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Occurrence of antimicrobial resistance in bacteria from diagnostic samples from dogs

Karl Pedersen[1,2]*, Kristina Pedersen[2,3], Helene Jensen[2,3], Kai Finster[3], Vibeke F. Jensen[2] and Ole E. Heuer[4]

[1]Department of Veterinary Pathobiology, Faculty of Life Sciences, University of Copenhagen, Stigbojlen 7, DK-1870 Frederiksberg C, Denmark; [2]National Veterinary Institute, Technical University of Denmark, Hangøvej 2, DK-8200 Aarhus N, Denmark; [3]Department of Microbial Ecology, University of Aarhus, Building 540, DK-8000 Aarhus C, Denmark; [4]National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark

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***Objectives*: To study the occurrence of antimicrobial resistance among common bacterial pathogens from dogs and relate resistance patterns to data on consumption of antimicrobials.**

***Methods*: The antimicrobial susceptibility patterns of 201 *Staphylococcus intermedius*, 37 *Streptococcus canis*, 39 *Pseudomonas aeruginosa*, 25 *Pasteurella multocida*, 29 *Proteus* spp. and 449 *Escherichia coli* isolates from clinical submissions from dogs were determined by a broth-dilution method for determination of minimal inhibitory concentration. Data for consumption of antimicrobials were retrieved from VetStat, a national database for reporting antimicrobial prescriptions.**

***Results*: The majority of the antimicrobials prescribed for dogs were broad-spectrum compounds, and extended-spectrum penicillins, cephalosporins and sulphonamides + trimethoprim together accounted for 81% of the total amount used for companion animals. Resistance to cephalosporins and amoxicillin with clavulanic acid was very low for all bacterial species examined, except for *P. aeruginosa*, and resistance to sulphonamides and trimethoprim was low for most species. Among the *S. intermedius* isolates, 60.2% were resistant to penicillin, 30.2% to fusidic acid and 27.9% to macrolides. Among *E. coli* isolates, the highest level of resistance was recorded for ampicillin, sulphonamides, trimethoprim, tetracyclines and streptomycin. Certain differences in resistance patterns between isolates from different sites or organs were noticed for *E. coli*, *S. intermedius* and *Proteus* isolates.**

***Conclusions*: This investigation provided data on occurrence of antimicrobial resistance in important pathogenic bacteria from dogs, which may be useful for the small animal practitioner. Resistance was low to the compounds that were most often used, but unfortunately, these compounds were broad-spectrum. Data on resistance and usage may form a background for the establishment of a set of recommendations for prudent use of antimicrobials for companion animals.**

Keywords: *Staphylococcus intermedius*, *Escherichia coli*, *Streptococcus canis*, pyoderma, companion animals

Introduction

Systematic surveillance of the occurrence of antimicrobial resistance among bacteria from food animals, food and humans has been established in several countries and results are published annually in national reports. These data often include results from susceptibility testing of pathogenic, zoonotic and indicator bacteria. Furthermore, data on the occurrence of antimicrobial resistance in bacteria from animals, foods and humans are published every year by the European Food Safety Authority on the basis of reporting from EU member states. Although data for many different animal species are reported through these reporting systems, data on the occurrence of antimicrobial resistance in bacteria from companion animals are absent or scarce.

The occurrence of antimicrobial resistance in companion animals may however be of significance to human health.

*Corresponding author. Tel: +45-35-33-27-03; E-mail: kape@life.ku.dk

Considering the shared environment of humans and companion animals, transfer of resistant bacteria or mobile resistance determinants between companion animals and humans is likely to occur and has been indicated in some studies.[1–3] However, the extent to which this exchange occurs is essentially unknown. In Denmark, few reports on the occurrence of antimicrobial resistance among bacteria from companion animals have been published and most of these investigations have focussed on *Staphylococcus intermedius*.[4,5]

