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Colistin-containing cement spacer for treatment of experimental carbapenemase-producing *Klebsiella pneumoniae* prosthetic joint infection ☆

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ABSTRACT

Carbapenemase-producing Enterobacteriaceae (CPE) are emerging multidrug-resistant bacteria responsible for invasive infections, including prosthetic joint infections (PJIs). Local administration of colistin may provide bactericidal concentrations in situ. This study evaluated the efficacy of a colistin-impregnated cement spacer, alone and in combination with systemic antibiotics, in a rabbit model of CPE-PJI. Elution of 3 MIU of colistimethate sodium (CMS) in 40 g of poly(methyl methacrylate) cement was studied in vitro. In vivo, 5×10^8 CFU of KPC-producing *Klebsiella pneumoniae* (colistin and meropenem MICs of 1 mg/L and 4 mg/L, respectively) were injected close to a prosthetic knee. Surgical debridement and prosthesis removal were performed 7 days later, and rabbits were assigned to six treatment groups (11–13 rabbits each): drug-free spacer; colistin-loaded spacer; colistin intramuscular (i.m.); colistin i.m. + colistin spacer; colistin i.m. + meropenem subcutaneous (s.c.); and colistin i.m. + meropenem s.c. + colistin spacer. Systemic treatment was administered at doses targeting pharmacokinetics in humans, and rabbits were euthanised 7 days later to evaluate bacterial counts in infected bones. In vitro, CMS elution was low (<0.1% at 24 h) but reached a local concentration of ≥20 mg/L (>20 × MIC). In vivo, combinations of local and systemic colistin, with or without meropenem, were the only regimens superior to the control group ($P \leq 0.05$) in terms of viable bacterial counts and the proportion of rabbits with sterile bone, with no emergence of colistin-resistant strains. Colistin-loaded cement spacer in combination with systemic antibiotics were the most effective regimens in this CPE-PJI model.

1. Introduction

Prosthetic joint infections (PJIs) cause significant morbidity and incur high treatment costs [1]. Carbapenemase-producing Enterobacteriaceae (CPE) are emerging multidrug-resistant bacteria [2] responsible for a broad range of difficult-to-treat invasive infections, including PJIs. As CPE increasingly present with resistance to almost all classes of available antibiotics, physicians are increasingly left with the use of old antibacterial agents, with limited data regarding their efficacy [2,3]. Although experts recommend various treatment combinations, data from clinical studies are scarce, and innovative experimental models are warranted to evaluate new medical and surgical treatments [4–7]. Colistin is often the only remaining option for treating carbapenemase-producing *Klebsiella pneumoniae* (CPKP). However, parenteral administration of colistin is associated with severe adverse effects, including nephrotoxicity and neurotoxicity [8]. Moreover, the emergence of colistin-resistant strains has been reported in

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patients treated with colistin [9], possibly promoted by suboptimal drug concentrations at the site of infection [10].

Local administration of antibiotics is a promising approach to reduce the risk of side effects and the emergence of resistant strains. In orthopaedic surgery, antibiotic-impregnated cement spacers are often used for local delivery of antibiotics following revision surgery, particularly in difficult-to-treat infections [11]. However, their use remains controversial because of concerns regarding their efficacy [12,13], tolerability [13,14] and the emergence of resistance in sites where the drug concentration is low [12]. Colistin elution from bone cement has been studied in vitro [15,16]. Cement loaded with colistin has been successfully used in case reports of *Pseudomonas aeruginosa* osteomyelitis [17,18] and PJI [19].

The aim of this study was to evaluate the efficacy of a colistin-impregnated cement spacer, alone or in combination with systemic colistin with or without systemic meropenem, using a rabbit model of PJI caused by CPKP that closely mimics human infection, adapted from a previous model [20]. Systemic colistin plus meropenem was selected because this combination is recommended for CPKP with meropenem minimum inhibitory concentrations (MICs) of $\leq$ 8 mg/L [21].

2. Methods

2.1. Bacterial strain

KPC99YC is a *K. pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* clinical strain susceptible to colistin (MIC = 1 mg/L), with intermediate susceptibility to meropenem and imipenem (MICs of 4 mg/L for both).

2.2. In vitro bactericidal activities of colistin and meropenem alone and in combination

Meropenem (AstraZeneca, Courbevoie, France) and colistimethate sodium (CMS) (Sanofi, Gentilly, France) were purchased from local drugs purchase companies and were prepared following the label instructions for clinical use in humans. The bactericidal activities of colistin and meropenem, alone or in combination, were determined in triplicate. An overnight bacterial culture was diluted in 10 mL of fresh Mueller–Hinton broth to yield an inoculum of 10^5 CFU/mL. The antibiotic concentrations used were equivalent to 4 × MIC. After 0, 3, 6, 9 and 24 h of incubation in a shaking water-bath at 37 °C, serial dilutions of 0.1 mL samples were subcultured on Mueller–Hinton agar plates (Bio-Rad, Marnes-la-Coquettes, France) and were incubated at 37 °C for 24 h before the number of CFU was enumerated. A bactericidal effect was defined as a >2 $\log_{10}$ decrease compared with the initial inoculum. Synergy was defined as a decrease of >2 $\log_{10}$ CFU/mL for the combination compared with its most active constituent.

