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Antimicrobial susceptibility monitoring of respiratory tract pathogens isolated from diseased dogs and cats across Europe (ComPath II, 2013-2014)

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Introduction

Under the umbrella of the Executive Animal Health Study Center (Centre Européen d'Etudes pour la Santé Animale; CEESA), global research-based veterinary pharmaceutical companies collaborate to organize microbial culture collections and to monitor antimicrobial resistance throughout Europe (de Jong *et al.*, 2013). CEESA's ComPath program is dedicated to predominantly aerobic bacterial pathogens in companion animals from three types of infections: skin, ear & soft tissue, urinary tract, and respiratory tract. The findings of the preceding study (ComPath I) for respiratory tract pathogens have been recently reported (Morrissey *et al.,* 2016). The results of isolates of ComPath II are presented hereafter.

Materials and Methods

Nasal, conjunctival swab or broncho-alveolar washing samples were collected according to a standardized protocol from animals with acute clinical signs not exposed to antibiotic treatment during the last 4 weeks prior to sampling, in 11 European countries: Belgium, Czech Republic, France, Germany, Hungary, Italy, the Netherlands, Poland, Spain, Switzerland and the UK. Chronically diseased animals were excluded and each animal could only be sampled once for a given infection. Pets from the same household or pound, cats from the same breeder, and dogs from the same kennel were excluded from sampling. Aerobic bacteria were isolated and identified by standard biochemical methods or by MALDI-ToF mass spectrometry in national laboratories. All susceptibility testing was conducted at a central laboratory (IHMA Europe Sàrl, Switzerland). On receipt at the central laboratory, subculturing was performed to confirm viability and purity of the strains and, if growth characteristics raised doubts on the identification, to verify the identity of those isolates by MALDI-ToF. Minimum inhibitory concentrations (MICs) were determined for 14 licensed antibiotics commonly used in European companion animal medicine by agar dilution in a two-fold concentration series according to CLSI VET01-A4 standards. Results were interpreted using CLSI VET01S, 3rd ed. veterinary clinical breakpoints for dogs and cats where available. In the absence of dog breakpoints, cat breakpoints were used and *vice versa*. If neither were available, human-derived breakpoints were used, but only if published in VET01S, 3rd ed. Additionally, all *Staphylococcus (pseud)intermedius* and coagulase-negative staphylococci with oxacillin MICs ≥ 0.5 mg/L as well as all *Staphylococcus aureus* with oxacillin MICs ≥ 4 mg/L (CLSI VET01S, 3rd ed.) were screened by PCR for the presence of *mecA* genes according to a method adapted from Zhang *et al.* (2012). Concurrently, the *mecA*-positive strain *S. aureus* ATCC 43300 was used as quality control. All *Enterobacteriaceae* with amoxicillin or ampicillin (MICs ≥ 16 mg/L) were tested for susceptibility to cefotaxime and ceftazidime with and without clavulanic acid in order to determine the presumptive ESBL or AmpC producers.

