



Usage of antimicrobials and occurrence of antimicrobial resistance among bacteria from mink

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Abstract

The usage of antimicrobials for treatment of mink and the occurrence of antimicrobial resistance among the most important bacterial pathogens in mink was investigated. The aim of the study was to provide data, which may serve as a basis for the formulation of recommendations for prudent use of antimicrobials for mink. A total of 164 haemolytic staphylococci, 49 haemolytic streptococci, 39 *Pseudomonas aeruginosa*, 13 *Pasteurella multocida*, and 1093 *Escherichia coli* isolates from Danish mink were included in the study. A high frequency of resistance among *S. intermedius* was found for tetracyclines (54.7%), followed by penicillin (21.7%), lincosamides (20.4%), macrolides (19.1%), and spectinomycin (18.5%). Very low frequencies of resistance were recorded for other antimicrobials. The highest frequency among the *E. coli* isolates was recorded for ampicillin, streptomycin, sulphonamides, and tetracyclines, whereas resistance to other antimicrobials was rare. All *P. aeruginosa* were sensitive to gentamicin and colistin and sensitive or intermediate to enrofloxacin, whereas most isolates were resistant to all other antimicrobials. All *P. multocida* and haemolytic streptococci were sensitive to penicillin.

There was a steady increase in the use of antimicrobials during the period 2001–2006, the majority of the prescribed amount being extended spectrum penicillins followed by aminoglycosides, sulphonamides with trimethoprim, and macrolides.

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1. Introduction

Mink may suffer from a number of infectious diseases, which demand therapy. The most important bacterial infections include *Escherichia coli* (enteritis, pneumonia, septicaemia), *Pseudomonas aeruginosa* (haemorrhagic pneumonia), haemolytic staphylo-

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cocci, most often *Staphylococcus intermedius* and occasionally *Staphylococcus aureus* (mastitis, pneumonia, pleuritis, dermatitis, metritis, urinary tract infection, septicaemia, and others), *Pasteurella multocida* (respiratory tract infection, pleuritis, wound infection, and others), haemolytic streptococci, mostly Lancefield's group G and less frequently group C (respiratory tract infection, pleuritis, dermatitis, wound infection, septicaemia, and others). Other bacterial infections occur occasionally, such as infections caused by *Salmonella*, *Plesiomonas shigelloides*, *Streptococcus bovis*, *Streptococcus pneumoniae*, *Aeromonas* spp., and others. Although viral infections cannot be treated with antibiotics, it is not unusual that such treatment is initiated in an attempt to reduce mortality due to secondary bacterial infections to specific viral diseases, such as mink virus enteritis, distemper, and "sticky kits". An increase in the prescription of antimicrobials for fur animals has been noted from 659 kg active compound in 2001 to 1694 kg in 2006 (Anon., 2002, 2007), but during the same period also an increase in production. So far, few investigations have been carried out on antimicrobial resistance in bacteria from fur animals and the current knowledge of antimicrobial resistance in important bacterial pathogens in mink is sparse. The present investigation was undertaken in order to elucidate the occurrence of antimicrobial resistance among important bacteria from infections in mink, and to present data on the consumption of antimicrobials in mink in Denmark.

2. Material and methods

2.1. Bacterial isolates and culture conditions

Bacterial isolates were obtained from clinical samples submitted to the National Veterinary Laboratory during the period 2000–2005. The isolates originated from more than 870 mink farms in Denmark and the number of isolates from each farm varied from 1 to 11, the majority of farms being represented by 1–4 isolates of different bacterial species. Therefore, the isolates were considered representative for the whole country.

The haemolytic staphylococci ($n = 164$), of which 157 were *S. intermedius* and 7 were *S. aureus* were

