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## **In vitro antibacterial activity of doripenem against clinical isolates from French teaching hospitals: proposition of zone diameter breakpoints**

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1 ***In vitro* antibacterial activity of doripenem against clinical isolates from**  
2 **French teaching hospitals. Proposition of zone diameters breakpoints**

3

4 **Running title:** doripenem, *in vitro* activity, breakpoints

5

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29

30 **ABSTRACT**

31 **Purpose:** The aims of the study were to determine the *in vitro* activity of  
32 doripenem, a new carbapenem, against a large number of bacterial  
33 pathogens and to propose zone diameter breakpoints for clinical  
34 categorization in France according to the EUCAST MIC breakpoints.

35 **Methods:** MICs of doripenem were determined by broth microdilution method  
36 against 1547 clinical isolates from 8 French hospitals. Disk diffusion test was  
37 performed (10- $\mu$ g discs) according to the CASFM method.

38 **Results:** MIC<sub>50/90</sub> (mg/L) values were as follows: methicillin-susceptible *S.*  
39 *aureus* (MSSA) (0.03/0.25), methicillin-resistant *S. aureus* (1/2) (MRSA),  
40 methicillin-susceptible coagulase-negative staphylococci (MSCoNS)  
41 (0.03/0.12), MRCoNS (2/8), *S. pneumoniae* (0.016/0.25), viridans group  
42 streptococci (0.016/2),  $\beta$ -haemolytic streptococci ( $\leq$ 0.008/ $\leq$ 0.008), *E. faecalis*  
43 (2/4), *E. faecium* (128/ $>$ 128), *Enterobacteriaceae* (0.06/0.25), *P. aeruginosa*  
44 (0.5/8), *A. baumannii* (0.25/2), *H. influenzae* (0.12/0.25), *M. catarrhalis*  
45 (0.03/0.06). According to the regression curve, zone diameter breakpoints  
46 were 24 and 19 mm for MICs of 1 and 4 mg/L.

47 **Conclusion:** This study confirms the potent *in vitro* activity of doripenem  
48 against *P. aeruginosa*, *Acinetobacter*, *Enterobacteriaceae*, MSSA, MSCoNS  
49 and respiratory pathogens. According to the EUCAST MIC breakpoints (mg/L)  
50 :  $\leq 1$ / $> 4$  for *Enterobacteriaceae*, *P. aeruginosa* and *Acinetobacter* and  $\leq 1$ / $> 1$  for  
51 streptococci, pneumococci and *Haemophilus*, zone diameter breakpoints  
52 could be (mm):  $\geq 24$ / $< 19$  and  $\geq 24$ / $< 24$ , respectively.

53 **Keywords:** doripenem, new carbapenem, *in vitro* activity, breakpoints

54

55 **INTRODUCTION**

56 The synthesis of new carbapenems remains an area of intense research  
57 because of the broad-spectrum antibacterial activity of this chemical class [1-  
58 3]. Doripenem (formerly S-4661) is a recently approved parenteral 1 $\beta$ -  
59 methlycarbapenem, originally discovered by Shionogi & Co., Ltd. (Osaka,  
60 Japan), is currently being developed in the United States by Johnson and  
61 Johnson Pharmaceutical Research and Development (Raritan, NJ) for the  
62 treatment of hospitalized patients with serious systemic bacterial infections.

63 Doripenem has a methyl group at position 4 and a sulfamoylaminomethylated  
64 pyrrolidinylthio group at position 3. Its *in vitro* antimicrobial potency is  
65 generally comparable to that of imipenem and meropenem, although  
66 doripenem is generally more active against Gram-positive organisms than  
67 meropenem and more active against Gram-negative organisms than  
68 imipenem. The activity of doripenem against *Pseudomonas* isolates is slightly  
69 better than that of other carbapenems. Doripenem is stable against human  
70 renal DHP-I and thus does not require co-administration of cilastatin.

71 Like other carbapenems, doripenem has stability to many  $\beta$ -lactamases, but  
72 remains labile to class B enzymes known as metallo-  $\beta$ -lactamases.

73 Pharmacokinetic parameters resemble to meropenem with T<sub>1/2</sub> of  
74 approximately 1h ; the serum protein binding is low (8-9%).

75 Previous surveillance studies indicate that doripenem is active *in vitro* against  
76 bacteria commonly associated with these indications [4-9,3] and is effective *in*  
77 vivo in murine models of bacteremia and pulmonary infection and in a rat  
78 intra-uterine infection model [10,9]. Doripenem is an agent that will be  
79 specially used to treat infections caused by Gram-negative bacteria resistant  
80 to a variety of antimicrobial agents. According to the European Medicines  
81 Agency (EMA), doripenem is indicated for the treatment of the following  
82 infections: nosocomial pneumonia, including ventilator-associated

83 pneumonia, complicated intra-abdominal infections, complicated urinary tract  
84 infections, including complicated and uncomplicated pyelonephritis and cases  
85 with concurrent bacteraemia. FDA only approved doripenem for complicated  
86 intra-abdominal and urinary tract infection.