Results and Discussion

A total of 464 isolates have been recovered (233 from dogs and 231 from cats). Additionally, minor pathogens have been retained, but results of species comprising less than 10 isolates per host are not reported here. Table 1 compiles MIC_{50} and MIC_{90} values of the major species, and percentage of clinical resistance (where CLSI resistance breakpoints are available) for the main canine and feline species, respectively. Among the canine and feline streptococci, 68.6% and 65.2% belonged to *Streptococcus canis*, respectively. The most prevalent bacterial species in dogs was *S. (pseud)intermedius* (34.3%); in cats *Pasteurella multocida* (36.4%). For the most prevalent bacterial species *S. (pseud)intermedius* and β-haemolytic streptococci in dogs, low $MIC_{50/90}$ values were observed for the β-lactam antibiotics. Streptococci were fully susceptible to penicillin and ampicillin (only S-breakpoint available). However, clinical resistance against penicillin was high for the staphylococci. Similar data were found in the isolates from cats; streptococci were 100% susceptible to penicillin and ampicillin. For both canine and feline isolates, clinical resistance was most apparent against tetracycline (11.4-46.7%). Resistance of staphylococci and streptococci to fluoroquinolones amounted to 0-7.5%, except for *S. (pseud)intermedius* in cats. We also noted a broad range of resistance to trimethoprim/sulfamethoxazole: from absence to 20.0%. For the Gram-negative organisms, a larger lack of CLSI breakpoints exists, which hampers the accurate interpretation of the results. Only for *E. coli* several breakpoints have been set and with respect to the other 3 bacterial species, only for *P. multocida* pradofloxacin breakpoints are available. All *P. multocida* isolates were fully susceptible to pradofloxacin. For *E. coli* the rate of resistance was variable among some β-lactam antibiotics, and varied for the fluoroquinolones from 13.6 in cats to 18.2% in dogs, comparable with the "older" molecules tetracycline and trimethoprim/sulfamethoxazole. The intrinsic resistance of *Pseudomonas aeruginosa* and *Bordetella bronchiseptica* (for both pathogens no breakpoints defined) to many antimicrobial agents including β-lactam antibiotics and their combination with β-lactamase inhibitors has been confirmed in the present survey. In all, 16 staphylococci strains were resistant to oxacillin (8 *S. (pseud)intermedius*; 7 coagulase-negative staphylococci; 1 *S. aureus*). Among those 16 strains, 6 originated from dogs and 10 from cats. The 6 strains from dogs (all *S. (pseud)intermedius*) and 7 of the 10 strains from cats harbored a *mecA* gene (oxacillin MICs ≥ 0.5 mg/L). In cats, 2 *S. (pseud)intermedius* (13.3%) and 5 coagulase-negative staphylococcus (14.3%) were *mecA* positive. Overall, the *mecA* positive strains correspond to approximately 5.8% of the recovered canine and 10.6% of the feline staphylococci strains. Six respiratory tract *E. coli* isolates were resistant or decreased susceptible to cefotaxime or ceftazidime. Among the 6 strains, 1 originated from a dog (Italy) and 5 from cats (Czech Republic, Netherlands, Switzerland, Poland (two isolates)). Phenotypical characterization showed that 2 isolates (33%) were categorized as presumptive extended spectrum β-lactamase (ESBL) and 4 isolates (66.6%) as AmpC producers. Genotypic typing will follow.

Table 1. Activity of various antimicrobials against the main bacterial species recovered from dogs and cats

