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

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

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25 **Running title:** Colistin spacer in prosthetic joint infection

26

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29

30 **ABSTRACT**

31 **Background:** Carbapenemase-producing *Enterobacteriaceae* (CPE) are emerging multidrug-  
32 resistant bacteria responsible for invasive infections, including prosthetic joint infections  
33 (PJI). Local administration of colistin may provide bactericidal concentration *in situ*.

34 **Objectives:** To evaluate the efficacy of a colistin-impregnated cement spacer, alone and in  
35 combination with systemic antibiotics, in a rabbit model of CPE PJI.

36 **Methods:** Elution of 3 M IU of colistimethate sodium (CMS) in 40 g of polymethyl  
37 metacrylate cement was studied *in vitro*. *In vivo*,  $5.10^8$  CFU of KPC-producing *Klebsiella*  
38 *pneumoniae* (colistin and meropenem MIC, 1 and 4 mg/L) were injected close to a prosthetic  
39 knee. Surgical debridement and prosthesis removal were performed 7 days later, and rabbits  
40 were assigned to one of six groups (12 rabbits each): i) drug-free spacer, ii) colistin-loaded  
41 spacer, iii) colistin i.m., iv) colistin i.m. + colistin spacer, v) colistin i.m. + meropenem s.c.,  
42 and vi) colistin i.m. + meropenem s.c. + colistin spacer. Systemic treatment was administered  
43 at doses targeting pharmacokinetic in humans, and rabbits were euthanized seven days later,  
44 to evaluate bacterial counts in infected bones.

45 **Results:** *In vitro*, CMS elution was low (<0.1% at 24 h), but reached local concentration of 20  
46 mg/L. *In vivo*, combinations of local and systemic colistin, with or without meropenem, were  
47 the only regimens superior to control group ( $P \leq 0.05$ ), in terms of viable bacteria counts, and  
48 proportion of rabbits with sterile bone, with no emergence of colistin-resistant strain.

49 **Conclusions:** Colistin-loaded cement spacer in combination with systemic treatment were the  
50 most active regimens in this model of CPE PJI.

51 **KEYWORDS:** Prosthetic joint infection; orthopedic surgery; Carbapenemase-producing  
52 *Enterobacteriaceae*; experimental model; colistin; meropenem

53        **1. Introduction**

54        Prosthetic joint infections (PJI) cause major morbidity and incur high treatment costs [1].  
55        Carbapenemase-producing *Enterobacteriaceae* (CPE) are emerging multidrug-resistant  
56        bacteria [2], responsible for a broad range of difficult-to-treat invasive infections, including  
57        PJI. As CPE increasingly present with resistance to almost all class of antibiotics available,  
58        physicians are increasingly left with the use of old antibacterial agents, with limited data on  
59        their efficacy [2, 3]. Although experts recommend various treatment combinations, data from  
60        clinical studies are scarce, and innovative experimental models are warranted to evaluate new  
61        medical and surgical treatments [4-7]. Colistin is often the only remaining option for treating  
62        carbapenemase-producing *Klebsiella pneumoniae* (CPKP). However, parenteral  
63        administration of colistin is associated with severe adverse effects, including nephrotoxicity  
64        and neurotoxicity [8]. Moreover, the emergence of colistin-resistant strains has been  
65        described in patients treated with colistin [9], possibly promoted by suboptimal drug  
66        concentration at the site of infection [10].

67        Local administration of antibiotics is a promising approach to reduce the risk of side  
68        effects and the emergence of resistant strains. In orthopedic surgery, antibiotic-impregnated  
69        cement spacers are often used for local delivery of antibiotics following revision surgery,  
70        particularly in difficult-to-treat infections [15]. However, their use remains controversial  
71        because of concerns about their efficacy [16,17], tolerability [17,18], and emergence of  
72        resistance in sites where drug concentration is low [16]. Colistin elution from bone cement  
73        has been studied *in vitro* [19,20]. Cement loaded with colistin has been successfully used in  
74        case reports of *Pseudomonas aeruginosa* osteomyelitis [21,22] or PJI [23].

75        The aim of this study was to evaluate the efficacy of a colistin-impregnated cement  
76        spacer alone or in combination with systemic colistin or systemic colistin + meropenem using  
77        a rabbit model of CPKP PJI that closely mimics human infection, adapted from a previous

78 model [24]. Systemic colistin + meropenem was selected because this combination is  
79 recommended for CPKP with meropenem minimum inhibitory concentration (MIC)  $\leq$  8 mg/L  
80 [25].

81

82

## 83 **2. Methods**

### 84 *2.1 Bacterial strain*

85 KPC99YC is a KPC-producing *Klebsiella pneumoniae* clinical strain susceptible to  
86 colistin (MIC, 1 mg/L), with intermediate susceptibility to meropenem and imipenem (MIC, 4  
87 mg/L for both).

88

### 89 *2.2 In vitro* bactericidal activities of colistin and meropenem alone and in combination

90 Meropenem (AstraZeneca, Courbevoie, France), and colistin (colistimethate sodium,  
91 Sanofi, Gentilly, France) were bought from local drug purchases companies and prepared  
92 following label instructions for clinical use in humans. The bactericidal activities of colistin,  
93 and meropenem, alone or in combination, were determined in triplicate. Overnight cultures  
94 were diluted in 10 mL of fresh Mueller-Hinton broth, to yield an inoculum of  $10^5$  colony-  
95 forming unit (CFU)/mL. The antibiotic concentrations used were equivalent to 4 MIC. After  
96 0, 3, 6, 9 and 24 h of incubation in a shaking water bath at 37°C, serial dilutions of 0.1 mL  
97 samples were subcultured on Mueller-Hinton agar plates (Bio-Rad, Marnes la Coquettes,  
98 France), and incubated at 37°C for 24 h before the number of CFUs was counted. A  
99 bactericidal effect was defined as a  $>2 \log_{10}$  decrease compared to the initial inoculum.  
100 Synergy was defined as a decrease of  $>2 \log_{10}$  CFU/mL for the combination, as compared to  
101 its most active constituent.

