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An evaluation of serum gentamicin concentrations and bacterial susceptibility to gentamicin in equine practice

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Background: Therapeutic drug monitoring and minimum inhibitory concentration (MIC) data allow more informed use of gentamicin.

Hypothesis/Objectives: To measure peak and trough serum gentamicin concentrations in horses after a 6.6 mg/kg dose of gentamicin given IV and the MIC of gentamicin of bacteria for which gentamicin might be selected.

Methods: Retrospective analysis of hospital records. Peak and trough plasma gentamicin concentrations were measured after 6.6 mg/kg gentamicin IV in 339 hospitalized horses. The MIC of gentamicin was measured for 503 isolates from ambulatory practice and 33 from hospital practice. The distribution of gentamicin concentrations and MIC results were compared to current recommendations for MIC breakpoints.

Results: The median serum gentamicin concentration at 60 minutes after administration (C_{60min}) was 21.4 μg/mL with a distribution indicating that bacteria with MIC ≥2 μg/mL were unlikely to be exposed to sufficient gentamicin for effective killing. Approximately 90% of isolates from ambulatory practice and 36% of hospital isolates had MICs at or below breakpoints for susceptibility with most of the remainder unlikely to be responsive, even to higher IV doses.

Conclusions and Clinical Importance: Gentamicin at a dosage of 6.6 mg/kg IV is likely to be effective against the majority of infections encountered in ambulatory practice, but less effective in an equine hospital. Because there was a dichotomy of most bacteria as being clearly susceptible or clearly resistant to gentamicin, it appears unlikely that higher doses would have been more efficacious, especially in the hospitalized population in our study.

KEYWORDS
equine, gentamicin, MIC, toxicity

1 | INTRODUCTION

Attempted prediction of antimicrobial drug efficacy in clinical infections requires knowledge of pharmacokinetic data indicative of drug concentration at the infected site, and pharmacodynamic data describing the association between drug exposure and bacterial killing.[1,2] The minimum inhibitory concentration (MIC) of an

Abbreviations: C_{24h}, serum gentamicin concentration at 24 hours following administration; C_{30min}, serum gentamicin concentration at 30 minutes following administration; C_{60min}, serum gentamicin concentration at 60 minutes following administration; CLSI, Clinical Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; MIC, minimum inhibitory concentration.

Work performed in Liphook Equine Hospital Laboratory.

antimicrobial for the infecting pathogen is regarded as the best pharmacodynamic indicator of antimicrobial activity. Several sources publish MIC breakpoints which are intended to discriminate between susceptible isolates (low MIC) for which clinical efficacy is likely using standard dosing regimens, versus resistant isolates (high MIC) for which clinical efficacy is not expected. An intermediate designation (mid-range MIC) is also sometimes used where clinical outcome is uncertain and dosage modification might increase the likelihood of success.[1] Unfortunately, a direct association between application of clinical breakpoint data and therapeutic outcome is not well established in equine medicine. Furthermore, measurement of antimicrobial drug concentrations and MIC results is not feasible in most cases in clinical equine practice.

Gentamicin is an aminoglycoside antimicrobial commonly used in horses at a dosage of 6.6 mg/kg IV once daily.[3,4] The drug exhibits concentration-dependent bacterial killing which is best predicted by the ratio of maximum plasma concentration of gentamicin (C_{max}) to the MIC of gentamicin for the bacteria being targeted. It has been suggested that a C_{max} : MIC ratio of between 8 and 10 is required for effective aminoglycoside therapy.[5–7] Therapeutic monitoring of plasma concentrations of gentamicin in equine clinical practice has become more routine in larger hospitals, allowing more informed and safe selection of drug and dosage regimens. However, in the light of such data, concern has been raised that standard dosing of 6.6 mg/kg gentamicin IV q24h often might not attain plasma concentrations adequate for effective treatment of infections in equine patients.[8]

The current study aimed to examine plasma gentamicin concentrations in a larger population of equine clinical patients than has been examined in previous studies and, furthermore, to determine the typical MIC results obtained from pathogens affecting horses to enable more informed assessment of the predicted efficacy of a dosage of 6.6 mg/kg gentamicin IV.

