Antimicrobial resistance in bacteria from horses: Epidemiology of antimicrobial resistance

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Summary

Antimicrobial resistance poses a significant threat to the continued successful use of antimicrobial agents for the treatment of bacterial infections. While the epidemiology of antimicrobial resistance in bacteria from man has been studied extensively, less work has been undertaken in companion animals, particularly horses. Methicillin-resistant Staphylococcus aureus has been identified as a cause of infections, with a low prevalence of nasal carriage by horses in the community but higher for hospitalised horses. Molecular characterisation has shown methicillin-resistant Staphylococcus aureus strains either to be predominantly of types associated with horses or of sequence type ST398. Antimicrobial-resistant Escherichia coli (including multidrug-resistant and extended spectrum β-lactamase-producing isolates) have caused infections and been documented in faecal carriage by horses, with many significant resistance mechanisms identified. More sporadic reports and molecular characterisation exist for resistance in other bacteria such as enterococci, Salmonella, Acinetobacter and Pseudomonas species. Limited work has been undertaken evaluating risk factors and much of the epidemiology of antimicrobial resistance in bacteria from horses remains to be determined.

Keywords: horse; antimicrobial resistance; epidemiology; Escherichia coli; extended spectrum β-lactamase; methicillin-resistant Staphylococcus aureus

Introduction

Bacterial resistance to antimicrobial agents represents a significant challenge for both human and veterinary medicine [1,2]. While acknowledged as an emerging problem in companion animals, the carriage of antimicrobial resistance bacteria by horses has only comparatively recently started to receive attention [3]. Much research has focused on methicillin-resistant Staphylococcus aureus (MRSA), but clinically significant antimicrobial resistance is encountered in many other bacterial species, notably in Gram-negative members of the Enterobacteriaceae, such as Escherichia coli.

Antimicrobials agents function by interrupting specific metabolic functions within bacterial cells. There are 4 primary targets for antimicrobial action; disruption of cell wall synthesis, inhibition of DNA/RNA synthesis, inhibition of protein biosynthesis or interference with a crucial metabolic pathway [4]. Antimicrobial resistance can be defined as the ability of a microbe to survive and reproduce in the highest concentrations of an antimicrobial that can be achieved in body tissues [5]. The mechanisms through which bacteria can achieve resistance to antimicrobials have been reviewed [6] and are summarised in Fig 1. Such mechanisms group into 3 major categories: protection or modification of the antimicrobial target site; exclusion of the antimicrobial agent from the cell interior (via reduced cell permeability or efflux pump expulsion); and production of antimicrobial inactivating enzymes [7]. Resistance mechanisms can be either intrinsic to the bacteria (arising from a particular trait common to all bacteria of that group) or represent acquired mechanisms found only in some members of a genus or species (as a consequence of some alteration to the bacterial genome).

Acquired resistance can arise through endogenous means via mutations in chromosomal genes, but is often achieved exogenously by horizontal acquisition of foreign genetic elements. The transferable genetic material involved in exogenous resistance can comprise resistance genes encoded on plasmids, gene cassettes linked to integrons, transposons and other mobile genetic components [8,9]. Pumps for drug efflux, enzymes for antimicrobial inactivation, alternative versions of the antimicrobial target site and sometimes factors providing protection for the molecular target can be encoded by these genetic elements [10–14]. Exogenous exchange of genetic material may take place between differing strains of the same species or even across genera, and can occur via bacterial transformation (incorporation of exogenous DNA from dead bacterial, conjugation (transfer of plasmids) or transduction (DNA transferred by viral bacteriophages that infect bacteria). Irrespective of their specific origin, acquired antimicrobial resistance mechanisms are of particular concern, as they allow both the emergence and rapid dissemination of resistance in previously susceptible bacterial populations. The majority of recognised antimicrobial resistance mechanisms have been documented in equine bacterial isolates. However, an appreciation of the epidemiology of such bacteria in horses is required.