102

### 103 *2.3 Selection of meropenem and colistin doses in rabbits*

104 Plasma antibiotic concentrations were measured in uninfected rabbits to select doses  
105 that will lead to plasma concentrations equivalent to those obtained in humans. Initial doses  
106 were selected based on previous experimental studies in rabbits, and we verified that they  
107 achieved pharmacokinetic (PK) and pharmacodynamic (PD) targets for the KPC99YC strain,

108 which are recommended for severe CPE infections in humans. Each antibiotic was tested on a  
109 group of 4 uninfected rabbits. Samples were obtained after 24 h of treatment (steady-state  
110 concentrations). Blood was drawn 15 min and 1, 2, 4, 6, 8, and 12 h after the injection, and  
111 centrifuged. Concentrations were measured by high-performance liquid chromatography  
112 (HPLC) - tandem-mass spectrometry using an electrospray ionization method. The limit of  
113 quantification was 0.25 mg/L. PK parameters were calculated using Monolix version 4.3.3  
114 (Lixoft SAS, Antony, France).

115

#### 116 **2.4 *In vitro* evaluation of colistin elution from the spacer**

117 For elution of antibiotics, 6 samples of spacer were immersed in 10 mL of an isotonic  
118 saline solution placed at 37°C with agitation at 125 rpm. Then, 250 µL of the saline solution  
119 was removed after 1, 4, 8, 24, 48, 72 h and 7 days. Colistin concentration was measured by  
120 HPLC with mass spectrometry detection, as described above. The results are expressed as  
121 percentages of colistin released from the cement spacers (amount released each day/total  
122 amount).

123

#### 124 **2.5 *Experimental prosthetic joint infection, and treatment groups***

125 New Zealand white female rabbits, each weighing 2.5 to 3 kg, were used. They were  
126 housed in individual cages with natural light-dark cycles. The experimental protocol was in  
127 keeping with French legislation on animal experimentation and was approved by the Animal  
128 Use Committee of Maisons Alfort Veterinary School. This model was previously described  
129 [24]. Briefly, each rabbit underwent partial right knee replacement with a tibial component by  
130 an orthopedic surgeon. The surgery was performed under general anesthesia induced by  
131 intramuscular (i.m.) injection of 25 mg/kg ketamine (Ketamine 1000; Virbac, Carros, France),  
132 and 25 mg/kg of 2% xylazine (Rompun; Bayer Santé, Division Santé Animal, Puteaux,

133 France), and then by continuous inhalation of 1% isoflurane (Vetflurane; Virbac, Carros,  
134 France). The skin overlying the right leg was shaved before the operation, and cleaned with  
135 an iodine solution prior to surgery. A longitudinal skin incision was made, and the knee joint  
136 was exposed. The epiphyseal plates were removed. The metaphysis was exposed. A silicone  
137 elastomer implant, commonly used in arthroplasty of the first metatarsophalangeal joint  
138 (Silastic, great toe implant HP; Swanson Design, Dow-Corning [provided by Wright Medical  
139 France, Créteil, France]), was implanted as a tibial prosthetic component. Immediately after  
140 surgery,  $5.10^8$  CFU KPC99YC in 0.5 mL was injected into the knee close to the prosthesis.  
141 Each rabbit was given patch analgesia (Durogesic; Janssen-CILAG SA, Issy Les Moulineaux,  
142 France). Blood cultures were performed 24 h after inoculation to identify the presence of early  
143 bacteremia.

144         Seven days after inoculation, surgical synovectomy was performed. Periprosthetic  
145 fluid culture was performed to ensure that all rabbits were infected. The prosthesis was  
146 removed and replaced by a cement spacer. At least 12 rabbits were randomly assigned to each  
147 control or treatment group, with six groups in total (treatments were for 7 days): i) Prosthesis  
148 replacement by drug-free cement spacer (controls); ii) Prosthesis replacement by  
149 colistimethate sulfate (colistin)-loaded (3 M IU/40 g of cement) cement spacer (Coli-Ce); iii)  
150 Prosthesis replacement by drug-free cement spacer, + colistin i.m., 12 mg/kg i.m. t.i.d.,  
151 equivalent to 3 M IU t.i.d. in humans (Coli S); iv) Prosthesis replacement by colistin-loaded  
152 cement spacer + colistin i.m. (Coli-Ce + Coli S); v) Prosthesis replacement by drug-free  
153 cement spacer + colistin i.m. and meropenem s.c., 80 mg/kg t.i.d., equivalent to 250 mg i.v.  
154 t.i.d. in humans (Coli-Mero S); vi) Prosthesis replacement by colistin-loaded cement spacer +  
155 colistin i.m. and meropenem s.c. (Coli-Ce + Coli-Mero S)

156         The antibiotic-loaded spacer was obtained by mixing 3 M IU colistimethate sulfate  
157 with 40 g of powdered cement polymer before the addition of methylmethacrylate, as done in

158 clinical practice and according to the manufacturer's instructions (CMW radiopaque bone  
159 cement; DePuy CMW, Blackpool, England). The dose of colistin in cement was similar to the  
160 dose previously studied *in vitro*.<sup>19-21</sup> It was then molded to reproduce a facsimile of the  
161 silicone prosthesis.

162

## 163 *2.6 Evaluation of therapy*

164 Fourteen days after inoculation, rabbits were euthanized by i.v. pentobarbital. Skin  
165 aspect and position of the prosthesis were noted. To quantify bacterial counts, the upper third  
166 of the tibia (3 cm long), including compact bone and marrow, was isolated, split with a bone  
167 crusher, weighed, cut into small pieces, and crushed in an autopulverizer (MM 200; Retsch  
168 GmbH, Haan, Germany). After 24 h of incubation at 37°C, the number of viable  
169 microorganisms was determined. The results are expressed as the median [IQR] log<sub>10</sub> CFU/g  
170 of bone and the number of animals with sterile bone. Bone was considered sterile when the  
171 culture showed no growth after incubation for 48 h at 37°C, and the number of CFUs  
172 recorded was the limit of detection (1.64 to 1.84 log<sub>10</sub> CFU/g of bone, depending on sample  
173 weight).

