Paper

Survey of susceptibility to marbofloxacin in bacteria isolated from diseased pigs in Europe

F. El Garch, S. Kroemer, D. Galland, I. Morrissey, F. Woehrle

A monitoring programme of marbofloxacin susceptibility of bacteria from Europe causing respiratory tract infection and meningitis in pigs has been active since 1994 and 2002, respectively. Monitoring digestive, metritis and urinary tract infection (UTI) in pigs has been active since 2005 and susceptibility results until 2013 are presented. Minimum inhibitory concentration (MIC) was determined by broth microdilution. For MIC interpretation, Vétoquinol-evaluated breakpoints were applied. For digestive pathogens, *Escherichia coli* and *Salmonella* species (1717 and 300 isolates, respectively) exhibited 7.5 per cent resistance in *E coli* and no resistance in *Salmonella* species. Similarly, *E coli* from metritis (369 isolates) had 7.0 per cent resistance to marbofloxacin. However, *E coli* from UTI (633 isolates) had higher resistance (10.4 per cent). For *Streptococcus suis* causing meningitis (585 isolates), marbofloxacin susceptibility was very high with only 0.5 per cent resistance and 0.4 per cent resistance was observed with *S suis* causing respiratory disease (729 isolates). Other respiratory pathogens were also highly susceptible to marbofloxacin with no resistance in *Actinobacillus pleuropneumoniae* (647 isolates) or *Bordetella bronchiseptica* (504 isolates), 0.1 per cent resistance in *Pasteurella multocida* (1373 isolates) and 1.4 per cent resistance in *Haemophilus parasuis* (145 isolates). There was no apparent change in marbofloxacin MIC over time for any bacterial pathogen based on $MIC_{50/90}$. These data confirm previously published MIC results from porcine and other animal infections.

Antimicrobial chemotherapy in both human and veterinary medicine, unlike most other disease therapies, is subject to resistance development in target pathogens. The rate of resistance development can be quite variable not only between antimicrobial agents, but also target pathogens, infection types and animal host. As recommended in various guidelines for prudent use of antimicrobials, the choice of an adequate antimicrobial should rely on individual antimicrobial susceptibility testing when possible or at least on relevant epidemiological data (European Commission Guidelines for prudent use of antimicrobials 2015-C299-04).

It is essential, therefore, that regular surveillance of antimicrobial susceptibility is undertaken to track any changes in susceptibility. This is particularly important for veterinary pathogens because of the potential to spread antimicrobial resistance to human via zoonotic bacteria and of special interest for critical agents, such as fluoroquinolones. Moreover, it is essential to update regularly the susceptibility epidemiological data on target pathogens in order to support further adaptations of treatment and development of improved dosing regimens.

There have been a number of veterinary resistance surveillance programmes initiated in Europe over recent years, such as those coordinated by the European Animal Health Study Centre (Centre Européen d'Etudes pour la Santé Animale (CEESA)) as described by De Jong and others (2013) and various national programmes in Germany (GERMAP 2012), the Netherlands (MARAN 2016), Norway (NORM/NORM-VET 2013), Sweden (SVARM 2015), France (RESAPATH 2015) and the UK (VARSS 2015).

Older first-generation quinolones (such as oxolinic acid and flumequine) have been licensed for use in food animals since the early 1980s and the first fluoroquinolone (enrofloxacin) during the late 1980s and early 1990s. Since then additional fluoroquinolone molecules have been authorised and a number of different veterinary medicines have become available (Committee For Medicinal Products For Veterinary Use (CVMP) 2007).

Fluoroquinolones are highly potent bactericidal substances that are well absorbed after oral administration and have a long elimination half-life with widespread distribution throughout the body. This makes fluoroquinolones attractive to be used in herd treatment of food-producing animals. Fluoroquinolones are effective in the treatment of serious infections like septicaemia, gastroenteritis and respiratory diseases caused by susceptible Gram-negative bacteria. They are also used to treat urinary tract and skin/soft-tissue infections caused by Gram-negative or some Gram-positive aerobic bacteria. They are effective for the treatment of *Mycoplasma* infections and, due to their ability to penetrate phagocytes, they have potential for the treatment of infections caused by atypical bacteria such as mycobacteria, *Chlamydia* species, *Ehrlichia* species or *Ricketsia* species (Wolfson and Hooper 1989).

Data from the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) programme indicate that fluoroquinolones are used in both food-producing animals and companion animals (ESVAC 2016).