2.3. Selection of meropenem and colistin doses in rabbits

Plasma antibiotic concentrations were measured in uninfected rabbits to select doses that will lead to plasma concentrations equivalent to those obtained in humans. Initial doses were selected based on previous experimental studies in rabbits. It was verified that they achieved pharmacokinetic (PK) and pharmacodynamic (PD) targets for strain KPC99YC that are recommended for severe CPE infections in humans. Each antibiotic was tested on a group of four uninfected rabbits. Samples were obtained after 24 h of treatment (steady-state concentration). Blood was drawn 15 min and 1, 2, 4, 6, 8 and 12 h after the injection and was centrifuged. Concentrations were measured by high-performance liquid chromatography (HPLC)–tandem mass spectrometry using an electrospray ionisation method. The limit of quantification was 0.25 mg/L. PK parameters were calculated using Monolix v.4.3.3 (Lixoft SAS, Antony, France).

2.4. In vitro evaluation of colistin elution from the spacer

For elution of antibiotic, six spacer samples were immersed in 10 mL of an isotonic saline solution placed at 37 °C with agitation at 125 rpm. Then, 250 µL of the saline solution was removed after 1, 4, 8, 24, 48 and 72 h and 7, 14 and 21 days. The colistin concentration was measured by HPLC with mass spectrometry detection as described above. Results are expressed as the percentage of colistin released from the cement spacer (amount released each day/total amount).

2.5. Experimental prosthetic joint infection and treatment groups

New Zealand white female rabbits, each weighing 2.5–3 kg, were used. They were housed in individual cages with natural light:dark cycles. The experimental protocol was in keeping with French legislation on animal experimentation and was approved by the Animal Use Committee of Maisons Alfort Veterinary School (Maisons-Alfort, France). This model has been described previously [20]. Briefly, each rabbit underwent partial right knee replacement with a tibial component by an orthopaedic surgeon. Surgery was performed under general anaesthesia induced by intramuscular (i.m.) injection of 25 mg/kg ketamine (Ketamine 1000; Virbac, Carros, France) and 25 mg/kg of 2% xylazine (Rompun®; Bayer Santé, Division Santé Animal, Puteaux, France) and then by continuous inhalation of 1% isoflurane (Vetflurane™; Virbac). The skin overlying the right leg was shaved before the operation and was cleaned with iodine solution prior to surgery. A longitudinal skin incision was made and the knee joint was exposed. The epiphyseal plates were removed and the metaphysis was exposed. A silicone elastomer implant, commonly used in arthroplasty of the first metatarsophalangeal joint [Silastic®, great toe implant HP; Swanson Design, Dow-Corning (provided by Wright Medical France, Créteil, France)] was implanted as a tibial prosthetic component. Immediately after surgery, 5×10^8 CFU of KPC99YC in 0.5 mL was injected into the knee close to the prosthesis. Each rabbit was given patch analgesia (Durogesic®; Janssen-Cilag SA, Issy-les-Moulineaux, France). Blood cultures were performed 24 h after inoculation to identify the presence of early bacteraemia.

Surgical synovectomy was performed 7 days after inoculation. Periprosthetic fluid culture was performed to ensure that all rabbits were infected. The prosthesis was removed and was replaced by a cement spacer. A total of 11–13 rabbits were randomly assigned to each control or treatment group (six groups in total), with treatments for 7 days: (i) prosthesis replacement by drug-free cement spacer (control); (ii) prosthesis replacement by colistin-loaded (3 MIU/40 g of cement) cement spacer (Coli-Ce); (iii) prosthesis replacement by drug-free cement spacer + colistin 12 mg/kg i.m. three times a day (t.i.d.), equivalent to 3 MIU t.i.d. in humans (Coli S); (iv) prosthesis replacement by colistin-loaded cement spacer + colistin i.m. (Coli-Ce + Coli S); (v) prosthesis replacement by drug-free cement spacer + colistin i.m. + meropenem 80 mg/kg subcutaneous (s.c.) t.i.d., equivalent to 250 mg intravenous (i.v.) t.i.d. in humans (Coli S + Mero S); and (vi) prosthesis replacement by colistin-loaded cement spacer + colistin i.m. + meropenem s.c. (Coli-Ce + Coli S + Mero S).

The antibiotic-loaded spacer was obtained by mixing 3 MIU of CMS with 40 g of powdered cement polymer before the addition of methyl methacrylate, as done in clinical practice and according to the manufacturer's instructions (CMW™ radiopaque bone cement; DePuy Synthes, Blackpool, UK). The dose of colistin in cement was similar to doses previously studied in vitro [15–17]. It was then moulded to reproduce a facsimile of the silicone prosthesis.

