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Antimicrobial susceptibility of bacteria isolated from neonatal foal samples submitted to a New Zealand veterinary pathology laboratory (2004 to 2013)

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Abstract

AIMS: To describe antimicrobial susceptibility, and identify antimicrobial resistance (AMR), in bacteria isolated from New Zealand foals.

METHODS: A database search was performed of submissions to a veterinary pathology laboratory between April 2004 and December 2013, for bacterial culture of samples from foals <3 weeks of age. Culture and susceptibility results were compiled with demographic information. Susceptibility results were as defined for the Kirby-Bauer disk diffusion susceptibility test based on Clinical Laboratory Standards Institute guidelines. Multi-drug resistance (MDR) was defined as non-susceptibility to ≥ 3 of a panel of antimicrobials (ceftiofur, enrofloxacin, gentamicin, penicillin, tetracycline, trimethoprim- sulfonamide); penicillin susceptibility was not included for Gram-negative isolates.

RESULTS: Submissions from 102 foals were examined, and 127 bacterial isolates were cultured from 64 (63%) foals. Of the 127 isolates, 32 (25%) were *Streptococcus* spp., 30 (24%) were *Staphylococcus* spp., 12 (10%) were *Enterococcus* spp. and 26 (21%) were *Escherichia coli*. Of 83 Gram-positive isolates, 57 (69%) were susceptible to penicillin. Over all isolates, 92/126 (73%) were susceptible to gentamicin and 117/126 (93%) to enrofloxacin; 62/82 (76%) of Gram-positive, and 22/42 (52%) of Gram-negative bacteria were susceptible to ceftiofur; 53/81 (65%) of Gram-positive, and 23/44 (52%) of Gram-negative bacteria were susceptible to tetracycline; 59/82 (72%) of Gram-positive, and 23/44 (43%) of Gram-negative bacteria were susceptible to trimethoprim-

sulfonamide. Of 126 isolates, 33 (26%) were MDR; >1 isolate with MDR was cultured from 24/64 (38%) foals, and ≥ 2 isolates with MDR were recovered from 8/64 (13%) foals.

CONCLUSIONS: Multi-drug resistance, including resistance to commonly used antimicrobials, was found in bacterial isolates from foals in New Zealand.

CLINICAL RELEVANCE: The results of this study are of concern from a treatment perspective as they indicate a potential for antimicrobial treatment failure. For future surveillance of AMR and the creation of national guidelines, it is important to record more data on samples submitted for bacterial culture.

KEY WORDS: Horse, foal, antimicrobial resistance, sepsis, antimicrobial stewardship.

AMR Antimicrobial resistance

MDR Multi- drug resistance

NZVP New Zealand Veterinary Pathology

TMPS Trimethoprim-sulfonamide

Introduction

Antimicrobial resistance (AMR) is a major medical, political and public issue of concern (Prescott 2014). The stewardship of antimicrobial drugs in the veterinary profession, including reporting and monitoring of AMR and implementation of coordinated interventions designed to improve and measure the use of antimicrobials, is an increasingly important part of the responsible use (Bowen 2013; Prescott 2014). Guidelines have the potential to prevent inappropriate antimicrobial use, and thus reduce prescribing practices that may select for resistance (Dunowska *et al.* 2006). The creation of guidelines for the rational use of antimicrobials enables practitioners to improve antimicrobial stewardship and is important to equine and human health (Wilson 2001; Bowen 2013). The aim of guidelines is to attempt to slow the development of AMR (Bengtsson *et al.* 2012); however these must be regionally relevant, and underpinned by evidence-based veterinary medicine (Morley *et al.* 2005).

New Zealand is geographically isolated from much of the rest of the world, and has stringent border biosecurity practices that have prevented many infectious equine diseases from entering the country (Crump *et al.* 2001; Rogers and Cogger 2010). However, this does not preclude the establishment of multi-drug resistant micro-organisms within the country, including some of importance to human health (Herdan *et al.* 2012). There are few published studies or reports encompassing the New Zealand equine population, but there have been recent concerns expressed about the emergence of multi-drug resistant bacteria (Herdan *et al.* 2012).