The use of antimicrobials in companion animals has received little attention and remains unregulated, whereas antimicrobial use in farm animals is regulated in many countries by guidelines or legal restrictions.[6] Some antimicrobials that are widely used in animals belong to classes of antimicrobials that are regarded as critically important for use in humans (e.g. cephalosporins and fluoroquinolones),[7] and the use of these antimicrobials in farm animals is restricted or prohibited in some countries. In Denmark, ~1100 kg of antimicrobials was prescribed for companion animals in 2005[8] and an estimated 80% of this amount was used in dogs. In addition, an estimated 750 kg of antimicrobials—mainly for injection—was used for companion animals in veterinary practice. Thus, the amount used for the population of ~600 000 dogs was more than twice the total usage for the production of 120 million broilers, 1.2 million turkeys and other poultry in Denmark in 2005.[7,8] There seems to be a tendency to use a broader selection of antimicrobials for companion animals than for food animals.[9] Recommendations for usage of antimicrobials in companion animals have been published in Norway, although only in the Norwegian language.[10] However, knowledge of antimicrobial use and occurrence of resistance in the most frequent bacterial pathogens from companion animals will be essential to establish prudent use guidelines in other countries. In the present investigation, we report data on the susceptibility patterns of *S. intermedius*, *Escherichia coli*, *Streptococcus canis*, *Pseudomonas aeruginosa*, *Pasteurella multocida* and *Proteus* spp. isolates from Danish dogs and compare the results with the registered usage of antimicrobials for companion animals in Denmark. These bacterial species were studied because they are some of the most frequently isolated pathogens from various infectious conditions in dogs.

Materials and methods

Bacterial isolates and culture conditions

Bacterial isolates were obtained from clinical submissions during the period 2000–05. All the *S. intermedius* isolates ($n = 201$) were associated with clinical disease: infection in the skin (mostly chronic pyoderma) ($n = 84$), in the ear (mostly otitis externa) ($n = 89$), or in the urogenital organs ($n = 28$). The *S. canis* isolates ($n = 37$) and the *P. multocida* isolates ($n = 25$) were derived from a variety of sites associated with the respiratory tract, integument, ear, or urogenital system, or from other organs in case of septicaemia. The *P. aeruginosa* isolates included in the study ($n = 39$) were all derived from cases of otitis externa. The *Proteus* isolates ($n = 29$) were either from ear infections ($n = 14$) or from infections in the urogenital tract ($n = 15$) and belonged to the species *Proteus mirabilis* or *Proteus vulgaris*. The *E. coli* isolates included in this investigation were obtained from the intestinal tract, including faeces, or from the urogenital organs. Of these, 121 were haemolytic and 328 were non-haemolytic. Unlike the other bacteria, *E. coli* isolates from faeces and the gastrointestinal tract may not have been causative pathogenic organisms. Primary cultures were made on blood agar (blood agar base, Oxoid, supplemented with 5% calf blood), Drigalski agar[11] and Enteric medium (Statens Serum Institut, Copenhagen, Denmark) (only samples from faeces and the gastrointestinal tract), and subcultured on blood agar. All media were incubated aerobically at 37°C for 18–24 h. Bacteria were identified from their appearance on agar media, swarming, haemolysis, odour, cell morphology, catalase and oxidase reaction and Gram properties. If necessary, identification kits were used (API ID 32E for *E. coli* and *Proteus*, API 20NE for *P. aeruginosa* and *P. multocida*, API ID 32 STAPH for *S. intermedius* and API rapid ID 32 STREP for *S. canis*; bioMérieux, Marcy l'Étoile, France). Identification of *S. intermedius* was confirmed with a positive test for coagulase and a negative test for hyaluronidase, and identification of *S. canis* with a positive test for reaction with Lancefield's group G antiserum (Oxoid Diagnostic Reagents).

Antimicrobial susceptibility testing

A semi-automated antimicrobial susceptibility testing system (Sensititre, Trek Diagnostic Systems, East Grinstead, UK), based on the broth-dilution method, was used together with customized ready-to-use microtitre plates containing 2-fold dilution amounts of antimicrobial compounds. Different panels were used for different bacterial species. The MIC breakpoints that were used in this study are listed in Table 1 and were adopted from published sources,[8,12–14] preferably CLSI (formerly NCCLS), when available. The ranges of antimicrobial concentrations in the panels used for testing different bacterial species are shown in Table 2. Only resistant isolates were counted as resistant, whereas intermediate ones were counted as susceptible. The method used was accredited by DANAK in accordance with ISO/EN 17025. *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213 and *Enterococcus faecium* ATCC 29212 were used as quality control reference strains and tested weekly. No deviations from their expected results were recorded.