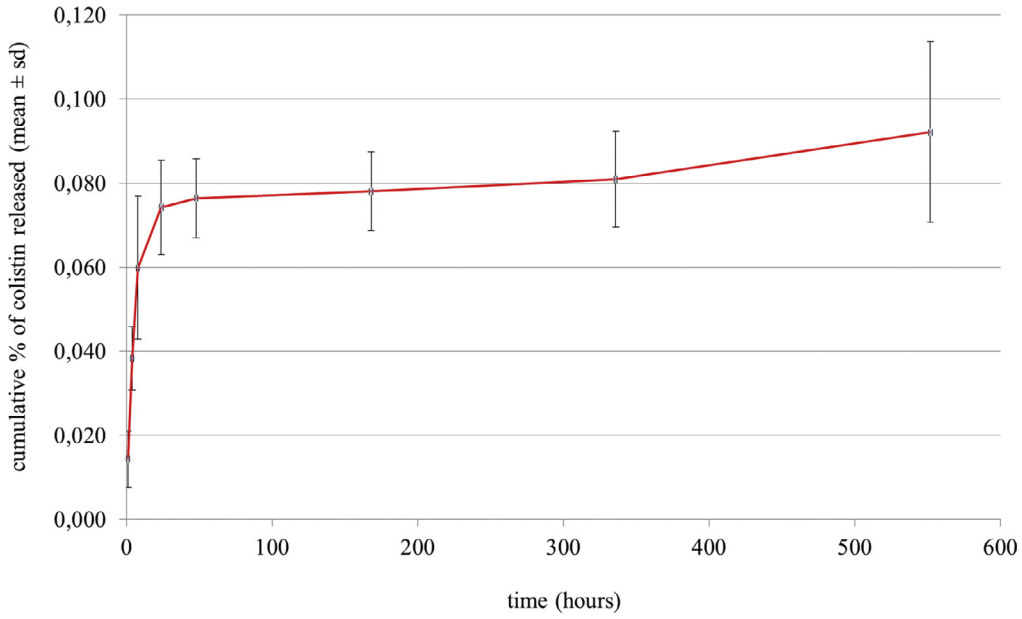

Fig. 1. Cumulative percentage of colistin released from the colistin-impregnated cement spacer over time. sd, standard deviation.

2.6. Evaluation of therapy

At 14 days after inoculation, rabbits were euthanised by i.v. pentobarbital. The skin aspect and position of the prosthesis were noted. To quantify bacterial counts, the upper third of the tibia (3 cm), including compact bone and marrow, was isolated and was split with a bone crusher, weighed, cut into small pieces and crushed in an automatic pulverizer (MM 200; Retsch GmbH, Haan, Germany). The number of viable micro-organisms was determined after 24 h of incubation at 37 °C. The results are expressed as the median [interquartile range (IQR)] $\log_{10}$ CFU/g of bone and the number of animals with sterile bone. Bone was considered sterile when the culture showed no growth following incubation for 48 h at 37 °C, and the number of CFU recorded was the limit of detection (1.64–1.84 $\log_{10}$ CFU/g of bone, depending on sample weight).

2.7. In vivo mutant selection

Subpopulation analysis was performed in untreated and treated rabbits to screen for the emergence of colistin-resistant strains. Each undiluted bone homogenate (50 µL) was plated onto Mueller–Hinton II agar and onto Mueller–Hinton II agar supplemented with 8 mg/L colistin and was incubated for 72 h at 37 °C. When bacterial growth was observed, the colonies were counted and *Klebsiella* identification was confirmed using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (Vitek MS; bioMérieux, La Balme-les-Grottes, France). Colistin MICs were determined by liquid broth dilution method based on the UMIC colistin reagents (Biocentric, Bandol, France). Mutants were defined as having a 3-fold greater MIC than that of the initial strain.

2.8. Statistics

Data were analysed using R software [22]. Due to the small sample size and asymmetric distribution of the analysed variables, exact non-parametric tests implemented in the 'coin' package were used [23]. Data for numeric variables were summarised by the minimum, maximum, median, range, IQR, mean and standard deviation, and categorical variables by numbers and percentages. The χ^2 test, Fisher's exact test and Kruskal–Wallis non-parametric method were used, as appropriate. The effect size of antibiotics and their 95% confidence interval (CI) on $\log_{10}$ CFU criteria were calculated by the Hodges–Lehmann estimator and the Bauer algorithm, respectively [24]. The effect size of antibiotics on 'sterility' criteria was estimated by the difference of 'sterility' proportion and 95% CI. Results of the bilateral statistical tests were considered significant at a P-value of <0.05.

3. Results

3.1. In vitro evaluation of colistin diffusion from the spacer (Fig. 1)

From 3 MIU (240 mg) of colistin spiked into the cement, only 0.1% was detected in immersion solution after 24 h. Steady-state was reached at 24–48 h. Although the elution coefficient was low, the concentration reached in the elution fluid was ≥20 mg/L (i.e. 20 times the MIC).

3.2. Plasma concentrations of antibiotics

For colistin, the PK/PD target is an area under the concentration–time curve (AUC)/MIC ratio of 25–50. This target was achieved with a dosing regimen of 12 mg/kg i.m. t.i.d., equivalent to 3 MIU t.i.d. in humans. The mean peak plasma concentration (C_{max}) at 2 h after injection in four uninfected animals was 2.9 mg/L. The mean AUC from 0–24 h (AUC_{0-24}) was 13.3 mg·h/L (Fig. 2).

For meropenem, the initial objective was to maintain the concentration above the MIC for ≥50% of the time between two injections. This target was achieved with 250 mg/kg t.i.d., but the treatment was very poorly tolerated, with severe diarrhoea in rabbits and a lethality >50%. Hence, a dosage of 80 mg/kg t.i.d., equivalent to 250 mg t.i.d. in humans, was finally selected. With this dosing schedule, the concentration was maintained above the MIC for 20% of the time between the two injections and no severe side effects were observed. Plasma meropenem concentrations measured in 12 infected rabbits after 24 h of treatment with 80 mg/kg t.i.d. (steady-state concentrations) were a mean C_{max} of 15.5 ± 12.2 mg/L and a mean trough plasma concentration (C_{min}) of 0.35 ± 0.6 mg/L, which is comparable with C_{max} and C_{min} values observed with 250 mg t.i.d. in humans.