derived from a variety of samples: skin, intestine or faeces, the urogenital tract, lung, brain, abscesses, pleural cavity, mammary glands, spleen or liver. The *E. coli* isolates included in this investigation ($n = 1093$) were obtained from the intestinal tract, including faeces, from the urogenital organs, mammary glands, lungs, liver, or spleen. When *E. coli* was isolated from internal organs, they were assumed to be causative organisms, whereas isolates from the intestine or from faeces could not with certainty be concluded to be causative agents, as *E. coli* is also a natural inhabitant of the intestinal tract. The haemolytic streptococci ($n = 49$) belonged to Lancefield's group G (*Streptococcus canis*) ($n = 35$) or C (*Streptococcus dysgalactiae* subsp. *equisimilis* or *Streptococcus equi* subsp. *zooepidemicus*) ($n = 14$) were derived from a variety of sites including the respiratory tract and pleural cavity, skin, mammary glands, urogenital system, or from liver or spleen in case of septicaemia. The *Pseudomonas aeruginosa* isolates included in the study ($n = 39$) were all derived from cases of haemorrhagic pneumonia. The *Pasteurella multocida* isolates ($n = 13$) were derived from a variety of sites, but mainly from the respiratory tract. Primary cultures were made on blood agar (blood agar base, OXOID, supplemented with 5% calf blood), Drigalski agar (Vulfson et al., 2001) and Enteric medium (Statens Serum Institut, Copenhagen, Denmark) 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, haemolysis, odour, cell morphology, catalase and oxidase reaction and Gram properties. Rapid identification kits were used as necessary (API ID 32E for *E. coli* and, API 20NE for *P. aeruginosa* and *P. multocida*, API ID 32 STAPH for *S. intermedius*, and API rapid ID 32 STREP for streptococci, bioMérieux, Marcy l'Étoile, France). Identification of haemolytic streptococci was supplemented with a test for positive reaction with Lancefield's group G or C antiserum (OXOID Diagnostic Reagents), and identification of *S. intermedius* and *S. aureus* with a positive test for coagulase and a negative and positive test, respectively, for hyaluronidase.

2.2. Antimicrobial susceptibility testing

A semi-automated antimicrobial sensitivity testing system (Sensititre, Trek Diagnostic Systems, East

Grinstead, UK), based on the broth dilution method, was used together with customised ready-to-use microtitre plates containing two-fold dilution amounts of antimicrobials. Different panels were used for different bacterial species. MIC breakpoints were as defined by CLSI (NCCLS, 2004) or, when not available here, Pedersen et al. (2007).

2.3. Usage of antimicrobials

The usage of antimicrobials for fur animals was based on data extracted from the Danish database on veterinary prescriptions of antimicrobials, VetStat. The prescription of specific antimicrobials for treatment of specific infectious diseases was sought elucidated through a survey among practicing veterinarians.

2.4. Statistics

Significance tests for differences between proportions of resistant isolates were calculated using StatCal in Epi-Info™ version 6 or SigmaStat version 3.0. A significance level of 5% was applied ($p < 0.05$). Fisher's exact test (2-tailed) was used when appropriate.

3. Results

3.1. Antimicrobial susceptibility

No *S. intermedius* isolate showed resistance to amoxicillin with clavulanic acid or fluoroquinolones, and resistance was also low for fusidic acid, cephalothin, kanamycin, potentiated sulphonamides, and chloramphenicol (Table 1). The far highest frequency of resistance was recorded for tetracycline with 54.7% of the isolates, while resistance to penicillin, macrolides, lincosamides and spectinomycin was around 20%. The *S. aureus* isolates were significantly more often resistant to penicillin ($p = 0.009$) and spectinomycin ($p = 0.030$) compared to the *S. intermedius* isolates. Among isolates that were resistant to tetracycline, a significantly higher proportion were also resistant to clindamycin than among tetracycline sensitive isolates, and vice versa, ($p = 0.04$). A similar correlation was recorded for clindamycin and penicillin ($p = 0.002$).

Table 1

Antimicrobial resistance among haemolytic staphylococci from Danish mink

Antimicrobial compound	% resistant isolates	
	<i>S. intermedius</i> (<i>n</i> = 157)	<i>S. aureus</i> (<i>n</i> = 7)
Tetracycline	54.7	28.6
Penicillin	21.7	71.4
Clindamycin	20.4	14.3
Erythromycin	19.1	14.3
Spectinomycin	18.5	57.1
Fusidic acid	3.8	14.3
Cephalothin	1.9	14.3
Kanamycin ^a	1.7	0
Chloramphenicol	0.6	0
Sulfamethoxazole with trimethoprim	0.6	0
Enrofloxacin	0	0
Amoxicillin with clavulanic acid	0	0

^a Kanamycin: for *S. intermedius* *n* = 57 and for *S. aureus* *n* = 3.

