



HAL
open science

Susceptibility testing of tulathromycin: interpretative breakpoints and susceptibility of field isolates

K.S. Godinho

► **To cite this version:**

K.S. Godinho. Susceptibility testing of tulathromycin: interpretative breakpoints and susceptibility of field isolates. *Veterinary Microbiology*, 2008, 129 (3-4), pp.426. 10.1016/j.vetmic.2007.11.033 . hal-00532367

HAL Id: hal-00532367

<https://hal.science/hal-00532367v1>

Submitted on 4 Nov 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: Susceptibility testing of tulathromycin: interpretative breakpoints and susceptibility of field isolates

Author: K.S. Godinho

PII: S0378-1135(07)00606-2
DOI: doi:10.1016/j.vetmic.2007.11.033
Reference: VETMIC 3909

To appear in: *VETMIC*

Received date: 6-10-2007
Revised date: 26-11-2007
Accepted date: 28-11-2007



Please cite this article as: Godinho, K.S., Susceptibility testing of tulathromycin: interpretative breakpoints and susceptibility of field isolates, *Veterinary Microbiology* (2007), doi:10.1016/j.vetmic.2007.11.033

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 [short communication for Veterinary Microbiology]

2

3 **Susceptibility testing of tulathromycin: interpretative breakpoints and**
4 **susceptibility of field isolates.**

5

6 K.S. Godinho

7

8 Veterinary Research & Development, Pfizer Ltd., Sandwich, Kent CT13 9NJ

9

10 Tel: 01304 645790

11 Fax: 01304 656257

12 E-mail: kevin.godinho@pfizer.com

13

14

15 **Abstract**

16 *In vitro* susceptibility tests were conducted on bovine and porcine respiratory
17 pathogens isolated from European countries during 2004-2006 for susceptibility to
18 tulathromycin using the recommended methodologies for broth microdilution. The
19 results were compared with data from a similar survey conducted prior to launch in
20 1998-2001 to monitor for any shift in susceptibility. The importance of maintaining
21 the pH of the culture media within the range 7.2-7.4 was re-affirmed as a key factor in
22 obtaining consistent minimum inhibitory concentration data. The use of recently
23 established interpretative breakpoints would indicate that to date there has been no
24 apparent decrease in susceptibility to tulathromycin since it became widely used
25 across Europe.

26

27

28 **1. Introduction**

29

30 Following the introduction of tulathromycin for veterinary use in the treatment
31 of respiratory disease in cattle and swine, a post-approval monitoring programme was
32 established in order to monitor any changes in the susceptibility of field isolates of the
33 key bacterial pathogens in countries where the product is approved. At the time of
34 approval, minimum inhibitory concentration (MIC) ranges for control organisms used
35 for quality control purposes had not been established, neither had interpretative
36 standards been accepted for *in vitro* susceptibility testing with tulathromycin.
37 Consequently it is thought probable that many laboratories have used the
38 interpretative breakpoints established for tilmicosin, another macrolide antimicrobial
39 used for the treatment of respiratory disease in cattle, and for which guidelines have
40 been previously established by the Clinical and Laboratory Standards Institute (CLSI,
41 2002). However, there are important structural differences between tilmicosin, which
42 has a 16-membered ring, and tulathromycin, which has a 15-membered ring with
43 three amine groups (Evans, 2005). These structural differences impart different
44 pharmacokinetic/pharmacodynamic properties rendering the use of the same
45 interpretative breakpoints inappropriate. Recently, breakpoints have been established
46 for susceptibility testing of bovine respiratory pathogens to tulathromycin (Table 1)
47 and these are expected to be incorporated into the next edition of the CLSI guidelines.
48 Generally, the breakpoints for tulathromycin are one dilution higher than those for
49 tilmicosin (CLSI, 2002). At this time the sponsor has developed similar guidance for
50 porcine respiratory pathogens but these have yet to be accepted by the CLSI

51 veterinary sub-committee and should be regarded as provisional. In addition, initial
52 investigation of the killing kinetics of tulathromycin indicated that susceptibility
53 testing was subject to error arising from methodological variation in conditions (Reese
54 et al., 2004; Evans, 2005). In order to ensure intra- and inter-laboratory consistency
55 and repeatability of results, it is essential that a common methodology is employed by
56 testing laboratories. This paper reports on a wide-ranging survey of the susceptibility
57 to tulathromycin of bacterial isolates obtained from field outbreaks of bovine and
58 porcine respiratory disease in Europe and reviews appropriate susceptibility testing
59 methodology for tulathromycin.