174

## 175 *2.7 In vivo selection of mutants*

176 Subpopulation analysis was performed in untreated and treated rabbits to screen for  
177 the emergence of colistin-resistant strains. Each undiluted bone homogenate (50 µL) was  
178 plated onto Mueller-Hinton II agar and onto Mueller-Hinton II agar supplemented with  
179 colistin, 8 mg/L, and incubated during 72 h at 37°C. When bacterial growth was observed, the  
180 colonies were counted, and *Klebsiella* identification was confirmed using matrix-assisted  
181 laser-desorption ionization - time of flight mass spectrometry (Vitek MS; bioMérieux, La  
182 Balme-les-Grottes, France). Colistin MICs were determined by liquid broth dilution methods

183 based on the UMIC colistin reagents (Biocentric, Bandol, France). Mutants were defined as  
184 having a 3-fold-greater MIC than that of the initial strain.

185

## 186 *2.8 Statistics*

187 Data were analyzed with R software (R Development Core Team, 2012) [26]. Due to  
188 the small sample size and asymmetric distributions of the analyzed variables, exact  
189 nonparametric tests implemented in the ‘coin’ package were used [27]. Data of numeric  
190 variables are summarized by the minimum, maximum, median range, interquartile range,  
191 mean and standard deviation and categorical variables by numbers and percentages. The Chi-  
192 square test, Fisher's exact test and the Kruskal-Wallis nonparametric method were used, as  
193 appropriate. The effect size of antibiotics and their 95% confidence intervals (CI) on log<sub>10</sub>  
194 CFU criteria were calculated by the Hodges-Lehmann estimator and the Bauer algorithm,  
195 respectively [28]. The effect size of antibiotics on ‘sterility’ criteria was estimated by the  
196 difference of ‘sterility’ proportion, and 95%CI. Results of the bilateral statistical tests were  
197 considered significant when  $P < 0.05$ .

198

199

## 200 **3. Results**

### 201 *3.1 In vitro evaluation of colistin diffusion from the spacer (Fig. 1)*

202 From 3 M IU (240 mg) of colistin spiked into the cement, only 0.1% were detected in  
203 immersion solution after 24 h. The steady state was reached at 24 to 48 h. Although the  
204 elution coefficient was low, the concentration reached in the elution fluid was  $\geq 20$  mg/L (i.e.,  
205 20 times the MIC).

### 206 *3.2 Plasma concentrations of antibiotics*

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

211 For meropenem, the initial objective was to maintain concentration above the MIC for  
212 at least 50% of the time between two injections. This target was achieved with 250 mg/kg  
213 t.i.d., but the treatment was very poorly tolerated, with severe diarrhea in rabbits and a  
214 lethality  $> 50\%$ . Hence, a dosage of 80 mg/kg t.i.d., equivalent to 250 mg t.i.d. in humans,  
215 was finally selected. With this dosing schedule, the concentrations were maintained above the  
216 MIC for 20% of the time between the two injections, and no severe side effect was observed.  
217 The plasma meropenem concentrations measured in 12 infected rabbits after 24 h of treatment  
218 with 80 mg/kg t.i.d. (steady-state concentrations) were as follows: Mean  $C_{max}$ ,  $15.5 \pm 12.2$   
219 mg/L, and mean  $C_{min}$ ,  $0.35 \pm 0.6$  mg/L, which is comparable to  $C_{max}$  and  $C_{min}$  observed  
220 with 250 mg t.i.d. in humans.

221

### 222 *3.3 In vitro bactericidal activity of colistin alone and in combination*

223 Time-kill studies obtained at 4 MIC (Fig. 3) showed that colistin alone was rapidly  
224 bactericidal in the first 6 h. However, regrowth occurred after 9 h of incubation. The MICs of

225 bacteria regrowing after 9 h were similar to the initial strain. Meropenem alone was poorly  
226 bactericidal, with a decrease of 2 log of the initial inoculum at 9 h. The addition of  
227 meropenem to colistin was synergistic, with no remaining viable bacteria after 6 h.

228

### 229 *3.4 Experimental prosthetic joint infection, and treatment groups*

230 Initially, each group included 12 animals. Rabbits who died during anesthesia (n=1),  
231 or before treatment start (n=3), or with sterile synovial fluid at D7 (n=2), were not included in  
232 the analysis, so that the final number of animals per group was  $\leq 12$  (Table 1). In the control  
233 group, peri-prosthetic joint infection was associated with severe local macroscopic changes at  
234 autopsy, with bone marrow involvement (yellow/white in half of infected animals), bone  
235 deformation in all animals, and purulent arthritis (Table 2). In the treatment groups, only a  
236 few (always <50%) had bone deformation or bone marrow involvement. Blood cultures  
237 performed 24 h after inoculation were positive in 44/72 rabbits (61%). All control rabbits  
238 except one (excluded from the analysis, as pre-specified) were infected at day 15 with a  
239 median bone bacterial count of 6.1 CFU/g.

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

246

### 247 *3.5 In vivo selection of mutants.*

248 Colistin-resistant mutants were searched for in the tibial bone of all rabbits with persistent  
249 infections (10 controls, 9 treated with local colistin, 7 treated with systemic colistin alone, 4

250 treated with systemic and local colistin, 10 treated with local colistin and systemic  
251 meropenem, and 4 treated with local and systemic colistin in combination with systemic  
252 meropenem). Only one rabbit, treated with local colistin alone, had colistin-resistant mutants  
253 with colistin MIC of 32 mg/L.

254

#### 255 4. Discussion

256 Our study showed that combinations of local and systemic colistin with or without  
257 systemic meropenem were the only regimens significantly more effective than the control on  
258 both number of rabbits with sterile bone, and bacterial counts. This study therefore supports  
259 the efficacy of local colistin in the treatment of PJI with CPKP in combination with systemic  
260 treatment. It should be noted that local treatment alone was insufficient, as it did not  
261 significantly decrease the bacterial density in the bone compared to controls. Furthermore, a  
262 colistin-resistant mutant strain emerged in one rabbit treated by local colistin alone.