Usage of antimicrobials

In Denmark, use of antimicrobials in animals is legal only after prescription by a veterinarian. All veterinary use of antimicrobials in animals is registered in the VetStat programme. VetStat is based on reporting from pharmacies, feed mills and from veterinary practitioners. All purchase at pharmacies and feed mills to the animal owners contain information on item identity and amount, identity of prescribing veterinarian, animal species, and for production animals, also on farm identity, disease group and age group. When purchased by a veterinary practice, information on item identity and amount together with veterinarian and practice identity is registered by the pharmacy. Information on species, farm identity, etc. is supplied by the veterinarian when used in production animals but not in companion animals.[15] The usage of antimicrobial compounds for companion animals was estimated from data from the pharmacies on sales to companion animals and sales to veterinary practice of drugs formulated and approved for use in companion animals (mainly tablets). These data do not comprise drugs sold for use in companion animals in veterinary practice, when the drugs may be used either for companion animals or production animals (mainly injectable and topical drugs).

Statistics

Significance tests for differences between proportions of resistant isolates were calculated using StatCal in Epi-Info™ version 6.

Table 1. Antimicrobials and breakpoints used in the investigation

Antimicrobial[(reference to breakpoints)]	Breakpoints (mg/L)											
	S. intermedius			*S. canis*			*E. coli*, *Proteus*			*P. aeruginosa*, *P. multocida*		
	R	S	I	R	S	I	R	S	I	R	S	I
Amoxicillin with clavulanic acid (1:2)[12,14]	≤4/2		≥8/4				≤8/4	16/8	≥32/16	≤8/4	16/8	≥32/16
Ampicillin[12]							≤8	16	≥32	≤8	16	≥32
Apramycin[8]							≤16		≥32[a]			
Ceftiofur[12]							≤2	4	≥8			
Cefalotin[13,14]	≤8	16	≥32	≤8	16	≥32	≤8	16	≥32	≤8	16	≥32
Chloramphenicol[12]	≤8	16	≥32	≤4	8	≥16	≤8	16	≥32	≤8	16	≥32
Ciprofloxacin[14]							≤1	2	≥4			
Clindamycin[12]	≤0.5	1–2	≥4	≤0.25	0.5	≥1				≤0.5	1–2	≥4
Colistin[8]							≤8		≥16	≤8		≥16
Enrofloxacin[13]	≤0.5	1	≥2	≤0.5	1	≥2				≤0.5	1	≥2
Erythromycin[12]	≤0.5	1–4	≥8	≤0.25	0.5	≥1				≤0.5	1–4	≥8
Florfenicol[8]							≤8	16	≥32			
Fusidic acid[c]	≤12	2	≥4	≤12	2	≥4						
Gentamicin[8]							≤2	4	≥8	≤2	4	≥8[b]
Kanamycin[12,14]	≤16	32	≥64	≤16	32	≥64				≤16	32	≥64
Nalidixic acid[14]							≤16		≥32			
Neomycin[8]							≤4	8	≥16			
Penicillin[12]	≤0.12		≥0.25	≤0.12	0.25–2	≥4						
Spectinomycin[12]	≤64		≥128	≤64		≥128	≤64		≥128	≤64		≥128
Streptomycin[8]							≤8	16	≥32			
Sulfamethoxazole[12,14]							≤256		≥512			
Sulfamethoxazole with trimethoprim[12,14]	≤38/2		≥76/4	≤38/2		≥76/4				≤38/2		≥76/4
Tetracycline[12,14]	≤4	8	≥16	≤4	8	≥16	≤4	8	≥16	≤4	8	≥16
Trimethoprim[14]							≤8		≥16			

[a]Breakpoint for apramycin was changed from 16 to 32 mg/L in 2003.
[b]Breakpoint for gentamicin was changed from 16 to 8 mg/L in 2004.
[c]Breakpoints for fusidic acid were given by the manufacturer (Leo Pharma Ltd, Denmark).

A significance level of 5% was applied ($P < 0.05$). Fisher's exact test (two-tailed) was used when appropriate.

Results

All *S. intermedius* isolates ($n = 201$) were susceptible to amoxicillin with clavulanic acid (Table 3). A low level of resistance to cefalotin, enrofloxacin, potentiated sulphonamides and spectinomycin was recorded. The highest level of resistance was recorded for penicillin (60.2% of the isolates). Resistance to macrolides and tetracycline was significantly higher among isolates from skin than from the ear ($P = 0.037$ and $P = 0.027$, respectively), and resistance to lincosamides was significantly higher among isolates from skin than from the urogenital tract ($P = 0.042$). For other compounds, differences between the three groups were not statistically significant. Resistance to macrolides, lincosamides and kanamycin was linked in most cases.