Compound	Parameter	Dogs							Cats							
		S. (pseud)-intermedius (n=80)	*S. aureus* (n=23)	*Streptococcus* spp. (35)	*B. bronchiseptica* (n=25)	*E. coli* (n=33)	*P. multocida* (n=14)	*P. aeruginosa* (n=23)	*Streptococcus* spp. (n=23)	*S. (pseud)-intermedius* (n=15)	*S. aureus* (n=16)	CNS (n=35)	*P. multocida* (n=84)	*B. bronchiseptica* (n=13)	*P. aeruginosa* (n=23)	*E. coli* (n=22)
Penicillin G	MIC_{50}	0.12	0.5	0.008	>8	>8	0.12	>8	0.008	0.06	0.12	0.06	0.25	>8	>8	>8
	MIC_{90}	0.25	1	0.015	>8	>8	0.5	>8	0.015	>8	1	2	0.5	>8	>8	>8
	R % [BP*]	20.0 [≥0.25]	65.2 [≥0.25	-	-	-	-	-	-	13.3 [≥0.25]	50.0 [≥0.25]	34.3 [≥0.25]	-	-	-	-
Amoxi/clav	MIC_{50}	0.12	0.5	≤0.015	4	4	0.25	>32	≤0.015	0.12	0.25	0.12	0.25	4	>32	4
	MIC_{90}	0.12	1	0.06	4	8	0.5	>32	0.03	16	1	1	0.25	4	>32	16
	R % [BP]	-	-	-	-	0.0 [≥32]	-	-	-	-	-	-	-	-	-	0.0 [≥32]
Ampicillin	MIC_{50}	0.12	1	≤0.06	32	4	0.25	>32	≤0.06	0.12	0.25	0.12	0.5	32	>32	4
	MIC_{90}	0.25	2	≤0.06	32	>32	1	>32	≤0.06	32	2	2	1	32	>32	>32
	R % [BP*]	-	-	-	-	36.4 [≥32]	-	-	-	-	-	-	-	-	-	40.9 [≥32]
Amoxicillin	MIC_{50}	0.25	1	≤0.015	32	4	0.25	>64	≤0.015	0.25	0.5	0.12	0.25	32	>64	4
	MIC_{90}	0.25	2	0.06	32	>64	0.5	>64	0.03	32	2	2	0.25	32	>64	>64
Cephalothin	MIC_{50}	≤0.06	0.25	0.25	16	8	0.25	>64	0.25	≤0.06	0.25	0.12	0.25	16	>64	8
	MIC_{90}	≤0.06	0.5	0.25	32	16	0.5	>64	0.25	64	0.5	1	0.5	32	>64	>64
	R % [BP]	-	-	-	-	9.1 [≥32]	-	-	-	-	-	-	-	-	-	18.2 [≥32]
Cephalexin	MIC_{50}	1	4	0.25	>32	8	2	>32	0.25	1	4	2	2	>32	>32	8
	MIC_{90}	2	8	0.5	>32	8	4	>32	0.5	>32	8	16	4	>32	>32	>32
Cefadroxil	MIC_{50}	1	4	≤0.25	>32	8	4	>32	≤0.25	1	4	1	4	>32	>32	4
	MIC_{90}	1	4	≤0.25	>32	8	8	>32	≤0.25	>32	8	8	8	>32	>32	>32
Trim/sulfa	MIC_{50}	0.25	0.06	0.12	8	0.25	0.06	8	0.12	0.12	0.06	0.12	0.06	2	4	0.12
	MIC_{90}	4	0.12	0.5	>8	>8	0.12	8	0.5	8	0.12	0.12	0.12	8	8	0.25
	R % [BP**]	11.3 [≥4]	0.0 [≥4]	-	-	21.2 [≥4]	-	-	-	20.0 [≥4]	0.0 [≥4]	2.9 [≥4]	-	-	-	-
Doxycycline	MIC_{50}	0.06	0.12	0.5	0.12	2	0.25	16	0.25	0.06	0.12	0.12	0.25	0.12	16	2
	MIC_{90}	8	2	16	8	16	0.25	32	16	8	1	2	0.25	0.12	32	16
Oxytetracycline	MIC_{50}	0.25	0.5	2	1	4	0.5	>8	4	0.25	0.5	0.5	0.5	1	>8	4
	MIC_{90}	>8	>8	>8	>8	>8	0.5	>8	>8	>8	>8	>8	0.5	1	>8	>8
Tetracycline	MIC_{50}	0.12	0.25	2	0.5	2	0.25	16	2	0.12	0.25	0.25	0.25	0.5	16	2
	MIC_{90}	>32	32	>32	>32	>32	0.5	32	32	>32	16	32	0.25	0.5	16	>32
	R % [BP]	45.0 [≥16]	13.0 [≥16]	40.0 [≥8]	-	18.2 [≥16]	-	-	21.7 [≥8]	46.7 [≥16]	12.5 [≥16]	11.4 [≥16]	-	-	-	13.6 [≥16]
Enrofloxacin	MIC_{50}	0.12	0.12	0.5	1	0.03	0.008	0.5	0.5	0.12	0.12	0.12	0.015	1	0.5	0.06
	MIC_{90}	0.5	0.25	1	1	>8	0.03	1	1	8	0.25	0.25	0.03	1	1	8
	R % [BP]	7.5 [≥4]	4.4 [≥4]	0.0 [≥4]	-	18.2 [≥4]	-	-	0.0 [≥4]	20.0 [≥4]	0.0 [≥4]	2.9 [≥4]	-	-	-	13.6 [≥16]
Marbofloxacin	MIC_{50}	0.25	0.25	1	1	0.03	0.03	0.5	1	0.25	0.5	0.25	0.03	1	0.25	0.03
	MIC_{90}	0.5	0.5	2	1	>8	0.06	2	1	8	0.5	0.5	0.06	1	0.5	>8
Pradofloxacin	MIC_{50}	0.03	0.06	0.12	0.5	0.015	0.004	0.5	0.12	0.03	0.06	0.03	0.008	0.5	0.5	0.03
	MIC_{90}	0.06	0.12	0.12	0.5	4	0.015	0.5	0.12	1	0.06	0.06	0.015	0.5	0.5	2
	R % [BP*†]	6.3 [≥2]	0.0 [≥2]	--	-	18.2 [≥2]	0.0 [≥2]	-	0.0 [≥2]	6.7 [≥2]	0.0 [≥2]	2.9 [≥2]	-	-	-	13.6 [≥2]
Neomycin	MIC_{50}	≤0.25	≤0.25	128	8	2	8	8	128	≤0.25	≤0.25	≤0.25	8	8	8	2
	MIC_{90}	16	0.5	>128	16	2	16	32	>128	32	0.5	≤0.25	8	8	8	2

MIC values are in mg/L; when no CLSI breakpoints were available, this is shown as '-'; resistance breakpoint (BP) according to CLSI VET01-A4 is indicated in parentheses.
***For *Streptococcus* spp. from dogs and cats a S-CLSI breakpoint is available - † For *P. multocida* from cats a S-CLSI breakpoint is available - ** For *E. coli* from cats a S-CLSI breakpoint is available**

Conclusions: The current survey is the first pan-European antimicrobial susceptibility monitoring program for companion animals using standardized methods and centralized MIC determination.
Resistance rates varied depending on compound and bacterial species. Responsible use of antibiotics is crucial to maintain susceptibility and continued resistance monitoring is important to support this goal.

ComPath

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