263 To overcome the limited efficacy of systemically delivered colistin, local colistin has  
264 been used in infections due to multidrug-resistant bacteria, such as *Pseudomonas aeruginosa*  
265 pneumonia in cystic fibrosis patients, or ventilator-associated pneumonia [11,12], extensively  
266 drug-resistant *Acinetobacter baumannii* ventriculitis, and meningitis [13]. Intravesical colistin  
267 irrigation has also been successful for *Acinetobacter baumannii* urinary tract infection [14]. In  
268 our study, colistin elution represented a low percentage of colistin introduced in bone cement,  
269 but achieved concentrations >20 MICs in the surrounding fluid, much higher than those  
270 achievable with systemic treatment [36], which should translate into significant bactericidal  
271 activity *in situ*. Cement loaded with colistin has been used in case reports of *Pseudomonas*  
272 *aeruginosa* osteomyelitis [21,22] or PJI [23]. Different doses of colistin in cement have been  
273 used in the literature (Supplementary Table 1), including higher doses than in our  
274 experiments. However, our study is the first to demonstrate the efficacy of local colistin  
275 cement spacer in combination with systemic colistin in CPE experimental PJI. Of note,  
276 although the proportion of colistin elution was low (<0.1%), local concentrations of colistin  
277 were > 20 MICs (i.e. 20 mg/L)

278 This study has several limitations. Firstly, a single strain of CPE was tested; these  
279 bacteria have different susceptibility profiles, and these results may not apply to all CPE

280 strains. Particularly, the strain was of intermediate susceptibility to meropenem (MIC, 4  
281 mg/L), and the findings may not apply to fully resistant strains. Secondly, we did not carry  
282 out a safety study. Although systemic effects of local administration of antibiotics are reduced  
283 as compared to systemic administration in humans, adverse events have been reported with  
284 local administration, as a consequence of systemic diffusion [17,18]. We performed  
285 histological studies of kidneys in three rabbits treated with local colistin, and two controls,  
286 and found no sign of nephrotoxicity (data not shown). Thirdly, we did not carry out a  
287 biomechanical study to test for the resistance of colistin-impregnated cements. Finally, we  
288 used a low dose of meropenem, i.e. 80 mg/kg s.c. t.i.d., equivalent to only 250 mg i.v. t.i.d. in  
289 humans, due to poor tolerability of higher doses in rabbits. Most experts would recommend  
290 meropenem doses of 2 g t.i.d. in humans with normal renal function for the treatment of  
291 multidrug-resistant PJI. However, even with this low dose, the combination of colistin +  
292 meropenem was effective, and the addition of local colistin was beneficial.

293

294 **5. Conclusions**

295 Optimal treatment of CPE PJI is poorly defined. The use of local colistin in a cement  
296 spacer in combination with systemic colistin, with or without meropenem, allowed for a  
297 marked reduction in the count of viable bacteria at the surgical site in this experimental  
298 model. This study suggests that local antibiotics, such as colistin-loaded cement spacer during  
299 a two-stage surgical procedure, should be considered in addition to systemic treatment, as it  
300 may increase the probability of treatment success in extensively durg-resistant PJI.

301

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304

305 **Declarations**

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308 **Competing Interests:** P. Tattevin has served as a scientific advisor for Astellas, AstraZeneca,  
309 Correvio, Gilead Sciences, MSD, Mylan, and Pfizer. A-C. Crémieux has received grants from  
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311 travel to meetings and accommodations.

312 **Ethical Approval:** The experimental protocol was in keeping with French legislation on  
313 animal experimentation and was approved by the Animal Use Committee of Maisons Alfort  
314 Veterinary School.

315

316 This study was presented during the 28<sup>th</sup> European Congress of Clinical Microbiology and  
317 Infectious Diseases (ECCMID), Madrid, Spain 2018.