Table 1
Number of rabbits excluded from the analysis in control and treatment groups, and reasons for exclusion.

Group	Enrolled	Excluded			Analysed
		Died during surgical procedure	Died before beginning of treatment	Non-infected	
Control	12	1	0	0	11
Coli-Ce	13	0	1	0	12
Coli S	11	0	1	0	10
Coli-Ce + Coli S	12	0	1	2	9
Coli S + Mero S	12	0	0	0	12
Coli-Ce + Coli S + Mero S	12	0	0	0	12

Coli-Ce, colistin-loaded cement spacer; Coli S, systemic colistin; Mero S, systemic meropenem.

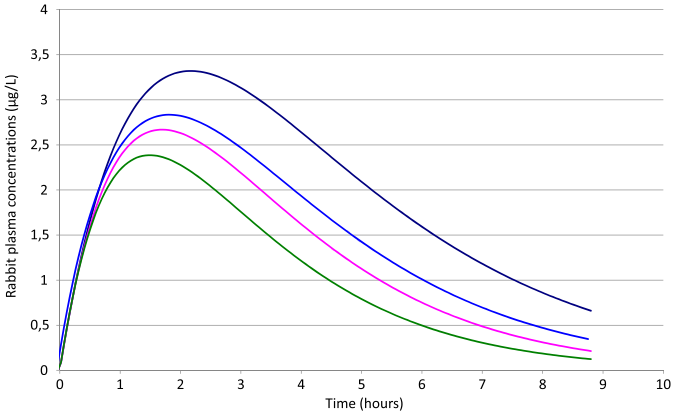

Fig. 2. Concentration–time profiles of colistin in rabbit plasma following a single intramuscular administration of 12 mg/kg ($n = 4$).

3.3. In vitro bactericidal activity of colistin alone and in combination

Time–kill studies at 4 × MIC (Fig. 3) showed that colistin alone was rapidly bactericidal in the first 6 h. However, re-growth occurred after 9 h of incubation. The MICs of bacteria re-growing after 9 h were similar to the initial strain. Meropenem alone was poorly bactericidal, with a decrease of 2 log of the initial inoculum at 9 h. Addition of meropenem to colistin was synergistic, with no remaining viable bacteria after 6 h.

3.4. Experimental prosthetic joint infection and treatment groups

Initially, each group included 11–13 animals. Rabbits who died during anaesthesia ($n = 1$) or before treatment initiation ($n = 3$) or those with sterile synovial fluid at Day 7 ($n = 2$) were not included in the analysis, thus the final number of animals per group was ≤12 (Table 1). In the control group, periprosthetic joint infection was associated with severe local macroscopic changes at autopsy, with bone marrow involvement (yellow/white in one-half of infected animals), bone deformation in all animals and purulent arthritis (Table 2). In the treatment groups, only a few animals had bone deformation or bone marrow involvement. Blood cultures performed 24 h after inoculation were positive in 44/72 rabbits (61%). All control rabbits except one (excluded from the analysis, as pre-specified) were infected at Day 15 with a median bone bacterial count of 6.1 CFU/g.

In contrast to local colistin, systemic colistin alone or combined with systemic meropenem was more effective than the control on bacterial counts in bone at the end of treatment ($P = 0.043$ and $P = 0.05$, respectively). However, only the combinations of local colistin and systemic colistin with or without meropenem were significantly more effective than the control both on proportion of rabbits with sterile bone ($P = 0.05$ and $P = 0.009$, respectively) and median bacterial counts ($P = 0.005$ and $P = 0.003$, respectively) (Fig. 4; Table 3).

3.5. In vivo selection of mutants

Colistin-resistant mutants were searched for in the tibial bone of all rabbits with persistent infections (10 controls, 9 treated with local colistin, 7 treated with systemic colistin alone, 4 treated with systemic and local colistin, 10 treated with local colistin and systemic meropenem, and 4 treated with local and systemic colistin in combination with systemic meropenem). Only one rabbit treated with local colistin alone had colistin-resistant mutants with a colistin MIC of 32 mg/L.

4. Discussion

This study showed that combinations of local and systemic colistin with or without systemic meropenem were the only regimens

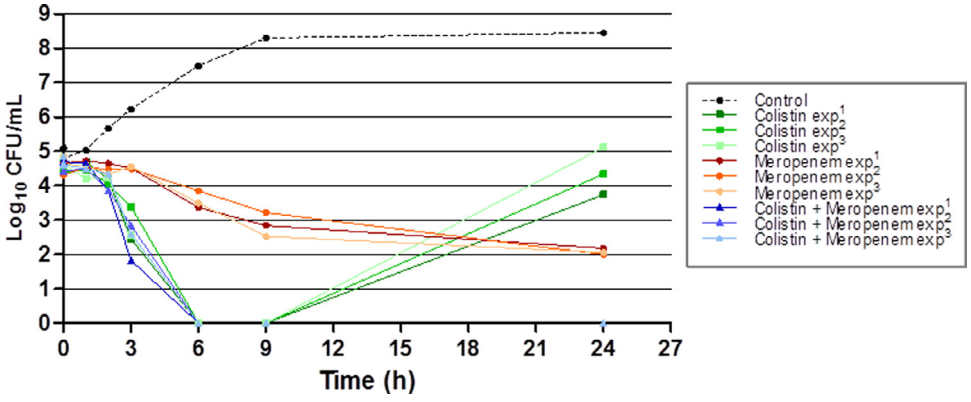

Fig. 3. In vitro killing curves for carbapenemase-producing *Klebsiella pneumoniae* strain KPC99YC using different antibacterial agents at 4 × MIC, alone and in combination. All experiments were performed in triplicate ($^{1-3}$). MIC, minimum inhibitory concentration.