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Marbofloxacin is a third-generation fluoroquinolone with a broad spectrum of activity against veterinary bacterial pathogens. Since 1994 Vétoquinol S.A. has run a surveillance programme in Europe to monitor the marbofloxacin susceptibility of bacteria from bovine, feline, canine and porcine diseases. Some initial reports for marbofloxacin have been published for pathogens from bovine infections (Meunier and others 2004a, Kroemer and others 2012) and infections from cats and dogs (Meunier and others 2004b, Kroemer and others 2014). In this study, the authors present susceptibility data for marbofloxacin against pathogens isolated from pigs in Europe between 1994 and 2013.

Materials and methods

Isolate collection

Veterinary surgeons or veterinary laboratories willing to participate in an antibiotic resistance monitoring programme were chosen to collect clinical samples from acutely sick pigs in eight European countries. Infections included respiratory, digestive, urinary, meningitis and metritis (Table 1).

The number of participating veterinary surgeons (vets) or laboratories (labs) varied slightly from year to year but the range of participants was as follows: France (3–4 vets and 3–4 labs), the Netherlands (1–2 vets and 1–2 labs), Belgium (1–3 vets and 1–3 labs), the UK (1 vet and 1–3 labs), Ireland (1–2 vets and 1 lab), Germany (1–2 vets and 1–4 labs), Italy (1–2 vets and 1 lab) and Spain (1–2 labs).

Samples were restricted to those from diseased animals before any antibiotic treatment (minimum three-week period between last antibiotic treatment and sampling) only. Samples were allowed from recently deceased animals (within 12 hours of death), with only one sample from any single farm to avoid testing epidemiologically related strains.

Isolated bacteria and case report forms including animal, clinical and sampling condition data were provided when available to the Vétoquinol central microbiology laboratory.

Isolation and identification of bacterial strains

Sample cultures were performed following standard methods. Identification of the bacterial isolates was performed by determining the following phenotypic characteristics: Gram-stained cell morphology, colony morphology and haemolysis on Columbia agar supplemented with 5 per cent defibrinated sheep blood (bioMérieux), catalase activity for Gram-positive cocci and oxidase activity for Gram-negative bacilli. Afterwards, the isolates were identified to species level by using API biochemical identification systems (bioMérieux). Any strains raising doubts about their identification at the central laboratory were identified by 16S rRNA sequencing (Thermo Fisher Scientific). Moreover, identification confirmation by 16S rRNA sequencing was performed randomly on 20 per cent of the collection each year as a quality control check. A total of 7002 isolates were sampled from eight European countries (Table 1).

Marbofloxacin minimum inhibitory concentration determination

The in vitro activity of marbofloxacin was determined by the standard microdilution broth method according to the Clinical and Laboratory Standards Institute guidelines (CLSI 2013a and preceding standards). Cation-adjusted Mueller Hinton broth (bioMérieux) was used as the test medium for *Enterobacteriaceae* (*Escherichia coli* and *Salmonella* species), *Bordetella bronchiseptica* and *Pasteurella multocida*, which was supplemented with 5 per cent sterile horse blood (bioMérieux) to test the susceptibility of *Streptococcus suis* and *P multocida* which failed to grow in cation-adjusted Mueller Hinton broth. *Actinobacillus pleuropneumoniae* and *Haemophilus parasuis* were tested in veterinary fastidious broth medium or in some cases when bacterial growth was weak in chocolate Mueller Hinton agar. Minimum inhibitory concentration (MIC) determinations were performed with 96-well microplates containing freeze-dried marbofloxacin solutions (Trek Diagnostic Systems) by the Vétoquinol central laboratory to ensure the consistency of susceptibility results. Using a Mac Farland standard, direct colony suspensions were used to inoculate microplates in order to obtain a final bacterial concentration of 10^5–10^6 CFU/ml in each well. Microplates were incubated for 18–24 hours at 35±2°C for *Enterobacteriaceae*, *B bronchiseptica, P multocida* and streptococci and at 35±2°C with 5 per cent CO_2 for *A pleuropneumoniae* and *H parasuis* isolates.

Staphylococcus aureus ATCC29213 (MIC range: 0.12–0.5 µg/ml), *E coli* ATCC25922 (MIC range: 0.008–0.03 µg/ml) and *A pleuropneumoniae* ATCC27090 (MIC range: 0.015–0.06 µg/ml) were used as reference strains for MIC quality control (CLSI 2013b and preceding standards).