Resistance among *E. coli* isolates ($n = 449$) was highest for sulphonamides, streptomycin, ampicillin, trimethoprim and tetracycline (Table 4). All isolates were susceptible to fluoroquinolones, and very low levels of resistance (<5%) were recorded to apramycin, amoxicillin with clavulanic acid, colistin, ciprofloxacin, gentamicin, ceftiofur and florfenicol. Among the isolates from faeces, the non-haemolytic isolates were significantly more often resistant than haemolytic ones to trimethoprim ($P = 0.001$), tetracycline ($P = 0.029$) and chloramphenicol ($P = 0.005$). For other compounds, the differences were not statistically significant.

All *P. aeruginosa* isolates ($n = 39$) were resistant to ampicillin, amoxicillin with clavulanic acid, cefalotin, clindamycin and erythromycin. Most isolates were resistant to chloramphenicol (89.7%), spectinomycin (97.4%), tetracycline (89.7%), sulphonamides with trimethoprim (92.3%) and kanamycin (95.0%). Only 35.9% of the isolates were resistant to enrofloxacin and 15.4% to gentamicin, while only a single isolate was resistant to colistin.

All *P. multocida* isolates ($n = 25$) were susceptible to penicillin and ampicillin, chloramphenicol, colistin, spectinomycin, tetracycline, sulphonamides with trimethoprim, cefalotin, enrofloxacin and kanamycin. In contrast, all isolates were resistant or intermediate susceptible to erythromycin and clindamycin.

Table 2. Range (mg/L) of antimicrobial agents used in the investigation

Antimicrobial agent	*S. intermedius*, *S. canis*	*E. coli*, *Proteus*	*P. aeruginosa*, *P. multocida*
Amoxicillin with clavulanic acid (1:2)	2–64	2–32	2–64
Ampicillin	0.06–32	1–32	0.5–32
Apramycin		4–32	
Ceftiofur		0.125–4	
Cefalotin	2–64	4–16	2–64
Chloramphenicol	2–64	2–64	2–64
Ciprofloxacin		0.03–4	
Clindamycin	0.12–64		0.25–8
Colistin		4–16	1–64
Enrofloxacin	0.12–8		0.12–8
Erythromycin	0.12–16		0.12–16
Florfenicol		2–64	
Fusidic acid	0.25–8		
Gentamicin		1–32	1–32
Kanamycin	2–128		4–128
Nalidixic acid		8–64	
Neomycin		2–32	
Penicillin	0.06–16		0.12–16
Spectinomycin	4–256	16–128	4–256
Streptomycin		4–64	
Sulfamethoxazole		64–1024	
Sulfamethoxazole with trimethoprim	0.25/4.74–16/304		0.25/4.74–16/304
Tetracycline	1–32	2–32	0.5–32
Trimethoprim		4–32	

All *Proteus* isolates ($n = 29$) were susceptible to ciprofloxacin and gentamicin (one isolate was only intermediate susceptible to gentamicin) and resistant to colistin and tetracycline. For other antimicrobials, variable levels of resistance were found (Table 5). Isolates from ear infections were more often resistant to apramycin than isolates from urogenital tract infections ($P = 0.017$). For other compounds, the differences were not statistically significant.

All *S. canis* ($n = 37$) isolates were susceptible to penicillin, cefalotin, spectinomycin, sulphonamides with trimethoprim and kanamycin. The highest level of resistance was recorded for tetracycline (27%), followed by enrofloxacin (13.5%), clindamycin (13.5%) and erythromycin (10.8%). Isolates resistant to erythromycin were also resistant to clindamycin.

Data on the usage of antimicrobials in companion animals are given in Table 6. The majority of these amounts (~80%) are administered as tablets formulated for use in dog or cats above 10 kg weight, which means that they are most likely used in dogs. The three most extensively used antimicrobial groups are extended-spectrum β-lactams, cephalosporins and sulphonamides/trimethoprim. Of the broad-spectrum β-lactams, 69% were in combination with the β-lactamase inhibitor clavulanic acid. The use of these broad-spectrum antimicrobial agents constituted 81% of the total amount.