Epidemiology of antimicrobial resistance in horses

Antimicrobial resistance in bacteria recovered from horses has been recognised since the 1970s [15] but in most cases the epidemiology of such resistance in horses has not been assessed. Many reports have concerned pathogenic bacterial isolates submitted for diagnostic bacteriology [16–24], with fewer studies monitoring resistance in commensal strains [25,26].

Exactly what constitutes antimicrobial resistance varies between studies; for the purposes of this review resistance was considered present if there was demonstration of phenotypic resistance (on the basis of antimicrobial susceptibility testing). In reports describing clinical infection this may also equate to demonstrated clinical resistance/treatment failure, but this will not apply to epidemiological studies surveying for prevalence.

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To date, most epidemiological studies of antimicrobial resistant bacteria from horses have focused on resistance in staphylococci and Escherichia coli. In particular, organisms such as MRSA and extended spectrum β-lactamase (ESBL)-producing E. coli have attracted much attention due to their often significant multidrug resistance. Additionally, this reflects the pathogenic potential of these bacteria, their prevalence
and significance in human medicine, and possible zoonotic nature. The occurrence, mechanisms and, where available, epidemiology of antimicrobial resistance in these and other bacteria relevant to equine medicine will be considered.

### Staphylococcal species

Methicillin-resistant *S. aureus* remains the best characterised of all antimicrobial resistant bacteria from horses, most likely reflecting its potentially pathogenic nature and the possible implications of zoonotic spread. However, significant resistance is seen in other staphylococcal species and may be clinically relevant. Staphylococci are considered among the most important Gram-positive pathogens in human and veterinary medicine [27,28]. Many of the genus are part of the commensal flora of the skin and mucous membranes of most mammals, with over 40 different species and subspecies described. *S. aureus* and *Staphylococcus pseudintermedius* are among the more frequent pathogenic staphylococci encountered in animals. These species and some strains of *Staphylococcus hyicus* are characterised by their ability to coagulate rabbit serum and are consequently named coagulase-positive, with the remainder of the genus (including species such as *Staphylococcus epidermidis* and *Staphylococcus sciuri*) termed coagulase-negative staphylococci (CNS). The CNS have been considered largely nonpathogenic, but some are recognised as causing opportunistic infections [29–31]. Coagulase-negative species are the predominant commensal staphylococci found in horses, with several species colonising their mucous membranes. Coagulase-positive staphylococci carriage appears less common, with a lower prevalence for *S. aureus* [32–34] and *S. pseudintermedius* only very rarely reported in horses [35]. Resistance to a variety of antimicrobial agents is common within the genus. However, resistance to the narrow spectrum β-lactam methicillin is considered of particular significance as it generally signifies resistance to all β-lactams drugs [36].

### Methicillin-resistant staphylococcal carriage in horses

In common with nonresistant members of the genus, methicillin-resistant staphylococci largely reside commensally on the mucous membranes, particularly within the nasal chambers [37–39]. Persistent recovery of the organisms from such sites indicates true commensal colonisation, whereas isolated or intermittent detection may simply reflect transient carriage. Carriage of MRSA has also been documented on the skin of horses hospitalised for prolonged periods [40]. Most MRSA colonised animals do

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**Table 1: Antimicrobial mechanisms of action, common acquired antimicrobial resistance mechanisms and their encoding genes.**