The risks of untreatable bacterial infections, including overwhelming sepsis, are especially great for neonatal equine populations (Palmer 2014; Theelen *et al.* 2014a). Bacterial translocation in the gastrointestinal tract soon after birth has the potential to cause life-threatening infection, especially when foals are immunologically compromised (Palmer 2014). This can occur through the failure of transfer of passive immunity, when an insufficient quality or quantity of colostrum is ingested after birth (McGuire *et al.* 1977; Vendrig and Fink-Gremmels 2012). Foals have been the subject of antimicrobial susceptibility studies and publications for this reason (Hirsh *et al.* 1993; Marsh and Palmer 2001; Russell *et al.* 2008). A recent North American study looking at temporal trends in culture results from septic foals showed a significant decrease of *in vitro* susceptibility to amikacin, ceftiofur, ceftiofime, gentamicin, imipenem and ticarcillin/clavulanic acid (Theelen *et al.* 2014a). However, the situation in New Zealand has yet to be described. This study aimed to describe the susceptibility of isolates from samples from equine neonates submitted to a New Zealand veterinary pathology laboratory between 2004 and 2013.

Materials and Methods

Data collection

Antimicrobial susceptibility records for bacterial isolates cultured from equine samples submitted to New Zealand Veterinary Pathology (NZVP; Hamilton and Palmerston North laboratories, New Zealand) between April 2004 and December 2013 were assessed. All equine culture and susceptibility records submitted to these laboratories between the study dates were available for selection. This represented a high proportion of available results from foal submissions made during the study period to all commercial veterinary microbiological laboratory companies in New Zealand (Leah Toombs-Ruane, unpublished data). Information identifying client, horse and veterinarian were removed from the records by NZVP in order to retain client confidentiality. Data collected included a unique accession number for each sample, the age of the horse at the time of sampling or submission, gender, breed, organ or tissue source of submission, geographical origin within New

Zealand and date of submission, along with the bacterial species of isolates cultured and the susceptibilities of these isolates to routine laboratory antimicrobial panels.

Case selection

A database search of the available records was conducted to extract information, using age as the selection criterion to identify foals <3 weeks old. A subset of samples submitted for research purposes, part of a previous perinatal mortality study at Massey University between 2007 and 2008, were excluded. Case information, excluding history, was limited to date of submission, age of foal, breed, animal species and sample source. Submissions were assumed to be from unique foals provided their sample accession numbers were different. Submissions listed as fetus or neonate were excluded from further review, as age was not defined. Submissions that were included were assumed to be from clinically affected cases. Horses with more than one sample submitted were assessed, and exclusion of isolates was made if two isolates from the same submission had an identical antibiogram. The time-periods assigned to the foal-year (1 August to 31 July the following year) were based upon the New Zealand Thoroughbred foaling season dates (Dicken *et al.* 2011).

Culture and susceptibility, identification and classification

Aerobic culture results were selected; anaerobic and fungal isolates were not assessed. Anaerobic susceptibilities were not part of the NZVP laboratory protocol. Kirby-Bauer disk-diffusion tests of cultured isolates based on a standardised protocol were documented (Bauer *et al.* 1966), and the definition of susceptible/intermediate (moderate)/resistant was based on the Clinical Laboratory Standards Institute's guidelines (CLSI, and formerly the National Committee for Clinical Laboratory Standards) for specific antimicrobial/bacterial isolate combinations (NCCLS 2002; CLSI 2008). The laboratory selection of antimicrobials for testing was based either on NZVP's standard protocols, with additional antimicrobials selected by an individual microbiologist, or at a clinician request. The number of antimicrobials tested against each isolate varied, and only ceftiofur, enrofloxacin, gentamicin, penicillin, tetracycline, trimethoprim-sulfonamide (TMPS), ampicillin, and amoxicillin-clavulanic acid are described in this study. Results indicating moderate/intermediate susceptibility, and resistant were both classified as not sensitive for the purpose of this study.