87 The aim of this multicenter study was to assess the *in vitro* activity of  
88 doripenem on a large number of isolates encountered in daily hospital  
89 practice in French hospitals. In addition, zone diameter breakpoints were  
90 determined for doripenem using the regression curve method by using the  
91 French national disk diffusion method calibrated to EUCAST MIC breakpoints,  
92 as recommended by EUCAST [11]

93

94

## 95 **MATERIALS AND METHODS**

### 96 **Bacterial strains**

97 Over a period of 3 months (October to December 2008), a total of 1,547 non-  
98 consecutive single-patient isolates, were collected from 8 French hospitals  
99 (six teaching and two general hospitals). 20 strains of each frequently isolated  
100 species was requested from every institution enrolled in the study. For rarely  
101 isolated species, all the strains collected during the study period were  
102 included. Clinical isolates were collected from the main types of pathological  
103 specimens from in-patients in hospital wards representing the different  
104 medical and surgical specialities: 36.5% were isolated from general medical  
105 ward, 22.3% from general surgical ward, 19.3% from intensive care unit  
106 (ICU), 14.8% from emergency room and the remaining (7.1%) from either  
107 other ward.

108 Isolates tested included: 173 *S. aureus* (90 methicillin-susceptible and 83  
109 methicillin-resistant strains), 104 coagulase-negative staphylococci (CoNS)  
110 (50 methicillin-susceptible and 54 methicillin-resistant strains), 63 *E. faecalis*,

111 40 *E. faecium*, 83 *S. pneumoniae*, 60  $\beta$ -haemolytic streptococci; 19 viridans  
112 group streptococci, 129 *E. coli*, 114 *K. pneumoniae*, 36 *K. oxytoca*, 90 *E.*  
113 *cloacae*, 49 *E. aerogenes*, 31 *Citrobacter spp.*, 114 *P. mirabilis*, 45 *M.*  
114 *morganii*, 39 *P. vulgaris*, 10 *P. rettgeri*, 15 *P. stuartii*, 99 *P. aeruginosa*, 71  
115 *Acinetobacter spp.*, 30 *S. maltophilia*, 7 *B. cepacia*, 71 *H. influenzae*, 18 *H.*  
116 *parainfluenzae* and 36 *M. catarrhalis*. Isolates were obtained from urinary  
117 tract infections (30%), respiratory tract infections (19.9%), blood cultures  
118 (22.8%), skin and soft tissues infections (11.9%) and from other origin  
119 (15.4%).

120

121

## 122 **Antimicrobial Susceptibility Testing**

123 All tests were carried out using the reference broth microdilution method  
124 according to the guidelines set forth in standard M7-A7 by the CLSI (formerly  
125 NCCLS) [12]. Cation Adjusted Mueller-Hinton II Broth (i.e. BBL™, Becton  
126 Dickinson, Le Pont de Claix, France) used for aerobic bacteria was modified  
127 for streptococci by supplementation with 5% lysed horse blood, whereas for  
128 *Haemophilus* the Haemophilus Test Medium (HTM) formulation (Oxoid,  
129 Dardilly, France) was used. The same batch of medium was used by each  
130 participating center. All tests followed CLSI technical details [12] for  
131 incubation temperature and environment.

132 In order to determine the correlation between MICs and the inhibition zone  
133 diameters, an agar diffusion antibiotic susceptibility testing was performed,  
134 according to the recommendations of the Comité de l'Antibiogramme de la  
135 Société Française de Microbiologie [13,14]. The discs with 10- $\mu$ g doripenem  
136 were supplied by Johnson and Johnson Pharmaceutical Research, Raritan,  
137 NJ, USA.

138

139 **Quality Control**

140 Analysis of doripenem MICs and inhibition zone diameters were performed  
141 against four reference strains: *K. pneumoniae* U2A2189, *K. pneumoniae*  
142 U2A2190, *P. aeruginosa* U2A2320 and *P. aeruginosa* U2A2321, provided by  
143 the French National Reference Center of Antibiotics (CRAB), Institut Pasteur,  
144 Paris. This indicated intercenter variabilities similar to those usually observed  
145 for these techniques. All centers provided results which were not significantly  
146 different from those of the reference center (with a 5% risk of error) (data not  
147 shown).

148 Results were examined to ensure that reported MICs were within acceptable  
149 standards set by EUCAST [11] or CLSI [15] based on the following ATCC  
150 quality control strains: *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *E.*  
151 *coli* ATCC 25922 and *P. aeruginosa* ATCC 27853.

