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Antibiotic susceptibility of bacteria isolated from infections in cats and dogs throughout Europe (2002–2009)

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ABSTRACT

A monitoring program of the pre-treatment susceptibility of clinical isolates of bacteria from diseased dogs and cats was active between the years 2002 and 2009. Susceptibility of each isolated strain to a panel of nine antibiotics (amoxicillin/clavulanic acid, ampicillin, penicillin, clindamycin, doxycycline, enrofloxacin, marbofloxacin, trimethoprim and trimethoprim/sulfamethoxazole) was assessed. The Minimum Inhibitory Concentration (MIC) of marbofloxacin was also determined by a standardized microdilution technique following CLSI recommendations. In total, 1857 bacterial strains were collected throughout Europe from cases of otitis, respiratory, urinary and dermatological infections. Although bacterial susceptibility varied for each of the antibiotics within the panel, patterns of susceptibility were similar to those described in the literature for comparable time periods and geographical areas. With a clinical resistance varying from 0 to 14.48% against the isolated strains, marbofloxacin susceptibility was very high and remains an effective antibiotic for the treatment of otitis, urinary, respiratory and dermatological infections in companion animals.

1. Introduction

It is widely accepted today that the intensive or inappropriate use of antimicrobial agents, such as antibiotics, in veterinary medicine may lead to an increased risk of bacterial resistance which may ultimately have a potential impact upon human health [1,2]. Maintaining the efficacy of antimicrobials has become a global public health concern and there are regular calls for recommendations for the prudent use of antibiotics in animal health [1,2]. Antibiotic therapies for veterinary diseases need to retain their efficacy in eliminating pathogens from an infected animal but should also prevent spread of these organisms to other animals and of resistance and eliminate the risk of transmission to humans.

Guidelines for the responsible use of antimicrobials have been developed by international organizations and veterinary associations [3,4] as well as at European level [1,2].

Regular reporting of bacterial sensitivity would facilitate better understanding of antimicrobial resistance and trends in this process over time to ensure long-term efficacy of the antibacterial products [5].

Such a monitoring program was implemented in 1994 by Vétoquinol S.A. throughout Europe in order to monitor the susceptibility to marbofloxacin of bacterial pathogens involved in swine, bovine, feline and canine diseases, prior to any therapeutic treatment. The data for 1994–2001

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has been published [6,7] and additional data for isolates obtained from bovine respiratory disease and mastitis between 2002 and 2009 are now available [8].

This article reports on the epidemiological survey carried out between 2002 and 2009 on the antibiotic susceptibility of common bacteria isolated from respiratory, dermatological (including otitis) and urinary infections in dogs and cats. The organisms monitored throughout the course of the study were: *Pasteurella multocida*, *Bordetella bronchiseptica*, *Pseudomonas aeruginosa*, *Staphylococcus* (*pseud*)*intermedius* (*S. intermedius* and/or *S. pseudintermedius*), *Staphylococcus aureus*, *Escherichia coli* and *Proteus mirabilis*.

Particular attention was paid to marbofloxacin throughout the study as it is part of the critically important class of antimicrobials, the fluoroquinolones. Marbofloxacin is a third generation fluoroquinolone, first marketed in Europe in 1995 and licensed for the treatment of bacterial infections affecting the urinary and respiratory tracts, skin, ear or soft tissues.

2. Materials and methods

2.1. Collection of bacterial strains

Bacterial isolates were collected from diseased cats and dogs throughout six European countries (France, Belgium, Germany, United Kingdom, Spain and the Netherlands) where marbofloxacin was licensed for use in these species. During the course of the epidemiological investigation, the total number of laboratories within the study area that contributed to the investigation varied between five and eight a year.

Bacterial isolates were recovered from collections of microbiology laboratories located in the targeted countries, where they were sampled by veterinary surgeons from sick animals as part of their daily practice. There was no direct contact between the survey network manager and veterinary investigators, in order to guarantee a random collection of microorganisms over the studied countries and pathologies.

