AJVR

Local anesthetics and antibiotics display synergistic and antagonistic drug interactions against pathogens causing septic arthritis in horses

Ditte M. T. Adler, DVM, PhD, DECVS[1*]; Ane T. Nielsen, DVM[1]; Christine Olsen, DVM[1]; Denis Verwilghen, DVM, PhD, DECVS[2,3]; Peter Damborg, DVM, PhD, DECVM[4]; Rikke H. Olsen, DVM, PhD[4]

[1]Department of Veterinary Clinical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Taastrup, Denmark
[2]Goulburn Valley Equine Hospital, Congupna, VIC, Australia
[3]Department of Clinical Sciences, School of Veterinary Medicine, University of Melbourne, Melbourne, VIC, Australia
[4]Department of Veterinary and Animal Science, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

*Corresponding author: Dr. Adler (dima@sund.ku.dk)

Objective
To investigate the in vitro efficacy of antibiotics (amikacin, ceftiofur, and gentamicin) in combination with local anesthetics (LAs; bupivacaine hydrochloride, lidocaine hydrochloride, and mepivacaine hydrochloride), a combination commonly performed for IA injectate and regional limb perfusion (RLP) in horses.

Methods
17 equine clinical isolates were tested by the checkerboard method for their minimum inhibitory concentration (MIC) against a combination of concentrations of LAs and antibiotics from August 2020 through December 2023.

Results
For the majority of combinations, the antibiotic efficacy was not affected. However, in a subset of combinations (n = 70), the addition of LA to the antibiotic solution displayed a synergistic (n = 14) or antagonistic (n = 56) effect, indicating that LA increased or decreased antibiotic activity, respectively. Increased MICs seen in most antagonistic combinations appeared to be without clinical relevance as MICs remained below or above clinically achievable concentrations. In contrast, antagonism observed for aminoglycoside-LA combinations resulted in MICs higher than the concentration achievable by RLP. In some synergistic combinations, MICs decreased from markedly above to below or near clinically achievable concentrations against a specific antibiotic.

Conclusions
The addition of LAs to antibiotic solutions for IA injections does not compromise the in vitro antibiotic effect. Conversely, the addition for RLPs compromises the in vitro antibiotic effect at clinical concentrations.

Clinical Relevance
This in vitro study suggests that LAs can be added to IA antibiotic solutions without compromising antibiotic effects against common equine pathogens. For RLP, the combination of tested aminoglycosides (amikacin and gentamicin) and LAs is discouraged without taking into account MICs of antimicrobial susceptibility testing.

Keywords: regional limb perfusion, IA injection, lameness, pain management, septic arthritis

Septic arthritis in horses is a life-threatening condition, which may lead to persistent lameness and euthanasia. Alongside joint lavage, antibiotics, such as amikacin, ceftiofur, and gentamicin, are crucial for treatment of septic arthritis.[1–3] Antibiotics are commonly administered either as IA injections or as regional limb perfusions (RLPs).[1,4–8] Such local administration can result in very high concentrations at the site of infection as exemplified by amikacin reaching 20,900 µg/mL in the synovial fluid (SF) upon IA injection.[6] Septic arthritis is a painful condition, typically manifested clinically as pronounced lameness. Furthermore, the RLP procedure is associated with pain and discomfort of the horse from the increased blood pressure of the limb distal to the

Received August 15, 2024
Accepted January 29, 2025
Published online February 19, 2025

doi.org/10.2460/ajvr.24.08.0214

applied tourniquet. Systemic NSAIDs are often not adequate to secure patient comfort during treatment, especially in the immediate postoperative period. Accordingly, for simultaneous pain management of a horse with septic arthritis and during the RLP procedure, local anesthetics (LAs) are often added to these local and regional antibiotic injections.[9–11] The LAs used most commonly in horses are the amide-type LAs: lidocaine, mepivacaine, and bupivacaine.[12] How this combination of antibiotics and LAs affects antimicrobial activity of the antibiotic is unknown. Aside from their analgesic effect, LAs themselves also possess antimicrobial properties against equine pathogens.[13] While their antimicrobial effect alone is not therapeutic at the concentrations achieved at injection sites, they could potentially broaden the antimicrobial spectrum when combined with antibiotics if synergism occurs. Conversely, combination of drugs may also lead to antagonism. In the presence of antagonism, the addition of LAs to antibiotics would reduce the efficacy of the antibiotic, potentially leading to treatment failure. Finally, a combination of drugs may not lead to any interaction between the drugs, a phenomenon known as indifference.[14]

In this study, we aimed to investigate antimicrobial interactions between 3 antibiotics and 3 LAs, which are often used in combination IA or by RLP in horses. We hypothesized that LAs and antibiotics can be combined without altering the clinical effect.

Methods

Bacterial isolates

A total of 17 equine clinical bacterial isolates identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Vitek MS RUO; bioMérieux) were included in the study **(Table 1)**. The isolates were selected to represent 7 bacterial species that are among the most common infectious agents associated with septic arthritis in horses.[3] The isolates had been obtained in the period of 2006 through 2012 from equine infections in joints (n = 4), liver (n = 1), nose (n = 1), and skin (n = 12). Two of the *Staphylococcus aureus* isolates were methicillin-resistant *S aureus* (MRSA), whereas 4 of the gram-negative isolates were extended-spectrum β-lactamase–producing *Escherichia coli* (n = 3) or *Enterobacter cloacae* (n = 1).