<table>
<thead>
<tr>
<th>Antimicrobial target within bacteria</th>
<th>Antimicrobial class</th>
<th>Type of acquired resistance mechanism</th>
<th>Specific resistance mechanism</th>
<th>Common encoding genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin binding protein (PBP)</td>
<td>Penicillins, other β-lactams</td>
<td>Target modification</td>
<td>Alternative PBP with reduced affinity (PBP2a)</td>
<td>mecA</td>
</tr>
<tr>
<td></td>
<td>Extended-spectrum β-lactams</td>
<td>Inactivating enzymes</td>
<td>β-lactamase enzymes (including AmpC enzymes)</td>
<td>blaTEM blaACT blaCMY blaZ</td>
</tr>
<tr>
<td></td>
<td>Cell wall peptidoglycans</td>
<td>Target modification</td>
<td>Extended-spectrum β-lactamases (ESBLs)</td>
<td>blaCTX-M blaOXA</td>
</tr>
<tr>
<td>DNA topoisomerase enzymes</td>
<td>Quinolones and Fluoroquinolones</td>
<td>Target modification</td>
<td>Altered QRDR of topoisomerase enzymes</td>
<td>Chromosomal mutations of gyrA and parC</td>
</tr>
<tr>
<td></td>
<td>Inactivating enzymes</td>
<td>Target protection</td>
<td>Pentapeptide molecule protection factor</td>
<td>qnrA, qnrB, qnrS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inactivating enzymes</td>
<td>Acetyltransferase enzymes</td>
<td>AAC(6’)-ib-cr</td>
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<tr>
<td></td>
<td>Antimicrobial efflux</td>
<td>Efflux pumps</td>
<td>Efflux pumps</td>
<td>qepA</td>
</tr>
<tr>
<td></td>
<td>Tetracyclines</td>
<td>Antimicrobial efflux</td>
<td>tet(A), tet(B), tet(C)</td>
<td></td>
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<tr>
<td></td>
<td>Aminoglycosides</td>
<td>Target protection</td>
<td>tet(M)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Macrolides and Lincosamides</td>
<td>Inactivating enzymes</td>
<td>Adenylyltransferase/ acetyltransferase enzymes</td>
<td>aadA1, aadA2, aadA4, aac(6’)-Ia, aac(6’)-Ib</td>
</tr>
<tr>
<td></td>
<td>Chloramphenicol</td>
<td>Target modification</td>
<td>Methylase enzymes</td>
<td>armA, ermB</td>
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<td></td>
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<td>Antimicrobial efflux</td>
<td>Esterase, acetyltransferase enzymes</td>
<td>mefA, msrD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inactivating enzymes</td>
<td>Acetyltransferase enzyme</td>
<td>mph(C)</td>
</tr>
<tr>
<td>Dihydrofolate reductase</td>
<td>Trimethoprim</td>
<td>Target modification</td>
<td>Alternative dihydrofolate reductase (DHFPR)</td>
<td>dfrA1</td>
</tr>
<tr>
<td>Dihydropteroic acid synthase</td>
<td>Sulfonamides</td>
<td>Alternative dihydrofolate acid synthase (DHPS)</td>
<td>sul1, sul2</td>
<td></td>
</tr>
</tbody>
</table>

Fig 1: Antimicrobial mechanisms of action, common acquired antimicrobial resistance mechanisms and their encoding genes.
not develop clinical infections, but colonisation is recognised as a risk factor for infection under some circumstances [41]. Various studies have evaluated equine carriage of MRSA for different horse populations (Supplementary Item 1). The prevalence appears highest for hospitalised horses, 2.3–16.4% under normal circumstances [41,42], and may be considerably higher during outbreaks of clinical infection [43]. Generally, lower prevalences from 1.9 to 10.9% have been found for horses at admission to equine hospitals. Studies of horses in the wider community from a number of countries have consistently identified a low prevalence of carriage of <2%. While a higher community prevalence has been identified by some in the USA and Canada [44,45], these studies used targeted or small convenience samples, and showed that prevalence can vary considerably between premises (with carriage ranging from 0 to 61% on individual yards). There has been more limited evaluation of methicillin-resistant CNS carriage in horses, but studies undertaken have generally reported much higher figures for prevalence of up to 42% [34,37,46].