Marbofloxacin MIC interpretation

Breakpoints for marbofloxacin have been established by Vétoquinol and validated for the aerobic pathogenic Gram-positive or Gram-negative bacteria isolated from cattle, pigs and pets, following Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI 2008)—although official marbofloxacin breakpoints are not published by CLSI. However, these breakpoints are accepted by the French Society of Microbiology (CA-SFM 2013). Marbofloxacin-resistant strains were determined as having a marbofloxacin MIC≥4 µg/ml, strains that had a marbofloxacin MIC=2 µg/ml were considered as intermediate and susceptible strains had a marbofloxacin MIC≤1 µg/ml. These breakpoints have been used previously in the analysis of marbofloxacin susceptibility in cattle (Meunier and others 2004a, Kroemer and others 2012) and cats and dogs (Meunier and others 2004b, Kroemer and others 2014) and also by the French monitoring programme on antibioresistance in animals (RESAPATH 2015).

Statistical analysis

Statistical analysis was performed to determine if trends in variation of bacterial susceptibility within the study period were significant, as described by Meunier (Meunier and others 2004a). A linear regression analysis of time versus difference between the MIC_{90} and the MIC_{50} (MIC_{90}–MIC_{50}) was performed for each bacterial species using SYSTAT software where N was ≥20 isolates. The following hypotheses were tested: H0: a=0 and H1: a≠0, with a as the slope. If H0 was not rejected, it can be concluded that there was no trend for a variation of Y (MIC_{90}–MIC_{50}) according to X (years).

Results

Distributions of marbofloxacin MIC and per cent of susceptible, intermediate and resistant for bacterial strains isolated from porcine digestive, meningitis, metritis and urinary pathologies are presented in Table 2, and a summary MIC data for these pathologies is presented in Table 3. These data for respiratory disease are given in Tables 4 and 5, respectively. MIC data for the reference strains were within the expected ranges (data not shown).

Digestive pathology

E coli

Table 2 shows a heterogeneous MIC distribution for marbofloxacin against *E coli* causing digestive disease. The majority of isolates belong to a highly susceptible population (MIC ≤0.06 µg/ml) and a moderately susceptible population (0.12–1 µg/ml). For the combined collection of *E coli* strains between 2005 and 2013, 92.3 per cent were susceptible to marbofloxacin. With the exception of 2011 (MIC_{90} of 8 µg/ml), the susceptibility of marbofloxacin in *E coli* from digestive disease remained unchanged between 2005 and 2013 with an MIC_{90} of 0.5 or 1 µg/ml (Table 2). No significant change of MIC_{50} and MIC_{90} was observed over the reported period (Tables 2 and 6). When comparing susceptibility data from individual countries (where N ≥20), there was generally similar marbofloxacin susceptibility in each country, but lower susceptibility for *E coli* from the UK and Ireland was observed (Fig 1).

TABLE 1: Bacterial strains isolated from porcine digestive, meningitis, metritis, respiratory and urinary pathologies in Europe

Pathology	Organism	All	Germany	Belgium	Spain	France	UK	Ireland	Italy	Netherlands
Digestive	*E coli*	1717	322	335	0	700	160	72	94	34
	Salmonella species	300	82	10	0	89	34	62	13	10
Meningitis	*S suis*	585	108	31	0	190	49	45	8	154
Metritis	*E coli*	369	127	60	18	78	78	2	6	0
Respiratory	*A pleuropneumoniae*	647	46	80	1	338	47	78	9	48
	B bronchiseptica	504	118	25	48	257	4	0	2	50
	H parasuis	145	43	0	0	86	2	0	0	14
	P multocida	1373	365	58	15	580	142	109	27	77
	S suis	729	177	70	8	364	364	42	4	64
Urinary	*E coli*	633	15	167	52	395	395	1	3	0
Total		7002	1404	836	1147	142	3077	411	166	451

Salmonella species

The marbofloxacin MIC distribution for *Salmonella* species causing digestive disease had a strong mode MIC at 0.03 µg/ml but some isolates with higher MICs were observed. Nevertheless, 100 per cent of the 2005–2013 collection were susceptible to marbofloxacin (Table 2) and MIC_{90} ranged between 0.06 and 0.25 µg/ml (Table 2). No trend for increasing MIC over the course of the study was observed (Tables 3 and 6).

Meningitis pathology

S suis

A unimodal MIC distribution was observed for marbofloxacin against *S suis* causing meningitis (Table 2). The majority of isolates were of a moderately susceptible population (0.25–1 µg/ml) with 0.5 per cent resistant. For the combined collection of *S suis* strains between 2002 and 2013, 97.1 per cent were susceptible to marbofloxacin (Table 2). Marbofloxacin-resistant isolates were only observed in three years of the study—2006, 2007 and 2010—and marbofloxacin MIC_{90} was consistently 1 µg/ml for each year (Table 3). MIC_{50} and MIC_{90} suggest that there was not any significant change in *S suis* susceptibility from 2002 to 2013 (Tables 3 and 6). There was >95 per cent susceptibility to marbofloxacin for isolates from all countries except Germany (92.6 per cent)—data not shown.