Local anesthetics and antibiotics

The antibiotics amikacin sulfate (Sigma-Aldrich), gentamicin sulfate (Sigma-Aldrich), and ceftiofur hydrochloride (Sigma-Aldrich) and the LAs bupivacaine hydrochloride (Fagron BV), lidocaine hydrochloride (Sigma-Aldrich), and mepivacaine hydrochloride (Fagron BV) were included in the study. These antibiotics and LAs were selected as they are commonly used for IA administration and RLP in equine clinical practice. All LAs and antibiotics were dissolved in ultrapure water, except from ceftiofur, which was dissolved in DMSO (Sigma-Aldrich), to achieve stock concentrations 4 times the highest concentrations in the assays for minimum inhibitory concentration (MIC) determinations. Test concentrations were in the range of 0.125 to 256 mg/L for the antibiotics, 2.5 to 80 mg/mL for lidocaine and mepivacaine, and 1.25 to 5 mg/mL for bupivacaine. For assessing drug interaction (see later), the test range for gentamicin was extended to a maximum test concentration of 1,024 mg/L.

Determination of minimum inhibitory concentration

The MICs of LAs and antibiotics were determined in all isolates by broth microdilution according to

Table 1—The minimum inhibitory concentration (MIC) for bupivacaine, lidocaine, and mepivacaine against 17 equine clinical isolates determined by the broth microdilution method.

		Concentration (mg/mL)		
		Bupivacaine	Lidocaine	Mepivacaine
Species	**Strain**	**MIC**	**MIC**	**MIC**
Gram-positive cocci				
Streptococcus equi subsp *zooepidemicus*	L158-1	2.5	10	20
	29021	1.25	5	10
Staphylococcus aureus	24760	5	20	40
	26571	5	20	40
	31598[a]	5	40	40
	26714[a]	5	40	40
Gram-positive rods				
Rhodococcus equi	L103-1	1.25	10	10
	L254	1.25	10	10
Gram-negative rods				
Escherichia coli	26718	2.5	5	10
	26638	5	10	10
	26363[b]	2.5	5	5
	26408[b]	2.5	10	5
Pseudomonas aeruginosa	26315	1.25	20	40
	28434	1.25	10	40
Enterobacter cloacae	29626[b]	5	10	10
Actinobacillus equuli	25453	5	10	10
	22173-1	1.25	5	10

[a]Methicillin-resistant *S aureus*. [b]Extended-spectrum β-lactamase–producing Enterobacteriaceae.

Table 2—The MICs for the antibiotics amikacin, ceftiofur, and gentamicin against 17 equine clinical isolates determined by the broth microdilution method.

		Concentration (ug/mL)		
		Amikacin	Ceftiofur	Gentamicin
Species	Strain	MIC	MIC	MIC
Gram-positive cocci				
S equi subsp *zooepidemicus*	L158-1	8	≤ 0.125	4
	29021	8	≤ 0.125	4
S aureus	24760	8	1	> 256
	26571	4	1	> 256
	31598[a]	4	128	128
	26714[a]	8	128	128
Gram-positive rods				
R equi	L103-1	2	4	0.5
	L254	2	4	0.5
Gram-negative rods				
E coli	26718[b]	2	128	256
	26638	2	0.25	256
	26363[b]	1	128	256
	26408[b]	2	128	256
P aeruginosa	26315	1	64	0.5
	28434	1	64	0.5
E cloacae	29626[b]	1	2	256
A equuli	25453	2	≤ 0.125	4
	22173-1	4	1	2

[a]Methicillin-resistant *S aureus*. [b]Extended-spectrum β-lactamase–producing Enterobacteriaceae.
> indicates that the exact MIC exceeded the maximum test concentration of the local anesthetic solution.

Table 3—Fractional inhibitory concentration (FIC) index for amikacin, ceftiofur, and gentamicin combined with lidocaine.

	Strain						
		Lidocaine/amikacin		Lidocaine/ceftiofur		Lidocaine/gentamicin	
Species		FIC min	FIC max	FIC min	FIC max	FIC min	FIC max
Gram-positive cocci							
S equi subsp *zooepidemicus*	L158-1	0.75	1.125	NI	NI	0.625	1.063
	29021	0.625	1.25	NI	NI	1	2.25
S aureus	24760	1	1.5	1	1.125	NI	NI
	26571	1.125	1.25	1.016	2.25	NI	NI
	31598[a]	1.25	2.625	0.313[c]	1.065	1	1.01
	26714[a]	0.625	1	0.375[c]	1.125	1.03	1.25
Gram-positive rods							
R equi subsp *equi*	L103-1	1.063	1.25	0.563	1.016	0.75	1.25
	L254	1	1.25	0.531	1.125	0.563	1.25
Gram-negative rods							
E coli	26718[b]	1.063	4.5[d]	0.501	1.5	1.004	4.5[d]
	26638	1.063	8.25[d]	1	4.25[d]	0.504	4.25[d]
	26408[b]	0.625	2.25	0.508	1.25	0.258[c]	2.125
	26363[b]	0.625	8.25[d]	0.501	1.25	1.004	4.25[d]
P aeruginosa	26315	0.531	4.5[d]	1.002	1.5	1.25	4.5[d]
	28434	0.375[c]	4.063[d]	1.004	2.125	1.031	4.5[d]
E cloacae	29626[b]	1.063	2.25	0.75	1.125	0.504	4.063[d]
A equuli	25453	0.375[c]	2.125	0.375[c]	1.031	1	1.5
	2217-1	0.531	1.125	0.625	2.063	1	1.5