152

153 **RESULTS**

154

155 ***Staphylococci***

156 Table 1 presents the *in vitro* activity of doripenem against Gram-positive  
157 microorganisms (542 isolates).

158 The *in vitro* activity of doripenem was examined against 173 *S. aureus*  
159 including 90 methicillin-resistant (MRSA) and 83 methicillin-susceptible  
160 (MSSA) isolates. Doripenem was potent against MSSA isolates with  
161 MIC<sub>50</sub>/MIC<sub>90</sub> values of 0.03/0.25 mg/L. By contrast they are 1/2 mg/L against  
162 MRSA and it is admitted that MRSA have to be considered resistant to  
163 carbapenems. The *in vitro* activity of doripenem was examined against 104  
164 coagulase negative staphylococci including 54 methicillin-resistant (MR-  
165 CoNS) and 50 methicillin-susceptible (MS-CoNS) isolates. Doripenem was as

166 potent against MS-CoNS isolates (MIC<sub>50/90</sub>: 0.03/0.12 mg/L), as against MS *S.*  
167 *aureus*. MR-CoNS isolates with MICs of 2/8 mg/L have to be considered  
168 resistant to carbapenems.

169

170 ***Enterococci***

171 *E. faecalis* susceptibility testing results for doripenem showed that MICs  
172 ranged from 0.06 to 8 mg/L with MIC<sub>50</sub> and MIC<sub>90</sub> values of 2 and 4 mg/L. For  
173 *E. faecium*, data showed that doripenem displayed no activity (MIC<sub>50/90</sub>:  
174 128/>128 mg/L) against this species.

175

176 ***Streptococci***

177 Doripenem MIC results for *S. pneumoniae* isolates were grouped by their  
178 susceptibility category to penicillin (Table 1). The doripenem MIC values vary  
179 according to the penicillin susceptibility with MIC<sub>50/90</sub> results at ≤0.008/0.25  
180 mg/L for penicillin-susceptible isolates and 0.25/0.5 mg/L for intermediate +  
181 resistant isolates, respectively.

182 Against viridans group streptococci, MICs ranged from ≤0.008 to 4 mg/L with  
183 MIC<sub>50</sub> and MIC<sub>90</sub> values of 0.016 and 2 mg/L, respectively. Doripenem  
184 inhibited all isolates of β-haemolytic streptococci at MIC≤0.03 mg/L.

185

186 ***Enterobacteriaceae***

187 Table 2 presents the *in vitro* activity of doripenem against *Enterobacteriaceae*  
188 (672 isolates).

189 Doripenem was particularly active against members of the  
190 *Enterobacteriaceae*: 70.8% of the isolates were inhibited by doripenem at  
191 concentrations of ≤0.06 mg/L.



192 The MIC<sub>50s</sub> and MIC<sub>90s</sub> for *E. coli*, *Klebsiella*, *Proteus*, *Providencia*,  
193 *Morganella*, *Citrobacter*, and *Enterobacter*, ranged from 0.03 to 0.25 mg/L  
194 and 0.03 to 0.5 mg/L, respectively.

195 Doripenem potency was high against *E. coli* and *Klebsiella*: 92.2% of *E. coli*  
196 isolates and 75% of *Klebsiella* isolates had a doripenem MIC  $\leq$ 0.03 mg/L and  
197  $\leq$ 0.06 mg/L, respectively. Isolates of *E. cloacae* and *C. freundii* were all  
198 susceptible to doripenem (100%) with MIC<sub>S50/90</sub> of 0.03/0.06 mg/L and  
199 0.03/0.25 mg/L for cefotaxime-susceptible isolates and 0.12/0.25 mg/L and  
200 0.06/0.5 mg/L for cefotaxime-non susceptible isolates, respectively. The *P.*  
201 *mirabilis*, *P. vulgaris* and *M. morgani* isolates the MICs of doripenem were  
202 slightly higher but still low with MIC<sub>50s</sub> ranging from 0.12 to 0.25 mg/L and  
203 MIC<sub>90s</sub> values of 0.5 mg/L.

204 Against cefotaxime-susceptible *Enterobacteriaceae*, doripenem had MIC<sub>50</sub>  
205 and MIC<sub>90</sub> of 0.06 and 0.25 mg/L, respectively. The level of doripenem activity  
206 against *Enterobacteriaceae* was essentially maintained against populations  
207 not susceptible to broad-spectrum cephalosporins (i.e., non-susceptible to  
208 cefotaxime). The MIC<sub>50</sub> and MIC<sub>90</sub> of doripenem against cefotaxime-non  
209 susceptible enterobacteria were similar or between 1 or 2 doubling dilutions  
210 when compared to those observed for cefotaxime-susceptible isolates,  
211 whatever the bacterial species was (Table 2). Against ESBL screen-positive  
212 isolates of *Enterobacteriaceae* (n =43), doripenem maintained the same level  
213 of activity as that observed against *Enterobacteriaceae* overall (MIC<sub>S50/90</sub>:  
214 0.06/0.25 mg/L). Among ESBL isolates, 69.8% of isolates were inhibited by  
215 doripenem at concentrations  $\leq$ 0.06 mg/L.

216

### 217 ***Pseudomonas aeruginosa* and *Acinetobacter spp***

218 Table 3 presents the *in vitro* activity of doripenem against non fermentative  
219 bacilli (207 isolates).

220 Doripenem demonstrated widely variable activity against the tested non-  
221 fermentative Gram-negative bacilli, as shown by 83.1% susceptibility at  
222 EUCAST breakpoint of  $\leq 1$  mg/L for *Acinetobacter* and 71.7% for *P.*  
223 *aeruginosa* but for *B. cepacia* and *S. maltophilia* MIC<sub>50/90</sub> were 2/8 and  
224  $>128/128$  mg/L.