Table 2
Macroscopic findings in rabbits at Day 15 of carbapenemase-producing *Klebsiella pneumoniae* strain KPC99YC experimental prosthetic joint infection.

Group	Bone deformation		Necrotic bone marrow		Purulent bone marrow		Open wound	
	n (%)	*P*[a]	*n* (%)	*P*[a]	*n* (%)	*P*[a]	*n* (%)	*P*[a]
Controls ($n=11$)	11 (100)	Ref.	11 (100)	Ref.	0 (0)	Ref.	6 (54.5)	Ref.
Coli-Ce ($n=12$)	6 (50.0)	0.014	9 (75.0)	0.217	1 (8.3)	>0.99	1 (8.3)	0.027
Coli S ($n=10$)	2 (20.0)	<0.001	2 (20.0)	<0.001	3 (30.0)	0.09	6 (60.0)	>0.99
Coli-Ce + Coli S ($n=9$)	7 (77.8)	0.189	6 (66.7)	0.074	5 (55.6)	0.008	5 (55.6)	>0.99
Coli S + Mero S ($n=12$)	5 (41.7)	0.005	6 (50.0)	0.014	8 (66.7)	0.001	4 (33.3)	0.414
Coli-Ce + Coli S + Mero S ($n=12$)	7 (58.3)	0.037	5 (41.7)	0.005	9 (75.0)	<0.001	4 (33.3)	0.414

Coli-Ce, colistin-loaded cement spacer; Coli S, systemic colistin; Mero S, systemic meropenem.
[a] *P*-value in comparison with the control group.