Metritis pathology

E coli

A similar heterogeneous marbofloxacin MIC distribution was observed for *E coli* causing metritis as that seen for digestive pathology (Table 2). For the combined collection of *E coli* strains between 2005 and 2013, 92.7 per cent were susceptible and 7.0 per cent resistant to marbofloxacin. Between 2005 and 2010 marbofloxacin MIC_{90} ranged from 0.25 to 1 µg/ml (Table 4). For 2011 and 2013 isolate numbers were too low to calculate MIC_{90} but data from 2012 showed a reduced MIC_{90} of 0.06 µg/ml (Table 3). Statistical analysis shows no trend of decreasing susceptibility to marbofloxacin (Tables 3 and 6). Comparing susceptibility data from individual countries (where N≥20), there was generally similar marbofloxacin susceptibility in each country, but lower susceptibility for *E coli* from Germany was observed (Fig 1).

Urinary pathology

E coli

As observed for digestive and metritis pathologies, a heterogeneous marbofloxacin MIC distribution was seen for *E coli* causing urinary pathology (Table 2). For the combined collection of *E coli* strains between 2005 and 2013, 89.4 per cent were susceptible, 0.2 per cent intermediate and 10.4 per cent resistant to marbofloxacin. Between 2005 and 2013 marbofloxacin MIC_{90} was quite variable. In 2006, 2007 and 2011–2013 marbofloxacin MIC_{90} ranged from 0.25 to 1 µg/ml, but was 2 µg/ml in 2009 and 8 µg/ml in 2005, 2008 and 2010 (Table 3). Nevertheless, statistical analysis did not suggest a global increasing trend over the whole period (Tables 3 and 6). Comparing susceptibility data from individual countries (where N≥20), there was generally similar marbofloxacin susceptibility in each country, but lower susceptibility for *E coli* from Spain was observed (Fig 1).

Respiratory pathology

A pleuropneumoniae

The marbofloxacin MIC distribution was also unimodal for *A pleuropneumoniae* causing respiratory disease (Table 4). For the combined collection of *A pleuropneumoniae* strains between 1994 and 2013, 99.4 per cent were susceptible and 0.6 per cent intermediate to marbofloxacin. The MIC_{90} for marbofloxacin ranged from 0.03 to 0.12 µg/ml in each year apart from 2006 where the MIC_{90} was 1 µg/ml (Table 5) but MIC ranges suggested there was not any significant change in *A pleuropneumoniae* susceptibility over the course of the study (Table 6). There was >95 per cent susceptibility to marbofloxacin for isolates from all countries—data not shown.

B bronchiseptica

As with other pathogens, the MIC distribution for marbofloxacin against *B bronchiseptica* causing respiratory disease was unimodal (Table 4). For the combined collection of *B bronchiseptica* strains between 2000 and 2013, 97.6 per cent were susceptible and 2.4 per cent intermediate to marbofloxacin. The MIC_{90} for

TABLE 2: Distributions of marbofloxacin minimum inhibitory concentration (MIC) (µg/ml) and % of resistance for bacterial strains isolated from porcine digestive, meningitis, metritis and urinary pathologies in Europe

						Distribution (numbers of isolates) of marbofloxacin MIC:															
Pathology	Organism	N	%S	%I	%R	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256
Digestive	*E coli*	1717	92.3	0.2	7.5	2	107	984	153	36	93	153	57	4	11	49	52	14	2		
	Salmonella species	300	100.0	0.0	0.0			211	61	13	3	6	5								
Meningitis	*S suis*	585	97.3	2.4	0.5				2	2	50	370	145	13	1		2				
Metritis	*E coli*	369	92.7	0.3	7.0	1	31	240	24	1	6	31	8	1	3	17	6				
Urinary	*E coli*	633	89.4	0.2	10.4	2	65	308	62	16	38	60	15	1	5	30	21	10			

Data from 2005 to 2013 combined, except for meningitis where data are from 2002 to 2013 combined
Vertical lines indicate susceptibility (≤1 µg/ml) and resistance (≥4 µg/ml) breakpoints
N, number of isolated strains

TABLE 3: Summary minimum inhibitory concentration (MIC) data for marbofloxacin (μg/ml) calculated for bacterial strains isolated from porcine digestive, meningitis, metritis and urinary pathologies in Europe