318

319 **References**

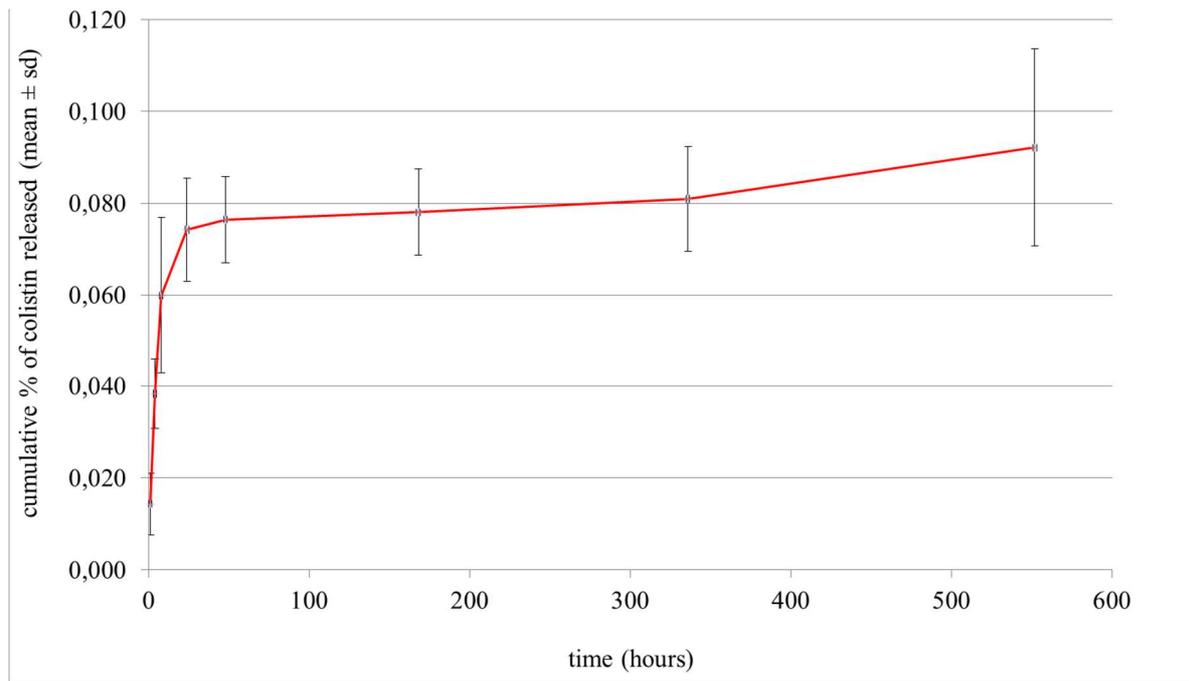
- 320 **1.** Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. *N Engl J Med.*  
321 2004;**351** : 1645–54.
- 322 **2.** Talbot GH, Bradley J, Edwards JE Jr, et al. Bad bugs need drugs: an update on the  
323 development pipeline from the Antimicrobial Availability Task Force of the Infectious  
324 Diseases Society of America. *Clin Infect Dis.* 2006;**42** : 657-68.
- 325 **3.** Boucher HW, Talbot GH, Bradley JS, et al. Bad bugs, no drugs: no ESKAPE! An  
326 update from the Infectious Diseases Society of America. *Clin Infect Dis.* 2009;**48** : 1-  
327 12.
- 328 **4.** Grammatico-Guillon L, Sabine Baron S, Gettner S, et al. Surveillance hospitalière des  
329 infections ostéo-articulaires en France: analyse des données médico-administratives,  
330 PMSI 2008. *Behaviour.* 2013;**4** : 39-44.
- 331 **5.** Nordmann P, Poirel L. The difficult-to-control spread of carbapenemase producers  
332 among Enterobacteriaceae worldwide. *Clin Microbiol Infect.* 2014;**20** : 821-30.
- 333 **6.** Castanheira M, Farrell SE, Krause KM, et al. Contemporary diversity of  $\beta$ -lactamases  
334 among Enterobacteriaceae in the nine U.S. census regions and ceftazidime-avibactam  
335 activity tested against isolates producing the most prevalent  $\beta$ -lactamase groups.  
336 *Antimicrob Agents Chemother.* 2014;**58** : 833-8.
- 337 **7.** de Sanctis J, Teixeira L, van Duin D, et al. Complex prosthetic joint infections due to  
338 carbapenemase-producing *Klebsiella pneumoniae* : a unique challenge in the era of  
339 untreatable infections. *Int J Infect Dis.* 2014;**25** : 73-8.
- 340 **8.** Karaiskos I, Souli M, Galani I, et al. Colistin: still a lifesaver for the 21st century?  
341 *Expert Opin Drug Metab Toxicol.* 2017;**13** : 59-71.
- 342 **9.** Macesic N, Nelson B, Uhlemann AC. Colistin resistance in carbapenem-resistant  
343 *Klebsiella pneumoniae*: de novo or drug exposure? *Clin Infect Dis.* 2017;**65** : 702–3.

- 344 **10.** Corona A, Cattaneo D. Dosing colistin properly: let's save "our last resort old drug!"  
345 *Clin Infect Dis.* 2017;**65** : 870.
- 346 **11.** Fiel SB. Aerosolized antibiotics in cystic fibrosis: an update. *Expert Rev Respir Med.*  
347 2014;**8** : 305-14.
- 348 **12.** Tumbarello M, De Pascale G, Trecarichi EM, et al. Effect of aerosolized colistin as  
349 adjunctive treatment on the outcomes of microbiologically documented ventilator-  
350 associated pneumonia caused by colistin-only susceptible gram-negative bacteria.  
351 *Chest.* 2013;**144** : 1768-75.
- 352 **13.** Karaiskos I, Galani L, Baziaka F, et al. Intraventricular and intrathecal colistin as the  
353 last therapeutic resort for the treatment of multidrug-resistant and extensively drug-  
354 resistant *Acinetobacter baumannii* ventriculitis and meningitis: a literature review. *Int*  
355 *J Antimicrob Agents.* 2013;**41** : 499-508.
- 356 **14.** Volkow-Fernández P, Rodríguez CF, Cornejo-Juárez P. Intravesical colistin irrigation  
357 to treat multidrug-resistant *Acinetobacter baumannii* urinary tract infection: a case  
358 report. *J Med Case Rep.* 2012;**6** : 426.
- 359 **15.** Anagnostakos K. Therapeutic use of antibiotic-loaded bone cement in the treatment of  
360 hip and knee joint infections *JBJS.* 2017;**2** : 29-37.
- 361 **16.** Walker LC, Baker P, Holleyman R, et al. Microbial resistance related to antibiotic-  
362 loaded bone cement: a historical review. *Knee Surg Sports Traumatol Arthrosc.*  
363 2017;**25** : 3808-17.
- 364 **17.** Iarikov D, Demian H, Rubin D, et al. Choice and doses of antibacterial agents for  
365 cement spacers in treatment of prosthetic joint infections: review of published studies.  
366 *Clin Infect Dis.* 2012;**55** : 1474-80.
- 367 **18.** James A, Larson T. Acute renal failure after high-dose antibiotic bone cement: case  
368 report and review of the literature. *Ren Fail.* 2015;**37** : 1061-6.

