Veterinary Microbiology 191 (2016) 44–51

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Antimicrobial susceptibility monitoring of bacterial pathogens isolated from respiratory tract infections in dogs and cats across Europe: ComPath results

Ian Morrissey[a], Hilde Moyaert[b,c], Anno de Jong[b,d,*], Farid El Garch[b,e], Ulrich Klein[b,f], Carolin Ludwig[b,d], Julien Thiry[b,g], Myriam Youala[b,h]

[a] IHMA Europe Sàrl, Monthey, Switzerland
[b] ComPath Study Group, CEESA, Brussels, Belgium
[c] Zoetis, Zaventem, Belgium
[d] Bayer Animal Health GmbH, Leverkusen, Germany
[e] Vétoquinol S.A., Lure, France
[f] Elanco Animal Health, Basel, Switzerland
[g] MSD Animal Health, Schwabenheim, Germany
[h] Virbac S.A., Carros, France

ARTICLE INFO

Article history:
Received 2 April 2016
Received in revised form 27 May 2016
Accepted 30 May 2016

Keywords:
Respiratory tract infection
Resistance
Antimicrobial susceptibility monitoring
Companion animals

ABSTRACT

ComPath is a pan-European resistance monitoring programme collecting bacterial pathogens from dogs and cats. We present data for respiratory tract infection (RTI) isolates collected between 2008 and 2010. Antimicrobial minimal inhibitory concentrations (MICs) were determined and susceptibility calculated following Clinical Laboratory Standards Institute (CLSI) standards for veterinary medicine. The main pathogen from dogs was *Staphylococcus intermedius* Group (49/215, 22.8%) which was >90% susceptible to most antimicrobials (including oxacillin – 93.9%; 3 isolates confirmed *mecA*-positive) but only 59.2%, 73.5% and 87.8% susceptible to tetracycline, chloramphenicol and penicillin. *Bordetella bronchiseptica* (48/215, 22.3%), streptococci (36/215, 16.7%), *Escherichia coli* (24/215, 11.2%) and *Pasteurella multocida* (23/215, 10.7%) were also found in dog RTI. There are no breakpoints for *Bordetella bronchiseptica*. Most streptococci were penicillin- chloramphenicol-, ampicillin- and pradofloxacin-susceptible. None were enrofloxacin-resistant but 6 isolates (16.7%) were of intermediate susceptibility. The least active agent against streptococci was tetracycline (47.2% susceptible). For *E. coli*, 37.5% were ampicillin-susceptible but 83.3% were amoxicillin/clavulanic acid-susceptible. Only chloramphenicol showed susceptibility >90% against *E. coli*, with 66.7% tetracycline-susceptible and 79.2% to 87.5% susceptibility to enrofloxacin, trimethoprim-sulfamethoxazole or pradofloxacin. *P. multocida* were susceptible to pradofloxacin (no other breakpoints are available). The main pathogen from cats was *P. multocida* (82/186, 44.1%), where only pradofloxacin has breakpoints (100% susceptible). Streptococci were also collected from cats (25/186, 13.4%) and were >90% susceptible to all antimicrobials except tetracycline (36% susceptible). Most susceptibility was calculated with human-derived breakpoints and some antimicrobials had no breakpoints. Therefore predictions of clinical utility for dog and cat RTI will remain problematical unless specific breakpoints are set.

1. Introduction

Surveillance of antimicrobial resistance plays an important role in both human and veterinary medicine to assist clinicians and veterinarians to make appropriate, often empirical, antibiotic choices. Although there are a number of national veterinary surveillance programmes in place in Europe (e.g., GE*RM*-Vet in Germany, the Swedish Veterinary Antimicrobial resistance Monitoring (SVARM) and RESAPATH in France), comparisons are difficult to make due to a lack of harmonisation and standardisation (Silley et al., 2011; de Jong et al., 2013). Particularly sampling conditions, methodology to determine the antimicrobial

* Corresponding author at: ComPath Study Group, CEESA, 168 Av de Tervueren, 1150 Brussels, Belgium.
E-mail address: anno.jong@bayer.com (A. de Jong).

http://dx.doi.org/10.1016/j.vetmic.2016.05.020
0378-1135/

susceptibility and interpretive criteria applied to analyse the data, are differing among the surveys. To help address this problem monitoring programmes are currently commissioned by the Executive Animal Health Study Centre (CEESA) investigating pathogens from both farm and companion animals (de Jong et al., 2013).

With respect to the interpretive criteria, the Clinical and Laboratory Standards Institute (CLSI) is the only organisation to provide internationally-available breakpoints specifically for bacteria isolated from animals (CLSI, 2015). These breakpoints have evolved since first being introduced in 1997 (NCCLS, 1997). Initially breakpoints used were those available for human infection but these are sub-optimal due to differing dosing regimens, pharmacokinetics/pharmacodynamics (PK/PD) and other factors in animals compared to humans. As data has become available, clinical breakpoints have been set for various specific veterinary hosts, including dogs and cats. This process remains far from complete, so analysis for some antimicrobials are animal-specific whereas others still rely on human-derived breakpoints (CLSI, 2015). This is further complicated because human-derived breakpoints can be applied irrespective of the animal host or infection type, whereas host-specific breakpoints are specific not only for the animal host but often also for the infection type. In addition, different interpretative criteria are often applied in Europe, for example the RESAPATH study (RESAPATH, 2016) uses national breakpoints (disk diffusion inhibition zones; Société Française de Microbiologie, 2015) and SVARM in Sweden uses a combination of epidemiological cut-off values set by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), CLSI breakpoints and MIC distributions found in the SVARM programme (SVARM, 2015).