60

61

62 **2. Materials and methods**

63

64 2.1 *Source of isolates*

65

66 Bacterial isolates were obtained from field studies conducted in various
67 countries (Belgium, Czech Republic, Denmark, France, Germany, Italy, the
68 Netherlands, Poland, Spain and the United Kingdom) as well as from library
69 collections. All isolates were sourced from animals that had not received treatment
70 with any antimicrobial. Species identification was performed using standard
71 techniques. Prior to susceptibility testing all mycoplasmal isolates were stored at
72 -70°C in vials containing 10% v/v glycerol and 1 mL Mycoplasma Medium™
73 (Mycoplasma Experience Ltd, Reigate, Surrey, RH2 9BY, UK). All other bacterial
74 isolates were stored on cryopreservative beads (Protect Beads™, Technical Services
75 Consultants Ltd., Heywood, Lancashire, OL10 1DS, UK) at -75°C to -85°C.

76

77 2.2 *Determination of minimum inhibitory concentrations*

78

79 Susceptibility testing of the bacterial isolates to tulathromycin was performed
80 in accordance with CLSI guidelines (CLSI, 2002) using a broth microdilution
81 technique. For non-fastidious species the medium used was cation-adjusted Mueller-
82 Hinton broth (CAMHB) with *N*-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic
83 acid (TES) buffer (Sensititre[®], Trek Diagnostic Systems, Cleveland, OH, USA) in
84 standard round-bottomed 96-well microdilution plates (Alpha Laboratories Sterile
85 Microtitre plates, Hampshire, UK). Each well contained 50 µL and the dilution range
86 for determination of MICs for tulathromycin was 0.06 to 64 µg/mL. Each plate
87 contained three untreated wells for growth control purposes. MIC determinations for
88 fastidious species (*Histophilus somni* and *Actinobacillus pleuropneumoniae*) were
89 made using Veterinary Fastidious Medium (VFM) in a carbon dioxide enriched
90 atmosphere (5% CO₂). The test system was validated and routinely monitored by
91 concurrently determining the MICs of *Escherichia coli* American Type Culture
92 Collection (ATCC) 25922 (MIC 2-8 µg/mL), *H. somni* ATCC 700025 (MIC 4-32
93 µg/mL), *A. pleuropneumoniae* ATCC 27090 (MIC 16-64 µg/mL) and *Staphylococcus*
94 *aureus* ATCC 29213 (MIC 2-8 µg/mL) as quality control organisms. Immediately
95 prior to each test the pH of the media was checked using a calibrated pH meter, to
96 ensure it was within the pH range 7.2-7.4, and media found to be outside this range
97 were discarded.

98 Bacterial isolates were retrieved from storage and sub-cultured onto Columbia
99 sheep blood agar, incubated overnight at 35±1°C and checked visually for
100 morphology and purity. Fastidious species, retrieved from storage, were sub-cultured

101 onto chocolate agar and incubated overnight at $35\pm 1^\circ\text{C}$ in a carbon dioxide enriched
102 atmosphere (5-7% CO_2), prior to visual checking for morphology and purity. 0.5
103 McFarland suspensions of test bacteria in saline were prepared by suspending 3-5
104 bacterial colonies in 5 mL of physiological saline and the optical density adjusted to
105 0.08-0.1 at 625 nm to give approximately 5×10^5 cfu/mL (as per CLSI guidelines).

106 An equilibrated stock solution of tulathromycin was prepared (Trek Diagnostic
107 Systems, Cleveland, OH, USA) and used for all susceptibility testing. Standardised
108 cell suspensions were inoculated into the test plates within 30 minutes of preparation
109 to give a final inoculum in each well of approximately 5×10^5 cfu/mL. A number of
110 randomly selected inocula were titrated for confirmation of the approximate levels of
111 cfu/mL. The colony counts obtained ranged from 4.3×10^5 to 8.8×10^5 cfu/mL and
112 were regarded as acceptable. Diluted inoculum was kept at -4°C or on ice until
113 microtitre plates were inoculated. Each well was inoculated with 50 μL of bacterial
114 suspension. Plates were sealed to prevent desiccation and incubated for 16-20 hours
115 (20-24 hours for fastidious species) at 35°C to ensure adequate growth in the drug-
116 free control wells. Tests with poor growth in the drug-free wells were invalid and
117 were repeated.