2 | METHODS

2.1 | Therapeutic monitoring of gentamicin

Clinical records of in-patients at The Liphook Equine Hospital were searched between July 2012 and July 2015 for data related to therapeutic monitoring of gentamicin. Cases were selected where both a peak and subsequent trough sample were collected from the same horse after the first dose of 6.6 mg/kg gentamicin sulfate IV, calculated from body mass as measured on a calibrated weighbridge. Horses <1 year of age were excluded. Peak concentrations were measured at 1 hour (C_{60min}) and trough concentrations were measured at 24 hours (C_{24h}) after dosing. Free plasma gentamicin was measured using an enzyme immunoassay method (Cedia Gentamicin II, Microgenics, Fremont, California), determined using a Prestige 24i analyser (Tokyo Boeki, Tokyo, Japan). The assay was validated previously yielding intra-assay and inter-assay variability <9% and measured recovery of 106%-112% of spiked gentamicin. The lower limit of detection for the assay is 0.24 μg/mL gentamicin.

2.2 | Antimicrobial susceptibility testing

Records at The Liphook Equine Hospital Laboratory were searched for submitted bacteriologic samples for which the MIC of gentamicin was measured in aerobic gram-negative bacilli and *Staphylococcus sp*. between October 2014 and July 2015. Although some of the bacteriologic data came from the same horses sampled for serum gentamicin concentrations, not all horses were sampled for both serum and bacteriologic data, and many bacteriologic samples were obtained from horses where serum samples were not taken or included in the study. Additionally, samples obtained from hospitalized horses were considered separately from samples collected in ambulatory practice.

Samples typically were first plated onto Columbia blood agar and MacConkey's agar, Colistin-Nalidixic acid agar, or both depending on sample type. Subcultures sometimes were prepared, depending on the purity of primary growth, to obtain pure cultures before suspending individual colonies in saline to a McFarlane standard of 0.5. The suspension then was processed using a Vitek 2 analyser (BioMerieux, Basingstoke, Hampshire, UK) for bacterial identification. Staphylococci were examined for gentamicin susceptibility across a range of dilutions between 0.5 and 16 μg/mL (AST-GP73, BioMerieux) whereas gram-negative bacilli were examined across a range of gentamicin dilutions between 1 and 16 μg/mL (AST-GN65, BioMerieux). For the purposes of further data examination, isolates then were classified as Enterobacteriaceae, *Pseudomonas sp*., "other gram-negative bacilli" and *Staphylococcus sp*. isolates were further classified according to coagulase expression (positive/negative) and susceptibility to 6 μg/mL cefoxitin (susceptible/resistant).

Bacterial resistance to gentamicin was compared between samples derived from ambulatory practices and those obtained from an equine hospital by comparing median MIC results for each bacterial group using the Mann-Whitney test with a P value <.05 to indicating a significant difference.

3 | RESULTS

3.1 | Therapeutic monitoring of gentamicin

A total of 339 horses each had 1 pair of plasma samples assayed for gentamicin concentration (C_{60min} and C_{24h}) after an initial dose of 6.6 mg/kg IV gentamicin sulfate. A variety of breeds, types, and ages were included with a variety of clinical problems ranging from critical care cases to simple wounds in systemically healthy individuals. The median age was 11 years, with a range of 1–30 years. The median C_{60min} and C_{24h} were, respectively, 21.4 μg/mL (range, 4.4–42.6 μg/mL) and 0.2 μg/mL (range, 0–5.7 μg/mL). The distribution of plasma concentrations among the population of tested horses is detailed in Figure 1A,B.

The likelihood of attaining adequate serum gentamicin concentrations to kill bacteria falling into the various MIC categories (≤0.5 to ≥16 mg/mL) was calculated based on an estimated target C_{60min} of 8–10 times the MIC (Table 1). Almost all treated horses attained serum gentamicin concentrations expected to be efficacious against bacteria with MIC ≤1 μg/mL and most horses (between 60.6 and 82.2%) attained serum gentamicin concentrations expected to be efficacious against