Max = Maximum. Min = Minimum. NI = Not investigated.
[a]Methicillin-resistant *S aureus*. [b]Extended-spectrum β-lactamase–producing Enterobacteriaceae. [c]Drug synergism (FIC index ≤ 0.5). [d]Drug antagonism (FIC index > 4).
The FIC index is used for determining the presence of drug interactions and is calculated as the MIC of the lidocaine in combination with the antibiotic divided by the MIC of lidocaine alone and likewise for the antibiotic. Fractional inhibitory concentration indices were then derived from the summation of individual FICs as previously described.[14] Drug interactions are classified as synergistic (FIC index ≤ 0.5), antagonistic (FIC index > 4), or indifferent (FIC index > 0.5 to 4). The FIC index was not investigated for a number of combinations (NI) as the MICs of the antibiotic were above the highest tested concentration of the checkerboard, hence limited clinical relevance was expected.

the Clinical and Laboratory Standards Institute.[15] Briefly, stock solutions of LAs and antibiotics (see above) were diluted to appropriate concentrations in Mueller-Hinton broth (MH). This was followed by 8 consecutive 2-fold dilutions in MH in a standard 96-well microtiter plate with a final volume of 100 µL/well. A McFarland standard 0.5 saline suspension of each test isolate (corresponding to 10^8 CFU/mL) was then diluted 1:100 in MH before 100 µL of the adjusted bacterial inoculum was added to each well, for a final concentration of 5 X 10^5 CFU/mL. A negative control with MH and LA or antimicrobial and a positive growth control with bacterial inoculum and MH but without LA or antimicrobial were also included in each assay (plate). Plates were incubated for 16 to 20 hours at 37 °C prior to reading the MICs.

Drug interactions

Interactions between the 9 combinations of LA and antibiotic (lidocaine-amikacin, lidocaine-ceftiofur, and lidocaine-gentamicin; mepivacaine-amikacin, mepivacaine-ceftiofur, and mepivacaine-gentamicin; and bupivacaine-amikacin, bupivacaine-ceftiofur, and bupivacaine-gentamicin) were determined against the 17 bacterial isolates by the checkerboard method as previously described.[16] Briefly, for each LA/antibiotic combination, 2-fold dilutions of each LA and each antibiotic were prepared using wells H through B and 12 through 2, respectively, in a standard 96-well microtiter plate, for a final volume of 50 µL in each well. Then, 50 µL of test bacterium adjusted to 10^6 CFU/mL in MH (see above) was added to provide a bacterial concentration of 5 X 10^5 CFU/mL in each well, followed by incubation for 24 hours at 37 °C. The concentrations of each LA and antibiotic were based on the initial MIC determinations, so the highest concentration in the final assay would be 4 times the initial MIC value. In this setup, all wells in row A were LA-free and were used to confirm the MIC for the antibiotic, whereas all wells in row 1 were antibiotic-free and were used to determine the MIC for the LA.

For each well not yielding any visible growth after incubation, the fractional inhibitory concentration (FIC) was calculated as the MIC of the LA in combination with the antibiotic divided by the MIC of the LA alone and likewise for the antibiotic. Fractional inhibitory concentration indices were then derived from summation of individual FICs.[14] Drug interactions were classified as synergistic (FIC index ≤ 0.5), antagonistic (FIC index > 4), or indifferent (FIC index > 0.5 to 4).[14]

Results

Table 1 and **Table 2** depict MIC values of the LAs and antibiotics, respectively. **Tables 3–5** depict FIC indices for the combination of LAs and antibiotics.

Table 4—Fractional inhibitory concentration index for antibiotics combined with mepivacaine.

	Strain						
		Mepivacaine/amikacin		Mepivacaine/ceftiofur		Mepivacaine/ gentamicin	
Species		FIC min	FIC max	FIC min	FIC max	FIC min	FIC max
Gram-positive cocci							
S equi subsp *zooepidemicus*	L15-1	1	1.25	NI	NI	0.75	1.125
	29021	1	1.25	NI	NI	1.063	2.25
S aureus	24760	0.53	1	0.516	1.063	NI	NI
	26571	0.625	1	1	1.25	NI	NI
	31598[a]	1.125	2.03	0.281[c]	1.031	1.02	1.50
	26714[a]	0.563	1	0.188[c]	1.031	2.05	2.50
Gram-positive rods							
R equi subsp equi	L10-1	1.063	2.5	0.375[c]	0.583	1	1.25
	L254	1.031	2.25	0.583	1.031	0.531	1.125
Gram-negative rods							
E coli	26718	0.5[c]	2.031	0.625	1.125	NI	NI
	26638	1.125	8.5[d]	1.063	1.5	0.508	8.25[d]
	26408[b]	1.031	4.25[d]	1	1.25	0.504	4.25[d]
	26363[b]	0.75	4.25[d]	1.001	1.5	1.004	4.125[d]
P aeruginosa	26315	1.016	8.5[d]	1.002	1.5	1.016	2.5
	28434	1.016	8.25[d]	1.004	2.25	1.016	2.25
E cloacae	29626[b]	1.125	2.25	0.5[c]	2.031	0.502	2.25
A equuli	25453	0.563	2.25	0.5[c]	1.063	1.125	2.5
	22173-1	0.75	2.125	0.55	2.125	0.75	1.25

Max = Maximum. Min = Minimum. NI = Not investigated.