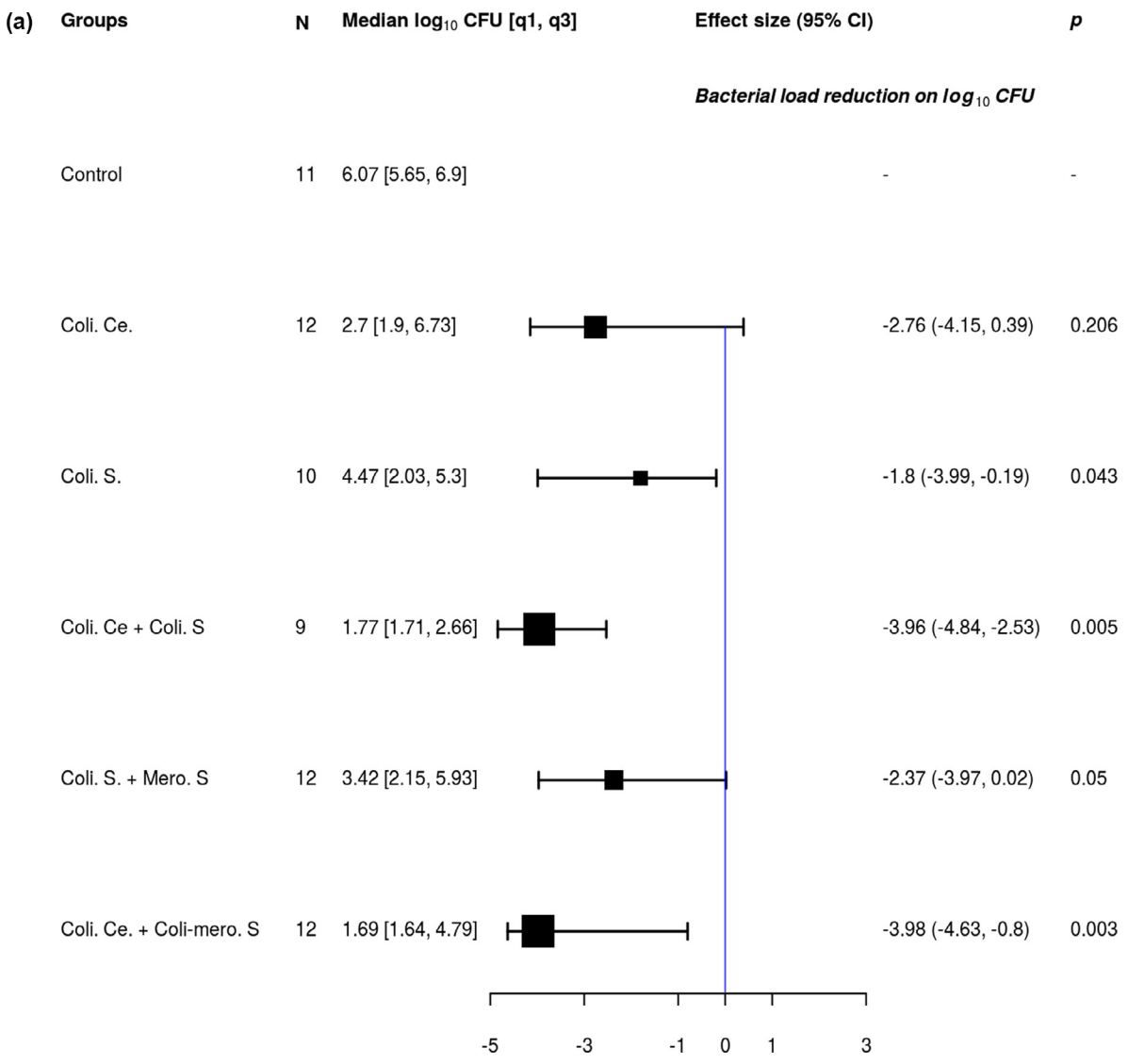

Fig. 4. Efficacy of colistin-loaded cement spacer alone (Coli Ce) or combined with systemic colistin (Coli S), with or without systemic meropenem (Mero S), for the treatment of carbapenemase-producing *Klebsiella pneumoniae* strain KPC99YC experimental prosthetic joint infection: (a) bacterial load reduction; and (b) percentage of sterile bones. q1, q3, first and third quartiles; CI, confidence interval.

significantly more effective than the control both on the number of rabbits with sterile bone and bacterial counts. This study therefore supports the efficacy of local colistin in the treatment of PJI caused by CPKP in combination with systemic treatment. It should be noted that local treatment alone was insufficient as it did not significantly decrease the bacterial density in the bone compared with the control. Furthermore, a colistin-resistant mutant strain emerged in one rabbit treated with local colistin alone.

To overcome the limited efficacy of systemically delivered colistin, local colistin has been used in infections due to multidrug-resistant bacteria such as *P. aeruginosa* pneumonia in cystic fibrosis patients [25], Gram-negative ventilator-associated pneumonia [26], and extensively drug-resistant *Acinetobacter baumannii* ventriculitis and meningitis [27]. Intravesical colistin irrigation has also been successful for treatment of *A. baumannii* urinary tract infection [28]. In the current study, colistin elution represented a low percentage of colistin introduced in bone cement but achieved

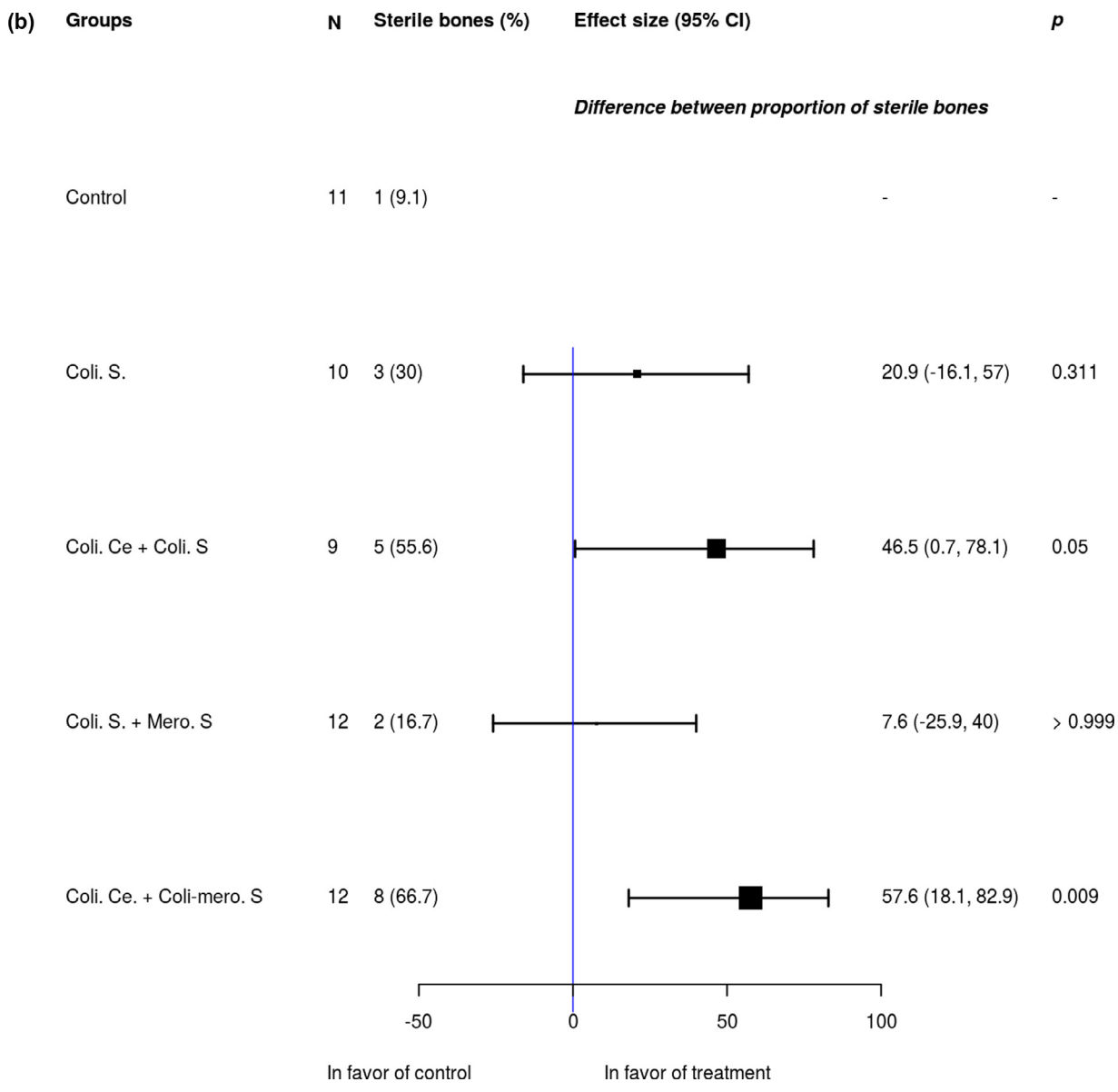

Fig. 4. Continued

Table 3
Pairwise comparisons for evaluation of the efficacy of colistin-loaded cement spacer (Coli-Ce) and systemic colistin (Coli S) with or without systemic meropenem (Mero S) for the treatment of carbapenemase-producing *Klebsiella pneumoniae* strain KPC99YC experimental prosthetic joint infection.