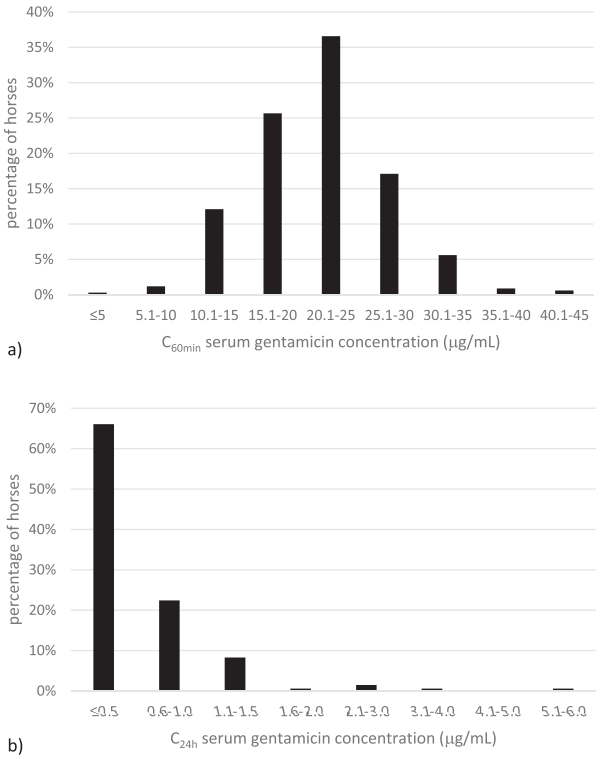

FIGURE 1 Distribution of (A) C_{60min} and (B) C_{24h} gentamicin concentrations among 339 horses treated with 6.6 mg/kg gentamicin sulfate IV q24h

bacteria with MIC of 2 μg/mL. However, <3.4% of horses attained gentamicin concentrations predictive of efficacy versus bacteria with MIC of 4 μg/mL, and none attained gentamicin concentrations predictive of efficacy versus bacteria with MIC ≥8 μg/mL. The C_{24h} was >1 μg/mL in 40/348 (11.5%) horses and >2 μg/mL in 9/348 (2.6%) horses.

3.2 | Antimicrobial susceptibility testing

A total of 536 gram-negative and Staphylococcal bacterial isolates was identified and had MIC results determined. Distribution of bacteria among groups and anatomic sites of origin are described in Table 2. Of the 536 isolates, 503 (94%) were submitted from ambulatory practices across the United Kingdom and the remaining 33 (6%) from in-patients at The Liphook Equine Hospital. Isolates from these 2 groups were separated before determining the MIC distributions of both data sets (Table 3).

Comparison of MIC results derived from ambulatory and hospital isolates indicated significantly higher MIC results for hospital isolates of Enterobacteriaceae ($P = .001$) and Staphylococci ($P < .001$), but not for "other gram-negative bacilli" ($P = .79$). No hospital *Pseudomonas* isolates were available for comparison.

4 | DISCUSSION

Our study found that horses treated with gentamicin sulfate at a dosage of 6.6 mg/kg IV q24h attained a median C_{60min} of 21.4 μg/mL, with 335/339 (98.8%) samples reaching a C_{60min} of ≥10 μg/mL (Figure 1A) and 330/339 (97.3%) samples having a C_{24h} of ≤2 μg/mL (Figure 1B), corresponding to recommendations for therapeutic gentamicin monitoring in horses.[9] After examination of bacterial isolates from clinical equine patients, the gentamicin MIC results were significantly higher for hospital-derived isolates of Enterobacteriaceae and Staphylococci as compared to those obtained from ambulatory practice, implying greater resistance to gentamicin in samples from the hospital sources. In both populations, however, the MIC distribution was approximately bimodal with most isolates being unequivocally susceptible or resistant (Table 3).

The setting of MIC breakpoints in human medicine is a contentious and evolving issue without international consensus. Breakpoints have been proposed by numerous organizations across the world, including the Clinical Laboratory Standards Institute (CLSI) in the United States and 6 separate national European groups that are coordinated by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The CLSI is the only organization thus far to have produced recommendations for veterinary species (Table 4). The CLSI breakpoint recommendations for humans frequently are higher than those of EUCAST, with a greater likelihood of bacteria being judged susceptible using CLSI than EUCAST recommendations.[10,11] Breakpoints are derived from multiple data sources and considerations including pharmacokinetic data and how the drug is used in clinical practice, typical bacterial MIC distributions and resistance mechanisms, predictive computer algorithms, and feedback from clinical outcomes.[1,2,12,13] Unfortunately equine-specific recommendations are quite sparse, leading to inter-species assumptions that might prove to be inappropriate given different clinical and pharmacokinetic factors. In fact, even the equine-specific gentamicin data produced by the CLSI[10] refers to IM dosing with 6.6 mg/kg, which might differ slightly compared with the standard IV dosing used in horses. Inevitably, setting of these breakpoints is work in progress in equine medicine.