**Molecular epidemiology of MRSA in horses**

Methicillin-resistance in MRSA and other staphylococci generally results from carriage of one of a number of SCCmec gene cassettes. This large but variably-sized DNA fragment incorporates the mecA gene that encodes a conventional penicillin binding protein (designated PBP2a), a key enzyme required for cell wall synthesis. PBP2a provides a substitute for penicillin binding proteins and its reduced affinity for β-lactam antimicrobials affords resistance to methicillin and most other β-lactam agents [47]. Resistance to β-lactam can also be mediated through staphylococcal production of a penicillinase enzyme (encoded by the blaz β-lactamase gene) and although widespread and encountered in staphylococci from horses [48,49], only a narrow spectrum of resistance is conferred. A range of other genes can be included within the SCCmec unit and results in its variable size; in some cases these incorporated genes confer resistance to other unrelated antimicrobials [50]. SCCmec cassettes can be assigned to groups based on their integrated genes, with at least 8 major types (SCCmec types I to VIII) and several subtypes defined [51].

Identification of the specific SCCmec type carried by an isolate forms the basis of one of several typing systems used for molecular characterisation of MRSA strains, although the limited number of identifiable types means it is not a highly discriminatory technique [52]. Other typing schemes involve gene sequencing of a single chromosomal gene locus encoding staphylococcal protein A (spa gene typing) or sequencing fragments of 7 well-conserved chromosomal housekeeping genes (multilocus sequence typing [MLST]) [53,54]. Multilocus sequence typing analysis assigns an isolate a sequence type (ST) based on allelic profiles and these can be grouped together in related clonal complexes (CC). Good correlation is seen between MLST and spo-gene typing, and their high discrimination makes both suitable tools for investigating the molecular epidemiology of MRSA [55].

Over 25 different spa-types have been recovered from horses, the most frequent types encountered being t011, 064a and 451 (Supplementary Item 2). Most isolates are assigned by MLST to CC398 or CC8, with over two-thirds of studies identifying equine isolates as belonging to these 2 CC. However, the molecular epidemiology of MRSA in horses may be changing. Prior to 2007, MRSA strain types from horses appeared largely restricted to the species. In contrast, many of the MRSA types recovered from dogs are highly similar or identical to the most commonly identified human strains [56,57]. Equine MRSA isolates typically represent rarer human types or equine-specific strains, mainly sequence types ST8 or ST254 (both CC8) carrying SCCmec IV/ cassette types [56-58]. However, following the identification of the first equine isolate of the livestock-associated clone ST398 (belonging to CC398) [59], spa-types of this sequence type (mostly t011 and occasionally t034) now predominate among reports of both carriage and infection. In contrast to most other MRSA types, this sequence type appears to have a high prevalence of colonisation in large animals, especially pigs [60]. This has led to the suggestion that ST398 may represent the first truly animal adapted strain of MRSA. Most studies involving horses from European countries have now recognised ST398 among equine MRSA isolates (Supplementary Item 2). High prevalences for ST398 of 9–11% have been detected in horses at admission to equine hospitals [43,61] and clusters of clinical infection have been reported [62,63]. This may be particularly significant; ST398 has been the cause of outbreaks of clinical infection in people working with animals [64,65] and carriage by a recently hospitalised foal has resulted in documented zoonotic human infection [66]. Healthy horse-owners and workers have also been identified as carrying the same ST398 MRSA types as their animals [67].

Strains in both clonal complexes 8 and 398 typically carry variants of SCCmec IV and this SCCmec type has consistently predominated among MRSA isolates recovered from horses. Isolates from horses harbouring other SCCmec types are unusual and there are only sporadic reports of equine MRSA possessing SCCmec types V and VI in Europe and the USA [61,68–72]. Interestingly, all of these SCCmec types represent the smaller versions of this gene cassette. It is suggested that such strains are better adapted for survival outside of situations of high antimicrobial selection pressure, as the carriage of a smaller SCCmec unit results in a lesser fitness cost to the organism [73].