Pathology	Organism	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	Total
Digestive	*E coli*	No data before 2005												
	MIC_{50}				0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
	MIC_{90}				0.5	1	1	1	0.5	1	8	1	0.5	1
	MIC_{MIN}				0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015
	MIC_{MAX}				32	16	32	16	32	64	32	32	64	64
	N				141	191	208	209	232	243	199	181	113	1717
	Salmonella species													
	MIC_{50}				–	0.06	0.03	0.03	0.03	0.06	0.03	–	0.03	0.03
	MIC_{90}				–	0.25	0.06	0.06	0.06	0.12	0.12	–	0.06	0.06
	MIC_{MIN}				0.03	0.03	0.03	0.015	0.03	0.03	0.03	0.03	0.03	0.03
	MIC_{MAX}				0.5	1	1	1	0.12	0.5	1	0.25	1	1
	N				10	35	47	50	42	47	28	17	24	300
Meningitis	*S suis*													
	MIC_{50}	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1	0.5	0.5	0.5
	MIC_{90}	1	1	1	1	1	1	1	1	1	1	1	1	1
	MIC_{MIN}	0.25	0.25	0.12	0.06	0.25	0.25	0.25	0.25	0.5	0.25	0.25	0.5	0.06
	MIC_{MAX}	1	1	2	2	16	4	1	2	16	1	1	1	16
	N	38	67	56	76	54	53	65	42	46	32	32	24	585
Metritis	*E coli*	No data before 2005												
	MIC_{50}				0.03	0.03	0.03	0.03	0.03	0.03	–	0.03	–	0.03
	MIC_{90}				0.5	1	1	0.25	0.5	1	–	0.06	–	0.5
	MIC_{MIN}				0.015	0.008	0.015	0.015	0.03	0.015	0.015	0.015	0.03	0.015
	MIC_{MAX}				0.5	16	16	8	8	16	0.5	4	16	16
	N				25	49	56	44	75	72	14	23	11	369
Urinary	*E coli*													
	MIC_{50}				0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
	MIC_{90}				8	0.5	0.25	8	2	8	0.5	1	0.5	4
	MIC_{MIN}				0.015	0.015	0.008	0.015	0.03	0.015	0.015	0.015	0.015	0.015
	MIC_{MAX}				16	32	32	32	32	32	32	32	8	32
	N				64	63	63	52	45	204	57	47	38	633

MIC_{50} and MIC_{90} have not been calculated when the number of strains is lower than 20
MIC_{MAX}, MIC maximal; MIC_{MIN}, MIC minimal; N, number of studied strains

marbofloxacin ranged from 0.5 to 2 μg/ml (Table 5) with no significant change over the time period investigated (Table 6). Comparing susceptibility data from individual countries (where N≥20), there was generally similar marbofloxacin susceptibility in each country, but lower susceptibility for *B bronchiseptica* from Spain was observed (Fig 1).

H parasuis

A bimodal marbofloxacin MIC distribution was observed for *H parasuis* causing respiratory disease (Table 4). The majority of isolates belong to a highly susceptible population (MIC≤0.06 μg/ml) and a moderately susceptible population (0.12–1 μg/ml). For the combined collection of *H parasuis* strains between 1999 and 2013, 97.2 per cent were susceptible, 1.4 per cent intermediate and 1.4 per cent resistant to marbofloxacin. Isolate numbers were too low in each individual period to analyse differences between the years (Table 5). There was >95 per cent susceptibility to marbofloxacin for isolates from separate countries, except Germany (93.0 per cent)—data not shown.

P multocida

A heterogeneous marbofloxacin MIC distribution was observed for *P multocida* causing respiratory disease (Table 4). For the combined collection of *P multocida* strains between 1994 and 2013, 99.6 per cent were susceptible, 0.3 per cent intermediate and 0.1 per cent resistant to marbofloxacin. Between 1999 and 2013 marbofloxacin MIC_{90} was 0.03–0.12 μg/ml in most years but higher at 0.25 in 2010 and 0.5 μg/ml in 1999 (Table 5) with no trend of decreasing susceptibility over the survey period (Table 6). There was >95 per cent susceptibility to marbofloxacin for isolates from separate countries—data not shown.