Group	Reduction in $\log_{10}$ CFU/mL (95% CI)	*P*-value
Coli-Ce + Coli S + Mero S compared with:		
Coli S + Mero S	−1.28 (−2.56, 0.13)	0.193
Coli-Ce + Coli S	−0.04 (−0.6, 2.79)	0.715
Coli S	−0.85 (−3.53, 0.1)	0.174
Coli S + Mero S compared with:		
Coli-Ce + Coli S	1.4 (−0.16, 3.87)	0.115
Coli. S	−0.06 (−2.19, 1.69)	0.959
Coli-Ce + Coli S compared with:		
Coli S	−1.56 (−3.55, 0.08)	0.108

CI, confidence interval.

concentrations >20 × MIC in the surrounding fluid, much higher than those achievable with systemic treatment [29], which should translate into significant bactericidal activity in situ. Cement loaded with colistin has been used in case reports of *P. aeruginosa* osteomyelitis [17,18] or PJI [19]. Different doses of colistin in cement have been reported in the literature (Supplementary Table S1), including higher doses than in the experiments in the current study. However, this study is the first to demonstrate the efficacy of a local colistin cement spacer in combination with systemic colistin in experimental CPE-PJI. Of note, although the proportion of colistin elution was low (<0.1%), local concentrations of colistin were >20 × MIC (i.e. 20 mg/L).

This study has several limitations. First, only a single strain of CPE was tested. These bacteria have different susceptibility profiles and therefore these results may not apply to all CPE strains. In particular, the strain was of intermediate susceptibility to meropenem (MIC = 4 mg/L) and the findings may not apply to fully resistant strains. Second, a safety study was not performed. Although the systemic effects of local antibiotic administration are reduced compared with systemic administration in humans, adverse events have been reported with local administration as a consequence of systemic diffusion [13,14]. Histological studies of the kidneys were performed in three rabbits treated with local colistin as well as

two controls but no signs of nephrotoxicity were observed (data not shown). Third, a biomechanical study was not performed to test for the resistance of colistin-impregnated cements. Finally, a low dose of meropenem was used, i.e. 80 mg/kg s.c. t.i.d., equivalent to only 250 mg i.v. t.i.d. in humans, owing to poor tolerability of higher doses in rabbits. Most experts would recommend meropenem doses of 2 g t.i.d. in humans with normal renal function for the treatment of multidrug-resistant PJI. However, even with this low dose, the combination of colistin plus meropenem was effective and the addition of local colistin was beneficial.

5. Conclusions

The optimal treatment of CPE-PJI is poorly defined. Use of local colistin in a cement spacer in combination with systemic colistin, with or without meropenem, allowed for a marked reduction in viable bacterial counts at the surgical site in this experimental model. This study suggests that local antibiotics, such as a colistin-loaded cement spacer during a two-stage surgical procedure, should be considered in addition to systemic treatment as they may increase the probability of treatment success in extensively drug-resistant PJI.

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Declaration of Competing Interest

PT has served as a scientific advisor for Astellas, AstraZeneca, Correvio, Gilead Sciences, MSD, Mylan and Pfizer; ACC has received grants from Janssen-Cilag, Novartis, AstraZeneca, Aventis and Heraeus for consultancies, workshops and travel to meetings and accommodations. All other authors declare no competing interests.

Ethical approval

The experimental protocol was in keeping with French legislation on animal experimentation and was approved by the Animal Use Committee of Maisons Alfort Veterinary School (Maisons-Alfort, France).

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2019.07.009.