Table 3. Antimicrobial resistance among *S. intermedius* isolates ($n = 201$) from Danish dogs

Antimicrobial compound	Percentage of resistant isolates			
	skin ($n = 84$)	ear ($n = 89$)	urogenital tract ($n = 28$)	total ($n = 201$)
Penicillin	64.3	57.3	57.1	60.2
Fusidic acid	27.4	36.0	25.0	30.9
Kanamycin[a]	31.0	28.4	35.0	30.3
Clindamycin	34.5	24.7	14.3	27.4
Erythromycin	36.9	22.5	17.9	27.9
Tetracycline	29.8	15.7	32.1	23.9
Chloramphenicol	14.3	14.6	3.6	12.9
Spectinomycin	9.5	3.4	3.6	6.0
Sulfamethoxazole with trimethoprim	3.6	2.3	0	2.5
Enrofloxacin	1.2	1.1	0	1.0
Cefalotin	0	1.1	0	0.5
Amoxicillin with clavulanic acid	0	0	0	0

[a]For skin $n = 71$, ear $n = 74$, urogenital tract $n = 20$ and total $n = 165$.

Discussion

In dogs, common bacterial infections treated with antimicrobials include pyoderma, ear infections, wound infections, gastroenteritis and urinary tract infections. A broad spectrum of antimicrobials is prescribed for these infections in dogs, including almost all main antimicrobial groups, both narrow- and broad-spectrum ones. Certain infections, such as ear infections and pyoderma, can be long-standing problems that may predispose for development of resistance due to repeated or prolonged antimicrobial treatment.

It is noteworthy that 81% of the total amount of antimicrobials prescribed for companion animals were the broad-spectrum compounds, cephalosporins, extended-spectrum penicillins (69% with clavulanic acid) and sulphonamides + trimethoprim, which is much in contrast to human medical practice. Tetracyclines accounted for 5% and lincosamides—mostly clindamycin—for 2% of the total usage in companion animals. The majority of these amounts were used for dogs. The widespread use of broad-spectrum antimicrobials does not seem fully justified by the present results, and it may indicate that the practitioner is uncertain about either the involved microorganism or its susceptibility pattern.

The isolates were tested in panels that were designed for different groups of bacteria from both therapeutic and surveillance criteria. Thus, not all compounds are licensed for dogs in Denmark and some relevant compounds were probably not tested. It is unknown to what extent preparations that are not registered for dogs are prescribed for use in dogs. Apramycin, florfenicol and ciprofloxacin are probably not used, whereas enrofloxacin probably accounts for most of the fluoroquinolones used. Some reports have indicated that bacteria isolated from dogs that had been treated for an infection were more likely to be resistant than isolates from untreated dogs.[16–18] The isolates included in this study were from clinical submissions to the

Table 4. Antimicrobial resistance among *E. coli* isolates from Danish dogs

Antimicrobial compound	Haemolytic isolates, % resistant				Non-haemolytic isolates, % resistant				Total ($n = 449$)
	faeces ($n = 69$)	intestine ($n = 26$)	urogenital tract ($n = 26$)	total ($n = 121$)	faeces ($n = 246$)	intestine ($n = 55$)	urogenital tract ($n = 27$)	total ($n = 328$)	
Sulfamethoxazole	18.8	23.1	19.2	19.8	27.6	38.2	18.5	28.7	26.7
Streptomycin	21.7	26.9	19.2	22.3	28.1	32.7	18.5	28.1	26.5
Ampicillin	17.4	38.5	15.4	21.5	26.0	40	22.2	28.1	26.3
Trimethoprim	7.3	19.2	11.5	10.7	25.2	16.4	18.5	23.2	19.8
Tetracycline	8.7	11.5	19.2	11.6	19.9	30.9	25.9	22.3	19.2
Nalidixic acid	7.3	0	7.7	5.8	15.5	10.9	18.5	14.9	12.5
Spectinomycin	2.9	3.9	11.5	5.0	9.4	16.4	3.7	10.1	8.7
Neomycin	5.8	0	3.9	4.1	5.7	7.3	3.7	5.8	5.8
Chloramphenicol	0	0	7.7	1.7	6.1	10.9	3.7	6.7	5.3
Cefalotin[a]	6.8	0	5.3	4.1	4.2	10.6	5.6	5.4	5.1
Apramycin	5.8	0	3.9	4.1	4.5	3.6	0	4.0	4.0
Amoxicillin with clavulanic acid (2:1)	0	0	3.8	0.8	3.7	10.9	3.7	4.9	3.8
Colistin	4.35	0	3.9	3.3	4.5	1.8	0	3.7	3.6
Ciprofloxacin	0	0	0	0	4.5	0	7.4	4.0	2.9
Gentamicin	1.45	0	0	0.8	4.1	1.8	3.7	3.7	2.9
Ceftiofur	1.45	0	3.9	1.7	1.2	1.8	3.7	1.5	1.6
Florfenicol	0	0	3.9	0.8	0.81	0	0	0.6	0.7