225 According to the EUCAST breakpoints of  $\leq 1$  and  $>4$  mg/L, 71.7% of the *P.*  
226 *aeruginosa* were susceptible, 15.2% were intermediate and 13.1% were  
227 resistant.

228 Doripenem activity was evaluated against imipenem-susceptible and non-  
229 susceptible *P. aeruginosa* isolates (Table 3). The MIC<sub>50/90s</sub> of doripenem for  
230 imipenem-susceptible isolates were of 0.25 and 2 mg/L and 87% of them  
231 were susceptible to doripenem.

232 Against imipenem-non susceptible isolates, MICs of doripenem were notably  
233 increased, as the MIC<sub>50</sub> and MIC<sub>90</sub> were 8 and 16 mg/L respectively but with a  
234 wide range of MICs (0.12 to 64 mg/L). Of note, 18.2% of imipenem-resistant  
235 isolates were susceptible to doripenem.

236 Doripenem was active against all imipenem-susceptible *A. baumannii* isolates  
237 with MIC<sub>50/90</sub>: of 0.25 and 1 mg/L and intermediate or resistant against  
238 imipenem-resistant isolates with MIC values range from 2 to 32 mg/L.

239

#### 240 ***Haemophilus spp* and *M. catarrhalis***

241 Table 4 presents the *in vitro* activity of doripenem against respiratory Gram-  
242 negative pathogens (126 isolates).

243 Doripenem has potent *in vitro* activity against *H. influenzae*, *H.*  
244 *parainfluenzae*, and *M. catarrhalis*. The MIC range for doripenem against the  
245 71 *H. influenzae* was  $\leq 0.008$  to 1 mg/L; MIC<sub>50</sub> and MIC<sub>90</sub> values were exactly  
246 the same for ampicillin-susceptible isolates as for ampicillin non-susceptible  
247 isolates (MIC<sub>50/90</sub>=0.12/0.25 mg/L).

248 The MIC range for doripenem against the 18 *H. parainfluenzae* isolates tested  
249 was  $\leq 0.008$  to 0.12 mg/L, doripenem potency was almost similar for ampicillin  
250 susceptible or non-susceptible isolates (Table 5).

251 The MIC range for doripenem towards the 36 *M. catarrhalis* isolates was  
252  $\leq 0.008$  to 0.06 mg/L; MIC<sub>50</sub> and MIC<sub>90</sub> values were 0.03 and 0.06 mg/L,  
253 respectively,

254 Finally, all the 125 isolates (100%) (71 *H. influenzae*, 18 *H. parainfluenzae*  
255 and 36 *M. catarrhalis*) were susceptible to doripenem according to the  
256 EUCAST breakpoint of  $\leq 1$  mg/L.

257

258

259

#### 260 **Correlation between inhibition zone diameters and MICs**

261 The regression curve between the MIC values (logarithm to the base 2) on  
262 the x-axis and the inhibition diameters (arithmetic scale) in the y-axis was  
263 determined by the least-square method on 1,547 isolates whose distribution  
264 is depicted in Figure 1, where the width of the lines is proportional to the  
265 number of isolates .

266 The zone diameters breakpoints can then be deduced from the doripenem  
267 EUCAST MIC breakpoints: 1/4 for *Enterobacteriaceae*, *Pseudomonas* and  
268 *Acinetobacter* and 1/1 for streptococci (including *S. pneumoniae*), and  
269 *Haemophilus spp* and *M. catarrhalis*.

270 Zone diameter breakpoints with a 10- $\mu$ g doripenem disc content could thus  
271 be 24 and 19 mm ( $\geq 24$ , susceptible; 19-23, intermediate and  $< 19$ , resistant)  
272 for *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter* and 24 mm ( $\geq 24$ ,  
273 susceptible and  $< 24$ , resistant) for streptococci (including *S. pneumoniae*),  
274 *Haemophilus spp* and *M. catarrhalis*.

275 For instance, using a zone diameter breakpoint of  $\geq 24$  mm to denote  
276 susceptibility, a false susceptibility rate (Rs) of 0.32% (5 isolates) and a false  
277 resistant (Sr) rate of 0.19% (3 isolates) were seen in each case (Figure 1)

278

## 279 **DISCUSSION**

280 Doripenem was potent against oxacillin-susceptible staphylococci with the  
281 same MIC<sub>50/90</sub> than imipenem [8]. Nevertheless, according to the EUCAST  
282 recommendations, “susceptibility of staphylococci to carbapenems is inferred  
283 from the methicillin susceptibility”. So methicillin-resistant staphylococci have  
284 to be considered resistant to carbapenems including doripenem. Most  
285 enterococci isolates were not susceptible to doripenem.

286 Doripenem was potent against penicillin-susceptible *S. pneumoniae*. The  
287 decrease of its activity coincided with decreased susceptibility to penicillin as  
288 already shown in literature [16].