[a]Methicillin-resistant *S aureus*. [b]Extended-spectrum β-lactamase–producing Enterobacteriaceae.[c]Drug synergism (FIC index ≤ 0.5). [d]Drug antagonism (FIC index > 4).

The FIC index is used for determining the presence of drug interactions and is calculated as the MIC of the lidocaine in combination with the antibiotic divided by the MIC of lidocaine alone and likewise for the antibiotic. Fractional inhibitory concentration indices were then derived from the summation of individual FICs as previously described.[14] Drug interactions are classified as synergistic (FIC index ≤ 0.5), antagonistic (FIC index > 4), or indifferent (FIC index > 0.5 to 4). The FIC index was not investigated for a number of combinations (NI) as the MICs of the antibiotic were above the highest tested concentration of the checkerboard, hence limited clinical relevance was expected.

Table 5—Fractional inhibitory concentration index for antibiotics combined with bupivacaine.

	Strain	Bupivacaine/amikacin		Bupivacaine/ceftiofur		Bupivacaine/gentamicin	
Species		**FIC min**	**FIC max**	**FIC min**	**FIC max**	**FIC min**	**FIC max**
Gram-positive cocci							
S equi subsp *zooepidemicus*	L15-1	0.508	1.25	NI	NI	0.562	1.016
	29021	1.008	1.5	NI	NI	1.063	2.25
S aureus	24760	1	1.5	1.016	1.5	NI	NI
	26571	1.01	1.5	1.063	0.531	NI	NI
	31598[a]	1.25	2.625	1	1.25	0.625	1
	26714[a]	1.02	1.50	1.03	1.50	1	1.25
Gram-positive rods							
R equi subsp *equi*	L10-1	1.062	4.5[d]	0.582	1.016	0.582	1.5
	L254	1.063	4.5[d]	1.031	2.5	0.625	1.25
Gram-negative rods							
E coli	26718	1.031	4.125[d]	1.001	1.5	1.004	2.5
	26638	1.125	8.031[d]	0.531	1.25	1.004	2.25
	26408[b]	0.625	4.25[d]	0.504	1.25	1.004	4.125[d]
	26363[b]	1.125	4.5[d]	0.502	1.5	1.004	4.25[d]
P aeruginosa	26315	NI	NI	NI	NI	NI	NI
	28434	NI	NI	NI	NI	NI	NI
E cloacae	29626[b]	1	1.25	1.031	2.25	1.004	1.5
A equuli	25453	1	1.25	1.125	2.5	0.75	2.125
	22173-1	0.75	4.031[d]	1.125	2.5	1	1.25

Max = Maximum. Min = Minimum. NI = Not investigated.

[a]Methicillin-resistant *S aureus*. [b]Extended-spectrum β-lactamase–producing Enterobacteriaceae. [d]Drug antagonism (FIC index > 4).

The FIC index is used for determining the presence of drug interactions and is calculated as the MIC of the lidocaine in combination with the antibiotic divided by the MIC of lidocaine alone and likewise for the antibiotic. Fractional inhibitory concentration indices were then derived from the summation of individual FICs as previously described.[14] Drug interactions are classified as synergistic (FIC index ≤ 0.5), antagonistic (FIC index > 4), or indifferent (FIC index > 0.5 to 4). The FIC index was not investigated for a number of combinations (NI) as the MICs of the antibiotic were above the highest tested concentration of the checkerboard, hence limited clinical relevance was expected.

A total of 153 checkerboard assays were conducted with 64 unique combinations of LA-antibiotic plus bacterial isolates in each checkerboard.

Synergistic and antagonistic drug interactions, as indicated by FIC indices ≤ 0.5 or > 4, were established for 18% of checkerboards, representing a total of 70 combinations. Synergism (FIC index ≤ 0.5) was observed in 14 of 70 combinations and antagonism (FIC index > 4) in 56 of 70 combinations. No interactions (indifference) were observed for the majority of investigated antibiotic-LA combinations (82%) as indicated by FIC indices > 0.5 to ≤ 4.

For the 56 combinations displaying an antagonistic drug interaction, the majority (n = 52) were observed for gram-negative pathogens, especially *E coli*. Antagonism was mainly evident for amikacin or gentamicin in combination with either lidocaine, mepivacaine, or bupivacaine (Tables 3–5). The antagonistic drug interactions resulted in 2 to 3 2-fold increases of the antibiotics' MIC value.

