a high prevalence of resistance to tetracycline in β-haemolytic *Streptococcus* spp. (66.7-100%) in a recent study of clinical respiratory submissions from horses in the UK, although MDR was low (<1%) [53]. The higher prevalence of tetracycline resistance in the study by Fonseca *et al*. is in contrast to the current study despite both studies undertaken in UK horses. This may reflect a temporal change in susceptibility patterns as the previously published study was conducted between 2002-2012 while the current study only included isolates sampled in 2018. Other differences may represent international variation which may be driven by different equine populations,

antimicrobial use or differences in emerging resistance in *Streptococcus* spp. and highlights the necessity of local surveillance for informing current antimicrobial guidelines [54]. These results could also suggest possible differences in methodology and interpretation of results between these studies, which highlights the need for harmonisation of susceptibility testing amongst laboratories at country or European level for enhanced AMR surveillance.

There are several bacterial isolates with high levels of IR leaving only two treatment options available in adult horses, particularly for *Enterococcus* spp*., Pseudomonas* spp., and α-haemolytic *Streptococcus* spp., which are only considered susceptible to tetracyclines and fluoroquinolones; aminoglycosides and fluoroquinolones; 3GC and fluoroquinolones, respectively. These bacteria are considered susceptible to few other antimicrobials (e.g. ampicillin/macrolides/extended spectrum penicillins/β-lactamase inhibitors/4GC), which are not safe or available for use in horses in the UK. Thus, using a classification of MDR of resistance to 3 or more classes may not be suitable for organisms such as *Pseudomonas* spp. and *Enterococcus* spp., which have multiple inherent resistance mechanisms and very few antimicrobials to which they are expected to be susceptible to. 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 [55]. 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 [56], from a variety of equine samples in Ireland [57], from companion animals [58] and from human cystic fibrosis patients [59]. *Enterococcus* spp*.* are common pathogens in hospital-acquired infections in humans [60], equine synovial infections [61] and companion animals [62] and have been associated with increased mortality in foals [63]. However, they are often present in human and animal gut flora [64], on skin [64] and urogenital mucosa [65] and therefore are often present in clinical specimens as contaminants [66,67]. 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 sepsis [68] and have been reported in bacteraemia and pneumonia in a neonatal foals [69] and companion animals [70]. 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 to 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., & *Pantoea* spp. (25.3%). This is similar to other studies in the UK where MDR in clinical *E. coli* from horses was 39.9% [9]. 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 were 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* gene [73] 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 ceftiofur 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 other [74–78] for the majority of antimicrobials against bacterial species such as *Salmonella* spp., *Enterobacteriaceae*, *A. baumannii* [77,79–81]. 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 agar [84] 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 utilised

by combining some bacterial species based on their similarities in intrinsic resistance patterns. This is similar to human studies [8] 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 [41], 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 (e.g. for amoxicillin in *Enterobacteriaceae* in dogs), but it is not relevant to include these breakpoints as they were not utilised 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 trans-tracheal 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 prudency 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 multi-laboratory 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 amongst 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 is 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 utilise 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.

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 recommended to determine differences in seasonality and to detect emerging trends in AMR.

Authors' declarations of interest

No competing interests have been declared.

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.

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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Authorship

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.

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Figure legends:

Fig 1: Map showing the spread of postcodes of the 208 veterinary practices that contributed to 3926 equine diagnostic submission in this study during 2018 in the UK.

Fig 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, (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.

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, S., Prescott, J.F. and Dowling, P.M. (Eds.). (2013). Antimicrobial Therapy in Veterinary Medicine. John Wiley & Sons.) GC- Generation Cephalosporin. Lincosamides such as Clindamycin and Lincomycin were not included where relevant (*Pasteurella spp.& Actinobacillus spp; Staphylococcus spp;* α and *β-haemolytic Streptococcus spp.; Corynebacterium spp.& Bacillus spp.)* as they were only rarely tested for (approx. 1% of isolates) and there is no readily available treatment option in adult horses in the UK. Intrinsic resistance (IR) to antimicrobial agents for each genus/species are listed at the bottom of each group.