289 Doripenem was highly potent against all *Enterobacteriaceae* isolates tested,  
290 including those resistant to “advanced-generation” cephalosporins and  
291 screen-positive ESBL isolates. This pattern of doripenem activity against  
292 *Enterobacteriaceae* was similar to that reported in previous studies [4-7,9].

293 Doripenem maintained the same activity against all cefotaxime-non  
294 susceptible or susceptible isolates of *Enterobacteriaceae* and also against  
295 ESBL positive isolates with the exception of *E. cloacae* for which the  
296 doripenem MIC<sub>50/90</sub> were four-fold higher for cefotaxime-non susceptible  
297 isolates in comparison to cefotaxime susceptible isolates .

298 The maintained activity of doripenem and other carbapenems against  
299 enterobacteria resistant to other  $\beta$ -lactams is largely due to their stability to  
300 hydrolysis by  $\beta$ -lactamases commonly encountered among these organisms.

301 Against *P. aeruginosa* imipenem-susceptible. Doripenem was highly potent,  
302 as already shown in other studies [4,5,7,17,9,3]. It is two- and four-fold more

303 potent than meropenem and imipenem respectively [8,16]. Against imipenem-  
304 resistant isolates with MICs  $\geq 8$  mg/L, 22.4 % have MIC of doripenem  $< 4$  mg/L  
305 [7].

306 Overall, 71.7% of isolates tested in this study were susceptible to doripenem.  
307 Though the *in vitro* activities of doripenem and imipenem show parallel  
308 variation against Gram-negative bacilli as described above, imipenem has  
309 been shown to be an acceptable surrogate marker for determining the  
310 susceptibility of Gram-negative clinical isolates to carbapenems including  
311 doripenem [18]. However, this type of testing does not account for clinical  
312 isolates which are non-susceptible to imipenem but remain susceptible to  
313 doripenem. For example, 18% of *P. aeruginosa* isolates tested in this study  
314 as non-susceptible to imipenem were susceptible to doripenem.

315 This is consistent with the view that doripenem is less affected by the  
316 deficiency of OprD porin protein than imipenem [19]. On the other hand, a few  
317 imipenem-susceptible strains (3%) have MICs of doripenem  $> 1$  mg/L and are  
318 thus considered non-susceptible according to EUCAST MIC breakpoints,  
319 what can be mainly explained by the difference between the susceptibility  
320 breakpoints concentrations established by EUCAST for imipenem and  
321 doripenem ( $\leq 4$  and  $\leq 1$  mg/L, respectively). Nevertheless, it was reported that  
322 doripenem, even if in a lesser degree than meropenem, is affected by the  
323 efflux mechanism and overexpression of efflux pumps in *P. aeruginosa*  
324 results in moderate resistance to doripenem, whereas imipenem largely  
325 escapes this mechanism [20].

326 Although cross-resistance exists between imipenem and doripenem, like  
327 meropenem, doripenem appears to be *in vitro* more active than imipenem  
328 against OprD-deficient *P. aeruginosa* strains.

329 The activity of doripenem against imipenem-susceptible *A. baumannii* was  
330 similar to that of imipenem-susceptible *P. aeruginosa* but in contrast,

331 doripenem was not active against any imipenem-non susceptible isolates of  
332 *Acinetobacter spp.*, which may be due to several mechanisms of resistance  
333 present in these strains such as carbapenemase phenotype, membrane  
334 impermeability or variable porin/outer membrane protein expression [21].  
335 No activity was observed against *S. maltophilia* isolates, and *B. cepacia* was  
336 inconstantly susceptible to doripenem (only 28.6% susceptible isolates).  
337 Doripenem was also active against respiratory pathogens as *H. influenzae*, *H.*  
338 *parainfluenzae* or *M. catarrhalis*, regardless of ampicillin resistance.

339

340

#### 341 **CONCLUSION**

342

343 Doripenem is a broad-spectrum carbapenem with a good *in vitro* activity  
344 against Gram-positive cocci and against challenging Gram-negative  
345 pathogens, including resistant *Enterobacteriaceae* and *P. aeruginosa*. This  
346 study performed on a large number of isolates confirms its potent *in vitro*  
347 activity against such clinically isolates. Based on the activity profile presented  
348 in this study and others, doripenem appears to be a promising new agent for  
349 the treatment of infections caused by severe Gram-negative pathogens  
350 commonly encountered in the hospital including cephalosporin resistant  
351 enteric bacilli and multidrug-resistant *P. aeruginosa* or *Acinetobacter spp.*  
352 Because its primary use will be associated with the hospital where plasmid-  
353 mediated resistance to carbapenems has already been documented among  
354 some Gram-negative bacterial isolates, it is important to continue to monitor  
355 the activity of doripenem throughout its clinical development and after its  
356 introduction into clinical use.

357 This study allowed us to determine zone diameter breakpoints, which are 24  
358 and 19 mm for MICs of 1 and 4 mg/L, used for clinical categorization according to  
359 the EUCAST MIC breakpoints.