Susceptibility to penicillin and gentamicin together was defined as susceptibility of the cultured bacterial species to penicillin or gentamicin alone for each bacterial isolate. Multi- drug resistance (MDR) was defined as non-susceptibility to three or more of a core panel of antimicrobials determined by the laboratory's protocol (ceftiofur, enrofloxacin, gentamicin, penicillin, tetracycline and TMPS). Penicillin was removed from the susceptibility results for Gram-negative bacteria, and

those isolates were required to be non-sensitive to three out of the remaining five antimicrobials. This is a modification of a previously described definition of MDR (Beard 2010).

Data analysis

Data were stored and manipulated in Microsoft Excel (Microsoft Corporation, Redmond, WA, USA, 2010). Submissions were stratified by foal-level signalment and demographic variables. These were age, gender and breed of animal, geographical region of submission, sample source, and culture type. The records for bacterial isolates were then examined with respect to susceptibility to antimicrobials, MDR and demographic factors. Summative data were described by using counts, percentages and 95% CI .

Results

Overall submissions

Over the 10-year study period there were 102 foal submissions. Of these, 64 (63%) returned a positive aerobic bacterial culture result.

Of these 64 foals that provided at least one isolate for susceptibility testing, 18 (28%) were fillies, 22 (34%) were colts, and 24 (38%) had no reported gender; 45 (70%) were from Thoroughbreds; 12 (19%) were from foals known to be <7 days old, 5 (8%) were from foals aged 8–21 days old, and the remainder (47/64; 73%) were submitted as being of unknown age, but were <3 weeks old. The locations from which submissions were made centred on three major regions: Auckland, Waikato, and Manawatu-Wanganui. These three regions accounted for 56/64 (88%) culture-positive submissions.

Of the 64 submissions, 35 (55%) were of unspecified anatomical origin. Orthopaedic (joint or bone) samples accounted for 15/64 (23%) positive submissions, and 14/64 (22%) were of ophthalmic, gastrointestinal, respiratory or urogenital origin.

Culture results

Overall, 127 isolates were cultured from 64 foals and subjected to antimicrobial panel testing. Of these, 83 (65%; 95% CI=57–74%) were Gram-positive and 44 (35%; 95% CI=26–43%) were Gram-negative. *Streptococcus* spp. were identified in 32/127 (25%; 95% CI=18–33%) isolates, *Staphylococcus* spp. in 30/127 (24%; 95% CI=16–31%), *Enterococcus* spp. in 12/127 (10%; 95% CI=4–15%) and *Escherichia coli* in 26/127 (21%; 95% CI=14–28%) isolates. The remaining 27 isolates included Gram positive *Bacillus* species, *Rhodococcus equi*, *Corynebacterium* species, and *Micrococcus* species; Gram negative isolates included *Klebsiella* species, *Enterobacter* species

(Including *E. cloacae*), *Acinetobacter* species (including *A. baumannii*), *Morexella* species, and *Pseudomonas* species.

Susceptibility results

The number of antimicrobials each isolate was tested against ranged from six to 16, with a median of seven. A summary of selected *in vitro* susceptibilities for the most commonly isolated bacterial species are given in Table 1.

No single antimicrobial was tested against all 127 isolates. In total, 126 isolates were subjected to susceptibility testing against penicillin and/or gentamicin; 102 (81%; 95% CI=74–88%) of these isolates were susceptible to one or the other of these antimicrobials.

Of the 127 isolates, 126 met the criteria for identification of possible MDR. Four isolates were resistant to all five or six of the major antimicrobials tested. Results for the isolates with MDR are shown in Table 2. The isolates with MDR came from 24/64 (38%; 95% CI=26–49%) culture-positive foals. More than one isolate with MDR was cultured from 8/64 (13%; 95% CI=4–21%) foals.

Discussion

Previously unreported AMR was found in bacterial cultures from samples collected from foals in New Zealand. This included non-susceptibility to many of the antimicrobials licensed for use in horses in this country (Anonymous 2013). MDR was found in 26% of isolates. The lack of susceptibility to commonly used antimicrobials may have a profound effect on the survival of compromised or sick individuals, particularly foals (Palmer 2014). Consequently it is important that these findings are used to support the creation of regionally relevant guidelines for the rational use of antimicrobials, to improve antimicrobial stewardship by equine veterinarians.