	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 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		
		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

	Bacterial genus or species	**Antimicrobial Class**	**Antimicrobial Agent**
	Pasteurella spp. &, Actinobacillus spp.	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.	
	Citrobacter spp., Enterobacter spp., Klebsiella spp., Serratia spp., & Pantoea spp.	Extended-spectrum β-lactamase inhibitor combinations	Ticarcillin-clavulanic 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 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 †	Ceftazidime Cefquinome
		Aminoglycosides	Gentamicin Amikacin Neomycin Framycetin Tobramycin

	Bacterial genus or species	**Antimicrobial Class**	**Antimicrobial Agent**
		Fluoroquinolones	Enrofloxacin Ciprofloxacin Marbofloxacin
		Intrinsic resistance: benzyl and amino penicillins, 1&2nd GCs, tetracyclines, folate pathway inhibitors, phenicols and macrolides. . † Ceftazidime/Cefquinome only	
	Acinetobacter spp.	Extended-spectrum β-lactamase inhibitor combinations	Ticarcillin-clavulanic acid Piperacillin-tazobactam
		3rd and 4th GCs‡	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. ‡Cefotaxime/Ceftazidime/Cefquinome only	
	Proteus spp., Morganella spp., & Providencia spp.,	Extended-spectrum β-lactamase inhibitor combinations	Ticarcillin-clavulanic acid Piperacillin-tazobactam
		3rd and 4th GCs	Cefotaxime Ceftazidime Cefpodoxime Ceftiofur Cefquinome
		Aminoglycosides	Gentamicin[đ] 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. đ -Gentamicin excluded for *Providencia* spp.	
	Staphylococcus spp. (coagulase positive and negative)	Anti-staphylococcal β-lactam	Oxacillin[Φ] Cefoxitin[Φ]
		Aminoglycosides	Gentamicin Amikacin Neomycin Framycetin Tobramycin
		Tetracyclines	Oxytetracycline Doxycycline

	Bacterial genus or species	**Antimicrobial Class**		**Antimicrobial Agent**
Gram-positive		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. $^{\Phi}$ Diagnostic purpose (no treatment option horses)		
	Beta-haemolytic Streptococcus 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 GC		Cefotaxime Ceftazidime Cefpodoxime Ceftiofur Cefquinome
		Tetracycline		Doxycycline Oxytetracycline
		Folate pathway inhibitors		Trimethoprim sulphadiazine Trimethoprim-sulfamethoxazole
		Fluoroquinolones		Enrofloxacin Ciprofloxacin Marbofloxacin
		Macrolides		Erythromycin Clarithromycin Azithromycin
		Phenicols		Chloramphenicol
		Intrinsic resistance: aminoglycosides		
	Alpha-haemolytic Streptococcus 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.		

	Bacterial genus or species	**Antimicrobial Class**		**Antimicrobial Agent**
	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	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: none		

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Table 2: Proportion of resistance (in %) of 5698 bacteria isolated from clinical infections in horses classified by sample site. 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). GC-Generation Cephalosporin, *Penicillin and Aminopenicillin combined for *Pasteurella* spp. †- Ceftazidime/Cefquinome only, ‡ Cefotaxime/Ceftazidime/Cefquinome only, ⸸ -Gentamicin excluded for *Providencia* spp. Bacterial isolates where there was <100 in a genus were not included (n=320) from the original 6018. *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.

				Proportion of resistant isolates by sample site, % (total tested)					
Pathogen (n)	**Antimicrobial**	**Total number of isolates tested**	**Proportion of resistance (% and 95%CI)**	Respiratory (2187)	Urogenital (1227)	Skin/Wound (1163)	SSI/CRI/Orthopaedic (526)	Unknown & Other (595)	P value
Gram-negative bacteria		2499		45.4 (992)	52.2 (641)	34.3 (400)	41.1 (216)	42.0 (250)	
Escherichia coli (958)	**Total**	958		8.4 (183)	31.9 (391)	13.1 (152)	18.8 (99)	22.4 (133)	
	Aminopenicillins	627	35.4 (31.8-39.2)	39.0 (141)	27.3 (300)	44.9 (91)	64.6 (48)	29.8 (47)	<0.001
	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 (f)
	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	945	44.3 (41.2-47.5)	37.0 (181)	38.1 (381)	53.3 (152)	60.2 (98)	50.4 (133)	<0.001

				Proportion of resistant isolates by sample site, % (total tested)					
Pathogen (n)	**Antimicrobial**	**Total number of isolates tested**	**Proportion of resistance (% and 95%CI)**	Respiratory (2187)	Urogenital (1227)	Skin/Wound (1163)	SSI/CRI/Orthopaedic (526)	Unknown & Other (595)	P value
	inhibitors								
	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
	Phenicols	204	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)	
	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 (f)
	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 (f)
	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 (f)
	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 (f)
	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 (f)
	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 (f)
	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
	Phenicols	93	5.4 (2.3-12.0)	6.7 (60)	0.0 (5)	0.0 (13)	0.0 (6)	11.1 (9)	<0.001 (f)
	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 (f)
Citrobacter spp., *Enterobacte*	Total	423		7.2 (158)	9.9 (121)	5.5 (64)	8.7 (46)	5.9 (34)	
	Extended spectrum penicillins /β-	16	0 (0.0-19.4)	0.0 (6)	0 (4)	0 (2)	0 (5)	0 (4)	>0.9 (f)