**MRSA infection and risk factors**

Although studies determining the true prevalence of equine MRSA infections are lacking, cases of MRSA seen in horses appear relatively infrequent in comparison with human infections. Most reported equine cases have been infections of soft tissues; mainly traumatic wounds and post surgical incisions, but cases of osteomyelitis and joint sepsis are also repeatedly identified (clinical cases are summarised in Supplementary Item 3). The incidence of infection in equine horses appears to be low, with reported infection rates of 1.8 [41] and 4.8 cases [58] per 1000 hospital admissions. However, there have also been clusters or outbreaks of infection reported in equine clinics [43,74]. In contrast, methicillin-resistant CNS have only very rarely been implicated as the cause of clinical infection [75].

The low prevalence of clinical disease has hampered determination of risk factors for MRSA infections in horses. However, a large study identified horses with nasal colonisation detected on hospital admission as being more likely to develop nosocomial clinical MRSA infections during hospitalisation [41]. Another multicentre study evaluated factors associated with community- versus hospital-acquired infection and clinical outcome in 115 infected horses [76]. The majority of MRSA infected horses [83,83] survived to discharge, similar to those with methicillin-susceptible S. aureus infections. However, prior i.v. catheterisation, a community-acquired infection and dissemination of infection to other body sites were significantly associated with nonsurvival. The only risk factor identified for hospital-acquired infection was incisional sepsis and the large number of post surgical infections reported in horses [62,74,76–78] would seem to support invasive surgery as a likely risk factor.

Risk factors for nasal carriage or colonisation with MRSA (as opposed to infection) have also been assessed. Prior antimicrobial treatment (with penicillin and trimethoprim), originating from an MRSA-positive premises, previously documented MRSA colonisation and admission for neonatal care were recognised as risk factors for carriage detected at hospital admission [79]. Similarly, antimicrobial administration has been associated with development of nosocomial MRSA colonisation during hospitalisation [41]. For horses in the community, residing on a stable yard premises with more than 20 horses was the only risk factor identified for MRSA nasal colonisation in a further study [44]. Conversely, no risk factors were identified for MRSA carriage by another study [80], leading to the suggestion that horses may function more as contaminated vectors rather than truly colonised animals. Some support for this is provided by the demonstration of spontaneous loss of MRSA carriage in untreated horses followed longitudinally [81]. However, horses with previous clinical infections of other sites have shown persistent nasal carriage for up to 711 days, with a median of 143 days [82].

**Escherichia coli**

*Escherichia coli* is considered part of the normal gastrointestinal tract of most mammals, with an almost universal prevalence in horses [83]. Despite a predominantly commensal nature, many strains of *E. coli* are capable of
causing disease of both the gastrointestinal tract and extraintestinal sites [84].

Antimicrobial resistance in E. coli

Antimicrobial resistance is commonly encountered in this species, with β-lactam resistance representing a particular concern. E. coli are intrinsically resistant to penicillin as it is unable to penetrate their outer membrane [85] and there is widespread acquired resistance to other β-lactams, mostly through the production of inactivating β-lactamase enzymes such as TEM-1, TEM-2 and SHV-1, or AmpC β-lactamases, all encoded by various bla resistance genes [13,86,87]. The extended spectrum β-lactam antimicrobials (including cefotaxime, ceftriax and cefquinome), were developed in response to resistance seen to the early β-lactams. Resistance to these agents is conferred by bacterial production of ESBL enzymes, of which there are many types [88]. Many ESBL enzymes are simple mutations of the original TEM/SHV β-lactamases; only a small number of amino acid substitutions are necessary to extend their spectrum of activity to include the newer compounds [89]. However, the last 2 decades have seen the emergence of a family of ESBL enzymes that are of activity to include the newer compounds [89].