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377 **Potential conflicts of interest:**

378 No conflict for all authors is declared.

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381 **REFERENCES**

382

383 1. Nomura S, Nagayama A. *In vitro* antibacterial activity of S-4661, a new  
384 parenteral carbapenem, against urological pathogens isolated from patients  
385 with complicated urinary tract infections. *J Chemother* 2002;14(2):155-60.

386 2. Ohiba F, Nakamura-Kamigo M, Watanabe N, Katsu K. *In vitro* and *in vivo*  
387 antibacterial activities of ER-35786, a new antipseudomonal carbapenem.  
388 *Antimicrob Agents Chemother* 1997;41:298-307.

389 3. Watanabe A, Takahashi H, Kikuchi T, et al. Comparative *in vitro* activity of  
390 S-4661, a new parenteral carbapenem, and other antimicrobial agents  
391 against respiratory pathogens. *Chemotherapy* 2000;46(3):184-7.

392 4. Brown SD, Traczewski MM. Comparative *in vitro* antimicrobial activity of a  
393 new carbapenem, doripenem: tentative disc diffusion criteria and quality  
394 control. *J Antimicrob Chemother* 2005;55(6):944-9.

395 5. Fritsche TR, Stilwell MG, Jones RN. Antimicrobial activity of doripenem (S-  
396 4661): a global surveillance report (2003). *Clin Microbiol Infect*  
397 2005;11(12):974-84.

398 6. Ge Y, Wikler MA, Sahm DF, Blosser-Middleton RS, Karlowsky JA. *In vitro*  
399 antimicrobial activity of doripenem, a new carbapenem. *Antimicrob Agents*  
400 *Chemother* 2004;48(4):1384-96.

401 7. Jones RN, Huynh HK, Biedenbach DJ. Activities of doripenem (S-4661)  
402 against drug-resistant clinical pathogens. *Antimicrob Agents Chemother*  
403 2004;48(8):3136-40.

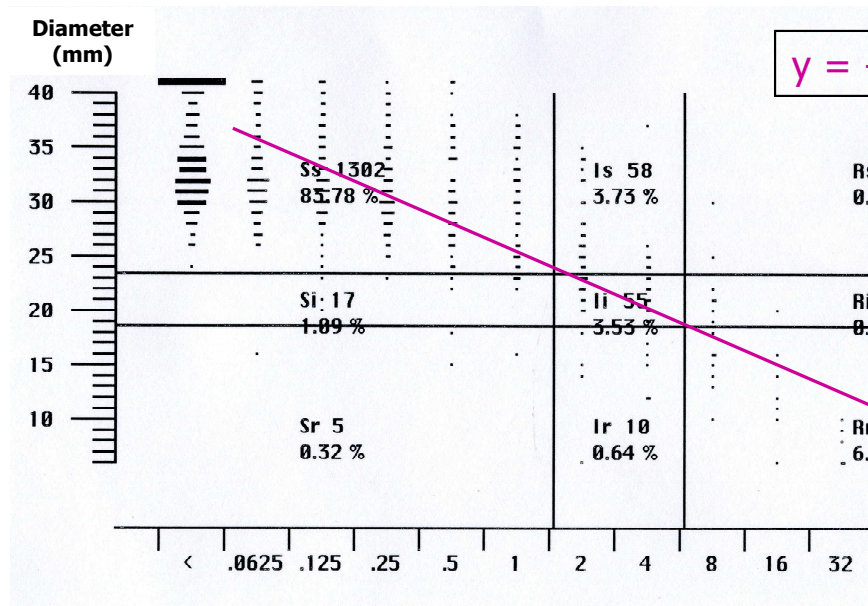
404 8. Jones RN, Huynh HK, Biedenbach DJ, Fritsche TR, Sader HS. Doripenem  
405 (S-4661), a novel carbapenem: comparative activity against contemporary  
406 pathogens including bactericidal action and preliminary *in vitro* methods  
407 evaluations. *J Antimicrob Chemother* 2004;54(1):144-54.



- 408 9. Tsuji M, Ishii Y, Ohno A, Miyazaki S, Yamaguchi K. In vitro and in vivo  
409 antibacterial activities of S-4661, a new carbapenem. *Antimicrob Agents*  
410 *Chemother* 1998;42(1):94-9.
- 411 10. Mikamo H, Izumi K, Hua YX, Hayasaki Y, Sato Y, Tamaya T. In vitro and  
412 in vivo antibacterial activities of a new injectable carbapenem, S-4661,  
413 against gynecological pathogens. *J Antimicrob Chemother* 2000;46(3):471-4.
- 414 11. European Committee on Antimicrobial Susceptibility Testing. Clinical  
415 Breakpoints. 2009. <http://www.eucast.org>.
- 416 12. Clinical Laboratory Standards Institute. Methods for Dilution Antimicrobial  
417 Susceptibility Tests for Bacteria that Grow Aerobically. 7th Edition. Approved  
418 Standard M7-A7. CLSI, Wayne, PA, USA, 2006.
- 419 13. Comité de l'Antibiogramme de la Société Française de Microbiologie.  
420 Communiqué 2009. Société Française de Microbiologie.  
421 <http://www.sfm.asso.fr>
- 422 14. Courvalin P., Soussy C.J. Report of the Comité de l'Antibiogramme de la  
423 Société Française de Microbiologie. *Clinical Microbiology and Infection*.  
424 1996,2, suppl. 1, SI-S49.
- 425 15. Clinical Laboratory Standards Institute. Performance Standards for  
426 Antimicrobial Susceptibility Testing. 17th Informational Supplement M100-17.  
427 CLSI, Wayne, PA, USA, 2007.
- 428 16. Pillar CM, Torres MK, Brown NP, Shah D, Sahm DF. In vitro activity of  
429 doripenem, a carbapenem for the treatment of challenging infections caused  
430 by gram-negative bacteria, against recent clinical isolates from the United  
431 States. *Antimicrob Agents Chemother* 2008;52(12):4388-99.
- 432 17. Jones RN, Sader HS, Fritsche TR. Comparative activity of doripenem and  
433 three other carbapenems tested against Gram-negative bacilli with various