For the 14 combinations displaying a synergistic drug interaction, the majority were observed for ceftiofur combined with either lidocaine or mepivacaine (Tables 3–5). The synergistic drug interactions resulted in 3 to 5 2-fold decreases in the MIC value of the antibiotics. Two isolates (*S aureus* 31598 and 26714, both MRSA) with a ceftiofur MIC of 128 µg/mL prior to the addition of LAs decreased to a ceftiofur MIC of 4 to 16 µg/mL after the addition of lidocaine/mepivacaine.

Discussion

For the majority of investigated antibiotic-LA combinations (82%), there was no drug interaction against equine clinical isolates according to the determined FIC indices. This indicates that the antimicrobial activity of the antibiotic is not affected by the combination with an LA.

Nonetheless, in a subset of combinations, antagonism (n = 56) or synergism (n = 14) was observed. Whether or not these elucidated changes in antimicrobial activity translate into clinical relevance is uncertain. Other factors, like: MICs different from those detected in the present study, the presence of fibrin, pus or biofilm in the joint, and the tissue concentration obtained at the injection site, may also influence clinical efficacy of antibiotics.

With regard to the antibiotic tissue concentration of antibiotics at injection sites, multiple studies[5,6,17–20] have investigated antibiotic concentrations achieved in SF following IA administration. Common clinical IA amikacin doses to equine joints are 125 to 500 mg, which can result in maximal SF concentrations of 1,680 to 20,900 µg/mL.[6] Since concentration-dependent drugs like aminoglycosides exert a maximum effect at 10 to 12 times the MIC,[21] this high concentration in SF after IA administration theoretically results in pathogens with MICs as high as 140 to 1,742 µg/mL (1,680/12 to 20,900/12) being susceptible to amikacin (**Table 6** depicts theoretical MIC limits in SF). In other words,

Table 6—Theoretical MIC limits calculated from previously published studies investigating antibiotic concentrations in synovial fluid after IA and regional limb perfusion.

	Concentration (µg/mL)		
Type of administration	**Amikacin MIC**	**Gentamicin MIC**	**Ceftiofur MIC**
IA administration	140[a]	150[b]	5[c]
Regional limb perfusion	38[a]	11[b]	5[c]

[a]Minimum inhibitory concentration limits in synovial fluid of horses calculated based on studies[6,17] establishing amikacin concentrations in synovial fluid of horses after the minimal clinical IA dose of amikacin (125 mg) and administration of 1,000 mg amikacin as a regional limb perfusion, taking into consideration that the concentration should be 12 times the MIC for an aminoglycoside to be effective. [b]Minimum inhibitory concentration limits in synovial fluid of horses calculated based on studies[4,19] establishing gentamicin concentrations in synovial fluid of horses after the clinical IA dose of gentamicin (150 mg) and administration of 500 mg gentamicin as a regional limb perfusion, taking into consideration that the concentration should be 12 times the MIC for an aminoglycoside to be effective. [c]Minimum inhibitory concentration limits in synovial fluid of horses after 24 hours calculated based on studies[5] establishing ceftiofur concentrations in synovial fluid of horses after the clinical IA dose of ceftiofur (150 mg), taking into consideration that the concentration should be > MIC for approximately 50% of the dosing interval to be effective, which is not fulfilled when ceftiofur is administered as a RLP.

even the lowest-used dose of amikacin is likely to be effective in killing bacteria with MICs ≤ 140 µg/mL, including all isolates belonging to the 7 genera of the present study and analyzed at our laboratory within the last 16 years (P Damborg, DVM, PhD, Vet Diagnostics, Department of Veterinary Sciences, University of Copenhagen, Denmark, unpublished data, 2007 through 2023).

Clinically reported amikacin doses used for RLPs vary from 500 to 2,500 mg,[18] but most commonly 500 to 1,000 mg are used.[22] In a study by Godfrey et al,[17] the SF concentration after RLP with 1,000 mg diluted to a total volume of 60 mL for the distal limb was 459 µg/mL 30 minutes after injection while the tourniquet was still applied and 14 µg/mL after 24 hours. This means that in theory, bacteria with MICs as high as 38 µg/mL would be susceptible to amikacin, considering the limit of up to 12 times the MIC to reach a concentration-dependent effect (459/12 = 38).[21] Within the past 16 years, 98% of equine isolates belonging to the included 7 genera and analyzed at our laboratory had MICs < 38 µg/mL (P Damborg, DVM, PhD, Vet Diagnostics, Department of Veterinary Sciences, University of Copenhagen, Denmark, unpublished data, 2007 through 2023) and were therefore likely susceptible to amikacin when administered as an RLP without LAs (Table 6 depicts theoretical MIC limits).

The observed antagonistic drug interaction for the combination of amikacin with LAs resulted in a 2-to-3 2-fold increase of the MIC of specific combinations. These antagonistic drug interactions are without clinical implications for the isolates included in this study as a 2-to-3 2-fold increase in the MIC would still be below the MIC limits (see Table 6 for theoretical MIC limits). The maximum amikacin MIC value of isolates of the present study was 8 µg/mL (Table 2), and other studies[23,24] have reported common equine pathogens to have amikacin MICs ≤ 16 µg/mL. Such Isolates with MICs of 16 µg/mL (as reported in other studies) would in the presence of an antagonistic drug interaction equivalent to the drug interaction displayed in the present study still be susceptible to the lowest IA amikacin dose used. Nonetheless, the opposite applies for RLPs as the increased MIC (a 2-to-3 2-fold increase of MIC of 64 to 128 µg/mL) is higher than the RLP MIC limit of SF. Therefore, caution is warranted when combining amikacin with LAs for RLPs for the treatment of bacteria with MICs > 8 µg/mL since potential antagonistic drug interactions result in MIC increases above concentrations achieved in SF. This underlines the importance of susceptibility testing prior to using amikacin-LA combinations for RLPs. Nonetheless, septic arthritis in horses needs prompt intervention for horses to survive. Therefore, aggressive treatment, including antibiotic administration, is commonly initiated based on clinical manifestations and SF analysis before culture results are available. In these cases, the addition of LAs to amikacin for RLPs should ideally be avoided, and pain should be managed alternatively (eg, through sedation and/or perineural analgesia).

