SHORT COMMUNICATION

Antibiotic resistance profiles of isolates from respiratory samples of horses from the UK

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Introduction

Bacteria are important causes of upper and lower respiratory disease in horses, which often result in poor performance and exercise intolerance (Reuss & Giguère, 2015; Corinne R. Sweeney, Timoney, Newton, & Hines, 2005). 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 (Reuss & Giguère, 2015; Corinne R. Sweeney et al., 2005). Delays in the initiation of treatment can lead to poor clinical outcomes (Racklyeft & Love, 2000), so antibiotics are often initiated on an empirical basis when a bacterial infection is suspected.Selection of empirical therapy should be based on current knowledge of the prevalence and antibiotic susceptibility patterns of the bacteria isolated from affected horses.

Materials and methods

We conducted a retrospective study of respiratory specimens collected from horses suspected of respiratory disease and processed at Dechra Laboratory Services (United Kingdom) between May 2002 and May 2012. Researchers obtained data on bacterial cultureantibiotic susceptibility testing results, cytology reports and age and sex of the patients.

erobic and anaerobic semi-quantitative bacterial cultures of the respiratory samples 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 4 sectors). Microorganism identification was done by

Antimicrobial susceptibility testing was performed by the Kirby-Bauer method in accordance with the guidelines from the Clinical and Laboratory Standards Institute (CLSI) and the results

interpreted based on contemporary criteria published by the same organisation. Herein, multiple drug resistance (MDR) was defined as resistance to three or more antimicrobial drug classes.

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

Results

Electronic records search identified a total of 615 samples for which bacterial culture results were available: 120 tracheal washes (TW), 2 bronchoalveolar lavages (BAL), 473 nasal swabs (NS), 19 nasopharyngeal swabs (NPS) and 1 guttural pouch wash (GPW). Bacteria of pathogenic potential were isolated from 91 (75.8%) of the TW, 2 (100%) of the BAL, 450 (95.1%) of the NS, 17 (89.5%) of the NPS and 1 (100%) of GPW samples. 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,*E. coli* (17.5%), coagulase-negative staphylococci (17.3%), and *Streptococcus equi* subspecies *equi* (14.1%) were the bacterial species most frequently isolated.

Polymicrobial growth was observed in 53 (58.2%) TW, 0 (0%) BAL, 311 (69.1%) NS, 13 (76.5%) NPS and 0 (0%) GPW samples. In TW, the most common combinations involved *S. zooepidemicus* (present in 11 samples), *Pasteurella* spp (12) or both (8). In NS and NPS samples with mixed growth, *S. zooepidemicus* (74) and *E. coli* (67) 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* spp (20.0%) and *Pseudomonas* spp (15.0%) were the most frequently isolated in lower respiratory samples and *S. zooepidemicus* (23.1%) and *S. equi* (18.9%) in

upper respiratory samples. Anaerobes were only present in TW samples (5.5%), mainly in combination with aerobic bacteria (60%).

Cytolog reports were available for 78 samples from which 26 had evidence of bacterial infection (i.e. increased numbers of degenerate neutrophils and presence of intracellular bacteria). Twenty two of these samples (18 from TW and 4 from NS) were culture-positive. Similarly to the results presented above, *Pasteurella* spp (22.2%) and *S. zooepidemicus* (14.8%) were the most prevalent bacterial species in culture- and cytology-positive TW samples and *S. zooepidemicus* (25%), coagulase-negative staphylococci (12.5%) and *E. coli* (12.5%) in NS samples.

The antibiotic resistance profiles of the respiratory isolates are presented on Tables 2 and 3. Two *S. zooepidemicus* and four *S. 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% of *S. zooepidemicus* and 2/3 of *S. equi* were resistant).

Enrofloxacin showed good *in vitro* activity against Gram-negative isolates except those belonging to *Pseudomonas* spp (approximately half were resistant). Also in Gram-negative isolates, resistance to gentamycin, trimethoprim-sulfamethoxazole and tetracycline was prevalent. From the 1,342 isolates included in our study, only 1% were MDR. Multiple drug resistance was observed in *E. coli* (7 isolates), *Acinetobacter* spp (4) and *Pseudomonas* spp (3).**Discussion**

Our findings are largely in agreement with previous reports on the aetiology of bacterial respiratory tract infections in horses, albeit with slight differences in terms of the prevalence of each bacterial species (Boguta et al., 2002; Racklyeft & Love, 2000; Reuss & Giguère, 2015; C R Sweeney, Holcombe, Barningham, & Beech, 1991; Whitwell & Greet, 1984; Wood, Newton, Chanter, & Mumford, 2005). 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 is generally considered to be more likely to represent true infection (Reuss &

Giguère, 2015). However, a considerable proportion of cytology-positive samples (41.2%) in our study only yielded the growth of small numbers of bacteria. This highlights the importance of cytology in the evaluation of these patients.