A key and fundamental principle of setting MIC breakpoints is that they correspond to realistically achievable antimicrobial concentrations at the site of infection, using standard dosages in vivo.[14] Because very little data is available on concentrations of antimicrobials at the various potential sites of infection, plasma drug concentrations generally are used as surrogate markers for tissue concentrations despite the fact that most infections occur at extravascular sites. Almost all horses in the present study achieved C_{60min} commensurate with killing bacteria with MIC of 1 μg/mL (C_{60min} >8–10 μg/mL), and most (between 61%

TABLE 1 Percentage of C_{60min} plasma samples reaching predicted therapeutic concentrations for each minimum inhibitory concentration (MIC) category based on thresholds of 8 and 10 times MIC concentrations

MIC breakpoint (μg/mL)	≤0.5	≤1	2	4	8	≥16
Proportion of C_{60min} serum samples reaching ×8 MIC threshold	100%	99.70%	82.20%	3.40%	0	0
Proportion of C_{60min} serum samples reaching ×10 MIC threshold	99.70%	98.90%	60.60%	0.60%	0	0

TABLE 2 Anatomic sites of origin of the 536 bacterial isolates arranged by category

	Origin of sample															
	Skin/wounds		Respiratory		♀ Reproductive		Abscesses		Urinary		Ocular		Other		TOTAL	
Bacterial group	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Enterobacteriaceae	75	31.3%	25	26.3%	54	71.1%	15	25.4%	6	40.0%	2	20.0%	20	48.8%	197	36.8%
Pseudomonas sp.	5	2.1%	5	5.3%	1	1.3%					1	10.0%	1	2.4%	13	2.4%
Other gram neg.	34	14.2%	41	43.2%	12	15.8%	14	23.7%	5	33.3%	5	50.0%	8	19.5%	119	22.2%
Staph (Coag+ Cef-S)	74	30.8%	17	17.9%	3	3.9%	18	30.5%			1	10.0%	6	14.6%	119	22.2%
Staph (Coag− Cef-S)	34	14.2%	7	7.4%	6	7.9%	10	16.9%	3	20.0%	1	10.0%	5	12.2%	66	12.3%
Staph (Coag+ Cef-R)	7	2.9%													7	1.3%
Staph (Coag− Cef-R)	11	4.6%					2	3.4%	1	6.7%			1	2.4%	15	2.8%
TOTAL	240	44.8%	95	17.7%	76	14.2%	59	11.0%	15	2.8%	10	1.9%	41	7.6%	536	

and 82%) reached a C_{60min} predictive of efficacy versus bacteria with MIC of 2 μg/mL (C_{60min} >16–20 μg/mL). Because fewer than 3.4% of serum samples had gentamicin concentrations thought adequate to kill bacteria with MIC of 4 μg/mL (C_{60min} >32–40 μg/mL), it seems unlikely that a dosage of 6.6 mg/kg gentamicin IV would be efficacious against such isolates unless the infection was at a site of known gentamicin accumulation such as the urinary tract.[15] Given the plasma concentrations of gentamicin typically seen in our study and a target C_{60min} of 8 to 10 times the MIC of gentamicin for the bacteria concerned, it is difficult to justify MIC breakpoint recommendations > 2 μg/mL, at least in this population of horses. This conclusion is consistent with CLSI equine-specific recommendations indicating that for Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Actinobacillus pleuropneumoniae*, MIC ≤2 μg/mL should be taken to indicate probable susceptibility, although possible susceptibility (intermediate) is attributed to bacteria with MIC of 4 μg/mL.[10]