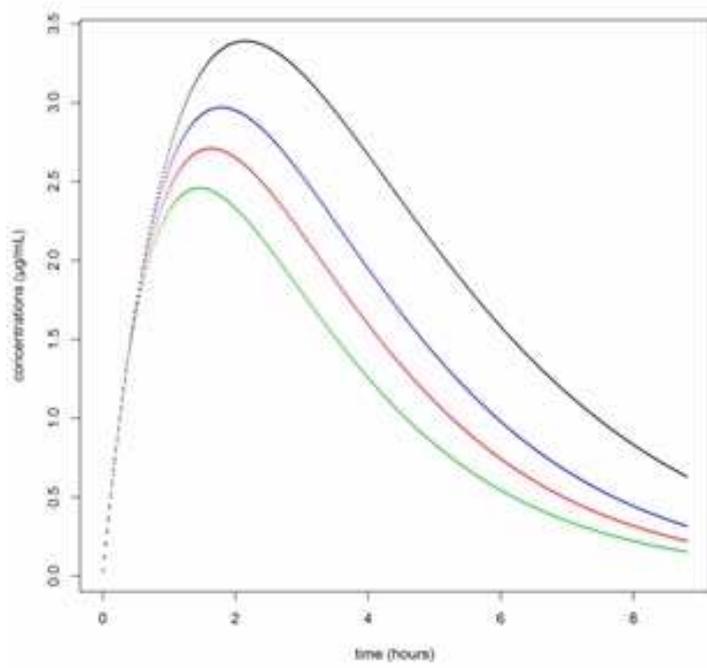
- 369 **19.** Gasparini G, De Gori M, Calonego G, et al. Drug Elution From High-Dose Antibiotic-  
370 Loaded Acrylic Cement: A Comparative, in vitro study. *Orthopedics*. 2014;**37** : e999-  
371 e1005.
- 372 **20.** Rosenthal AL, Rovell JM, Girard AE. Polyacrylic bone cement containing  
373 erythromycin and colistin. I. In vitro bacteriological activity and diffusion properties  
374 of erythromycin, colistin and erythromycin/colistin combination. *J Int Med Res*.  
375 1976;**4** : 296–304.
- 376 **21.** Krajewski J, Bode-Böger SM, Tröger U, et al. Successful treatment of extensively  
377 drug-resistant *Pseudomonas aeruginosa* osteomyelitis using a colistin- and  
378 tobramycin-impregnated PMMA spacer. *Int J Antimicrob Agents*. 2014;**44** : 363-6.
- 379 **22.** Stanzani M, Tumietto F, Giannini MB, et al. Successful treatment of multi-resistant  
380 *Pseudomonas aeruginosa* osteomyelitis after allogeneic bone marrow transplantation  
381 with a combination of colistin and tigecycline. *J Med Microbiol*. 2007;**56** : 1692–5.
- 382 **23.** Papagelopoulos PJ, Mavrogenis AF, Giannitsioti E, et al. Management of a multidrug-  
383 resistant *Pseudomonas aeruginosa* infected total knee arthroplasty using colistin. A  
384 case report and review of the literature. *J Arthroplast*. 2007;**22** : 457-63.
- 385 **24.** Belmatoug N, Crémieux AC, Bleton R, et al. A new model of experimental prosthetic  
386 joint infection due to methicillin-resistant *Staphylococcus aureus*: a microbiologic,  
387 histopathologic, and magnetic resonance imaging characterization. *J Infect Dis*.  
388 1996;**174** : 414-7.
- 389 **25.** Bassetti M, Giacobbe DR, Giamarellou H, et al. Management of KPC-producing  
390 *Klebsiella pneumoniae* infections. *Clin Microbiol Infect*. 2018;**24** : 133-44.
- 391 **26.** *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R  
392 Foundation for Statistical Computing. ISBN 3-900051-07-0. <[http://www.R-  
393 project.org/.11](http://www.R-project.org/.11)>.

- 394 **27.** Hothorn T, Hornik K, van de Wiel MA, et al. Implementing a Class of Permutation  
395 Tests: the coin Package. *J Stat Softw.* 2008;**28** : 1-23.
- 396 **28.** Bauer DF. Constructing confidence sets using rank statistics. *J Am Stat Assoc.*  
397 1972;**67** : 687-90.
- 398 **29.** Crane DP, Gromov K, Li D, et al. Efficacy of colistin-impregnated beads to prevent  
399 multidrug-resistant *A. baumannii* implant-associated osteomyelitis. *J Orthop Res.*  
400 2009;**27** : 1008-15.
- 401 **30.** Mountziaris PM, Shah SR, Lam J, et al. A rapid, flexible method for incorporating  
402 controlled antibiotic release into porous polymethylmethacrylate space maintainers for  
403 craniofacial reconstruction. *Biomater Sci.* 2016;**4** : 121-9.
- 404 **31.** Shi M, Kretlow JD, Nguyen A, et al. Antibiotic-releasing porous  
405 polymethylmethacrylate constructs for osseous space maintenance and infection  
406 control. *Biomaterials.* 2010;**31** : 4146-56.
- 407 **32.** Shi M, Kretlow JD, Spicer PP, et al. Antibiotic-releasing porous  
408 polymethylmethacrylate/gelatin/antibiotic constructs for craniofacial tissue  
409 engineering. *J Control Release.* 2011;**152** : 196-205.
- 410 **33.** Rodeheaver GT, Rukstalis D, Bono M, et al. A new model of bone infection used to  
411 evaluate the efficacy of antibiotic-impregnated polymethylmethacrylate cement. *Clin*  
412 *Orthop Relat Res.* 1983; **178** : 303-11.
- 413 **34.** Back DA, Bormann N, Calafi A, et al. Testing of antibiotic releasing implant coatings  
414 to fight bacteria in combat-associated osteomyelitis - an in-vitro study. *Int Orthop.*  
415 2016; **40** : 1039-47.
- 416 **35.** Ferry T, Desmarchelier R, Magréault S, et al. Gentamycin-vancomycin-colistin local  
417 antibiotherapy in a cement spacer in a 54-year-old haemophilic patient with relapsing  
418 plurimicrobial severe prosthetic joint infection. *BMJ Case Rep.* 2017: 221743.