S suis

As observed for meningitis a unimodal MIC distribution was seen for marbofloxacin against *S suis* causing respiratory disease (Table 4). For the combined collection of *S suis* strains between 1994 and 2013, 97.9 per cent were susceptible, 1.6 per cent intermediate and 0.4 per cent resistant to marbofloxacin. Marbofloxacin-resistant isolates were only observed in three years of the study—2004, 2006 and 2008—and marbofloxacin

TABLE 4: Distributions of marbofloxacin minimum inhibitory concentration (MIC) (μg/ml) and of resistance for bacterial strains isolated from porcine respiratory pathologies in Europe (years 1994–2013 combined)

					Distribution (numbers of isolates) of marbofloxacin MIC (μg/ml)														
Organism	N	%S	%I	%R	≤0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32
A pleuropneumoniae	647	99.4	0.6	0.0			3	210	323	59	11	16	11	10	4				
B bronchiseptica	504	97.6	2.4	0.0						2	4	145	317	24	12				
H parasuis	145	97.2	1.4	1.4	2	4	26	49	28	8	6	2	6	10	2	1	1		
P multocida	1373	99.6	0.3	0.1		9	51	682	425	101	46	29	17	8	4		1		
S suis	729	97.9	1.6	0.4					3	1	12	58	452	188	12		1	1	1

Data from 1994 to 2013 combined.
Vertical lines indicate susceptibility (≤1 μg/ml) and resistance (≥4 μg/ml) breakpoints
N, number of isolated strains

TABLE 5: Summary minimum inhibitory concentration (MIC) data for marbofloxacin (μg/ml) calculated for bacterial strains isolated from respiratory pathologies in Europe.

Organism	1994	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	Total
A pleuropneumoniae		No data in 1997																	
MIC_{50}	–		–	0.015	0.015	–	0.015	0.03	0.03	0.015	0.03	0.03	0.03	0.03	0.03	0.03	0.03	–	0.03
MIC_{90}	–		–	0.03	0.03	–	0.03	0.03	0.03	0.03	1	0.03	0.12	0.12	0.06	0.06	0.06	–	0.06
MIC_{MIN}	0.015		0.015	0.015	0.015	0.015	0.008	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.03	0.015	0.008	0.03	0.015
MIC_{MAX}	0.03		0.12	0.03	2	2	0.5	0.06	2	1	1	0.5	2	1	0.5	1	0.5	0.06	2
N	10		2	24	25	15	39	55	57	67	49	72	62	43	44	26	40	17	647
B bronchiseptica			No data 1997–1999																
MIC_{50}				0.25	–	–	0.5	0.25	0.25	0.5	–	–	0.5	0.5	0.5	0.5	–	–	0.5
MIC_{90}				1	–	–	1	0.5	0.5	0.5	–	–	0.5	2	0.5	0.5	–	–	0.5
MIC_{MIN}				0.25	0.25	0.25	0.25	0.25	0.12	0.25	0.06	0.25	0.25	0.25	0.12	0.25	0.25	0.25	0.12
MIC_{MAX}				1	1	2	1	0.5	2	2	0.5	0.5	1	2	1	1	1	1	2
N				23	12	6	23	23	34	29	17	18	146	81	39	28	18	7	504
H parasuis		No data before 1999																	
MIC_{50}				–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.015
MIC_{90}				–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.5
MIC_{MIN}				8	0.008	0.12	0.008	0.004	0.008	0.004	0.015	0.015	0.008	≤0.002	0.008	0.015	0.015	≤0.002	≤0.002
MIC_{MAX}				8	1	0.12	1	2	0.12	0.5	0.5	2	4	0.06	0.12	0.03	0.03	0.12	8
N				1	16	1	11	15	19	12	12	13	17	8	9	3	2	6	145
P multocida																			
MIC_{50}	–	–	–	0.015	0.03	0.03	0.03	0.03	0.03	0.03	0.015	0.03	0.015	0.015	0.015	0.015	0.015	0.015	0.015
MIC_{90}	–	–	–	0.5	0.06	0.06	0.06	0.12	0.12	0.06	0.06	0.06	0.03	0.03	0.25	0.03	0.03	0.03	0.06
MIC_{MIN}	0.015	0.015	0.015	0.004	0.015	0.008	0.004	0.008	0.015	0.008	0.004	0.008	0.004	0.008	0.008	0.008	0.008	0.008	0.008
MIC_{MAX}	0.25	1	0.06	2	0.12	1	1	0.5	1	0.5	2	0.5	2	8	0.5	0.06	0.25	0.12	8
N	18	10	12	31	33	22	52	89	131	139	103	136	168	130	91	86	82	40	1373
S suis			No data in 1998																
MIC_{50}	–	–		–	0.5	–	–	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
MIC_{90}	–	–		–	0.5	–	–	1	1	1	1	1	1	1	1	1	1	1	1
MIC_{MIN}	0.25	0.5		0.25	0.25	0.25	0.12	0.25	0.03	0.03	0.25	0.25	0.25	0.12	0.25	0.12	0.25	0.12	0.12
MIC_{MAX}	1	0.5		0.5	1	0.5	1	2	16	2	32	1	8	1	1	2	1	2	32
N	8	1		8	26	12	18	68	45	65	47	53	69	78	64	77	46	44	729