Given the limitations with respect to clinical data in the current study, comparisons with other published studies describing AMR in isolates from foals are not meaningful. In contrast to data available in the current study, previous studies had more precise case definition (e.g. neonatal sepsis) which make their findings more readily interpreted in a clinical context (Marsh and Palmer 2001; Russell *et al.* 2008; Theelen *et al.* 2014a). In the current study, samples were submitted from a variety of primary and secondary referral veterinary practices, with frequently incomplete signalment and case history, limiting the ability to make specific clinical recommendations based on the culture results. Broad-spectrum antimicrobial use is commonly recommended for the treatment of at-risk foals or those suspected of sepsis, in order to attempt to reduce morbidity and mortality

(Palmer 2014). Consequently, there are various recommendations for the most efficacious initial treatment, most often beta-lactam/aminoglycoside combinations (Corley and Hollis 2009; Palmer 2014; Theelen *et al.* 2014a). In the current study 81% of tested isolates were sensitive to either penicillin or gentamicin. This result was higher than for most other broad-spectrum antimicrobials when used as a single drug therapy; overall susceptibility to TMPS was 65%; and susceptibility to tetracycline was 59%. The results from the current study indicate that at the isolate level the most rational initial therapy for sepsis in neonatal foals in New Zealand would be a combination of penicillin and gentamicin, while awaiting results of culture and susceptibility. In the United States of America, the recommendations are also for a beta-lactam/aminoglycoside combination, although ampicillin and amikacin are specifically recommended on the basis of susceptibilities (Theelen *et al.* 2014a).

The greatest susceptibility amongst the bacterial isolates in the current study was to enrofloxacin. However it should be noted that this is listed as a critically important antimicrobial by the World Health Organisation (Bowen 2013). It is therefore not acceptable for this antimicrobial to be used as a first choice treatment, and should only be used on the basis of culture and susceptibility results (Anonymous 2012). Quinolones also have the potential to cause arthropathies experimentally in foals (Vivrette *et al.* 2001) and therefore are not recommended as a first-choice treatment for neonatal sepsis (Wilson 2001). The detection of AMR to enrofloxacin in Gram-negative and -positive isolates in these foals supports the need for the development of and adherence to specific antimicrobial use guidelines, to preserve the efficacy of this drug.

Isolates with MDR were cultured from 38% of culture-positive foal submissions, a similar finding to results in Australia and the United Kingdom (Russell *et al.* 2008; Johns and Adams 2015). However, the definition of MDR used in the current study is limited and is not likely to accurately reflect the true phenotype or genotype of the bacteria (Schwarz *et al.* 2010). Further knowledge and molecular characterisation of these isolates would be useful in future monitoring of resistance, especially with respect to risk factors.

Previous studies in North America and Australia have identified changes in the types of bacteria isolated in septic foals, with a decrease in the proportion of Gram-negative bacteria cultured, although overall more Gram-negative isolates have been cultured in these populations (Marsh and Palmer 2001; Russell *et al.* 2008; Theelen *et al.* 2014b). In the current study, a higher proportion of Gram-positive than negative bacteria were cultured, and may reflect the lack of detail on the origin of some of the samples in the records. The results reflect the susceptibility of the bacteria these foals were exposed to, but do not give an accurate representation of the clinical significance or patient

outcomes. As relevant clinical information, including history was not available from the data set, the authors had no knowledge of previous treatments or current disease processes, which constitutes a clear limitation of the study. There has been limited research into the susceptibility of pathogenic microorganisms found in clinically affected equines in New Zealand, despite work carried out in other species (Petrovski *et al.* 2011; Pleydell *et al.* 2012). The current study provides an initial insight into the current situation in New Zealand, and is a starting point for the design of prospective bacterial susceptibility monitoring.