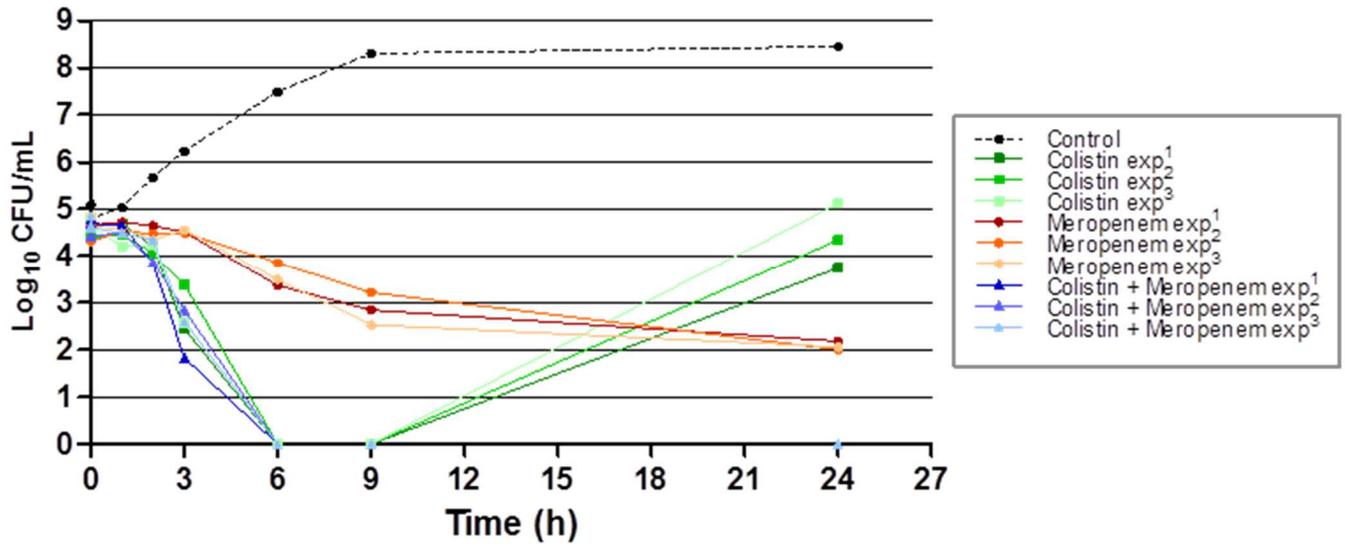
419 **36.** Lebeaux D, Ghigo JM, Beloin C. Biofilm-related infections: bridging the gap between  
420 clinical management and fundamental aspects of recalcitrance toward antibiotics.  
421 *Microbiol Mol Biol Rev.* 2014; **78** : 510-43.



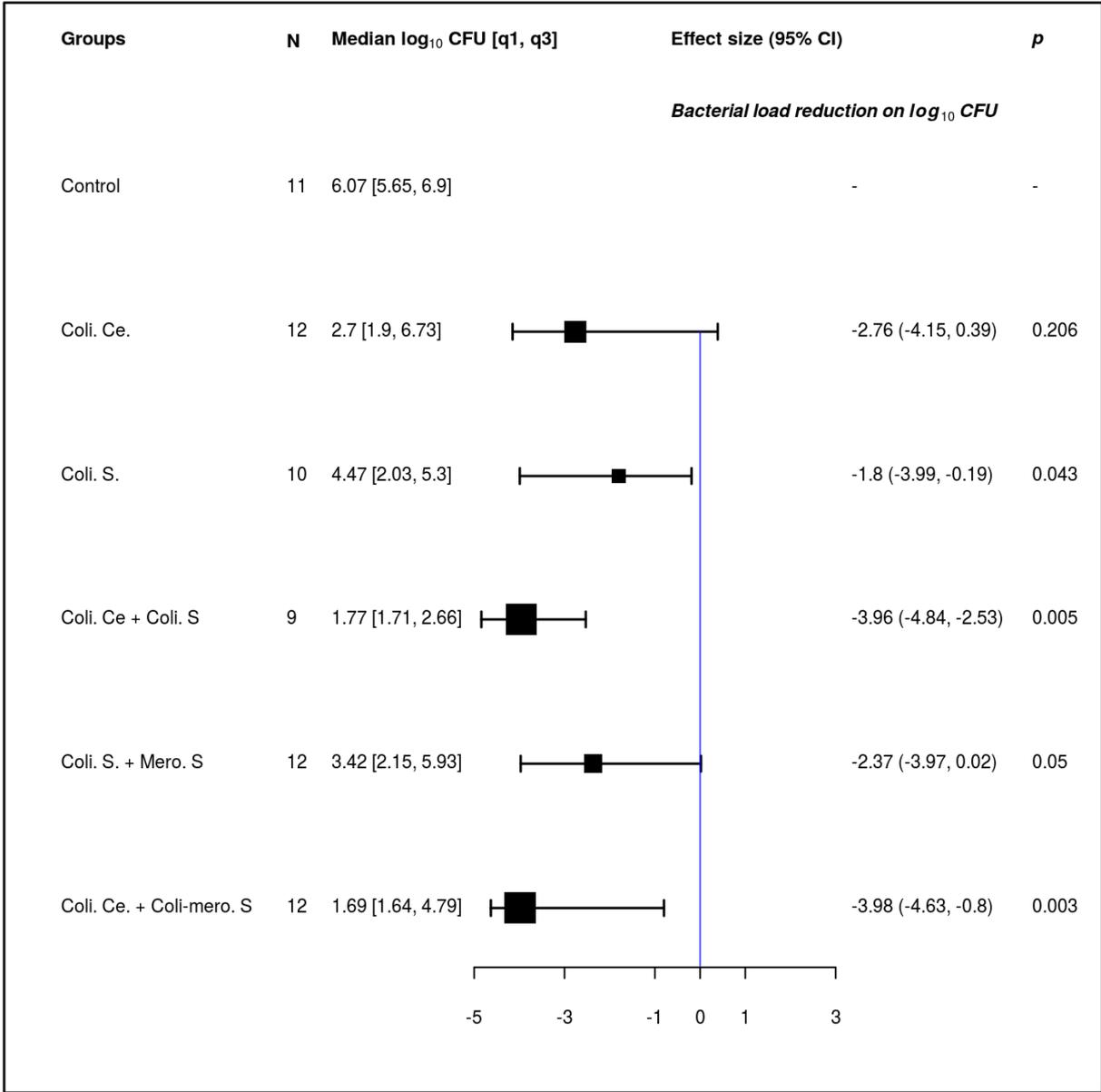
**Fig. 1:** Cumulative percentage of colistin released from the colistin-impregnated cement spacer over time