References

[1] Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. N Engl J Med 2004;351:1645–54.
[2] Talbot GH, Bradley J, Edwards JE Jr, Gilbert D, Scheld M. Bartlett JG; Antimicrobial Availability Task Force of the Infectious Diseases Society of America. Bad bugs need drugs: an update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. Clin Infect Dis 2006;42:657–68.
[3] Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis 2009;48:1–12.
[4] Grammatico-Guillon L, Baron S, Gettner S, Lecuyer AI, Gaborit C, Rosset P, et al. Surveillance hospitalière des infections ostéo-articulaires en France: analyse des données médico-administratives, PMSI 2008 [Hospital surveillance of osteoarticular infections in France: analysis of medico-administrative data, PMSI 2008]. Behaviour 2013;4:39–44.
[5] Nordmann P, Poirel L. The difficult-to-control spread of carbapenemase producers among Enterobacteriaceae worldwide. Clin Microbiol Infect 2014;20:821–30.
[6] Castanheira M, Farrell SE, Krause KM, Jones RN, Sader HS. Contemporary diversity of β-lactamases among Enterobacteriaceae in the nine U.S. census regions and ceftazidime–avibactam activity tested against isolates producing the most prevalent β-lactamase groups. Antimicrob Agents Chemother 2014;58:833–8.
[7] de Sanctis J, Teixeira L, van Duin D, Odio C, Hall G, Tomford JW, et al. Complex prosthetic joint infections due to carbapenemase-producing *Klebsiella pneumoniae*: a unique challenge in the era of untreatable infections. Int J Infect Dis 2014;25:73–8.
[8] Karaiskos I, Souli M, Galani I, Giamarellou H. Colistin: still a lifesaver for the 21st century? Expert Opin Drug Metab Toxicol 2017;13:59–71.
[9] Macesic N, Nelson B, Uhlemann AC. Colistin resistance in carbapenem-resistant *Klebsiella pneumoniae*: de novo or drug exposure? Clin Infect Dis 2017;65:702–3.
[10] Corona A, Cattaneo D. Dosing colistin properly: let's save 'our last resort old drug!'. Clin Infect Dis 2017;65:870.
[11] Anagnostakos K. Therapeutic use of antibiotic-loaded bone cement in the treatment of hip and knee joint infections. J Bone Jt Infect 2017;2:29–37.
[12] Walker LC, Baker P, Holleyman R, Deehan D. Microbial resistance related to antibiotic-loaded bone cement: a historical review. Knee Surg Sports Traumatol Arthrosc 2017;25:3808–17.
[13] Iarikov D, Demian H, Rubin D, Alexander J, Nambiar S. Choice and doses of antibacterial agents for cement spacers in treatment of prosthetic joint infections: review of published studies. Clin Infect Dis 2012;55:1474–80.
[14] James A, Larson T. Acute renal failure after high-dose antibiotic bone cement: case report and review of the literature. Ren Fail 2015;37:1061–6.
[15] Gasparini G, De Gori M, Calonego G, Della Bora T, Caroleo B, Galasso O. Drug elution from high-dose antibiotic-loaded acrylic cement: a comparative, in vitro study. Orthopedics 2014;37:e999–1005.
[16] Rosenthal AL, Rovell JM, Girard AE. Polyacrylic bone cement containing erythromycin and colistin. I. In vitro bacteriological activity and diffusion properties of erythromycin, colistin and erythromycin/colistin combination. J Int Med Res 1976;4:296–304.
[17] Krajewski J, Bode-Böger SM, Tröger U, Martens-Lobenhoffer J, Mulrooney T, Mittelstädt H, et al. Successful treatment of extensively drug-resistant *Pseudomonas aeruginosa* osteomyelitis using a colistin- and tobramycin-impregnated PMMA spacer. Int J Antimicrob Agents 2014;44:363–6.
[18] Stanzani M, Tumietto F, Giannini MB, Bianchi G, Nanetti A, Vianelli N, et al. Successful treatment of multi-resistant *Pseudomonas aeruginosa* osteomyelitis after allogeneic bone marrow transplantation with a combination of colistin and tigecycline. J Med Microbiol 2007;56:1692–5.
[19] Papagelopoulos PJ, Mavrogenis AF, Giannitsioti E, Kikilas A, Kanellakopoulou K, Soucacos PN, et al. Management of a multidrug-resistant *Pseudomonas aeruginosa* infected total knee arthroplasty using colistin. A case report and review of the literature. J Arthroplasty 2007;22:457–63.
[20] Belmatoug N, Crémieux AC, Bleton R, Volk A, Saleh-Mghir A, Grossin M, et al. A new model of experimental prosthetic joint infection due to methicillin-resistant *Staphylococcus aureus*: a microbiologic, histopathologic, and magnetic resonance imaging characterization. J Infect Dis 1996;174:414–17.
[21] Bassetti M, Giacobbe DR, Giamarellou H, Viscoli C, Daikos GL, Dimopoulos G, et al. Management of KPC-producing *Klebsiella pneumoniae* infections. Clin Microbiol Infect 2018;24:133–44.
[22] *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org/.
[23] Hothorn T, Hornik K, van de Wiel MA, Zeileis A. Implementing a class of permutation tests: the coin package. J Stat Softw 2008;28:1–23.
[24] Bauer DF. Constructing confidence sets using rank statistics. J Am Stat Assoc 1972;67:687–90.
[25] Fiel SB. Aerosolized antibiotics in cystic fibrosis: an update. Expert Rev Respir Med 2014;8:305–14.
[26] Tumbarello M, De Pascale G, Trecarichi EM, De Martino S, Bello G, Maviglia R, et al. Effect of aerosolized colistin as adjunctive treatment on the outcomes of microbiologically documented ventilator-associated pneumonia caused by colistin-only susceptible Gram-negative bacteria. Chest 2013;144:1768–75.
[27] Karaiskos I, Galani L, Baziaka F, Giamarellou H. Intraventricular and intrathecal colistin as the last therapeutic resort for the treatment of multidrug-resistant and extensively drug-resistant *Acinetobacter baumannii* ventriculitis and meningitis: a literature review. Int J Antimicrob Agents 2013;41:499–508.
[28] Volkow-Fernández P, Rodríguez CF, Cornejo-Juárez P. Intravesical colistin irrigation to treat multidrug-resistant *Acinetobacter baumannii* urinary tract infection: a case report. J Med Case Rep 2012;6:426.
[29] Lebeaux D, Ghigo JM, Beloin C. Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. Microbiol Mol Biol Rev 2014;78:510–43.