Antibiotic resistance in bacteria associated with equine respiratory disease in the United Kingdom

Joana D Fonseca,[1] Daphne E Mavrides ,[2] Alice L Morgan,[1] Jea G Na,[1] Peter A Graham,[3] Timothy D McHugh [1]

Abstract

Introduction Respiratory diseases account for the highest number of clinical problems in horses compared with other body systems. While microbiological culture and sensitivity testing is essential for certain cases, knowledge of the most likely bacterial agents and their susceptibilities is necessary to inform empirical antibiotic choices.

Methods A retrospective study of microbiological and cytological results from upper and lower respiratory samples (n=615) processed in a commercial laboratory between 2002 and 2012 was carried out. A further study of lower respiratory samples from horses with clinical signs of lower respiratory disease from May to June 2012 was undertaken.

Results Both studies revealed *Streptococcus equi* subspecies *zooepidemicus*, *Pseudomonas aeruginosa*, *Pasteurella* species, *Escherichia coli* and *Bordetella bronchiseptica* as the most frequently isolated species. *S equi* subspecies *zooepidemicus* and subspecies *equi* were susceptible to ceftiofur (100 per cent) and erythromycin (99 per cent). Resistance to penicillin (12.5 per cent of *S equi* subspecies *equi* from upper respiratory tract samples) and tetracycline (62.7 per cent) was also detected. Gram-negative isolates showed resistance to gentamicin, trimethoprim-sulfamethoxazole and tetracycline but susceptibility to enrofloxacin (except *Pseudomonas* species, where 46.2 per cent were resistant). Multiple drug resistance was detected in 1 per cent of isolates.

Conclusion Resistance to first-choice antibiotics in common equine respiratory tract bacteria was noted and warrants continued monitoring of their susceptibility profiles. This can provide information to clinicians about the best empirical antimicrobial choices against certain pathogenic bacteria and help guide antibiotic stewardship efforts to converse their efficacy.

Introduction

Bacteria are important causes of upper and lower respiratory diseases in horses, which often result in poor performance and exercise intolerance.[1 2] Bacterial respiratory disease is mostly initiated by a viral infection which weakens the respiratory immune mechanisms.[3] A noteworthy exception to this is strangles, where *Streptococcus equi* subspecies *equi* can cause disease of the upper respiratory tract without any predisposing factors.[4] Frequently isolated opportunistic respiratory pathogens are *Streptococcus equi* subspecies *zooepidemicus*, *Pasteurella* species and *Bordetella bronchiseptica*, but *Pseudomonas aeruginosa* and *Escherichia coli* are also commonly detected in respiratory tract samples.[5]

The cytological examination and bacterial culture of respiratory specimens are useful tools for the diagnosis of these infections, the determination of their aetiology and the selection of adequate antibiotic treatment.[1 2] Unfortunately culture and sensitivity of lower airway fluid samples is not always performed and treatment is often initiated on an empirical basis when a bacterial infection is suspected.[6] This is warranted as delays in the initiation of treatment can lead to poor clinical outcomes.[5] Selection of empirical therapy should be based on current knowledge of the prevalence and antibiotic susceptibility patterns of the bacteria most commonly isolated from affected horses.

Veterinary Record (2020) doi: 10.1136/vr.105842

[1]Centre of Clinical Microbiology, University College London, London, UK
[2]Department of Veterinary Medicine, University of Cambridge, Cambridge, UK
[3]School of Veterinary Medicine and Science, University of Nottingham, Nottingham, UK

E-mail for correspondence: Dr Daphne E Mavrides, Department of Veterinary Medicine, University of Cambridge, Cambridge CB3 0ES, UK; daphne.mavrides@gmail.com

Provenance and peer review Not commissioned; externally peer reviewed.

Received January 2, 2020
Revised March 17, 2020
Accepted April 24, 2020

The aim of this study was to provide evidence on the aetiology and antibiotic resistance of bacteria isolated from horses with respiratory disease using retrospective data from a diagnostic laboratory as well as prospective data from a substudy and evaluation of their relevant comparisons.

Materials and methods

A retrospective review of respiratory specimens collected from horses suspected of respiratory disease and processed at NationWide Laboratories (NWL; UK) was conducted between May 2002 and May 2012. Data on bacterial culture, antibiotic susceptibility testing results, cytology reports and age and sex of the patients were obtained by record review. Samples from the same patient that had the same organisms with identical antibiotic susceptibilities and were submitted within three months of the original sample were excluded. The analysis focused on the aetiology of the lower and upper respiratory infections and their antibiotic susceptibilities.