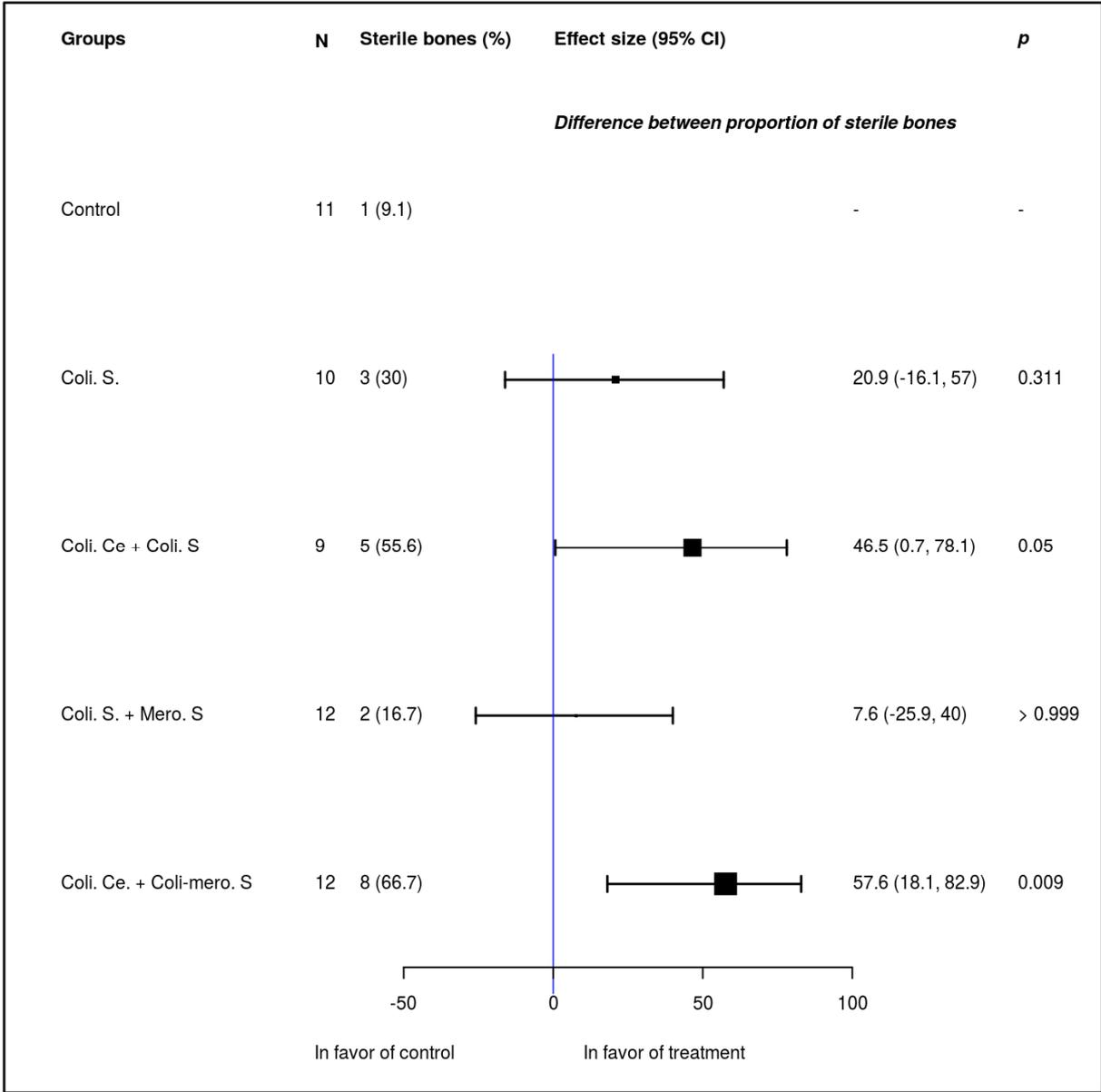
**Fig. 2:** Time–concentration profiles of colistin in rabbits plasma following a single i.m. administration of 12 mg/kg (n=16)



**Fig. 3:** *In vitro* killing curves for carbapenemase-producing *Klebsiella pneumoniae* strain KPC99YC, using different antibacterial agents alone and in combinations, at 4 MICs. All experiments were performed in triplicate.



**Fig. 4a:** 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* experimental prosthetic joint infection KPC99YC (bacterial load reduction).



**Figure 4b:** 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* experimental prosthetic joint infection KPC99YC (% of sterile bones).

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

<b>Groups</b>	<b>Enrolled</b>	<b>Excluded</b>			<b>Analyzed</b>
		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

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

Groups	Bone deformation		Necrotic bone marrow		Purulent bone marrow		Open wound	
	n (%)	<i>P</i>	n (%)	<i>P</i>	n (%)	<i>P</i>	n (%)	<i>P</i>
Controls (n=11)	11 (100)	ref	11 (100)	ref	0 (0)	ref	6 (54.5)	ref
Coli-Ce (n=12)	6 (50)	0.014	9 (75)	0.217	1 (8.3)	>0.99	1 (8.3)	0.027
Coli S (n=10)	2 (20)	<0.001	2 (20)	<0.001	3 (30)	0.09	6 (60)	>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)	0.014	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)	<0.001	4 (33.3)	0.414

*P* is in comparison with the control group

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

**Table 3.** Pairwise comparisons for evaluation of efficacy of colistin-loaded cement spacer, and systemic colistin, for the treatment of carbapenemase-producing *Klebsiella pneumoniae* experimental prosthetic joint infection.

	Reduction on log <sub>10</sub> , CFU/mL [95% CI]	<i>P</i>
<b>Coli-Ce + Coli-Mero S, compared to</b>		
- 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
<b>Coli S + Mero S, compared to</b>		
- Coli-Ce + Coli S	1.4 [-0.16, 3.87]	0.115
- Coli. S.	-0.06 [-2.19, 1.69]	0.959
<b>Coli-Ce + Coli S, compared to</b>		
Coli S	-1.56 [-3.55, 0.08]	0.108

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