MIC_{50} and MIC_{90} have not been calculated when the number of strains is lower than 20
MIC_{MAX}, MIC maximal; MIC_{MIN}, MIC minimal; N, number of studied strains

TABLE 6: Determination of linear regression (Y=aX+b) of time versus MIC_{90}–MIC_{50}, Fisher's test (F-test) and probability (P=0.05) of each bacterial species studied

Pathology	Bacterial species	a	b	F-test	P value
Respiratory	*A pleuropneumoniae*	0.004	−7.993	0.04	0.837
	B bronchiseptica	−0.022	44.487	0.39	0.551
	P multocida	−0.011	21.642	2.21	0.161
	*S suis**	–	–	–	–
Meningitis	*S suis*	−0.012	25.026	1.03	0.334
Urinary	*E coli*	−0.467	940.698	0.98	0.354
Metritis	*E coli*	−0.068	137.452	1.08	0.347
Digestive	*E coli*	0.233	−467.185	0.53	0.490
	Salmonella species	−0.038	77.032	3.01	0.143

*Not determined as the difference MIC_{90}–MIC_{50} was stable since 2003
MIC, minimum inhibitory concentration

MIC_{90} was 1 μg/ml for each year except 2000, where the MIC_{90} was 0.5 μg/ml (Table 5). No significant change in MIC_{50} and MIC_{90} was observed over the reporting period (Table 6). There was >95 per cent susceptibility to marbofloxacin for isolates from separate countries—data not shown.

Discussion

This study evaluated the susceptibility of porcine bacterial isolates in Europe isolated from five separate pathologies: respiratory, digestive, urinary, meningitis and metritis. The analysis of seven bacterial pathogens indicated no evidence of marbofloxacin susceptibility reduction over the study periods investigated, including 20-year surveillance data for respiratory pathogens. Furthermore, susceptibility to marbofloxacin was 89.4 per cent or higher for all bacterial isolates from all pathologies. For *Salmonella* species (digestive), *P multocida* (respiratory) and *A pleuropneumoniae* (respiratory) more than 99 per cent were marbofloxacin susceptible. Overall there was little difference in marbofloxacin susceptibility by country, apart from a few minor exceptions.

A recently published study of VetPath data (a CEESA study) for respiratory isolates from pigs collected in 2002–2006 (De Jong and others 2014) showed marbofloxacin MIC_{50} and MIC_{90} values for *A pleuropneumoniae* (0.03 and 0.06 μg/ml, respectively), *P multocida* (0.03 and 0.06 μg/ml, respectively) and *S suis* (0.5 and 1 μg/ml, respectively), which are virtually identical to the data presented in this study.

The marbofloxacin data from the previous porcine study (de Jong and others 2014) and also from Resapath (2015) and GERMAP (2012) (including similar enrofloxacin and danofloxacin data) compare well with the data presented in this study and confirm the excellent activity of marbofloxacin against veterinary bacterial pathogens.

Virtually all bacterial pathogens were susceptible to marbofloxacin, but some variability in susceptibility was observed in *E coli* between pathologies. For example in this study, marbofloxacin resistance in *E coli* from porcine urinary pathologies was higher at 10.4 per cent than that for *E coli* from porcine digestive or metritis pathologies (7.5 per cent and 7.0 per cent, respectively). This difference may not have any particular clinical relevance but indicates the importance of continued surveillance of marbofloxacin susceptibility.

MIC for *E coli* isolated from digestive, metritis and urinary infections shows a trimodal distribution. The moderately susceptible population (0.12–1 μg/ml) can be considered as first-step mutants as the resistance to fluoroquinolone appears through stepwise accumulation of mutation in the genes coding for DNA gyrase and topoisomerase IV. This population requires a particular attention when defining a dosing protocol for *E coli* infection treatment. It is important to target this population since fluoroquinolones treatment of infections caused by first-step mutants can lead to the selection of resistant isolates and consequently in the treatment failure. The expected efficacy of the treatment should therefore be assessed based on a pharmacokinetics-pharmacodynamic (PKPD) approach taking into account the MIC of the mutants population and not limiting on MIC_{50} or MIC_{90} measured from the overall population.

