**In vitro** antimicrobial activity of marbofloxacin and enrofloxacin against bacterial strains isolated from companion animals

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**Summary**

Fluoroquinolones were originally developed for the Gram-negative aerobic spectrum, but the newer generation agents are also highly effective against some Gram-positive pathogens and cause few adverse effects. Owing to these characteristics, fluoroquinolones are often used in first line therapy in small animal practice. However, their widespread use has raised concern over emerging bacterial resistance. In this study we evaluated the *in vitro* efficacy of two fluoroquinolones, marbofloxacin and enrofloxacin, on field strains isolated from clinical infections between 2002 and 2005. Our data show that most of the isolates are still sensitive to both antimicrobials and marbofloxacin was more effective than enrofloxacin, especially against *P. aeruginosa* and β-Streptococci (*P < 0.01*). β-Streptococci demonstrated the greatest resistance to the two study drugs.

Keywords: marbofloxacin, enrofloxacin, antimicrobial sensitivity *in vitro*, dog, cat

**Introduction**

The emergence of antibiotic-resistant bacteria is a growing concern in both human and veterinary medicine. The prophylactic and therapeutic uses of these drugs (Prescott et al., 2002) are the known risk factors for selection of antibiotic-resistant strains. Considerable data exist concerning antimicrobial drug resistance in bacteria of food animal origin, and quantities of antimicrobial drug use in food animals, while useful data on antimicrobial drug use and resistance in pets is lacking (Schwarz et al., 1998; Van den Bogaard et al., 1999). The possible transfer of resistant bacteria from companion animals to humans has been drawing more attention to the issue of antimicrobial drug resistance originating from pets (Damborg et al., 2004; Heuer et al., 2005). Several scientific publications have reported the occurrence of some resistance genes in companion animals and humans, as well as the possible transfer of bacteria between companion animals and humans (Guardabassi et al., 2004; Rodriguez et al., 2004; Van Immerseel et al., 2004). However, most of the problems as regards resistance in human medicine are correlated to the use of antimicrobials in humans and the infections are predominantly caused by organisms unrelated to animals (EMEA, 2006). Fluoroquinolones represent a class of antimicrobials, which is very important in the treatment of severe infections in humans and animals. These drugs were ranked by the U.S. Food and Drug Administration as being critically important in human medicine and for this reason the presence of resistant bacteria is especially undesirable (Heuer et al., 2005).

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The resistance of *Staphylococcus aureus* and *Escherichia coli* to fluoroquinolones is limited in comparison with other classes of antimicrobials (McGowan, 1996). However, the incidence of resistance to fluoroquinolones is limited in comparison with other classes of antimicrobials (Goodmann and Gilman’s, 2001).

Created in 1990, enrofloxacin was the first fluoroquinolone developed exclusively for veterinary medicine, while marbofloxacin has been recently introduced in a number of countries for use in animals (Spreng, 1995). Pharmacokinetics and susceptibility data are generally used to compare different antimicrobial agents (Heinen, 2002). Enrofloxacin and marbofloxacin have a limited protein binding, 15–25% and 9% respectively (Petzinger, 1991). In the dog after oral administration the maximum serum concentration (C\text{max}) and the time to achieve C\text{max} (t\text{max}) are respectively 1.4–1.7 µg/ml and 1.7–2 hours for enrofloxacin (Walker et al., 1992; Frazier et al., 2000; Heinen 2002). For marbofloxacin C\text{max} is 1.4–2.5 µg/ml and t\text{max} is 1–2.5 hours (Schneider et al., 1996; Frazier et al., 2000; Heinen, 2002). The area under serum concentration – time curve from 0 to 24 hours (AUC\text{0–24}) is 8.74 µg · h/ml for enrofloxacin (Heinen, 2002) and 13–23 for marbofloxacin (Cester et al., 1996; Heinen, 2002).

After oral or parenteral administration bioavailability ranges from 62 to 100% for marbofloxacin (Marbofloxacin reference book, 1999) and 53% for enrofloxacin (Schneider et al., 1996). About 40% of enrofloxacin is further metabolized to ciprofloxacin and this active metabolite is then biotransformed into four or more additional compounds (Cester and Toutain, 1997). Marbofloxacin is eliminated essentially in the native form (Schneider et al., 1996; Frazier et al., 2000) and metabolites are formed in limited quantities, less than 5% of the administered dose (Marbofloxacin reference book, 1999). Both drugs are excreted in the urine and bile. In the dog, enrofloxacin has an elimination half-life of 2–5 hours (Schneider et al., 1996; Walker et al., 1992; Frazier et al., 2000; Heinen, 2002), marbofloxacin 9–12 hours (Schneider et al., 1996; Frazier et al., 2000; Heinen, 2002).