[a]For haemolytic *E. coli*: faeces $n = 59$, intestine $n = 26$, urogenital tract $n = 19$ and total $n = 104$.

laboratory from the whole country, and they were assumed to be representative and not epidemiologically related. Unfortunately, information about duration of the condition, previous treatments, age, breed and gender of the dogs was rarely given by the practitioner, and our data therefore does not allow any meaningful comparison of these data.

Table 5. Antimicrobial resistance of *Proteus* spp. ($n = 29$) isolates from Danish dogs

Antimicrobial compound	Perentage resistant		
	ear ($n = 14$)	urogenital tract ($n = 15$)	total
Colistin	100	100	100
Tetracycline	100	100	100
Streptomycin	42.9	40	41.4
Spectinomycin	21.4	33.3	27.6
Trimethoprim	28.6	20	24.1
Sulfamethoxazole	21.4	20	20.7
Ampicillin	28.6	13.3	20.7
Chloramphenicol	21.4	20	20.7
Apramycin	35.7	0	17.2
Neomycin	14.3	13.3	13.8
Nalidixic acid	21.4	0	10.3
Ceftiofur	14.3	0	6.9
Amoxicillin with clavulanic acid (2:1)	14.3	0	6.9
Ciprofloxacin	0	0	0
Gentamicin	0	0	0

Table 6. Antimicrobials (kg active compound) sold for use in companion animals in Denmark[8] in 2005[a]

Antimicrobial class	Active compound (kg)
Penicillins, extended-spectrum[b]	440
Cephalosporins	313
Sulphonamides + trimethoprim	140
Tetracyclines	57
Penicillins, β-lactamase sensitive	69
Lincosamides	20
Fluoroquinolones	14
Aminoglycosides	7
Tiamulin	4
Macrolides	7
Amphenicols	1
Quinolones	0
Others	31
Total	1103

[a]An estimated 750 kg of active compound—mainly injectables and topical drugs—purchased for use in small animal practice is not included.
[b]Including 303 kg of amoxicillin/clavulanic acid combination.

The low levels of resistance to cephalosporins, despite a high consumption, are encouraging, but it may merely reflect that cephalosporins for oral use were not approved until 1998 in Denmark, to our knowledge, did not enter common use in small animal practice until around 2001.

The site of isolation has been reported to influence resistance levels of *S. intermedius*. In a study by Hoekstra and Paulton,[19] more isolates from the ear were resistant to cefalotin, lincomycin, sulphonamides with trimethoprim or amoxicillin with clavulanic acid than those from other sites of the body. In another study, it was found that *S. intermedius* from the skin was more often resistant (28% susceptible) to penicillin than from the ear (49% susceptible).[20] Lloyd *et al.*[21] reported higher levels of resistance to penicillin among staphylococci from the skin (84.1%) and ear (72.5%) than from the nasal and oral cavity (100%). We only found statistically significant differences for macrolides, clindamycin and tetracyclines where the highest levels of resistance were observed among isolates from skin. The explanation for this may be that, in particular, pyoderma but also otitis externa are infections that tend to become chronic and be treated for extended periods, thereby selecting for antimicrobial resistance.

Fusidic acid has for a number of years been the drug of choice for treatment of canine ear infections in Denmark. However, although isolates from ear infections were numerically more often resistant to fusidic acid than isolates from the urogenital tract and skin, these differences were not statistically significant.

The absence of resistance to amoxicillin with clavulanic acid in this study is encouraging, albeit surprising, since this drug combination for many years has been one of the most often prescribed antimicrobials for dogs, in particular for skin infections, due to the very frequent resistance to penicillin.

A Norwegian survey indicated that resistance levels among canine *S. intermedius* isolates were high and apparently increasing for penicillin (86%), fusidic acid (59%) and tetracycline (53%), but low or moderate for other compounds.[22]

In the Swedish surveillance programme for antimicrobial resistance, *S. intermedius* from clinical submissions from the skin of dogs is included.[23] The resistance to penicillin reported there was very high, 84%, compared to 60.2% in our investigation, whereas resistance to most other antimicrobials was comparable to the levels in the present investigation.