- 434 beta-lactamase resistance mechanisms. *Diagn Microbiol Infect Dis*  
435 2005;52(1):71-4.
- 436 18. Jones RN, Sader HS, Fritsche TR, Janecek MJ. Selection of a surrogate  
437 beta-lactam testing agent for initial susceptibility testing of doripenem, a new  
438 carbapenem. *Diagn Microbiol Infect Dis* 2007;59(4):467-72.
- 439 19. Mushtaq S, Ge Y, Livermore DM. Doripenem versus *Pseudomonas*  
440 *aeruginosa in vitro*: activity against characterized isolates, mutants, and  
441 transconjugants and resistance selection potential. *Antimicrob Agents*  
442 *Chemother* 2004;48(8):3086-92.
- 443 20. Masuda N, Sakagawa E, Ohya S, Gotoh N, Tsujimoto H, Nishino T.  
444 Substrate specificities of MexAB-OprM, MexCD-OprJ, and MexXY-oprM  
445 efflux pumps in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother*  
446 2000;44(12):3322-7.
- 447 21. Poirel L, Nordmann P. Carbapenem resistance in *Acinetobacter*  
448 *baumannii*: mechanisms and epidemiology. *Clin Microbiol Infect*  
449 2006;12(9):826-36.
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**Figure 1: Regression curve and scattergram of doripenem MICs/zone diameters with 10-μg disks**

488 **Table 1. Antimicrobial activity of doripenem tested against Gram-**  
 489 **positive microorganisms (572 isolates)**  
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| Organism (no. tested)                  | MIC (mg/L) |        |             | % Suscep |
|--|------------|--------|-------------|----------|
|  | 50 %       | 90 %   | Range       |          |
| <i>Staphylococcus aureus</i> (173)     |            |        |             |          |
| OXA S (90)                             | 0.03       | 0.25   | ≤0.008-0.5  | -        |
| OXA R (83)                             | 1          | 2      | 0.03-16     | -        |
| Coagulase Negative Staphylococci (104) |            |        |             |          |
| OXA S (50)                             | 0.03       | 0.12   | ≤0.008-0.12 | -        |
| OXA R (54)                             | 2          | 8      | 0.25-64     | -        |
| <i>Enterococcus faecalis</i> (63)      | 2          | 4      | 0.06-8      | -        |
| <i>Enterococcus faecium</i> (40)       | 128        | >128   | 4->128      | -        |
| <i>Streptococcus pneumoniae</i> (83)   |            |        |             |          |
| PEN S (48)                             | ≤0.008     | 0.03   | ≤0.008-0.25 | 83.      |
| PEN I + R(35)                          | 0.25       | 0.5    | ≤0.008-1    |          |
| Streptococci A, B, C and G (60)        | ≤0.008     | ≤0.008 | ≤0.008-0.03 | 96.      |
| Other Streptococci (49)                | 0.016      | 2      | ≤0.008-4    | 86.      |

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OXA: oxacillin, PEN: penicillin

nd: not determined

\*Determination of doripenem susceptibility according MIC EUCAST breakpoints (not available for staphylococci and enterococci, ≤ 1 mg/L for streptococci)

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**Table 2. Antimicrobial activity of doripenem tested against *Enterobacteriaceae* (672 isolates)**