All the samples included in the study were collected from patients prior to treatment in order to obtain an accurate field representation of the bacterial susceptibility. Isolates from animals which had received antibiotic therapy in the three weeks prior to sampling were excluded from the study.

2.2. Isolation and identification of bacterial strains

Bacterial isolates were identified using a standardized protocol. Preliminary characterization was based upon the following phenotypic characteristics: cellular morphology; Gram staining; colony morphology and haemolysis on Columbia agar supplemented with 5% defibrinated sheep blood (BioMérieux, Marcy l'Étoile, France). Gram-positive cocci were further characterized by catalase activity and Gram-negative bacilli were tested for oxidase activity. Species classification was then performed using API® biochemical identification systems (BioMérieux). Once bacterial isolates had been identified, the strains were stored on cryobeads (AES, Combourg, France) and shipped in dry ice at −80 °C to the Vétoquinol central laboratory in France.

As the laboratories enrolled in the study were unable to discriminate between *S. intermedius* and *S. pseudintermedius*, both species were given the designation "*S.* (*pseud*)*intermedius*" for the purposes of this study [9].

2.3. Antimicrobial susceptibility testing

The in vitro susceptibility of isolated strains was assessed by using a standardized agar disk diffusion method compliant with Clinical and Laboratory Standards Institute (CLSI) guidelines [10–12]. Mueller-Hinton agar (BioMérieux) was used as growth medium which was supplemented with 5% sterile defibrinated sheep blood (BioMérieux) to enable susceptibility testing for *Pasteurellaceae*. Agar plates were inoculated by streaking the agar surface with a swab that had been dipped into Mueller-Hinton broth cultures. Broth cultures have been incubated for 2–6 h and adjusted to 0.5 Mac Farland turbidity standard prior to being used as an inoculum. Antibiotic-impregnated paper disks (Bio-Rad, Marnes-la-Coquette; France) were placed on the agar surface; test plates were then incubated for 16–24 h at 35 ± 2 °C. Inhibition zone diameters were interpreted according to CLSI registered breakpoints [10–12]. All the susceptibility assays were performed by the Vétoquinol central laboratory to ensure the consistency of disk diffusion susceptibility results.

Isolate susceptibility data were obtained for the following antibiotics: amoxicillin/clavulanic acid (20 μg/10 μg), ampicillin (10 μg), penicillin (10 U), clindamycin (2 μg), doxycycline (30 μg), enrofloxacin (5 μg), marbofloxacin (5 μg), trimethoprim (5 μg) and trimethoprim/sulfamethoxazole (1.25 μg/23.75 μg).

2.4. Marbofloxacin MIC determination

Additional testing to determine the in vitro activity of marbofloxacin was also performed using a standardized microdilution broth method compliant with CLSI guidelines [10–12]. Mueller-Hinton broth (BioMérieux) was used as growth medium which was supplemented with 5% sterile horse serum (BioMérieux) to test the susceptibility of *Pasteurellaceae*. MIC values were determined by inoculating 96-well microplates which contained freeze-dried marbofloxacin solution (Trek diagnostic systems, Cleveland, USA) with a direct colony suspension. A final bacterial concentration of 10^5–10^6 CFU/mL was obtained in each well of microplates which were then incubated for 18–24 h at 35 ± 2 °C.

S. aureus ATCC29213 (MIC range: 0.12–0.5 μg/mL), *P. aeruginosa* ATCC27853 (MIC range: 0.5–2.0 μg/mL), and *E. coli* ATCC25922 (MIC range: 0.008–0.03 μg/mL) were used as reference strains for quality control purposes [10–12].

As with the antibacterial susceptibility tests previously described, all marbofloxacin MIC determinations were performed by the Vétoquinol central laboratory in order to ensure consistency of methodology and reporting.