In a prospective substudy, 200-µl aliquots of 13 bronchoalveolar lavage (BAL) and one tracheal wash (TW) samples from horses with clinical signs of lower respiratory disease received by NWL for routine microbiological analysis from May to June 2012 were frozen at −20°C for processing at the University College London Centre for Clinical Microbiology (CCM).

Samples were processed by NWL and CCM using standard protocols for aerobic and anaerobic semiquantitative bacterial culture using the calibrated loop method. The level of bacterial growth was reported as follows: no growth, scanty (colonies limited to the initial sector), moderate (colonies on sectors 1–3) or profuse (colonies on all four sectors).

Microorganism identification was done by NWL using Gram staining and standard biochemical procedures, while CCM used matrix-assisted laser desorption/ionisation (MALDI-TOF) and proceeded to standard biochemical procedures for the isolates where MALDI-TOF was unsuccessful. The Biotyper 3 Wizard program was employed to analyse the mass spectrum profiles of each isolate and parallel them against the Bruker taxonomy library to identify each organism by pattern matching. Isolates with a match log score of over 2 were considered to have a valid genus and species identification, while a score between 1.7 and 2 was marked as a valid identification of the genus only and the species were written in brackets.

The identity of only one isolate of each bacterial species was confirmed, and testing for susceptibility was only carried out when the organism was isolated in moderate or profuse growth. Antimicrobial susceptibility testing was performed by the Kirby-Bauer method in accordance with the guidelines from the Clinical and Laboratory Standards Institute.[7–9] Herein, multiple drug resistance (MDR) was defined as resistance to three or more antimicrobial drug classes as proposed by Magiorakos *et al*.[10]

Cytology slides were prepared by cytocentrifugation, stained with Wright-Giemsa and examined by clinical pathologists at NWL. The cytology reports reviewed by the researchers included overall cellularity, cell types and appearances, and interpretation of findings.

Results

Bacterial culture results were available for 615 samples: 120 TW, 2 BAL, 473 nasal swabs (NS), 19 nasopharyngeal swabs (NPS) and 1 guttural pouch wash (GPW). Bacteria were isolated from 91 (75.8 per cent) of the TW samples, both of the BAL samples, 450 (95.1 per cent) of the NS samples, 17 (89.5 per cent) of the NPS samples and the GPW sample. The mean age of horses with culture-positive lower respiratory samples (TW and BAL) was 8.2±6.7 years in females and 10.0±9.0 years in males. For those with culture-positive upper respiratory samples (NS, NPS and GPW), the mean age at diagnosis was 10.6±6.8 years in females and 9.4±7.3 years in males.

In samples from the upper respiratory tract, *S equi* (37 per cent) (subspecies *zooepidemicus* 22.9 per cent, subspecies *equi* 14.1 per cent), *E coli* (17.5 per cent), coagulase-negative staphylococci (17.3 per cent) and *S equi* subspecies *equi* (14.1 per cent) were the bacterial species most frequently isolated. In lower respiratory samples, there was a predominance of *S equi* (29.7 per cent) (subspecies *zooepidemicus* 25.3 per cent, subspecies *equi* 4.4 per cent), *Pasteurella* species (28.6 per cent), *Pseudomonas* species (20.9 per cent) and *E coli* (13.2 per cent) (table 1). In the prospective study (May–July 2012), *S equi* was demonstrated to be the most common isolate, eight of the 14 processed samples (57 per cent).

Polymicrobial growth was observed in 53 (58.2 per cent) TW, 311 (69.1 per cent) NS and 13 (76.5 per cent) NPS samples. In TW samples, the most common combinations involved *S equi* subspecies *zooepidemicus* (present in 11 samples), *Pasteurella* species (12 samples) or both (8 samples). In NS and NPS samples with mixed growth, *S equi* subspecies *zooepidemicus* (74 samples) and *E coli* (67 samples) were the bacterial species most frequently isolated. These were often in combination with staphylococci, particularly *Staphylococcus aureus* or coagulase-negative staphylococci. When only one organism was present, *Pasteurella* species (20.0 per cent) and *Pseudomonas* species (15.0 per cent) were the most frequently isolated in lower respiratory samples and *S equi* subspecies *zooepidemicus* (23.1 per cent) and *S equi* subspecies *equi* (18.9 per cent) in upper respiratory samples. Anaerobes were only present in TW samples (5.5 per cent), mainly in combination with aerobic bacteria (60 per cent) (table 1). Polymicrobial growth was noted in 13 out of the 14 TW and BAL prospective samples, with the most