| Organism (no. tested)               | MIC (mg/L) |      |             | % Susceptible* |
|-------------------------------------|------------|------|-------------|----------------|
|                                     | 50 %       | 90 % | Range       |                |
| <i>All Enterobacteriaceae</i> (672) |            |      |             |                |
| CTX S (578)                         | 0.06       | 0.25 | ≤0.008-2    | 99.8           |
| CTX R (94)                          | 0.06       | 0.25 | 0.016-2     |                |
| <i>Escherichia coli</i> (129)       |            |      |             |                |
| CTX S (118)                         | 0.03       | 0.03 | ≤0.008-0.12 | 100            |
| CTX R (11)                          | 0.03       | 0.06 | 0.016-0.06  |                |
| <i>Citrobacter freundii</i> (31)    |            |      |             |                |
| CTX S (22)                          | 0.03       | 0.25 | ≤0.008-0.5  | 100            |
| CTX R (9)                           | 0.06       | 0.5  | 0.03-0.5    |                |
| <i>Klebsiella pneumoniae</i> (114)  |            |      |             |                |
| CTX S (97)                          | 0.03       | 0.06 | ≤0.008-0.12 | 100            |
| CTX R (17)                          | 0.03       | 0.06 | 0.03-0.5    |                |
| <i>Klebsiella oxytoca</i> (36)      |            |      |             |                |
| CTX S (35)                          | 0.03       | 0.06 | ≤0.008-0.5  | 100            |
| CTX R (1)                           | nd         | nd   | nd          |                |
| <i>Enterobacter cloacae</i> (90)    |            |      |             |                |
| CTX S (57)                          | 0.03       | 0.06 | ≤0.008-0.25 | 100            |
| CTX R (33)                          | 0.12       | 0.25 | 0.016-0.5   |                |
| <i>Enterobacter aerogenes</i> (49)  |            |      |             |                |
| CTX S (36)                          | 0.06       | 0.12 | ≤0.008-0.25 | 100            |
| CTX R (13)                          | 0.12       | 0.25 | 0.06-0.25   |                |
| <i>Proteus mirabilis</i> (114)      |            |      |             |                |
| CTX S (112)                         | 0.12       | 0.5  | ≤0.008-2    | 99.1           |
| CTX R (2)                           | nd         | nd   | nd          |                |
| <i>Morganella morganii</i> (46)     |            |      |             |                |
| CTX S (42)                          | 0.25       | 0.5  | ≤0.008-1    | 100            |
| CTX R (4)                           | 0.25       | 0.5  | 0.12-0.5    |                |
| <i>Proteus vulgaris</i> (38)        |            |      |             |                |
| CTX S (37)                          | 0.25       | 0.5  | ≤0.008-1    | 100            |
| CTX R (1)                           | nd         | nd   | nd          |                |
| <i>Providencia stuartii</i> (15)    |            |      |             |                |
| CTX S (13)                          | 0.12       | 0.25 | ≤0.008-0.25 | 100            |
| CTX R (2)                           | nd         | nd   | nd          |                |
| <i>Providencia rettgeri</i> (10)    |            |      |             | 100            |

|            |      |      |             |
|------------|------|------|-------------|
| CTX S (10) | 0.06 | 0.12 | ≤0.008-0.25 |
|------------|------|------|-------------|

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CTX: cefotaxime

nd: not determined

\*Determination of doripenem susceptibility according MIC EUCAST breakpoints (≤ 1 mg/L)

**Table 3. Antimicrobial activity of doripenem tested against non fermentative bacilli (207 isolates)**

| Organism (no. tested)                    | MIC (mg/L) |      |         |                |
|--|------------|------|---------|----------------|
|  | 50 %       | 90 % | Range   | % Susceptible* |
| <i>Pseudomonas aeruginosa</i> (99)       |            |      |         |                |
| IPM S (77)                               | 0.25       | 2    | 0.016-8 | 71.7           |
| IPM R (22)                               | 8          | 16   | 0.12-64 |                |
| <i>Acinetobacter baumannii</i> (71)      |            |      |         |                |
| IPM S (65)                               | 0.25       | 1    | 0.03-4  | 83.1           |
| IPM R (6)                                | nd         | nd   | 2-32    |                |
| <i>Stenotrophomonas maltophilia</i> (30) | >128       | >128 | 64->128 | 0              |
| <i>Burkholderia cepacia</i> (7)          | 2          | 8    | 1-128   | 28.6           |

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IPM: imipenem

nd: not determined

\*Determination of doripenem susceptibility according MIC EUCAST breakpoints (≤ 1 mg/L)

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**Table 4. Antimicrobial activity of doripenem tested against Gram-negative respiratory pathogens (125 isolates)**

| Organism (no. tested)                  | MIC (mg/L) |      |             |         |
|--|------------|------|-------------|---------|
|  | 50 %       | 90 % | Range       | % Susce |
| <i>Haemophilus influenzae</i> (71)     |            |      |             |         |
| AMP S (54)                             | 0.12       | 0.25 | ≤0.008-1    | 100     |
| AMP R (17)                             | 0.12       | 0.25 | ≤0.008-1    |         |
| <i>Haemophilus parainfluenzae</i> (18) |            |      |             |         |
| AMP S (9)                              | 0.12       | 0.12 | ≤0.008-0.12 | 94.7    |
| AMP R (9)                              | 0.06       | 0.12 | ≤0.008-0.12 |         |
| <i>Moraxella catarrhalis</i> (36)      |            |      |             |         |
| AMP S (2)                              | nd         | nd   | 0.016-0.03  | 100     |
| AMP R (34)                             | 0.03       | 0.06 | ≤0.008-0.06 |         |

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AMP: ampicillin  
nd: not determined  
\*Determination of doripenem susceptibility according MIC EUCAST breakpoints (≤ 1 mg/L)

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