118 Mycoplasma isolates (stored at -70°C) were used to initiate further cultures
119 which were vortexed after a one in ten dilution, and then further diluted to 10^{-5} and
120 inoculated onto plates to allow estimation of the number of colony forming units (cfu)
121 in the broth culture. Mycoplasma suspensions for MIC determination in the range 10^3 -
122 10^5 cfu/mL were prepared in fresh media at pH 7.5. Titres were determined by adding
123 0.1 mL of suspension to 0.1 mL volumes of medium, and inoculating the mixture onto
124 agar media. After 7 days incubation to allow for development of all colonies present,
125 the number of colonies were counted.

126 The susceptibility of mycoplasmal isolates was determined using a
127 modification of the method described by Tanner and Wu (1992) using Cellstar® plates
128 (Greiner) with a single reading to determine end-points. Susceptibility testing was
129 carried out using pre-counted, actively growing cultures. Proprietary media
130 (Mycoplasma Media™, Mycoplasma Experience Ltd, Reigate, Surrey, RH2 9BY,
131 UK) were composed of a broth base supplemented with bovine or porcine serum and
132 yeast extract, and contained phenol red as a pH indicator and ampicillin as a bacterial
133 inhibitor. Dilutions of mycoplasma broth culture were prepared in fresh media, prior
134 to incubation at 36±1°C. Stock drug solutions were prepared fresh as described above.
135 Each microtitre plate contained wells with uninoculated medium, medium at pH 6.8
136 (to provide an end-point control) and inoculated medium alone as challenge controls.
137 Plates were sealed with Falcon® (Becton Dickinson, 1 Becton Drive, Franklin Lakes,
138 New Jersey 07417, USA) sealing strips, prior to incubation at 36±1°C. Growth was
139 apparent as a pH dependent colour change in wells where growth was evident, caused
140 by a growth-induced pH shift in the medium (changing from red to orange/yellow for
141 glucose fermenters). Plates were inspected at the start, middle and end of each day
142 until a colour change in the drug-free challenge control well was equal to or exceeded
143 that for the end-point control well (pH 6.8) at which time the tests were then read.
144 When end-points were not achieved or if the mycoplasma inocula fell outside the
145 range 10³-10⁵ cfu/mL the tests were repeated. The test system was validated and
146 routinely monitored by concurrently testing *M. bovis* reference strain NC10131 and
147 *M. hyopneumoniae* reference strain NC10110 with each set of susceptibility tests.

148 The MIC for any particular isolate was defined as the lowest concentration of
149 tulathromycin to completely inhibit visible growth. The MIC₅₀ and MIC₉₀ values were

150 defined as the lowest concentrations capable of inhibiting the growth of 50% and 90%
151 of isolates, respectively.

152

153 3. Results

154

155 A total of 170 isolates were of bovine origin (*Mannheimia haemolytica* 33,
156 *Pasteurella multocida* 44; *H. somni* 30; and 63 *M. bovis*) and 133 isolates were of
157 porcine origin (*A. pleuropneumoniae* 53; *P. multocida* 53; and 27
158 *M. hyopneumoniae*). The MIC results for each species are summarised in Table 1 and
159 the interpretative breakpoints would indicate that at these MIC₉₀ values, the bacterial
160 species tested were susceptible to tulathromycin. However, no adequate interpretation
161 of breakpoints has, as yet, been determined for *M. bovis* or *M. hyopneumoniae*,
162 although the low MIC data obtained for *M. hyopneumoniae* would suggest general
163 susceptibility. The results for *M. bovis* would indicate an MIC₉₀ of >64 µg/mL, but
164 this is not necessarily predictive of a lack of clinical efficacy. Data has been
165 previously presented to demonstrate that under controlled conditions, tulathromycin
166 was clinically efficacious in treatment of respiratory disease in calves inoculated with
167 a strain of *M. bovis* known to have an MIC for tulathromycin of >64 µg/mL (Godinho
168 et al., 2005b). The MIC₉₀ values (and MIC range) for tulathromycin across all
169 countries for bovine isolates determined in the current survey conducted with isolates
170 collected during 2004-2006 were compared with those determined in the period 1998-
171 2001 (Godinho et al., 2005a,b; McKelvie et al., 2005; Nanjiani et al., 2005) prior to
172 the European product launch (Table 2).