The finding that the displayed antagonism after the combination of amikacin with LAs in our study (for isolates with an amikacin MIC of ≤ 8 µg/mL) is unlikely to have clinical implications is supported by an equine ex vivo study.[9] Colbath et al[9] found that the antimicrobial activity of amikacin was unaffected when SF, after RLP with 1,000 mg of amikacin combined with 500 mg of mepivacaine, was investigated against *E coli* and *S aureus*. The finding of an unaltered clinical antimicrobial activity for this RLP administration is likely caused by administration of amikacin in excess combined with low MICs of included isolates. This is corroborated by findings of the same study[9] revealing a median amikacin concentration in SF of 104 µg/mL (IQR, 26.0 to 269.0) and an amikacin MIC of their 2 included reference strains of 0.5 to 4 µg/mL.[25,26]

A common clinical dose of gentamicin to a medium-sized joint (carpal joints, metacarpophalangeal and metatarsophalangeal joints) is 150 mg,[4] whereas a common dose for regional perfusion of the distal limb is 500 mg in dilution.[19,22] Immediately after the administration of 150 mg to the radiocarpal joint, a mean peak SF concentration of 1,828 µg/mL[4] was measured, and the mean SF concentration of gentamicin in the metacarpophalangeal joint was 125.9 µg/mL 30 minutes after the administration of a 500-mg distal limb regional perfusion.[19] The high concentration in SF after IA and RLP administration theoretically results in the killing of pathogens with MICs as high as 150 µg/mL (1,828/12 = 152) and 11 µg/mL (125.9/12 = 11) upon IA and RLP administration of gentamicin, respectively.

In our study, the observed antagonistic drug interactions for combinations of gentamicin with LAs resulted in a 2-to-3 2-fold MIC increase for specific isolates. The presented antagonistic drug interactions are unlikely to be clinically relevant for isolates included in the present study as a 2-to-3 2-fold gentamicin MIC increase would still render isolates either susceptible or resistant to gentamicin, equivalent to results prior to combining antibiotics with LAs, albeit many common clinical equine pathogens that are considered to be susceptible to gentamicin have gentamicin MICs higher than those included in our study.[27] In such cases, the presence of antagonistic drug interactions may have clinical implications. Isolates with gentamicin MICs of up to 16 µg/mL would, in the presence of equivalent drug antagonism as displayed in the present study, still be susceptible to common IA administration of gentamicin. On the other hand, for RLP, only isolates with gentamicin MICs < 2 µg/mL would still be susceptible to gentamicin as an increased MIC (3 2-fold increases of 2 = 16 µg/mL) exceeds theoretical SF MIC limits (Table 6).

A common IA dose of ceftiofur for medium-sized joints is 150 mg, and a previous study[5] revealed that the maximum IA concentration of ceftiofur was 5,825.08 µg/mL 15 minutes after administration of this dose to the radiocarpal joint. At 24 hours after administration, the concentration was 4.94 µg/mL.[5] This concentration is above the MIC of the majority (91%) of isolates belonging to the included 7 genera analyzed at our laboratory during a 16-year period (P Damborg, DVM, PhD, Vet Diagnostics, Department of Veterinary Sciences, University of Copenhagen, Denmark, unpublished data, 2007 through 2023). Because the concentration remained above this MIC for the entire dosing interval of 24 hours, IA administration of this time-dependent β-lactam alone should be successful in treating clinical joint infections from common equine ceftiofur-susceptible isolates at this dose.[21]

Only 1 isolate (*E coli* 26638) displayed an antagonistic drug interaction when ceftiofur was combined with lidocaine. The MIC of that specific isolate increased 2 2-fold dilutions. While this increase did not result in any clinically relevant findings of the present study as the increased MIC (= 2 µg/mL) remained below the MIC limit of ceftiofur (Table 6), potential clinical implications for other isolates cannot be ruled out. Interestingly, ceftiofur did, as the only antibiotic of the 3 included, reveal promising synergistic effects when combined with lidocaine and mepivacaine. The observed synergistic drug interactions for combinations of ceftiofur and lidocaine/mepivacaine resulted in a 3-to-5 2-fold reduction of MICs. These observed decreases of MICs are likely to be clinically relevant, particularly for the 2 MRSA strains. For these 2 isolates, the combination of ceftiofur and lidocaine and mepivacaine consistently reduced MICs with 3 to 5 2-fold dilutions to final concentrations either below MIC limits (4 µg/mL) or in the near clinical range (8 to 16 µg/mL). Although concentrations of 8 to 16 µg/mL are above the MIC limit, the IA ceftiofur combination with mepivacaine/lidocaine might also be effective against these MRSA as the theoretical MIC limit is based on achieved concentrations of ceftiofur in the SF of joints (4.94 µg/mL) 24 hours after IA injection. Based on the fact that ceftiofur only needs to exceed the MIC for approximately 50% of the 24-hour dosing interval to be effective and that the maximal measured concentration is 5,825.08 µg/mL[5] after a standard IA ceftiofur dose, concentrations are likely to be higher than 16 µg/mL for 50% of the dosing interval. Nonetheless, elaborate pharmacokinetic studies are needed to firmly conclude this.