Table 1 Bacterial species most commonly isolated from respiratory samples from horses

	Lower respiratory samples				Upper respiratory samples			
	Samples with moderate/profuse growth (n=43)		Total number of samples (n=91)		Samples with moderate/profuse growth (n=359)		Total number of samples (n=468)	
Bacterial species	n	%	n	%	n	%	n	%
Acinetobacter species	4	9.3	5	5.5	38	10.6	57	12.2
α-haemolytic streptococci	2	4.7	9	9.9	31	8.6	47	10.0
Anaerobe	2	4.7	5	5.5	0	0	0	0
β-haemolytic streptococci	0	0	5	5.5	0	0	2	0.4
Bordetella species	3	7.0	6	6.6	4	1.1	6	1.3
Enterobacter species	7	16.3	9	9.9	21	5.8	29	6.2
Escherichia coli	4	9.3	12	13.2	60	16.7	82	17.5
Pasteurella species	15	34.9	26	28.6	35	9.7	44	9.4
Pseudomonas species	7	16.3	19	20.9	32	8.9	58	12.4
Staphylococcus aureus	1	2.3	4	4.4	54	15.0	76	16.2
Coagulase-negative staphylococci	1	2.3	2	2.2	55	15.3	81	17.3
Coagulase-positive staphylococci	1	2.3	2	2.2	39	10.9	63	13.5
Staphylococcus pseudintermedius	0	0	1	1.1	7	1.9	58	12.4
Streptococcus dysgalactiae subspecies *equisimilis*	1	2.3	2	2.2	11	3.1	12	2.6
Streptococcus equi subspecies *equi*	1	2.3	4	4.4	55	15.3	66	14.1
Streptococcus equi subspecies *zooepidemicus*	15	34.9	23	25.3	80	22.3	107	22.9

Only one isolate of each bacterial species was identified, and testing for susceptibility was only carried out when the organism was isolated in moderate or profuse growth.
%, number of isolates of each bacterial species divided by the number of samples; n, number of isolates.

common combinations including *Streptococcus* and *Staphylococcus* species.

Cytology reports were available for 78 samples, from which 26 had evidence of bacterial infection (ie, increased numbers of degenerate neutrophils and presence of intracellular bacteria). Twenty-two of these samples (18 from TW samples and four from NS samples) were culture-positive. *Pasteurella* species (22.2 per cent) and *S equi* subspecies *zooepidemicus* (14.8 per cent) were the most prevalent bacterial species in culture-positive and cytology-positive TW samples, and *S equi* subspecies *zooepidemicus* (25 per cent), coagulase-negative staphylococci (12.5 per cent) and *E coli* (12.5 per cent) in NS samples.

The antibiotic resistance profiles of the respiratory isolates are presented in tables 2 and 3. Two *S equi* subspecies *zooepidemicus* and four *S equi* subspecies *equi* isolates from NS samples were resistant to penicillin. All isolates of these β-haemolytic group C streptococci were susceptible to ceftiofur and (with the exception of one isolate from each species) to erythromycin. In contrast, resistance to tetracycline was common, particularly in isolates from lower respiratory samples (more than 90 per cent of *S equi* subspecies *zooepidemicus* and 66.7 per cent of *S equi* subspecies *equi* were resistant).

Enrofloxacin showed good in vitro activity against Gram-negative isolates except those belonging to

Table 2 Antibiotic resistance patterns of bacteria isolated from lower respiratory samples