173

174 **4. Discussion**

175

176 This is the first report to include interpretation of the antimicrobial
177 susceptibility of respiratory pathogens to tulathromycin following its introduction
178 onto the veterinary market. These data would indicate that despite the usage of the
179 antimicrobial, in cattle and pigs there has as yet been no shift in susceptibility
180 amongst the key pathogenic target species. Nevertheless, it is important that the
181 situation continues to be monitored regularly. In recently published work comparing
182 the MICs of porcine isolates of *P. multocida* collected over a two year period for a
183 battery of antimicrobials by Kaspar and others (2007), the MIC₉₀ for tulathromycin
184 was 2 µg/mL in 2004-5 and 1 µg/mL in 2005-6. Using the proposed new
185 interpretative guidelines, with the exception of one resistant isolate from 2004-5
186 (MIC = 64 µg/mL), all other isolates (639 isolates in 2004-5 and 471 in 2005-6) from
187 both years were susceptible to tulathromycin (MIC range 0.03-4 µg/mL) (Kaspar et
188 al., 2007).

189 In the work presented here, the sampling methodology utilised was broadly in
190 agreement with that suggested by Wallmann (2006) for the monitoring of
191 antimicrobial resistance in pathogenic bacteria from farm animals. As with any survey
192 of susceptibility data it is accepted that there are a small number of shortcomings in
193 the methodology employed. It was not possible, for example, to identify isolates from
194 premises known to have been exposed to tulathromycin in the intervening period
195 between the two periods surveyed. Neither was it possible to obtain isolates from the
196 same farms or indeed in many instances the same countries. However, bias was
197 reduced by not using data solely from herds where the product was known to either
198 have been used or not to have been used. The only stipulation was that the isolate had

199 to be collected prior to administration of any antimicrobials during the current
200 outbreak of respiratory disease. Surveys of antimicrobial susceptibility based upon
201 isolates received by laboratories from practitioners are likely to be biased if the data
202 include isolates from samples collected from problem animals/herds that have not
203 responded to treatment and such data automatically selects in favour of a reduced
204 susceptibility. It is not unknown for data biased in this manner to be used to support
205 arguments in favour of restricting the veterinary usage of antimicrobials, especially in
206 food producing species. While antimicrobials can provide valuable benefits in terms
207 of animal welfare and control of clinical disease, it is important in relation to the
208 development of resistance to monitor the field susceptibility of key pathogens to
209 specific antimicrobials particularly if their use is widespread.

210 In order to ensure comparability of results, it is also important that a common
211 methodology is employed during the conduct of susceptibility testing. Previous
212 reports have indicated that alterations in the pH and variability between batches of
213 media during susceptibility tests for tulathromycin against fastidious species
214 (*H. somni* and *A. pleuropneumoniae*), could result in MIC values that differed by up
215 to 4-fold (Reese et al., 2004; Evans, 2005; Godinho et al., 2005a). In addition, it was
216 found that the results could be influenced by the presence of serum, with the range of
217 MIC and MIC₉₀ considerably reduced in the presence of serum by a factor of at least
218 4-fold for the aerobic species and 6 to 10-fold for the fastidious species, which was
219 thought to be related to the increased buffering capacity provided by the serum
220 (Godinho et al., 2005a). At this time it is not known whether the presence of serum
221 could also affect susceptibility testing of mycoplasma species. Similar changes in
222 MIC due to reduction of pH have been reported for erythromycin, azithromycin and
223 clarithromycin (Pruul and McDonald 1992; Edney et al., 1998). The recently

224 established CLSI methodology for control organisms and interpretative guidelines for
225 bovine isolates and the suggested guidance for porcine pathogens should provide
226 improved repeatability of susceptibility testing for tulathromycin.

227

228

229 **Acknowledgements**

230

231 The author would like to thank the staff of the many contributing laboratories
232 for their assistance in supplying the isolates used. The author is grateful for the
233 technical assistance of Sue Keane and David Windsor and would like to thank Jeffrey
234 Watts for reviewing the manuscript.

235

236

237 **References**

238

239 CLSI - Clinical and Laboratory Standards Institute, 2002. *Performance standards for*
240 *antimicrobial disk and dilution susceptibility tests for bacteria isolated from farm animals;*
241 *Approved Standard – second edition*. CLSI (formerly National Committee of Clinical
242 Laboratory Standards – NCCLS), 940 West Valley Road, Suite 1400, Wayne, Pennsylvania
243 19087 USA. Doc. M31-A2. 22.