Prevalence of ESBL enzymes

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For clinically derived isolates of E. coli from horses [17,18,20,22,26,94]. Studies have examined E. coli recovered from the faeces of horses without disease (both hospitalised and non-hospitalised horses) and clinical isolates causing disease. The prevalence of faecal carriage of E. coli with resistance to at least one antimicrobial is generally higher for hospitalised horses compared with nonhospitalised horses in the community. Prevalence of 60.5–81.7% has been identified for hospitalised horses [95–97], whereas a lower prevalence of 13.4–24.5% has been reported for community horses [96,98,99], although a prevalence of 69.5% was identified by one large study [34]. For resistance to specific antimicrobial agents, some broad similarities are apparent for both hospitalised and nonhospitalised horses, with a higher prevalence of resistance to trimethoprim/sulphamethoxazole, tetracyclines and the nonextended spectrum β-lactams, and somewhat lower prevalence for amoxicillin and fluoroquinolone resistance. However, this difference is less marked for hospitalised horses, where resistance to amoxicillin and fluoroquinolones is more frequently encountered [26,34,97–100]. The estimated prevalence of carriage of multidrug resistant isolates with considerably lower for horses in the community (2.6–37.6%) compared with hospitalised horses (13–53.5%) [34,96–100]. The prevalence of faecal ESBL-producing E. coli appears low among horses in the community; identified as 6.7% by one study [34], but may be as high as 27.3% in hospitalised horses [96,97].

For clinically derived isolates of E. coli, resistance to β-lactams and trimethoprim/sulphamethoxazole has also tended to predominate [21,101,102]. However, multidrug-resistant and ESBL-producing E. coli isolates have been identified as the cause of infections in horses on multiple occasions. Most of these infections have been of soft tissues or wounds, but involvement in uterine and synovial infections have also been reported [16,22,103,104].

Molecular characterisation of resistance in E. coli

Many antimicrobial resistance determinants or their encoding genes have been recovered from both clinically derived and faecal commensal E. coli from horses (summarised in Supplementary Item 4). Multidrug resistant, and more recently ESBL-producing isolates, have been recovered from faecal samples of clinically normal horses, as well as from soft tissue and wound infections [22,95,105]. Of particular note is the frequent occurrence of CTX-M-type ESBL-producing E. coli from horses, and especially the recent emergence of E. coli of sequence type (ST) ST131 producing CTX-M-15 [16]. The ST131 sequence type has rapidly become globally disseminated and is a significant cause of human disease [106]. Also relatively recently, there has been identification of the aminoglycoside modifying acetyltransferase enzyme (AAC(6’)-Ib-cr), conveying low level ciprofloxacin resistance [16], and several qnr genes encoding efflux pumps for quinolone (fluoroquinolone) resistance [107].

Epidemiology of antimicrobial resistant E. coli

Some epidemiological aspects of antimicrobial-resistant E. coli in horses have been examined. As noted previously, the prevalence in hospitalised animals is higher than for those in the community. Moreover, this prevalence has been shown to increase significantly during hospitalisation, with this effect being particularly profound for multidrug-resistant and ESBL-producing E. coli [97,108,109]. Some studies including hospitalised horses have noted a somewhat consistent effect of antimicrobial exposure being associated with increased risk of resistance in faecal E. coli [26,95,97], although this does not appear true for all antimicrobial types or all resistance types. One study also associated overall hospital-level treatment with antimicrobials with increased prevalence of resistance, even in animals not actually being treated [97]. Other studies have not identified significant effects for antimicrobial exposure in hospitalised horses [100,109], suggesting hospitalisation even without treatment as a likely further risk factor. Two studies identified hospitalisation for gastrointestinal disease as a risk factor [97,110] and a further study found increased risk of resistance in horses with gastrointestinal disease treated with antimicrobials compared with those treated similarly for non-gastrointestinal disease [95].

Outside of the hospital setting, fewer studies have evaluated risk factors for carriage of antimicrobial-resistant E. coli. Recent hospitalisation, recent antimicrobial treatment, contact with non- Сантических animals and some specific premise types were associated with various antimicrobial resistances by some studies [95,111]. Prevalence of carriage of ESBL-producing E. coli appears low (<7%) among horses in the community [34,95], but similar risk factors have been recognised for carriage of such bacteria. However, interestingly being stable on the same yard as a recently hospitalised horse was identified as a further risk factor for ESBL-producing E. coli carriage [111]. Supportive of this finding has been the identification of continued carriage of multidrug resistant bacteria by horses discharged from hospital on returning to the community [95].