% resistant isolates													
Bacterial species	**Growth level**	**P**	**AMP**	**SXT**	**CEF**	**TE**	**ENR**	**MAR**	**CN**	**S**	**C**	**E**	**RD**
Escherichia coli	Moderate/profuse	N/A	25	0	0	0	0	0	25	76.6	0	N/A	N/A
	Total	N/A	9.1	28.6	0	42.9	0	0	14.3	77.8	0	N/A	N/A
Pasteurella species	Moderate/profuse	N/A	0	16.7	0	0	0	0	23	57	0	N/A	N/A
	Total	N/A	9.1	14.3	0	0	5	0	26.3	57.9	0	N/A	N/A
Pseudomonas species	Moderate/profuse	N/A	84.6	80	80	50	50	5.7	12.5	75.8	65	N/A	N/A
	Total	N/A	80.9	58.3	69.2	30.8	46.2	2.6	10.9	76.2	67.6	N/A	N/A
Coagulase-negative staphylococci	Moderate/profuse	76.6	N/A	0	0	34.7	0	0	0	0	0	0	0
	Total	75	N/A	0	0	33.3	0	0	0	0	0	0	0
Streptococcus equi subspecies *equi*	Moderate/profuse	0	N/A	50	0	100	N/A	N/A	100	100	0	0	0
	Total	0	N/A	25	0	66.7	N/A	N/A	100	100	0	0	0
Streptococcus equi subspecies *zooepidemicus*	Moderate/profuse	0	N/A	30	0	90.9	N/A	N/A	100	100	0	0	40
	Total	0	N/A	21.4	0	92.9	N/A	N/A	100	100	0	0	30
Total	Moderate/profuse	29	35.2	34.3	26.2	29.9	11.5	1.6	13.9	53.1	9.5	14.9	47.4
	Total	30.5	34.3	32.5	24.1	28.8	12	3.1	16.8	56.7	13	18.2	37.3

Moderate growth: colonies on sectors 1–3; profuse growth: colonies on all 4 sectors.
Results are shown as percentage of resistant isolates per total number of isolates tested.
AMP, ampicillin; C, chloramphenicol; CEF, ceftiofur; CN, gentamicin; E, erythromycin; ENR, enrofloxacin; MAR, marbofloxacin; N/A, not available; P, penicillin; RD, rifampicin; S, streptomycin; SXT, trimethoprim-sulfamethoxazole; TE, tetracycline.

Table 3 Antibiotic resistance patterns of bacteria isolated from upper respiratory samples

% resistant isolates

Bacterial species	Growth level	P	AMP	SXT	CEF	TE	ENR	MAR	CN	S	C	E	RD
Escherichia coli	Moderate/profuse	N/A	50	30.5	2.1	33.3	0	0	5.1	59.3	5.9	N/A	N/A
	Total	N/A	42	26.2	2.9	30	0	0	6.2	53.7	5.3	N/A	N/A
Pasteurella species	Moderate/profuse	N/A	8.6	12.1	5.6	4.3	17.4	0	5.7	40	0	N/A	N/A
	Total	N/A	9.5	12.5	8.3	4	16	0	7.1	42.9	0	N/A	N/A
Pseudomonas species	Moderate/profuse	N/A	87.5	62.9	68.2	49.3	37.5	5.3	9.4	40.6	56.2	N/A	N/A
	Total	N/A	86	63.2	64.6	42.9	25	2.9	5.4	36.8	58.6	N/A	N/A
Coagulase-negative staphylococci	Moderate/profuse	21.8	N/A	3.7	6.7	5.6	13.5	2.9	1.9	13.7	0	9.4	1.9
	Total	19.8	N/A	6.2	4.4	7.7	9.3	4.1	1.3	10.5	0	12.8	2.5
Streptococcus equi subspecies *equi*	Moderate/profuse	12.5	N/A	16.4	0	36.8	N/A	N/A	89.5	91.7	0	0	9.1
	Total	6.1	N/A	13.8	0	33.3	N/A	N/A	91.1	92.7	0	1.5	7.7
Streptococcus equi subspecies *zooepidemicus*	Moderate/profuse	0	N/A	10.3	0	79.5	N/A	N/A	80	92.1	2.6	0	6.5
	Total	1.9	N/A	12.4	0	74	N/A	N/A	86	92.6	2	1	5.9
Total	Moderate/profuse	23.9	36.6	18.6	7.1	25	12.4	2	16.8	49.9	6.1	18.2	30.2
	Total	24.3	32.2	17.7	8.6	20.7	12	1.8	15.6	45.7	8.1	19.1	30.6

Moderate growth: colonies on sectors 1–3; profuse growth: colonies on all 4 sectors.
Results are shown as percentage of resistant isolates per total number of isolates tested.
AMP, ampicillin; C, chloramphenicol; CEF, ceftiofur; CN, gentamicin; E, erythromycin; ENR, enrofloxacin; MAR, marbofloxacin; N/A, not available; P, penicillin; RD, rifampicin; S, streptomycin; SXT, trimethoprim-sulfamethoxazole; TE, tetracycline.