The purpose of this study was to evaluate the in vitro relative efficacy of these two fluoroquinolones on field strains isolated from clinical cases.

**Animals, Material and Methods**

Strains were isolated from 390 dogs and cats with clinical infections between January 2002 and December 2005 at the Veterinary Medicine Teaching Hospital in the Faculty of Veterinary Medicine in Grugliasco (Turin). Samples were collected from urine, tonsils, conjunctiva, skin, ear, bone, faeces, vagina, prostate and bronchial secretions by sterile swabs or sterile urine containers. The swabs were then placed directly into
transport tubes (Becton Dickinson Microbiology Systems Europe, France) containing Amies media and transported to the Bacteriology Laboratory within 8 h for processing. The swabs were plated onto Columbia agar and Colistin-Nalidixic Acid agar containing both 5% sheep’s blood and MacConkey agar (Oxoid GmbH, Wesel, Germany). The plates were incubated for up to 48 h at 37°C. The urine samples were obtained by cystocentesis and plated onto Trislide E (Oxoid GmbH, Wesel, Germany), a support with three solid agars (Colistine-Lactose-Electrolyte Deficient, MacConkey and Bile-Esculine). Bacterial isolates were identified according to standard laboratory practice by biochemical tests and/or a commercial identification system (BBL Crystal Enteric/Non-fermenter ID kit and Gram-Positive ID System, Becton Dickinson, Sparks, MD).

Susceptibilities to enrofloxacin (ENO 5 μg, Bayer, Germany) and marbofloxacin (MAR 5 μg, Vetoquinol, France) were tested by the disk diffusion method according the National Committee for Clinical Laboratory Standards (NCCLS, 1999) recommendations. Briefly, about 106 CFU of bacterial cells were inoculated onto Mueller-Hinton agar plates (90 mm in diameter), and antibiotic-containing discs (Oxoid GmbH, Wesel, Germany) were applied. The plates were incubated at 35 ± 1°C for 18 h. Interpretation was carried out according to the drug manufacturer’s instructions, and inhibition zone diameters were recorded and compared with breakpoint values (ENO: sensitivity ≥ 22 mm; intermediate 18–21 mm; resistance ≤ 17 mm; MAR: sensitivity ≥ 18 mm; intermediate 14–18 mm; resistance ≤ 14 mm) in order to classify the strains as sensitive or resistant to antimicrobials. For the purpose of our study, intermediate strains were considered as resistant. Four hundred and twenty strains were identified and of these, 44 Pseudomonas aeruginosa, 95 Escherichia coli, 84 Staphylococci (St. ausus, St. epidermidis, St. intermedius) and 118 β-Streptococci were used.

Significance testing of differences in proportions was performed using the χ² test and the comparison between two proportions test (Stanton A. Glanz, 1988). Differences were considered significant at P < 0.05.

Results

The agar diffusion method was used to evaluate ENO and MAR resistance in 341 isolates: 147 strains resulted sensitive to both study drugs, 6 were sensitive only to ENO, 71 were sensitive only to MAR, and 117 were resistant to both fluoroquinolonones. From 2002 to 2005, the rate of susceptibility of isolated strains was 45% to ENO and 65% to MAR. Sensitivity to ENO was nearly stable, whereas sensitivity to MAR decreased from 71% in 2003 to 58% in 2005 (Fig 1). In particular, the decrease in sensitivity of E. coli to MAR from 2002 to 2005 was statistically significant (P < 0.05). The in vitro efficacy of the two study drugs against Gram-positive and Gram-negative isolates was compared. MAR showed greater in vitro efficacy against Gram-positive (n = 202) and Gram-negative bacteria than ENO (P < 0.01). Gram-positive bacteria sensitivity to the study drugs (60% versus MAR and 44% versus ENO) was lower than that of Gram-negative bacteria (69% versus MAR and 47% versus ENO); these data were not statistically significant.