Enterococci

Enterococci are Gram-positive members of the commensal flora of the gastrointestinal tract of many mammals, including man and horses [112]. However, they are also capable of causing disease and represent a serious clinical concern in nosocomial infections in people [113], with the most significant species being Enterococcus faecalis and Enterococcus faecium. All enterococci are intrinsically resistant to cephalosprins and aminoglycosides, limiting treatment options, which is complicated further by the increasing prevalence of transferable resistance to other antimicrobials (most critically to vancomycin) in some species [113]. Most vancomycin resistant enterococci (VRE) are E. faecalis and E. faecium, but resistance is occasionally seen in other members of the genus such as Enterococcus gallinarum, Enterococcus durans and Enterococcus casseliflavus [114,115].

Epidemiological studies regarding enterococci in horses are lacking. Transferable van genes for resistance to vancomycin have been documented in enterococcal isolates of several species from horses, with vanA and vanB genes detected, and sample prevalences of 6.7–9.6% [112,115,116]. Other resistance genes have also been identified, including for macrolides (ermB) and tetracycline (tet(L)) [112]. The equine situation somewhat parallels human medicine: a significant increase in both the prevalence and breadth of resistance has been seen in clinical enterococcal isolates over the last 2 decades [117]. Enterococcus faecalis and E. gallinarum isolates with extensive multidrug resistance have been the cause of infection in horses, but only currently in limited reports or as isolated cases [114].
**Pseudomonas**

Multidrug resistance is commonly encountered in *Pseudomonas* species, partly due to widespread intrinsic resistance to agents such as β-lactams and also the high prevalence of multidrug efflux pumps observed. *Pseudomonas aeruginosa* provides a prime example of this as it can host 4 major efflux systems known as MexAB-OprM, MexXY-OprM, MexCD-OprJ and MexEF-OprN [118]. MexAB-OprM and MexXY-OprM are expressed constitutively in wild type cells and provide intrinsic multidrug resistance. However, MexCD-OprJ and MexEF-OprN are hyperexpressed only in mutant strains and represent acquired multidrug resistance mechanisms. Other members of the genus, such as *Pseudomonas fluorescens*, less frequently demonstrate multidrug resistance.

Extensive multidrug resistance has been seen in *Pseudomonas* isolates recovered from foals with sepsis, with resistance to most of the antimicrobials tested and only amikacin, ticarcillin-clavulanic acid and imipenem remaining active against the majority of isolates [117]. With carbapenem such as imipenem considered critically important antimicrobials and largely reserved for human medicine [2], treatment options can be limited for horses. *Pseudomonas* species (particularly *P. aeruginosa*) are often involved in ulcerative keratitis, and extensive multidrug resistance has been seen in isolates from such cases, with increased prevalence of resistance seen over time [119]. The genes and mechanisms involved in antimicrobial resistance in equine *Pseudomonas* have not been well characterised. An outbreak of endometritis associated with *P. aeruginosa* with limited antimicrobial resistance showed the isolates involved to be genotypically identical [120], but few studies have examined the epidemiology of resistant *Pseudomonas* in horses.