Most strangles cases recover uneventfully without antibiotics but treatment is recommendedif the animal has prolonged high fever, severe lethargy and anorexia, and especially if it develops dyspnoea due to partial upper airway obstruction (Corinne R. Sweeney et al., 2005). It is also indicated for the decolonisation of subclinical *S. equi* carriers and could be used as prophylaxis during strangles outbreaks (Corinne R. Sweeney et al., 2005)Penicillin is currently regarded as the drug of choice for the treatment of infections by non-pneumococcal streptococci in horseshe emergence of penicillin-resistant strains of *S. equi* should be closely monitorednlike similar studies on isolates of equine origin conducted in the UK and elsewhere (Clark, Greenwood, Boison, Chirino-Trejo, & Dowling, 2008; Erol, Locke, Donahoe, Mackin, & Carter, 2012; Johns & Adams, 2015; Kirinus, Pötter, Gressler, Leite, & Vargas, 2011) we detected penicillin resistance in *S. equi*. This is a primary equine pathogen and the aetiological agent of strangles, a highly infectious disease of the upper respiratory tract that remains common in the UK (Parkinson, Robin, Newton, Slater, & Waller, 2011).

Tetracyclines are sometimes recommended as alternative agents for the treatment of upper respiratory infections in horses (British Equine Veterinary, 2016) but our results suggest that a significant proportion of *S. equi* and *S. zooepidemicus* responsible for these infections might be resistant.

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 (Reuss & Giguère, 2015). 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 our study (resistance ranging from 10.9-26.3%, depending on

the bacterial species). The emergence of gentamicin resistance in *E. coli* of equine origin was documented in a recent study (Johns & Adams, 2015) and should be further monitored. Gentamicin is sometimes substituted by enrofloxacin in adult horses (Reuss & Giguère, 2015). Whilst enrofloxacin resistance remained low amongst isolates from most Gram-negative species, approximately half of the *Pseudomonas* spp were refractory.

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. Continued monitoring of the susceptibility profiles of these infections is not only necessary to inform clinicians about the best empirical antibiotic choices but also to help guide antibiotic stewardship efforts to converse antibiotic efficacy.

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

Bacterial species	Lower respiratory samples				Upper respiratory samples			
	Samples with moderate/profuse growth		Total number of samples		Samples with moderate/profuse growth		Total number of samples	
	N	%	n	%	N	%	n	%
***Acinetobacter* spp.**	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 strep	0	0	5	5.5	0	0	2	0.4
***Bordetella* spp.**	3	7.0	6	6.6	4	1.1	6	1.3
***Enterobacter* spp.**	7	16.3	9	9.9	21	5.8	29	6.2
E. coli	4	9.3	12	13.2	60	16.7	82	17.5
***Pasteurella* spp.**	15	34.9	26	28.6	35	9.7	44	9.4
***Pseudomonas* spp.**	7	16.3	19	20.9	32	8.9	58	12.4
S. 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
S. pseudintermedius	0	0	1	1.1	7	1.9	58	12.4
S. dysgalactiae* subsp. *equisimilis	1	2.3	2	2.2	11	3.1	12	2.6
S. equi* subsp. *equi	1	2.3	4	4.4	55	15.3	66	14.1

S. equi* subsp. *zooepidemicus	15	34.9	23	25.3	80	22.3	107	22.9

N – number of isolates, % - number of isolates of each bacterial species divided by the number of samples.

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

Bacterial species	Growth level	P	AMP	SXT	CEF	TE	ENR	MAR	CN	S	C	E	RD
E. 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* spp.**	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* spp.**	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
S. 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
S. 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

P – penicillin, AMP – ampicillin, SXT – trimethoprim-sulfamethoxazole, CEF – ceftiofur, TE – tetracycline, ENR – enrofloxacin, MAR – marbofloxacin, CN – gentamicin, S – streptomycin, C – chloramphenicol, E – erythromycin, RD – rifampicin, N/A – not available. Results are shown as percentage of resistant isolates per total number of isolates tested.

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

Bacterial species	Growth level	P	AMP	SXT	CEF	TE	ENR	MAR	CN	S	C	E	RD
E. 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* spp.**	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* spp.**	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
S. 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
S. 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

P – penicillin, AMP – ampicillin, SXT – trimethoprim-sulfamethoxazole, CEF – ceftiofur, TE – tetracycline, ENR – enrofloxacin, MAR – marbofloxacin, CN – gentamicin, S – streptomycin, C – chloramphenicol, E – erythromycin, RD – rifampicin, N/A – not available. Results are shown as percentage of resistant isolates per total number of isolates tested.

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