We compared the in vitro efficacy of MAR and ENO against different isolates of bacterial species (Tab 1; Fig 2). P. aeruginosa and β-Streptococci showed a significantly higher sensitivity to MAR than to ENO (P < 0.01). No statistically significant differences were found between MAR and ENO in sensitivity of E. coli and Staphylococci. The resistance of P. aeruginosa, E. coli and β-Streptococci increased from 2002 (P. aeruginosa 25%; E. coli 8%; β-Streptococci 50%) to 2005 (P. aeruginosa 33%; E. coli 42%; β-Streptococci 60%), whereas Staphylococci resistance declined (from 40% to 22%); these data were not statistically significant.
Discussion

In agreement with previous reports (Caprioli et al., 2000), we found disk diffusion a useful method to describe the level of bacterial resistance to fluoroquinolones. With a few notable exceptions (Schwarz et al., 1998; Cohn et al., 2003; Guardabassi et al., 2004; Van Immerseel et al., 2004), data on the development of drug resistance in companion animal bacteria are lacking. However, the resistance reported by diagnostic laboratories may be overestimated, since it often represents treatment failures rather than treatment successes, which do not usually reach the laboratory (Prescott et al., 2002). Our data show that sensitivity to fluoroquinolones remained relatively stable from 2002 to 2005, even though these antimicrobials are frequently used in veterinary clinical therapy. The sensitivity of bacteria was higher to marbofloxacin than to enrofloxacin, an advantage possibly linked to marbofloxacin intrinsic molecular characteristics. Weber et al. (2000) suggested that marbofloxacin and enrofloxacin may act on two different bacterial DNA isomerases, topoisomerases I-III and topoisomerases IV, respectively.

In agreement with previous studies (Goodman and Gilman’s, 2001), we found that Gram-negative bacteria were more sensitive to marbofloxacin and enrofloxacin than Gram-positive bacteria. In fact, while all fluoroquinolones accumulate within bacteria very rapidly, Gram-positive bacteria have an energy-dependent efflux transport system that pumps these antimicrobials out of the bacterial cell (Brown, 1996). Marbofloxacin resulted more effective than enrofloxacin against P. aeruginosa (68% to MAR, 30% to ENO) and β-Streptococci (53% to MAR, 33% to ENO). In accordance with previous studies (Brown, 1996), β-Streptococci demonstrated greater resistance to fluoroquinolones than the other bacterial species we examined. Some strains of E. coli and Staphylococci isolates were sensitive only to enrofloxacin.

In conclusion, our results indicate that most of the isolates collected between 2002 and 2005 are still sensitive to the two study drugs. Although marbofloxacin was generally more effective than enrofloxacin, a recent decline in the sensitivity of bacteria, specifically of E. coli, was observed. This decline may be explained by the increased use of these antimicrobials in veterinary clinical therapy.

Table 1. Sensitivity (%) of Pseudomonas aeruginosa, Escherichia coli, Staphylococci and β-Streptococci strains versus marbofloxacin (MAR) and enrofloxacin (ENO)

<table>
<thead>
<tr>
<th></th>
<th>Strains</th>
<th>Year</th>
<th>MAR (%)</th>
<th>ENO (%)</th>
<th></th>
<th>Year</th>
<th>MAR (%)</th>
<th>ENO (%)</th>
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<tbody>
<tr>
<td>E. coli</td>
<td>24</td>
<td>2002</td>
<td>92</td>
<td>58</td>
<td>Staphylococci</td>
<td>20</td>
<td>2002</td>
<td>60</td>
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<td></td>
<td>26</td>
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<td>61</td>
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<td>21</td>
<td>2004</td>
<td>67</td>
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<td>73</td>
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<td>50</td>
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<td>70</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>8</td>
<td>2002</td>
<td>75</td>
<td>37</td>
<td>β-Streptococci</td>
<td>36</td>
<td>2002</td>
<td>50</td>
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<tr>
<td></td>
<td>7</td>
<td>2003</td>
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<td>67</td>
<td>34</td>
<td></td>
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<td>2005</td>
<td>40</td>
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</tbody>
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Figure 2. Sensitivity (%) of E. coli, P. aeruginosa, Staphylococcus spp., β-Streptococci versus marbofloxacin (MAR) and enrofloxacin (ENO).
by an increased use of this antimicrobial, since, owing to selective pressure, resistance to any antimicrobial agent increases with the frequency of use (McGowan, 1996). Our results confirm that fluoroquinolones resistance has not yet reached the crisis stage in small animals practice. Even so, these are early warning signs that more information is needed, along with a more careful use of antimicrobial agents. Antibiotics should be used only when necessary, for as short a time as possible with optimal dosage and possibly guided by tests of in vitro sensitivity to reduce the selection for resistance strains. Bearing this in mind we suggest avoiding the use of fluoroquinolones as a first line therapy reserving these agents to infections where susceptibility to drugs has been demonstrated.

References

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