Pseudomonas species (46.2 per cent resistant). Also in Gram-negative isolates, resistance to gentamicin, trimethoprim-sulfamethoxazole and tetracycline was prevalent. From the 1342 isolates included in this study, only 1 per cent were MDR. MDR was observed in *E coli* (seven isolates), *Acinetobacter* species (four isolates) and *Pseudomonas* species (three isolates).

Six *S equi* (subspecies not characterised during this study) were identified and isolated from the inhouse lab work (CCM) carried out on the BAL and TW samples. All of these were found to be sensitive to the β-lactams tested (penicillin and ampicillin), trimethoprim/sulfamethoxazole, erythromycin and cefoxitin, and resistant to tetracycline and streptomycin. These findings were in agreement with and reinforced the findings of the retrospective work carried out. There were variable susceptibilities to rifampicin, with four of the isolates being intermediately resistant, one being sensitive and another resistant. One of the *S equi* was found to be MDR, with resistance to tetracycline, streptomycin, rifampicin and kanamycin.

Discussion

The findings of this study are largely in agreement with previous reports on the detection of bacteria in the respiratory tracts of horses with respiratory disease, albeit with minor differences in terms of the relative proportion of each bacterial species in horses with clinical signs of respiratory disease.[1 5 11–15] Most isolates belonged to environmental or commensal species capable of opportunistic infection when the host's defence mechanisms are compromised, which complicates the interpretation of culture results. Moderate to heavy bacterial growth, especially if in pure culture, is generally considered to be more likely to represent true infection.[1] However, a considerable proportion of cytology-positive samples (41.2 per cent) in the present study only yielded the growth of small numbers of bacteria. *P aeruginosa* and *E coli* are often detected in upper respiratory tract samples from horses but are not necessarily the reason for clinical disease. Alternatively, *Pasteurella* species are often cultured with *S equi* subspecies *zooepidemicus* and are more likely to be associated with inflammation of the lower respiratory tract.[16] Furthermore, although the lower airways in a healthy horse are considered sterile, the passage of an endoscope during BAL sampling can introduce oropharyngeal contamination or nasopharyngeal bacteria during TW sampling. This indicates that antibiotic susceptibility tests should be analysed with caution depending on the organism(s) isolated and highlights the importance of cytology in the evaluation of these patients.

The use of antibiotics for the treatment of strangles remains controversial, and studies to indicate the use and appropriate timing of antibiotics are lacking. Prospective studies observing horses on antibiotic treatment and without treatment are warranted.[17] Nevertheless most strangles cases recover uneventfully without antibiotics, but are indicated in certain cases such as marked lymphadenopathy and dyspnoea. Their use is also advocated in acutely infected horses with high fever and lethargy in order to prevent abscess formation, as well as cases of 'bastard strangles' and guttural pouch infections to eliminate the carrier state.[2 18]

Penicillin is currently regarded as the drug of choice for the treatment of infections by non-pneumococcal streptococci in horses, and benzylpenicillin administration topically and systemically has appeared to improve treatment success rates for strangles carriers.[2 18] The emergence of penicillin-resistant strains of *S equi* subspecies *equi* should therefore be closely monitored, and contrary to similar studies on isolates of

equine origin conducted in the UK and elsewhere[15 19–21] the authors detected penicillin resistance in *S equi* subspecies *equi*.

Tetracyclines are sometimes recommended as alternative agents for the treatment of upper respiratory infections in horses,[22] but the results of the present study suggest that a significant proportion of *S equi* subspecies *equi* and subspecies *zooepidemicus* responsible for these infections might be resistant.

Ceftiofur has also been proposed for off-licence use in *S equi* infections. Studies have shown both *S equi* subspecies *zooepidemicus* and subspecies *equi* to be susceptible in vitro to ceftiofur, while another has indicated that sustained release ceftiofur suspension was effective against lower respiratory tract infections associated with *S equi* subspecies *zooepidemicus*.[23–25] From the findings of the present study, all β-haemolytic group C streptococci tested were susceptible to ceftiofur. In order to preserve their efficacy and ensure appropriate antibiotic cascade use, cephalosporins should be reserved for cases indicated by culture and where clinical signs and disease progression necessitate treatment.[18]