**Salmonella**

Salmonellosis is a significant cause of clinical disease in horses in some situations, with several of the over 2500 serotypes of *Salmonella enterica* having been associated with outbreaks of nosocomial disease in equine hospitals [121–123]. Antimicrobial-resistant serotypes have been recovered from horse faeces, such as Heidelberg, Newport and Typhimurium, (including the often multidrug-resistant definitive phage type DT104) [124–128]. Several of the more significant resistance mechanisms have been identified in equine *Salmonella* isolates. Extended spectrum β-lactamase-genes such as *blaCTX-M*, *blaOXA-23* and the plasmid-mediated *ampC* gene *blaCTX-M-1* have been identified [128–131]. Antimicrobial resistance genes associated with integrons are prevalent within *Salmonella*, particularly DT104 [127]. Several examples have been identified in *Salmonella* from horses including aminoglycoside-modifying adenyltransferase enzymes such as *aadA1*, *aadA2*, *aadB*, *sul1* for sulfonamide resistance and multiple alternative dihydrofolate reductase *dfr* genes providing trimethoprim resistance [126,132].

Although risk factors for shedding of *Salmonella* have received some attention [133], the specific epidemiology of antimicrobial-resistant *Salmonella* has not been characterised. However, over a 20 year period, a significant increase in minimum inhibitory concentration values for cephalosporins (a third generation cephalosporin) and decrease in proportion of isolates susceptible to gentamicin has been noted for *Salmonella* recovered from foals with sepsis [117]. *Salmonella* isolates have also been implicated as causal agents in some cases of diarrhoea associated with antimicrobial treatment [134]. *Salmonella* can be recovered from the faeces of horses without disease, but the shedding of antimicrobial resistant *Salmonella* in the general horse population appears to be very low [135].

**Acinetobacter**

Acinetobacter species are Gram-negative coccobacilli, and are considered opportunistic pathogens that are often associated with nosocomial infections in man [136]. One species in particular, *Acinetobacter baumannii*, frequently exhibits high-level resistance to a wide range of antimicrobials including aminoglycosides, cephalosporins, fluoroquinolone and tetracyclines [136], with less resistance seen in other members of the genus. Despite their significance in human medicine, they have received limited attention in veterinary species, particularly horses. Multidrug-resistant isolates of *A. baumannii* have been sporadically reported as causes of wound infections and bronchopneumonia [137,138], with sensitivity restricted to trimethoprim-sulfonamide and marbofloxacin in some cases. Specific resistance mechanisms have been documented in some equine cases; class 1 integrons containing multiple aminoglycoside resistance genes have been found in *A. baumannii* [137] and the carbapenemase OXA-23 has been documented in an equine *Acinetobacter* isolate [139].

**Conclusions**

Antimicrobial resistance is prevalent in bacteria from horses, particularly *E. coli*, and many of the most significant resistance mechanisms have been identified in equine isolates. For some bacteria, a relatively high prevalence of multidrug resistance has been identified and such bacteria have been the cause of infections in horses. Methicillin-resistant *S. aureus* has predominated as a concern, and while its prevalence in hospitalised horses can be high, it is less frequently present in the general equine population. However, the emergence of multidrug resistance in many other bacterial species, particularly the increasing prevalence of ESBL-producing bacteria, represents a huge challenge for society. For horses, little work has examined either the prevalence or mechanisms of resistance encountered in many of the significant bacterial species and the need for increased surveillance is clear. There has been limited evaluation of risk factors associated with either carriage or infection for some antimicrobial-resistant bacteria, with some consistent effects of antimicrobial treatment noted. However, antimicrobial exposure has not been the sole reason identified for resistance and many risk factors are currently undetermined. It is clear that these aspects will need to be more fully addressed if successful attempts are to be made to limit the problems associated with such organisms in the future.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s website:

Supplementary Item 1: Estimates of carriage of methicillin-resistant Staphylococcus aureus by horses in various equine populations.

Supplementary Item 2: Molecular characterisation of methicillin-resistant Staphylococcus aureus isolates recovered from horses. (Additionally many CC398 isolates were recovered from several countries of mainland Europe; the majority of these (over 80%) were characterised as spa type t011 carrying SCCmeC IV [154].)

Supplementary Item 3: Frequency and characteristics of reported clinical methicillin-resistant Staphylococcus aureus infections in horses.

Supplementary Item 4: Antimicrobial resistance genes identified in Escherichia coli recovered from horses.