A further issue is the potential transmission of pathogens including antimicrobial-resistant isolates or their resistance determinants to and from humans, (Ewers et al., 2010; Platell et al., 2011; Wieler et al., 2011; Damborg et al., 2015). It is therefore important to know the extent of resistance in bacteria causing infections in companion animals through surveillance. Various international guidelines have been produced to help assist rational and appropriate antimicrobial use in animals (WHO, 2011; Battersby, 2011) and these rely on data from resistance surveillance.

This current study (ComPath) is part of the CEESA monitoring programme to establish a pan-European collection of representative bacterial pathogens isolated from diseased dogs and cats not recently exposed to antimicrobials and to then determine MICs of relevant antimicrobials against these isolates in a central laboratory. Antimicrobial susceptibilities of pathogens recovered from respiratory tract infections (RTI) are presented here. Organisms were obtained from dogs and cats in nine European Union (EU) countries. Sampling and isolation were conducted over an approximately 3 year period commencing January 2008 and ending November 2010.

2. Methodology

2.1. Bacterial collection

Bacterial isolates were collected from dogs and cats with clinical symptoms of respiratory disease. However, isolates were excluded from the study if the host animal was known to be treated with antibiotics within the four weeks prior to the bacteriological sampling and if animals were chronically diseased. In all cases, only one sample per pet was allowed to help prevent the collection of strains that are epidemiologically related. Whenever possible, pets from the same household or pound, cats from the same breeder, and dogs from the same kennel were not sampled twice. The countries selected for sampling (n = 9) were a geographical selection representing a major part of the companion animals in the EU: Czech Republic, France, Germany, Hungary, Italy, The Netherlands, Poland, Spain and the United Kingdom.

Processing laboratories in the individual countries were responsible for identification of bacterial isolates. However, a full identification was performed at the central microbiology laboratory (LGC, Fordham, UK; formerly Quotient Bioresearch), using MALDI-ToF technology or biochemical confirmatory tests, if isolates were only identified to genus level by the collecting laboratory.

2.2. Antimicrobial testing

MICs were determined at the central laboratory using standardised agar dilution methodology as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2008). Results of these experiments were used to categorise isolates as susceptible, intermediate or resistant to antimicrobials according to MIC breakpoints published by the veterinary standard VET01S (CLSI, 2015). Of the agents investigated in this study only enrofloxacin has specific breakpoints for dog RTI and only pradofloxacin for cat RTI. These breakpoints were used to calculate susceptibility for both dogs and cats. Susceptibility to other agents was calculated using breakpoints derived from human data, as recommended in VET01S (CLSI, 2015). No breakpoints are available for cephalexin, ibafloxacin and marbofloxacin. Antimicrobial susceptibility was analysed for isolates where total number of available strains exceeded 20 isolates.

Staphylococcus intermedius Group isolates (n = 3) with oxacillin MICs $\geq$0.5 μg/ml and *S. aureus* isolates with MICs $\geq$4 μg/ml (n = 2) (CLSI, 2015) were screened by PCR for the presence of *mecA* genes according to a method adapted from Bignardi et al. (1996).

3. Results

3.1. Bacterial species

Isolate numbers collected by country for dogs and cats from RTI are shown in Tables 1 and 2, respectively. For dogs *Staphylococcus intermedius* Group was the most common pathogen (n = 49, 22.8%) closely followed by *Bordetella bronchiseptica* (n = 48, 22.3%). *Streptococcus* spp (n = 36, 16.7%), *Escherichia coli* (n = 24, 11.2%) and *Pasteurella multocida* (n = 23, 10.7%) were also well represented. The remaining pathogens made up less than 10% each (Table 1). For cats the most common organism was *P. multocida* (n = 82) at 44.1% of all isolates. The next most commonly analysed pathogen was *Streptococcus* spp. (n = 25) at 13.4% of isolates. As with dogs, the remainder represented less than 10% each (Table 2).

3.2. Antimicrobial susceptibility in dogs

Staphylococcus intermedius Group susceptibility to most agents, including oxacillin, for all isolates recovered from dogs was around 90% or above, except for chloramphenicol (73.5% susceptible) and tetracycline (59.2% susceptible), as shown in Table 3. Three *Staphylococcus intermedius* Group isolates found to be oxacillin-resistant, all with MICs >8 μg/ml, were confirmed to harbour *mecA* genes (6.1%).

No breakpoints have been set for any of the agents tested for *B. bronchiseptica* susceptibility, and hence without breakpoints it is not possible to relate antimicrobial susceptibility to a potential clinical effect. All MIC distributions are shown in Table 4. Against *B. bronchiseptica*, the beta-lactam agents penicillin and cephalexin had MIC values beyond the highest concentration tested and although amoxicillin/clavulanic acid had lower MICs, the MIC_{50}