244

245 Ednie, L.M., Jacobs, R.M., Appelbaum, P.C., 1998. Anti-anaerobic activity of erythromycin,
246 azithromycin and clarithromycin: effect of pH adjustment of media to compensate for pH
247 shift caused by incubation in CO₂. *J. Antimicrob. Chemother.*, 41, 387-389.

248

249 Evans, N.A., 2005. Tulathromycin: An overview of a new triamilide antimicrobial for
250 livestock respiratory disease. *Vet. Ther.*, 6, 83-95.

251

252 Godinho, K.S., Keane, S.G., Nanjiani, I.A., Benchaoui, H.A., Sunderland, S.J., Jones,
253 M.A., Weatherley, A.J., Gootz, T.D., Rowan, T.G., 2005a. Minimum inhibitory
254 concentrations of tulathromycin against respiratory bacterial pathogens isolated from
255 clinical cases in European cattle and swine and variability arising from changes in In
256 vitro methodology. *Vet. Ther.*, 6, 113-121.

257

258 Godinho, K.S., Rae, A., Windsor, G.D., Tilt, N., Rowan, T.G., Sunderland, S.J.,
259 2005b. Efficacy of tulathromycin in the treatment of bovine respiratory disease
260 associated with induced *Mycoplasma bovis* infections in young dairy calves. *Vet.*
261 *Ther.*, 6, 96-112.

262

263 Kaspar, H., Schröer, U., Wallmann, J., 2007. Quantitative resistance level (MIC) of
264 *Pasteurella multocida* isolated from pigs between 2004 and 2006: National Resistance
265 Monitoring by the BVL. *Berl. Münch. Tierärztl. Wochenschr.*, 120, 442-451.

266

267 McKelvie, J., Morgan, J.H., Nanjiani, I.A., Sherington, J., Rowan, T.G., Sunderland,
268 S.J., 2005. Evaluation of tulathromycin for the treatment of pneumonia following
269 experimental infection of swine with *Mycoplasma hyopneumoniae*. *Vet. Ther.*, 6,
270 197-202.

271

272 Nanjiani, I.A., McKelvie, J., Benchaoui, H.A., Godinho, K.S., Sherington, J.,
273 Sunderland, S.J., Weatherley, A.J., Rowan, T.G., 2005. Evaluation of the therapeutic

- 274 activity of tulathromycin against swine respiratory disease on farms in Europe. *Vet.*
275 *Ther.*, 6, 203-213.
- 276
- 277 Pruul, H., McDonald, P.J., 1992. Potentiation of antibacterial activity of azithromycin and
278 other macrolides by normal human serum. *Antimicrob. Agents Chemother.*, 36, 10-16.
- 279
- 280 Reese, C.P., Norcia, L.J., Skogerboe, T.L., 2004. Time killing kinetics and impact of culture
281 conditions (pH, CO₂ and serum) on MIC values of tulathromycin against *Haemophilus*
282 *somnus*. *Proc. 23rd World Buiatrics Congr.*, pp. 70-71.
- 283
- 284 Tanner, A.C., Wu, C-C., 1992. Adaptation of the Sensititre[®] broth microdilution
285 technique to antimicrobial susceptibility testing of *Mycoplasma gallisepticum*. *Avian*
286 *Dis.*, 36, 714-717.
- 287
- 288 Wallmann, J., 2006. Monitoring of antimicrobial resistance in pathogenic bacteria
289 from livestock animals. *Int. J. Med. Microbiol.*, 296, 81-86.

Table 1. Distribution and frequency of minimum inhibitory concentrations (MICs) for tulathromycin against bovine and porcine respiratory pathogens isolated from Europe during 2004-2006.