Other studies have indicated that gentamicin concentrations higher than the median C_{60min} of 21.4 μg/mL (range, 4.4–42.6 μg/mL) found in the current study can be achieved with standard dosing schedules. For example, pharmacokinetic studies in healthy horses have described mean peak plasma gentamicin concentrations of 71.9 μg/mL immediately post-dosing and 41.6 μg/mL 30 minutes after 6.6 mg/kg IV.[3,16] A postoperative study of colic cases found the mean plasma gentamicin concentration to be 40.7 μg/mL 20 minutes after 6.6 mg/kg IV gentamicin,[4] and a more recent study of hospitalized horses indicated a mean plasma gentamicin concentration of 26.7 μg/mL at 30 minutes, using a median dosage of 6.7 mg/kg gentamicin IV, with 17% of peak concentrations >32 μg/mL.[8] However, the different studies are not readily comparable given possible methodologic differences (eg, measurement of free or total drug) and, most importantly, differences in timing of sampling and state of health of the tested subjects. Clearly, peak plasma concentration is a time-dependent variable and different times of sampling will have a large and unpredictable effect on measured plasma concentrations.[17] The most appropriate time for determining the relevant plasma concentration for clinical therapeutic monitoring of horses has not been established. It is suggested to be between 30 and 60 minutes to allow adequate time for tissue distribution of drug, but not allowing too much time for substantial drug elimination.[9] In any case, it is likely that the most appropriate collection time would vary among different sites of infection, adding to the uncertainty about the best time interval. It is also evident that sick horses treated with gentamicin may demonstrate different pharmacokinetics, making it additionally difficult to extrapolate from studies of healthy experimental horses to those with clinical disease.[8,18] Unfortunately, in most clinical infections, no data are available on actual infection site drug concentrations, especially in diseased horses. Intuitively, these concentrations will be highly variable depending on the actual site and severity of systemic illness. Therefore, using peak plasma concentration at a given time point is always a compromise and most likely often inaccurately reflects actual peak concentration at the infection site. Thus, given these variables for measuring a clinically meaningful peak plasma concentration, the current study and a previous study[8] probably offer the most useful approximations and had similar findings, given the earlier sampling time of 30 minutes in the previous study.[8] Neither study supported the use of gentamicin at a dosage of 6.6 mg/kg IV for the treatment of infections caused by bacteria with MIC ≥4 μg/mL, and it seems inconceivable that success could be obtained against bacteria with MIC ≥8 μg/mL. This conclusion concurs with CLSI recommendations that bacteria with MIC ≥8 μg/mL should be regarded as resistant and that those with MIC 4 μg/mL as having intermediate sensitivity suggesting therapeutic success might only be possible using higher dosages or when infection is at sites of drug accumulation (eg, urine). The previous study[8] further investigated the effect of higher dosages of gentamicin between 7.7 and 15.0 mg/kg and found that although these were significantly more likely to attain C_{30min} >32 μg/mL (possibly adequate to kill bacteria with MIC of 4 μg/mL), they still failed to do so in the majority of cases.

It is also relevant that the current study, along with others,[19,20] found an essentially bimodal distribution of MICs in clinical isolates. Most isolates from both ambulatory and hospital samples in the current study had MICs that were either ≤2 μg/mL (susceptible) or ≥8 μg/mL (resistant), with only 9/536 (1.7%) isolates having an intermediate MIC

TABLE 3 Details of bacterial isolates with the distribution of their minimum inhibitory concentration (MIC) results for gentamicin for samples collected in ambulatory practices or the Liphook Equine Hospital