Given the multiplicity of agents that can cause lower respiratory tract infections in horses and the possibility of mixed aerobic/anaerobic infections, a broad-spectrum antibiotic regimen is usually recommended for the empirical treatment of more severe cases.[1] A combination of gentamicin for Gram-negative coverage and penicillin for Gram-positive and anaerobic coverage (with or without metronidazole) is often used. Gentamicin showed good to moderate in vitro activity against the Gram-negative isolates from lower respiratory samples included in this study (with resistance ranging from 10.9 per cent to 26.3 per cent, depending on the bacterial species). The emergence of gentamicin resistance in *E coli* of equine origin was documented in a recent study[20] and should be further monitored. Gentamicin is sometimes substituted by enrofloxacin in adult horses.[1] While enrofloxacin resistance remained low among isolates from most Gram-negative species, approximately half of the *Pseudomonas* species were refractory. Information on susceptibility patterns of Gram-negative isolates in equine respiratory tracts is useful in observing trends, but care should be taken in their interpretation as they may not be clinically significant to the specific case. There is a drive to reduce enrofloxacin and third-generation and fourth-generation cephalosporins in equine medicine, and since the introduction of antibiotic cascade guidelines by the British Equine Veterinary Association (PROTECT ME toolkit[26]) a 90–95 per cent decrease in prescribing enrofloxacin has been shown in one clinical setting without impacting clinical results and a 30 per cent decrease in national sales of third-generation and fourth-generation cephalosporins has been achieved.[27]

The findings of the present study provide evidence on the aetiology and antibiotic resistance of bacteria isolated from horses with respiratory disease in the UK and highlight the importance of cytology in the interpretation and analysis of these samples. The emergence and spread of antibiotic resistance in the bacterial agents most commonly implicated in infectious respiratory disease in horses can have serious impacts on animal welfare (higher morbidity and mortality associated with treatment failure) and increase the costs of treatment. It has become an important issue affecting public health, and antibiotic use by veterinarians has become a concern in recent years, leading to the introduction of antimicrobial protocols.[28] Research indicates that factors affecting veterinary prescribing behaviours and the judicious use of antimicrobials include costs of culture and sensitivity, lack of rapid and cost-effective diagnostic tests, and client pressure.[29 30] Further research into factors influencing these behaviours as well as continued monitoring of the susceptibility profiles of these infections are not only necessary to inform clinicians about the best empirical antibiotic choices but also to help guide antibiotic stewardship efforts to converse antibiotic efficacy.

Twitter Daphne E Mavrides @DaphneMavrides

Acknowledgements The authors acknowledge Pedro Serra, DVM FRCPath Dip ACVP (Clin Path) MRCVS (VPG Cork, Ireland), for facilitation of the study at NWL and for his helpful advice on its development.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information. However, further information can be obtained from TDM at University College London.

ORCID iDs
Daphne E Mavrides http://orcid.org/0000-0002-1249-9561
Timothy D McHugh http://orcid.org/0000-0003-4658-8594

References

1 Reuss SM, Giguère S. Update on bacterial pneumonia and pleuropneumonia in the adult horse. *Vet Clin North Am Equine Pract* 2015;31:105–20.
2 Sweeney CR, Timoney JF, Newton JR, *et al*. *Streptococcus equi* infections in horses: guidelines for treatment, control, and prevention of strangles. *J Vet Intern Med* 2005;19:123–34.
3 Dixon PM, Railton DI, McGorum BC. Equine pulmonary disease: a case control study of 300 referred cases. Part 3: ancillary diagnostic findings. *Equine Vet J* 1995;27:428–35.
4 Chapman PS, Green C, Main JP, *et al*. Retrospective study of the relationships between age, inflammation and the isolation of bacteria from the lower respiratory tract of thoroughbred horses. *Vet Rec* 2000;146:91–5.
5 Racklyeft DJ, Love DN. Bacterial infection of the lower respiratory tract in 34 horses. *Aust Vet J* 2000;78:549–59.
6 Christley RM, Rose RJ, Hodgson DR, *et al*. Attitudes of Australian veterinarians about the cause and treatment of lower-respiratory-tract disease in racehorses. *Prev Vet Med* 2000;46:149–59.
7 NCCLS. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. M31-A. USA: Wayne, PA, 1991.
8 NCCLS. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; informational supplement. M31-S1. USA: Wayne, PA, 2004.
9 CLSI. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. M31-A3. USA: Wayne, PA, 2008.
10 Magiorakos A-P, Srinivasan A, Carey RB, *et al*. Multidrug-Resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012;18:268–81.
11 Boguta L, Gradzki Z, Borges E, *et al*. Bacterial flora in foals with upper respiratory tract infections in Poland. *J Vet Med B Infect Dis Vet Public Health* 2002;49:294–7.