MIC ($\mu\text{g/mL}$)	Bovine isolates				Porcine isolates		
	<i>Mannheimia</i> <i>haemolytica</i>	<i>Pasteurella</i> <i>multocida</i>	<i>Histophilus</i> <i>somni</i>	<i>Mycoplasma</i> <i>bovis</i>	<i>Actinobacillus</i> <i>pleuropneumoniae</i>	<i>Pasteurella</i> <i>multocida</i>	<i>Mycoplasma</i> <i>hyopneumoniae</i>
≤ 0.004	-	-	-	-	-	-	1 (4%)
0.008	-	-	-	-	-	-	5 (19%)
0.015	-	-	-	-	-	-	7 (26%)
0.03	-	-	-	-	-	-	6 (22%)
0.06	-	-	-	-	-	-	4 (15%)
0.125	-	-	-	1 (2%)	-	-	4 (15%)
0.25	2 (6%)	2 (5%)	-	3 (5%)	-	2 (4%)	-
0.5	2 (6%)	8 (41%)	-	11 (17%)	-	19 (36%)	-
1	13 (39%)	20 (45%)	1 (3%)	8 (13%)	-	28 (53%)	-
2	15 (45%)	4 (9%)	18 (60%)	6 (10%)	-	4 (8%)	-
4	1 (3%)	-	11 (37%)	2 (3%)	-	-	-
8	-	-	-	-	8 (15%)	-	-
16	-	-	-	2 (3%)	44 (83%)	-	-
32	-	-	-	-	1 (2%)	-	-
64	-	-	-	-	-	-	-
>64	-	-	-	30 (48%)	-	-	-
Total No. of Isolates	33 ^a	44	30	63	53	53 ^a	27
Interpretative breakpoints for broth microdilution ($\mu\text{g/mL}$)							
S	$\leq 16^\dagger$	$\leq 16^\dagger$	$\leq 16^\dagger$	-	$\leq 32^\ddagger$	$\leq 16^\ddagger$	-
I	32^\dagger	32^\dagger	32^\dagger	-	-	32^\ddagger	-
R	$\geq 64^\dagger$	$\geq 64^\dagger$	$\geq 64^\dagger$	-	$\geq 64^\ddagger$	$\geq 64^\ddagger$	-

^aPercentages may not add to 100 due to rounding. [†]Standards approved by the CLSI subcommittee on veterinary antimicrobial susceptibility testing 2006.

[‡]Recommended standards, not currently approved by the CLSI. S = Susceptible, I = Intermediate, R = Resistant.

Table 2. Comparison of sensitivity results for tulathromycin determined for European bovine and porcine isolates collected during 1998-2001 with those collected during 2004-2006.

	Bovine isolates							
	<i>Mannheimia haemolytica</i>		<i>Pasteurella multocida</i>		<i>Histophilus somni</i>		<i>Mycoplasma bovis</i>	
	2004-2006	Pre-2002 [†]	2004-2006	Pre-2002 [†]	2004-2006	Pre-2002 [†]	2004-2006	Pre-2002 [‡]
	<i>n</i> =33	<i>n</i> =79	<i>n</i> =44	<i>n</i> =88	<i>n</i> =30	<i>n</i> =53	<i>n</i> =63	<i>n</i> =53
MIC ₅₀	1 µg/mL	1 µg/mL	1 µg/mL	0.5 µg/mL	2 µg/mL	2 µg/mL	4 µg/mL	0.25 µg/mL
MIC ₉₀	2 µg/mL	2 µg/mL	1 µg/mL	1 µg/mL	4 µg/mL	4 µg/mL	>64 µg/mL	>64 µg/mL
MIC range	0.25 – 4 µg/mL	0.25 – 4 µg/mL	0.25 – 2 µg/mL	0.25 - 2 µg/mL	1-4 µg/mL	1-4 µg/mL	0.125 - >64 µg/mL	≤0.063 - >64 µg/mL
	Porcine isolates							
	<i>Actinobacillus pleuropneumoniae</i>		<i>Pasteurella multocida</i>		<i>Mycoplasma hyopneumoniae</i>			
	2004-2006	Pre-2002 [†]	2004-2006	Pre-2002 [†]	2004-2006	Pre-2002 [§]		
	<i>n</i> =53	<i>n</i> =54	<i>n</i> =53	<i>n</i> =65	<i>n</i> =27	<i>n</i> =58		
MIC ₅₀	16 µg/mL	8 µg/mL	1 µg/mL	0.5 µg/mL	0.03 µg/mL	0.05 µg/mL		
MIC ₉₀	16 µg/mL	16 µg/mL	1 µg/mL	2 µg/mL	0.06 µg/mL	0.05 µg/mL		
MIC range	8 – 32 µg/mL	4 – 16 µg/mL	0.25 - 2 µg/mL	0.25 – 2 µg/mL	≤0.004 – 0.125 µg/mL	0.05 – 0.4 µg/mL		

[†] Godinho et al., 2005a. [‡] Godinho et al., 2005b. [§] McKelvie et al., 2005; Nanjiani et al., 2005.