				Proportion of resistant isolates by sample site, % (total tested)					
Pathogen (n)	**Antimicrobial**	**Total number of isolates tested**	**Proportion of resistance (% and 95%CI)**	Respiratory (2187)	Urogenital (1227)	Skin/Wound (1163)	SSI/CRI/Orthopaedic (526)	Unknown & Other (595)	P value
r spp., *Klebsiella* spp., *Serratia* spp., & *Pantoea* spp. (423)	lactamase inhibitors								
	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
	Phenicols	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)	
	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)	
	Extended spectrum penicillins/β-	6	0 (0.0-39.0)	0 (1)	0(0)	0 (1)	0 (4)	0 (1)	>0.9 (f)

				Proportion of resistant isolates by sample site, % (total tested)					
Pathogen (n)	**Antimicrobial**	**Total number of isolates tested**	**Proportion of resistance (% and 95%CI)**	Respiratory (2187)	Urogenital (1227)	Skin/Wound (1163)	SSI/CRI/Orthopaedic (526)	Unknown & Other (595)	P value
	lactamase inhibitors								
	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)
Proteus spp., *Morganella* spp., & *Providencia* spp., (120)	Total	120		0.1 (3)	1.0 (12)	4.5 (52)	4.0 (21)	5.4 (32)	
	Extended spectrum penicillins/β-lactamase inhibitors	7	0 (0-35.4)	0 (2)	0 (3)	0 (2)	0 (0)	0 (0)	>0.9 (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[d]	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)
	Phenicols	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		3199		54.6 (1195)	47.8 (586)	65.6 (763)	58.9 (310)	58.0 (345)	
Beta	**Total**	1467		31.3 (685)	29.5 (362)	20.0 (233)	10.3 (54)	22.0 (131)	

				Proportion of resistant isolates by sample site, % (total tested)					
Pathogen (n)	**Antimicrobial**	**Total number of isolates tested**	**Proportion of resistance (% and 95%CI)**	Respiratory (2187)	Urogenital (1227)	Skin/Wound (1163)	SSI/CRI/Orthopaedic (526)	Unknown & Other (595)	P value
haemolytic *Streptococcus* spp (1467)	Penicillin	1466	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 (30)	0.0 (145)	0.0 (26)	0.0 (22)	>0.9 (f)
	3/4th GCs	1467	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	1460	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	1465	15.0 (12.2-16.9)	15.7 (683)	15.8 (362)	10.7 (233)	20.4 (54)	14.3 (133)	0.5
	Fluoroquinolones	1467	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
	Phenicols	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	1467	8.3 (7.0-9.8)	7.5 (685)	3.9 (362)	6.4 (233)	18.5 (54)	21.8 (133)	<0.001
Staphylococcus spp. (916)	**Total**	916		7.1 (155)	8.8 (108)	32.2 (374)	28.1 (148)	22.0 (131)	
	Oxacillin/Cefoxitin	315	15.9$^{\Phi}$ (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)

				Proportion of resistant isolates by sample site, % (total tested)					
Pathogen (n)	**Antimicrobial**	**Total number of isolates tested**	**Proportion of resistance (% and 95%CI)**	Respiratory (2187)	Urogenital (1227)	Skin/Wound (1163)	SSI/CRI/Orthopaedic (526)	Unknown & Other (595)	P value
	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)	
	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)	
	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 (0.0-1.4)	0.0 (58)	0.0 (63)	0.0 (46)	0.0 (64)	0.0 (47)	>0.9 (f)
Corynebacterium spp. & *Bacillus* spp (185)	**Total**	185		0.9 (20)	.2.5 (31)	7.1 (82)	4.6 (24)	4.7 (28)	
	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 (f)
	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

				Proportion of resistant isolates by sample site, % (total tested)					
Pathogen (n)	**Antimicrobial**	**Total number of isolates tested**	**Proportion of resistance (% and 95%CI)**	Respiratory (2187)	Urogenital (1227)	Skin/Wound (1163)	SSI/CRI/Orthopaedic (526)	Unknown & Other (595)	P value
	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 (f)
	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 (f)
	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 (f)
	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 (f)
	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 (f)
	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

Φ = For *S. aureus* prevalence of oxacillin/cefoxitin resistance was 12.1% (30 of 247 isolates tested).