Concentrations of ceftiofur in SF after RLP with a full IV dose of 2 g ceftiofur revealed mean SF concentrations of 393 ± 103 µg/mL 30 minutes after injection and at 1.62 µg/mL after 24 hours.[5] The same study revealed that ceftiofur remained above 2 µg/mL for 8 hours after RLP. While obtained concentrations in SF after RLP are higher than those achieved after IV administration, 8 hours is < 50% of the time during the dosing interval (once daily), which is less than that necessary for a time-dependent antibiotic to have clinical success.[21] Consequently, independently of our results, ceftiofur is less likely to have clinical success in the treatment of septic arthritis when administered as RLP compared with an IA injection.

The main limitation of our study is inherent to its in vitro nature as the theoretical calculations provided need to be confirmed in vivo for final clinical conclusions. Further, due to economic and logistic reasons, we investigated a limited subset of equine isolates within 7 genera that most often cause septic arthritis in horses. Even though the inclusion of additional isolates would have increased the validity of our results, we ensured that the included isolates were representative of the MIC ranges observed in other studies. Some of the included isolates originated from infections other than joint infections. This was a necessity due to our limited strain database comprising only a few isolates from joints. Nevertheless, this is unlikely to have affected results negatively in view of the aforementioned representativeness of MICs.

In conclusion, our in vitro results suggest that commonly used antibiotics for the treatment of septic arthritis in horses can generally be combined with LAs for pain management during IA injections without compromising the clinical antimicrobial activity of the antibiotic. This information is pivotal for clinicians working in equine practice as our results show that these drugs can mostly be combined without compromising the antimicrobial activity of the antibiotic agent. For RLPs, caution is warranted when using aminoglycosides as drug antagonism was observed when combining LAs with gentamicin and amikacin in this in vitro study. Therefore, we suggest avoiding adding LAs to aminoglycosides for RLPs in the treatment of orthopedic infections caused by bacteria with unknown antimicrobial susceptibility.

The combination of ceftiofur with lidocaine and mepivacaine shows promising results by decreasing the MIC dramatically for the tested MRSA strains. The combination of ceftiofur with lidocaine or mepivacaine for IA injections could potentially be a treatment option for confirmed MRSA septic arthritis

cases. However, the described synergistic interaction alongside thorough pharmacokinetics of IA ceftiofur should be further investigated before making a firm conclusion on its potential clinical efficacy.

Acknowledgments

None reported.

Disclosures

The authors have nothing to disclose. No AI-assisted technologies were used in the composition of this manuscript.

Funding

The authors have nothing to disclose.