The information from records used for this study reflected the demographics of the major equine sub-populations in New Zealand in terms of location and breed (Rosanowski *et al.* 2013) but there was a lack of signalment information, including the high proportion of unknown/unspecified submission parameters (notably sex, age, sample source). There was a loss of submissions due to age not being specified at the time of sample submission. Consequently it is probable that the results from this study under represent the total number of submissions from foals during the study period. Further analyses, including temporal change in susceptibilities (and MDR) over time may have been possible if larger numbers of foal submissions were available in the dataset, and should be considered for future studies.

The findings of this study highlight the need for complete submission information at the laboratory level, in order to more closely monitor resistant organisms. This requires the participation, and acceptance of responsibility, of veterinarians in practice, and a workplace culture of accurate record-keeping, education of clients, and compliance monitoring (Hodgson *et al.* 2008; Wernli *et al.* 2011). Better compliance by submitting veterinarians with laboratory requirements not only improves the feedback and interpretations given to the clinician by the laboratory, but also provides valuable information for surveillance of MDR (Hodgson *et al.* 2008; Schwarz *et al.* 2010).

The results of this retrospective records-based descriptive study indicate the presence of antimicrobial resistant bacteria in foals under veterinary care in New Zealand. Multi-drug resistance was found, indicating a need for regionally relevant antimicrobial use recommendations to be developed, taking into account the New Zealand situation. These guidelines could attempt to slow the development of antimicrobial resistance. This study also highlights the importance of complete submission information to determine risk factors for multi-drug resistance.

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Table 1: Proportion (%) of bacterial isolates that were susceptible to selected antimicrobials, from samples submitted from 64 foals, aged ≤3 weeks, to a veterinary pathology laboratory in New Zealand between 2004 and 2013.

Bacterial category	Antimicrobial tested							
	Amox-clav	Ampicillin	Ceftiofur	Enrofloxacin	Gentamicin	Penicillin	Tetracycline	TMPS
All bacteria	12/18 (67)	36/67 (54)	84/124 (68)	117/126 (93)	92/126 (73)	NA	73/124 (59)	82/126 (65)
All Gram-positive bacteria	9/11 (82)	31/45 (69)	62/82 (76)	75/82 (91)	60/82 (73)	57/83 (69)	53/81 (65)	59/82 (72)
Staphylococcus spp.	3/3 (100)	7/14 (50)	22/29 (76)	28/29 (97)	23/29 (79)	12/30 (40)	21/29 (72)	24/29 (83)
Streptococcus spp.	NT	18/19 (95)	30/32 (94)	28/32 (88)	22/32 (69)	30/32 (94)	20/31 (65)	22/32 (69)
Enterococcus spp.	NT	NT	4/12 (33)	10/12 (83)	6/12 (50)	8/12 (67)	3/12 (25)	7/12 (58)
All Gram-negative bacteria	3/7 (43)	5/22 (23)	22/42 (52)	42/44 (95)	32/44 (73)	NA	20/43 (47)	23/44 (43)
Escherichia coli	2/6 (33)	5/15 (33)	13/24 (54)	26/26 (100)	20/26 (77)	NA	9/25 (46)	12/26 (33)

Amox-clav=amoxicillin/clavulanate; NA=Not applicable; NT=Not tested; TMPS=trimethoprim-sulfonamide

Table 2: Proportion of bacterial isolates that exhibited resistance to ≥ 3 selected antimicrobials ^a, from samples submitted from 64 foals, aged ≤ 3 weeks, to a veterinary pathology laboratory in New Zealand between 2004 and 2013.

Bacterial category	Proportion	Percentage (95% CI)
All bacteria	33/126	26 (19–34)
All Gram-positive bacteria	20/83	24 (15–33)
<i>Staphylococcus aureus</i>	6/23	26 (8–44)
<i>Streptococcus</i> spp.	5/32	15 (3–28)
<i>Enterococcus</i> spp.	6/12	50 (22–78)
All Gram-negative bacteria	13/43	30 (17–44)
<i>Escherichia coli</i>	6/25	24 (7–41)

^a Ceftiofur, enrofloxacin, gentamicin, penicillin, tetracycline and trimethoprim-sulfonamide, with penicillin not included for Gram-negative bacteria