Table 3: Proportions (in % with 95% CI) of antimicrobial resistance (AMR) in 5698 bacterial isolates from clinical infections from horses in the UK from 2018. Broadly susceptible isolates were susceptible to all antimicrobials tested. 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.

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)

Bacteria (total number of isolates)	Susceptibility patterns of isolates	Number of isolates	Proportion (% [95% CI])
	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. (1467)	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)
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)

*For α-haemolytic *Streptococcus* spp. and *Enterococcus* spp. only three classes on antimicrobials were considered hence multidrug resistance is the same as resistance to all classes of antimicrobials tested (XDR).

Table 4: Proportion of clinical submissions (n=3926) 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. 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.

Sample Site (n)					**P value**
	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)	
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

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 5564 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=2820)		First opinion practice (n=2744)		P value
	Total number of isolates	Proportion of MDR (% and 95%CI)	Total number of isolates	Proportion of MDR (% and 95%CI)	
Gram-negative bacteria (n=2422)					
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)*	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=3142)					
Beta haemolytic *Streptococcus* spp. (1455)	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)*	273	0.0 (0.0-1.4)	78	0.0 (0.0-4.7)	>0.9 (f)
Enterococcus spp. (271)*	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

*There are several bacterial isolates with high levels of IR leaving limited treatment options available in adult horses (for example *Enterococcus spp., Pseudomonas* spp., and α-haemolytic *Streptococcus* spp. Thus, using a classification of MDR of resistance to 3 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.

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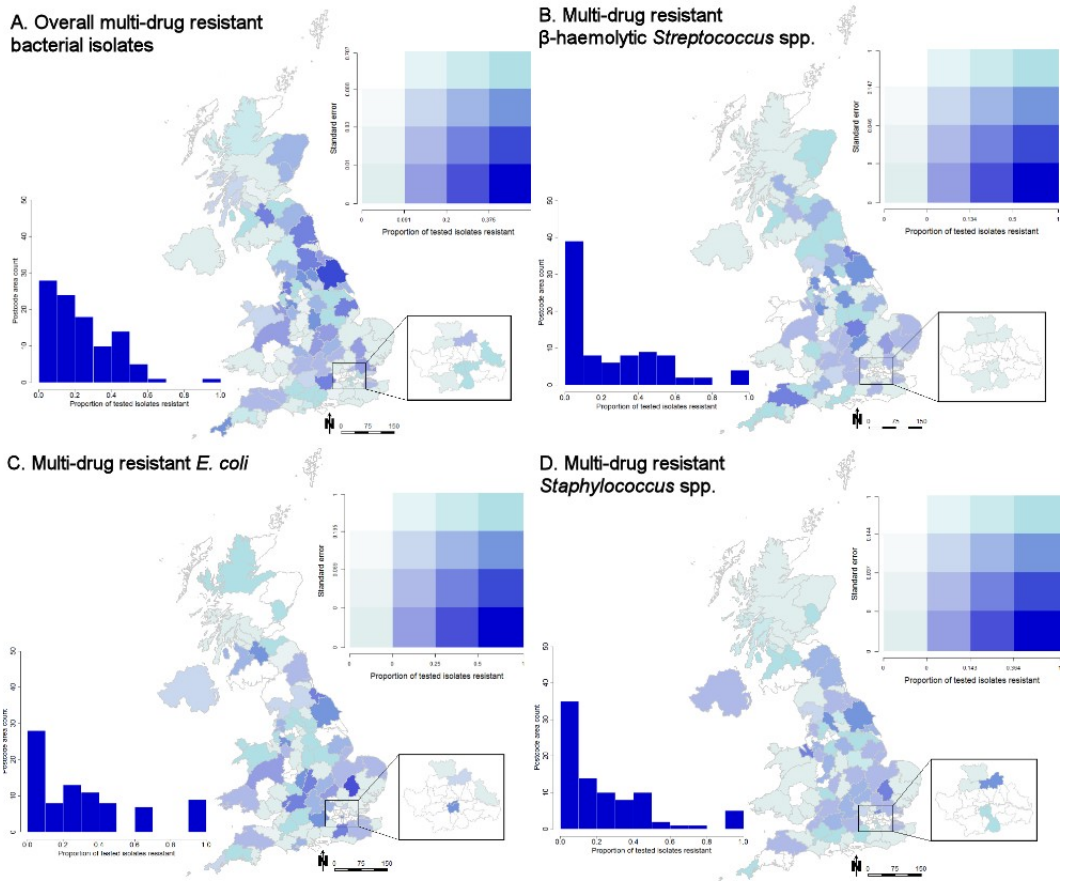

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