Available data on MIC distribution provide important information on the variability of bacterial susceptibility in isolates from clinical cases. These data should be analysed alongside antimicrobial pharmacokinetic data, which can be variable from one animal to another. Vilalta and others (2014) calculated, from a

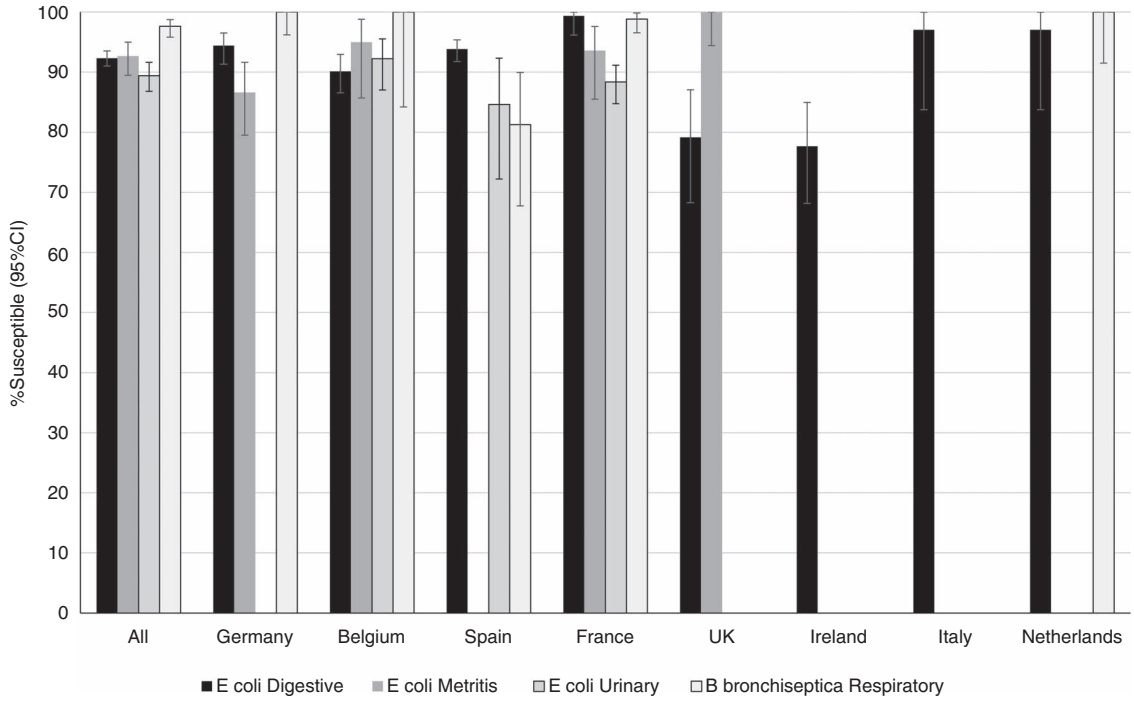

FIG 1: Percentage susceptibility to marbofloxacin by country and pathogen/infection. Data only shown where number of isolates per country was at least 20 and at least one country had susceptibility less than 90%

Monte Carlo simulation, the probability of PKPD target attainment taking into account the variability of drug exposure in pigs and the variability of MICs from respiratory bacterial isolates. This study also reported the cumulative fractions of response for several dosing schemes, meaning the proportion of animals in the population achieving a PKPD threshold values taking into account both the MIC distribution against the bacteria and the PK parameter distribution in the population.

Moreover, it should be noticed that fluoroquinolones are considered as antibiotic substances that are critically important in human medicine and therefore based on risk profiles for fluoroquinolones. CVMP (CVMP 2007) concluded, among other recommendations, that an appropriate level of risk mitigation would be to reserve them for the treatment of clinical conditions that have responded poorly, or are expected to respond poorly, to other antimicrobials. This recommendation is applicable in all European member states and has been implemented in legislation in some.

In conclusion, the data from this study indicate that marbofloxacin activity against bacterial isolates from pigs has remained constant over time and indicates a high marbofloxacin susceptibility rate in key porcine pathogens relevant to veterinary medicine. Although antimicrobial susceptibility testing is recommended as the optimal approach, in cases where empirical therapy is necessary these data indicate that porcine bacterial isolates from respiratory, digestive, metritis and urinary infection in Europe are very likely to be susceptible to marbofloxacin. Finally, the availability of these data is an important step towards further scientific work on PKPD determination of optimal dosing strategy.

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Notes