		Gentamicin MIC distribution											
		<0.5 μg/mL		<1 μg/mL		2 μg/mL		4 μg/mL		8 μg/mL		>16 μg/mL	
Bacterial isolates	**Total n**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Ambulatory practices													
ENTEROBACTERIACEA		NOT TESTED											
E. coli	111			101	91.0%							10	9.0%
Proteus sp	15			14	93.3%					1	6.7%		
Pantoea agglomerans	13			13	100.0%								
Enterobacter sp.	13			10	76.9%							3	23.1%
Klebsiella sp.	11			11	100.0%								
Serratia sp.	5			5	100.0%								
Raoultella planticola	4			4	100.0%								
Citrobacter sp.	3			2	66.7%							1	33.3%
Morganella sp.	3			2	66.7%			1	33.3%				
Salmonella sp.	3			3	100.0%								
Cronobacter sakazakii	1			1	100.0%								
Kluyvera intermedia	1			1	100.0%								
	183			167	91.3%			1	0.5%	1	0.5%	14	7.7%
PSEUDOMONAS SP.	13			12	92.3%	1	7.7%						
OTHER GRAM NEGATIVES													
Acinetobacter sp.	37			34	91.9%	1	2.7%					2	5.4%
Sphingomonas paucimobilis	29			25	86.2%			2	6.9%	1	3.4%	1	3.4%
Pasteurella sp	24			23	95.8%			1	4.2%				
Aeromonas caviae	7			7	100.0%								
Bordetella bronchiseptica	2							2	100.0%				
Moraxella sp	3			3	100.0%								
Achromobacter xylosoxid.	3					1	33.3%	1	33.3%			1	33.3%
Alcaligenes faecalis	1											1	100.0%
Brevundimonas diminuta	1			1	100.0%								
Burkholderia capacia	1											1	100.0%
Ralstonia pickettii	1											1	100.0%
Vibrio parahaemolyticus	1			1	100.0%								
Comamonas testosteroni	1									1	100.0%		
Myroides sp.	1											1	100.0%
	112			94	83.9%	2	1.8%	6	5.4%	2	1.8%	8	7.1%
STAPHYLOCOCCUS SP.													
Coag +, Cefoxitin susceptible	116	103	88.8%							2	1.7%	11	9.5%
Coag −, Cefoxitin susceptible	64	64	100.0%										
Coag +, Cefoxitin resistant	6	3	50.0%					1	16.7%	1	16.7%	1	16.7%
Coag −, Cefoxitin resistant	9	5	55.6%							1	11.1%	3	33.3%
All cefoxitin-susceptible	180	167	92.8%							2	1.1%	11	6.1%
All cefoxitin-resistant	15	8	53.3%					1	6.7%	2	13.3%	4	26.7%
All *Staphylococcus sp.*	195	175	89.7%					1	0.5%	4	2.0%	15	8.7%
TOTAL ALL ISOLATES	503	175	34.8%	273	54.3%	3	0.6%	8	1.7%	7	1.4%	37	7.4%
Liphook Equine Hospital													
ENTEROBACTERIACEA		NOT TESTED											
E. coli	10			3	30.0%							7	70.0%
Enterobacter sp.	3											3	100.0%
Citrobacter sp.	1			1	100.0%								
	14			4	28.6%							10	71.4%
OTHER GRAM NEGATIVES													
Pasteurella sp	4			4	100.0%								
Sphingomonas paucimobilis	2			1	50.0%			1	50.0%				
Acinetobacter sp.	1									1	100.0%		
	7			5	71.4%			1	14.3%	1	14.3%		
STAPHYLOCOCCUS SP.													
Coag +, Cefoxitin susceptible	3	1	33.3%									2	66.7%
Coag -, Cefoxitin susceptible	2	2	100.0%										
Coag +, Cefoxitin resistant	1											1	100.0%
Coag -, Cefoxitin resistant	6									2	33.3%	4	66.7%
All cefoxitin-susceptible	5	3	60.0%									2	40.0%
All cefoxitin-resistant	7									2	28.6%	5	71.4%
All *Staphylococcus sp.*	12	3	25.0%							2	16.6%	7	58.3%
TOTAL ALL ISOLATES	33	3	9.1%	9	27.3%			1	3.0%	3	3.0%	17	51.5%

TABLE 4 Published breakpoint recommendations for the use of gentamicin sulfate to treat bacterial infections

Bacteria	Breakpoints (μg/mL)			Derived from	Advisory body
	Susceptible	Intermediate	Resistant		
Enterobacteriaceae	≤2	4	≥8	Horses[a]	CLSI
Pseudomonas aeruginosa					
Actinobacillus pleuropneumoniae					
Other bacteria	≤4	8	≥16	Humans	CLSI
Enterobacteriacea	≤2	-	≥4	Multi-species	EUCAST
Pseudomonas sp.	≤4	-	≥4	Multi-species	EUCAST
Acinetobacter sp.	≤4	-	≥4	Multi-species	EUCAST
Staphylococcus sp.	≤1	-	≥1	Multi-species	EUCAST
Other bacteria	≤2	-	≥4	Multi-species	EUCAST

CLSI, Clinical Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing.
[a]6.6 mg/kg IM.[8,9]

of 4 μg/mL (Table 3). Overall, this finding suggests a very low likelihood of clinical benefit from increased dosages of gentamicin in the horses in our study, and that an alternative antimicrobial may be a better solution than an increased dosage in order to decrease the likelihood of sub-therapeutic high-dose gentamicin treatment with its implications for treatment failure, bacterial resistance, and toxicity.

Aminoglycoside-associated nephrotoxicity has been associated with multiple-daily versus once-daily dosing regimens and is proposed to be related to trough plasma drug concentrations, although it has been suggested that increased trough concentrations could be a result rather than a cause of renal dysfunction.[21,22] Interestingly, there is little mention of ototoxicity in horses, which may be a major complication in human patients.[23] Recommended target trough concentrations are variable although generally considered to be no higher than 1–2 μg/mL, although little evidence supports this concentration range.[9] In the present study, and in another recent report,[8] C_{24h} concentrations were rarely of concern, being >2 μg/mL in only 2.6% of cases, although more work needs to be done.