ORCID

D. M. T. Adler https://orcid.org/0000-0001-5042-0810

References

1. Pille F, De Baere S, Ceelen L, et al. Synovial fluid and plasma concentrations of ceftiofur after regional intravenous perfusion in the horse. *Vet Surg.* 2005;34(6):610–617. doi:10.1111/j.1532-950X.2005.00095.x
2. Gillespie CC, Adams SB, Moore GE. Methods and variables associated with the risk of septic arthritis following intra-articular injections in horses: a survey of veterinarians. *Vet Surg.* 2016;45(8):1071–1076. doi:10.1111/vsu.12563
3. Moore R, Schneider R, Kowalski J, Bramlage LR, Mecklenburg LM, Kohn CW. Antimicrobial susceptibility of bacterial isolates from 233 horses with musculoskeletal infection during 1979–1989. *Equine Vet J.* 1992;24(6):450–456. doi:10.1111/j.2042-3306.1992.tb02875.x
4. Lloyd KC, Stover SM, Pascoe JR, Adams P. Synovial fluid pH, cytologic characteristics, and gentamicin concentration after intra-articular administration of the drug in an experimental model of infectious arthritis in horses. *Am J Vet Res.* 1990;51(9):1363–1369. doi:10.2460/ajvr.1990.51.09.1363
5. Mills ML, Rush BR, Jean GS, et al. Determination of synovial fluid and serum concentrations, and morphologic effects of intraarticular ceftiofur sodium in horses. *Vet Surg.* 2000;29(5):398–406. doi:10.1053/jvet.2000.9141
6. Pezzanite L, Chow L, Hendrickson D, et al. Evaluation of intra-articular amikacin administration in an equine non-inflammatory joint model to identify effective bactericidal concentrations while minimizing cytotoxicity. *Front Vet Sci.* 2021;8:676774. doi:10.3389/fvets.2021.676774
7. Taintor J, Schumacher J, DeGraves F. Comparison of amikacin concentrations in normal and inflamed joints of horses following intra-articular administration. *Equine Vet J.* 2006;38(2):189–191. doi:10.2746/042516406776563233
8. Werner LA, Hardy J, Bertone AL. Bone gentamicin concentration after intra-articular injection or regional intravenous perfusion in the horse. *Vet Surg.* 2003;32(6):559–565. doi:10.1111/j.1532-950X.2003.00559.x
9. Colbath AC, Wittenburg LA, Gold JR, McIlwraith CW, Moorman VJ. The effects of mepivacaine hydrochloride on antimicrobial activity and mechanical nociceptive threshold during amikacin sulfate regional limb perfusion in the horse. *Vet Surg.* 2016;45(6):798–803. doi:10.1111/vsu.12515
10. Mahne AT, Rioja E, Marais HJ, Villarino LM, Rubio-Martinez LM. Clinical and pharmacokinetic effects of regional or general anaesthesia on intravenous regional limb perfusion with amikacin in horses. *Equine Vet J.* 2014;46(3):375–379. doi:10.1111/evj.12125
11. Mendez-Angulo JL, Granados MM, Modesto R, et al. Systemic and local effects of lidocaine or mepivacaine when used for intravenous regional anaesthesia of the distal limb in standing sedated horses. *Equine Vet J.* 2020;52(5):743–751. doi:10.1111/evj.13236
12. Bassage L, Ross M. Diagnostic analgesia. In: Ross M, Dyson S, eds. *Diagnosis and Management of Lameness in the Horse.* 2nd ed. Elsevier; 2010:100–135.
13. Adler DMT, Damborg P, Verwilghen DR. The antimicrobial activity of bupivacaine, lidocaine and mepivacaine against equine pathogens: an investigation of 40 bacterial isolates. *Vet J.* 2017;223:27–31. doi:10.1016/j.tvjl.2017.05.001
14. Odds FC. Synergy, antagonism, and what the chequerboard puts between them. *J Antimicrob Chemother.* 2003;52(1):1–1. doi:10.1093/jac/dkg301
15. Clinical and Laboratory Standards Institute (CLSI) Performance Standards for Antimicrobial Susceptibility Testing. CLSI Approved Standard M100. Clinical and Laboratory Standards Institute, Wayne; 2024. Accessed February 6, 2025. https://clsi.org/standards/products/microbiology/documents/m100/
16. Pillai SK, Moellering R, Eliopoulos G. Antimicrobial Combinations. In: Lorian V, ed. *Antibiotics in Laboratory Medicine.* 5th ed. Lippincott Williams & Wilkins; 2005:365–441.
17. Godfrey JL, Hardy J, Cohen ND. Effects of regional limb perfusion volume on concentrations of amikacin sulfate in synovial and interstitial fluid samples from anesthetized horses. *Am J Vet Res.* 2016;77(6):582–588. doi:10.2460/ajvr.77.6.582
18. Harvey A, Kilcoyne I, Byrne BA, Nieto J. Effect of dose on intra-articular amikacin sulfate concentrations following intravenous regional limb perfusion in horses. *Vet Surg.* 2016;45(8):1077–1082. doi:10.1111/vsu.12564
19. Hyde RM, Lynch TM, Clark CK, Slone DE, Hughes FE. The influence of perfusate volume on antimicrobial concentration in synovial fluid following intravenous regional limb perfusion in the standing horse. *Can Vet J.* 2013;54(4):363–367.
20. Lloyd K, Stover S, Pascoe JR, Baggot JD, Kurpershoek C, Hietala S. Plasma and synovial fluid concentrations of gentamicin in horses after intra-articular administration of buffered and unbuffered gentamicin. *Am J Vet Res.* 1988;49(5):644–649.
21. Kuti JL. Optimizing antimicrobial pharmacodynamics: a guide for your stewardship program. *Rev Méd Clín Las Condes.* 2016;27(5):615–624. doi:10.1016/j.rmclc.2016.08.001
22. Rubio-Martínez LM, Cruz AM. Antimicrobial regional limb perfusion in horses. *J Am Vet Med Assoc.* 2006;228(5):706–712. doi:10.2460/javma.228.5.706
23. Butt TD, Bailey JV, Dowling PM, Fretz PB. Comparison of 2 techniques for regional antibiotic delivery to the equine forelimb: intraosseous perfusion vs. intravenous perfusion. *Can Vet J.* 2001;42(8):617–622.
24. Levine D, Richardson D. Clinical use of the locking compression plate (LCP) in horses: a retrospective study of 31 cases (2004–2006). *Equine Vet J.* 2007;39(5):401–406. doi:10.2746/042516407X196555
25. Amikacin susceptibility and minimum inhibitory concentration (MIC) data. ECDC EARS-Net External Quality Assessment (EQA) 2011. Updated. Accessed February 6, 2025. https://www.ecdc.europa.eu/sites/default/files/documents/5-%20Derek%20Brown.pdf
26. Veterinary reference card. Sensititre. Accessed August 7, 2024. https://vetmed.illinois.edu/wp-content/uploads/2018/09/EQUIN1F-Veterinary-Reference-Card.pdf
27. Durham AE. An evaluation of serum gentamicin concentrations and bacterial susceptibility to gentamicin in equine practice. *J Vet Intern Med.* 2018;32(3):1194–1201. doi:10.1111/jvim.15078