12 Sweeney CR, Holcombe SJ, Barningham SC, *et al*. Aerobic and anaerobic bacterial isolates from horses with pneumonia or pleuropneumonia and antimicrobial susceptibility patterns of the aerobes. *J Am Vet Med Assoc* 1991;198:839–42.
13 Whitwell KE, Greet TR. Collection and evaluation of tracheobronchial washes in the horse. *Equine Vet J* 1984;16:499–508.
14 Wood JLN, Newton JR, Chanter N, *et al*. Association between respiratory disease and bacterial and viral infections in British racehorses. *J Clin Microbiol* 2005;43:120–6.
15 Erol E, Locke SJ, Donahoe JK, *et al*. Beta-hemolytic *Streptococcus* spp. from horses. *J VET Diagn Invest* 2012;24:142–7.
16 Wood JL, Burrell MH, Roberts CA, *et al*. Streptococci and *Pasteurella* spp. associated with disease of the equine lower respiratory tract. *Equine Vet J* 1993;25:314–8.
17 Ramey D. Does early antibiotic use in horses with 'strangles' cause metastatic *Streptococcus equi* bacterial infections? *Equine Vet Educ* 2010;19:14–15.
18 Boyle AG, Timoney JF, Newton JR, *et al*. Streptococcus equi infections in horses: guidelines for treatment, control, and prevention of Strangles-Revised consensus statement. *J Vet Intern Med* 2018;32:633–47.
19 Clark C, Greenwood S, Boison JO, *et al*. Bacterial isolates from equine infections in Western Canada (1998-2003). *Can Vet J* 2008;49:153–60.
20 Johns IC, Adams E-L. Trends in antimicrobial resistance in equine bacterial isolates: 1999-2012. *Vet Rec* 2015;176:334
21 Kirinus JK, Pötter L, Gressler LT, *et al*. Perfil fenotípico E susceptibilidade antimicrobiana de Streptococcus equi isolados de equinos dA região Sul do Brasil. *Pesquisa Veterinária Brasileira* 2011;31:231–8.
22 British Equine Veterinary. Protect Me - Practice policy, 2016. Available: https://www.beva.org.uk/Portals/0/Documents/Resources/1beva-antimicrobial-policy-template-distributed.pdf [Accessed 9 January 2019].
23 Bade D, Portis E, Keane C, *et al*. In vitro susceptibility of ceftiofur against *Streptococcus equi* subsp *zooepidemicus* and subsp *equi* isolated from horses with lower respiratory disease in Europe since 2002. *Vet Ther* 2009;10:E1–10.
24 Bade D, Sibert G, Hallberg J, *et al*. Ceftiofur susceptibility of *Streptococcus equi* subsp *zooepidemicus* isolated from horses in North America between 1989 and 2008. *Vet Ther* 2009;10:E1–7.
25 McClure S, Sibert G, Hallberg J, *et al*. Efficacy of a 2-dose regimen of a sustained release ceftiofur suspension in horses with *Streptococcus equi* subsp. *zooepidemicus* bronchopneumonia. *J Vet Pharmacol Ther* 2011;34:442–7.
26 BEVA. Protect me toolkit, 2018. Available: https://www.beva.org.uk/Resources-For-Vets-Practices/Medicines-Guidance/Protect-me [Accessed 9 December 2019].
27 Bowen M, Marr CM, Clegg PD. Letter to the editor: equine veterinary Journal's antimicrobial stewardship policy. *Equine Vet J* 2016;48:532–3.
28 Hardefeldt LY, Gilkerson JR, Billman-Jacobe H, *et al*. Antimicrobial labelling in Australia: a threat to antimicrobial stewardship? *Aust Vet J* 2018;96:151–4.
29 Norris JM, Zhuo A, Govendir M, *et al*. Factors influencing the behaviour and perceptions of Australian veterinarians towards antibiotic use and antimicrobial resistance. *PLoS One* 2019;14:e0223534.
30 Raidal SL. Antimicrobial stewardship in equine practice. *Aust Vet J* 2019;97:238–42.