As a bacterial group, cefoxitin-resistant Staphylococci appeared to have especially high rates of gentamicin resistance indicative of multiple drug resistance in this group. Cefoxitin resistance is predictive of resistance to all beta-lactam drugs and is a surrogate marker for the *mecA* gene. Only 8/22 (36.4%) cefoxitin-resistant Staphylococcal strains had gentamicin MIC ≤0.5 μg/mL versus 170/185 (91.8%) of those that were cefoxitin-susceptible (Table 3). Nevertheless, the same strongly bimodal distribution was still found with 13 of the remaining 14 cefoxitin-resistant strains (93%) having MIC results indicating resistance to gentamicin (MIC ≥ 8 μg/mL) and 1 sample most likely resistant with an MIC of 4 μg/mL.

Given that gentamicin is rarely used as monotherapy and generally is combined with benzylpenicillin, gentamicin resistance among cefoxitin-susceptible Staphylococci may have been clinically irrelevant if the isolates were sensitive to benzylpenicillin. However, examination of the MIC results obtained from the 15 gentamicin-resistant, cefoxitin-sensitive Staphylococci (all *Staphylococcus aureus*) indicated that only 1 (6.7%) was sensitive to benzylpenicillin (MIC, 0.25 μg/mL) suggesting frequent concurrent beta-lactamase production as has been reported previously.[24]

Increased resistance in bacteria derived from hospitalized populations has been reported previously[25] and also was clearly evident in the current study. Approximately 90% of isolates from ambulatory practices appeared susceptible to gentamicin (MIC ≤2 μg/mL) in contrast to approximately 40% of isolates from the hospital. Given that IV administration of antimicrobials is inconvenient in ambulatory practice, the hospital-derived bacteriology data (Table 3, Liphook Equine Hospital) may be more clinically relevant, and also more consistent with previous studies of bacterial gentamicin susceptibilities.[19,20,26,27] However, the different resistance patterns described in different studies do suggest a cautious approach to extrapolation of study findings to other circumstances and populations.

The present study provided details from all isolates from clinical samples and made no effort to separate unequivocal pathogens from possible contaminants or commensals, and it is likely that some (possibly many) isolates might not have had direct relevance to the horses' clinical problems. However, difficulties and incomplete knowledge in determining such clinical relevance led to inclusion of all isolates, necessitating that clinicians judge those isolates that are more or less likely to represent pathogens. For example, although coagulase-negative Staphylococci are commonly regarded as non-pathogenic, there clearly are clinical circumstances in which they may act as pathogens.[28] Additionally, given the retrospective nature of our clinical study, it proved difficult to determine with reasonable certainty that any isolates could be conclusively dismissed as contaminants.

All horses treated with gentamicin in the current study were weighed accurately, giving reasonable reassurance that dosing was consistent and precise. Indeed, in most cases, the drug was administered via an IV catheter, offering assurance of effective and complete administration. Although a few horses were treated via IV injection without a catheter, injection was always performed by experienced equine clinicians. Still, it remains possible that occasional partial perivascular injection or leakage could explain some low C_{60min} concentrations. Similarly, the laboratory analysis of plasma samples was performed as part of standard clinical care and duplicate samples were neither tested nor stored as might be done in an experimental study.

Thus, occasional laboratory error cannot be ruled out, but is expected to be very rare. Anecdotally, on occasion an unexpectedly low C_{60min} result was reanalyzed, but the findings always concurred with the original result.

Thus, the present study found that very few horses receiving 6.6 mg/kg IV q24h attained C_{60min} results considered adequate to kill bacteria with MIC $\geq$ 4 μg/mL as has been found previously.[8] Bacterial MIC $\leq$2 μg/mL are very common in infections of horses sampled in ambulatory practice and would be expected to respond well to standard gentamicin dosages. However, isolates sensitive to standard gentamicin dosages were significantly less common in hospitalized horses. Given the relatively limited increases in C_{60min} observed in response to dosage increases[8] and the rarity of isolates with intermediate MIC, there seems to be little justification for increasing the standard dosage of gentamicin from 6.6 mg.kg IV q24h, at least in this population of horses.

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CONFLICT OF INTEREST DECLARATION

Andy Durham serves as Associate Editor for the Journal of Veterinary Internal Medicine. He was not involved in review of this manuscript.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

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