Haemolysin is considered a virulence factor for *E. coli* in dogs.[24] In a previous investigation of *E. coli* from mink, haemolytic isolates were found to be more resistant than non-haemolytic isolates to tetracycline, amoxicillin, sulphonamides, trimethoprim and spectinomycin.[11] In the present study, we found a statistically significant difference among isolates from faeces for trimethoprim, tetracycline and chloramphenicol, but here the non-haemolytic isolates were in fact more resistant than haemolytic ones. The reason for this difference is unknown.

In the Swedish surveillance system, SWARM,[23] *E. coli* isolates from clinical submissions from canine urinary tract infections are included. The resistance levels reported in the SWARM report were in general lower than those obtained in our study, most notably 17% resistance to ampicillin, 7% to tetracyclines and 8% to sulphonamides.

Proteus is frequently isolated as the cause of urinary tract infection and otitis externa. Not many investigations have focussed on antimicrobial resistance of canine *Proteus* isolates. We found all isolates to be resistant to tetracyclines and polymyxins, but susceptible to ciprofloxacin and gentamicin. For other antimicrobials, resistance varied from 6.9% to 41.4%. Prescott *et al.*[25] reported that 13% of *Proteus* isolates from dogs and cats were susceptible to penicillin and 35% were susceptible to lincomycin and erythromycin. This observation is in contrast to the general belief that enterobacteria possess innate resistance to these compounds, and for the same reason they are not included in our test panel for Enterobacteriaceae. The results underline the importance of standardized test methods and breakpoints.

P. aeruginosa is reputed for its innate resistance to most antimicrobials. In dogs, this bacterial species is mostly isolated from chronic ear infections. A low level of resistance was only found for two compounds in this investigation: colistin and gentamicin. For enrofloxacin, 35.9% of the isolates were resistant, but for most susceptible isolates, MIC values were close to the breakpoint. Hirsh and Jang[26] also found most *P. aeruginosa* isolates susceptible to gentamicin and to another aminoglycoside, amikacin. In addition, they found most isolates were susceptible to the β-lactam ticarcillin with clavulanic acid. These authors had not included any polymyxins in their study. Petersen *et al.*[20] also found most or all isolates to be susceptible to gentamicin, amikacin and ticarcillin, but also close to 100% of the isolates were susceptible to enrofloxacin. Hirsh and Jang[26] found only 2% sensitive to enrofloxacin and we found 64.1% susceptible. This difference in susceptibility to fluoroquinolones is likely to be due to differences in methods or breakpoints in combination with MIC values close to breakpoints rather than real differences in susceptibility.

Rantala *et al.*[18] advocated for a systematic surveillance of antimicrobial resistance among both pathogenic and indicator bacteria from companion animals and suggested research in resistance mechanisms. We support these ideas, and additionally, suggest a systematic surveillance of the consumption of antimicrobial drugs for companion animals and the elaboration of a set of guidelines for prudent use of antimicrobials for companion animals. The form and extent of the surveillance is open for discussion, but optimally it should be based on pathogenic as well as indicator organisms, and isolates recovered from healthy as well as diseased animals.

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Transparency declarations

None to declare.

References

1. Guardabassi L, Schwarz S, Lloyd DH. Pet animals as reservoirs of antimicrobial-resistant bacteria. *J Antimicrob Chemother* 2004; **54**: 321–32.

2. Van Duijkeren E, Wolfhagen MJHM, Box ATA *et al*. Human-to-dog transmission of methicillin-resistant *Staphylococcus aureus*. *Emerg Infect Dis* 2004; **10**: 2235–7.

3. Simjee S, White DG, McDermott PF *et al*. Characterization of Tn*1546* in vancomycin-resistant *Enterococcus faecium* isolated from canine urinary tract infections: evidence of gene exchange between human and animal enterococci. *J Clin Microbiol* 2002; **40**: 4659–65.

4. Pedersen K, Wegener HC. Antimicrobial susceptibility patterns and rRNA gene restriction patterns among *Staphylococcus intermedius* from healthy dogs and from dogs suffering from pyoderma or otitis externa. *Acta Vet Scand* 1995; **36**: 335–42.

5. Wegener HC, Pedersen K. Variations in antibiograms and plasmid profiles among multiple isolates of *Staphylococcus intermedius* from pyoderma in dogs. *Acta Vet Scand* 1992; **33**: 391–4.

6. Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. http://europa.eu.int/eur-lex/pri/en/oj/dat/2003/l_268/l_26820031018en00290043.pdf (15 June 2007, date last accessed).

7. Heuer OE, Jensen VF, Hammerum AM. Antimicrobial drug consumption in companion animals. *Emerg Infect Dis* 2005; **11**: 344–5.

8. Anonymous. *DANMAP 2005—Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark*. Copenhagen, Denmark, 2006. ISSN 1600-2032.

9. Sternberg S. Antimicrobial resistance in bacteria from pets and horses. *Acta Vet Scand Suppl* 1999; **92**: 37–50.

10. Heiene R, Krontveit R, Barton L *et al*. Valg av antibakterielle midler til hund og katt [Choice of antibacterial drugs for dog and cat]. *Norsk Veterinærtidsskrift* 2004; **116**: 664–78.

11. Vulfson L, Pedersen K, Chriél M *et al*. Serogroups and antimicrobial susceptibility among *Escherichia coli* isolated from farmed mink (*Mustela vison* Schreiber) in Denmark. *Vet Microbiol* 2001; **79**: 143–53.

12. National Committee for Clinical Laboratory Standards. *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals—Second Edition: Approved Standard M31-A2*. NCCLS, Wayne, PA, USA, 2002.

13. National Committee for Clinical Laboratory Standards. *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals: Informational Supplement M31-S1*. NCCLS, Wayne, PA, USA, 2004.

14. National Committee for Clinical Laboratory Standards. *Performance Standards for Antimicrobial Susceptibility Testing: Fourteenth Informational Supplement M100-S14*. NCCLS, Wayne, PA, USA, 2004.

15. Stege H, Bager F, Jacobsen E *et al*. VETSTAT-the Danish system for surveillance of the veterinary use of drugs for production animals. *Prev Vet Med* 2003; **57**: 105–15.

16. Medleau L, Long RE, Brown J *et al*. Frequency and antimicrobial susceptibility of *Staphylococcus* species isolated from canine pyodermas. *Am J Vet Res* 1986; **47**: 229–31.

17. Noble WC, Kent LE. Antibiotic resistance in *Staphylococcus intermedius* isolated from cases of pyoderma in the dog. *Vet Dermatol* 1992; **3**: 71–4.

18. Rantala M, Lahti E, Pesonen S *et al*. Antimicrobial resistance in *Staphylococcus* spp., *Escherichia coli*, and *Enterococcus* spp. in dogs given antibiotics for chronic dermatological disorders, compared with non-treated control dogs. *Acta Vet Scand* 2004; **45**: 37–45.

19. Hoekstra KA, Paulton RJL. Antibiotic sensitivity of *Staphylococcus aureus* and *S. intermedius* of canine and feline origin. *Lett Appl Microbiol* 1996; **22**: 192–4.

20. Petersen AD, Walker RD, Bowman MM *et al*. Frequency of isolation and antimicrobial susceptibility patterns of *Staphylococcus intermedius* and *Pseudomonas aeruginosa* isolates from canine skin and ear samples over a 6-year period (1992–1997). *J Am Anim Hosp Assoc* 2002; **38**: 407–13.

21. Lloyd DH, Lamport AI, Feeney C. Sensitivity to antibiotics amongst cutaneous and mucosal isolates of pathogenic staphylococci in the UK, 1980–96. *Vet Dermatol* 1996; **7**: 171–5.

22. Anonymous. *NORM/NORM-VET* 2004. *Usage of antimicrobial agents occurrence of antimicrobial resistance in Norway*. Tromsø/Oslo, 2005. ISSN 1502-2307.

23. Anonymous. *SWARM 2005—Swedish Veterinary Antimicrobial Resistance Monitoring*. Uppsala, Sweden: National Veterinary Institute, 2006.

24. Holland RE, Walker RD, Sriranganathan N *et al*. Characterization of *Escherichia coli* isolated from healthy dogs. *Vet Microbiol* 1999; **70**: 261–8.

25. Prescott JF, Gannon VP, Kittler G *et al*. Antimicrobial drug susceptibility of bacteria isolated from disease processes in cattle, horses, dogs, and cats. *Can Vet J* 1984; **25**: 289–92.

26. Hirsh DC, Jang SS. Antimicrobial susceptibility of selected infectious bacterial agents obtained from dogs. *J Am Anim Hosp Assoc* 1994; **30**: 487–94.