Resistance among *E. coli* isolates was highest for ampicillin, streptomycin, sulphonamides, and tetracycline (Table 2) whereas very low frequencies of resistance (<5%) were recorded to fluoroquinolones, gentamicin, florfenicol, amoxicillin with clavulanic acid, ceftiofur, chloramphenicol, colistin, nalidixic acid, and apramycin. Among isolates that were resistant to ampicillin, a significantly higher proportion were also resistant to tetracycline than among ampicillin sensitive isolates, and vice versa, ($p < 0.001$). Similar connections were recorded for ampicillin and sulphonamides ($p < 0.001$), ampicillin and trimethoprim ($p < 0.001$), tetracycline and sulphonamides ($p < 0.001$), tetracycline and trimethoprim ($p < 0.001$), and sulphonamides and trimethoprim ($p < 0.001$). Haemolytic isolates were generally more often resistant than non-haemolytic ones (data not shown), but the differences were only statistically significant for ampicillin, streptomycin, sulphonamides, and tetracycline ($p < 0.001$ in all cases). Some differences in resistance patterns between isolates from different origins were observed. Non-haemolytic isolates from the lungs were significantly more often resistant to streptomycin ($p = 0.041$) and trimethoprim ($p = 0.009$) than isolates from faeces or intestines, and were also significantly more often resistant to trimethoprim

Table 2
Antimicrobial resistance of *E. coli* isolates from Danish mink

Antimicrobial compound	% resistant isolates				
	Faeces or intestine (<i>n</i> = 700)	Lung (<i>n</i> = 195)	Spleen or liver (<i>n</i> = 173)	Urogenital tract or mammary glands (<i>n</i> = 25)	Total (<i>n</i> = 1093)
Ampicillin	38.7	48.2	39.3	60.0	40.9
Streptomycin	26.3	36.4	30.1	36.0	28.9
Tetracycline	24.2	29.7	25.4	42.0	24.5
Sulfamethoxazole	24.0	30.2	20.8	32.0	24.4
Spectinomycin	11.3	11.2	9.2	4.0	10.7
Trimethoprim	9.0	16.4	7.0	8.0	10.0
Neomycin	7.3	6.7	6.3	8.0	7.1
Chloramphenicol	4.3	5.6	3.5	0	4.3
Cephalothin ^a	3.9	3.9	3.5	9.0	4.0
Nalidixic acid	3.0	3.1	2.3	8.0	3.0
Apramycin	2.3	2.1	1.7	4.0	2.2
Colistin	2.0	1.0	0.6	0	1.6
Gentamicin	0.4	0	1.1	4.0	0.5
Ceftiofur	0.6	0.5	0	0	0.4
Amoxicillin with clavulanic acid (2:1)	0.3	0	0	8.0	0.3
Florfenicol	0.5	0	0	0	0.3
Ciprofloxacin	0.2	0	0	0	0.1

^a Cephalothin: for faeces or intestine *n* = 462, for lungs *n* = 155, for spleen or liver *n* = 115, for urogenital tract or mammary glands *n* = 20, for total *n* = 752.

(*p* = 0.002) than isolates from the spleen or liver. For haemolytic isolates, frequencies of resistance to ampicillin (*p* = 0.017) were significantly higher in isolates from the urogenital tract or mammary glands than in isolates from faeces or intestine.

All *P. aeruginosa* isolates were sensitive to gentamicin and colistin and only 5.1% of the isolates were resistant to enrofloxacin. In contrast, all isolates were resistant to ampicillin, amoxicillin with clavulanic acid, cephalothin, chloramphenicol, lincosamides, macrolides, and spectinomycin and most isolates were resistant to sulphonamides with trimethoprim (92.3%), tetracycline (89.7%), and kanamycin (66.7%).

P. multocida isolates were invariably sensitive to penicillin and ampicillin, chloramphenicol, gentamicin, colistin, spectinomycin, tetracycline, sulphonamides with trimethoprim, cephalothin, enrofloxacin and kanamycin. In contrast, all isolates were resistant or intermediately sensitive to erythromycin and clindamycin, the majority being resistant (61.5% and 92.3%, respectively).

Isolates of haemolytic streptococci were all sensitive to penicillin, cephalothin, and sulphona-

mides with trimethoprim. The highest frequency of resistance was recorded for clindamycin and erythromycin, followed by tetracycline (Table 3). Although a difference was noticed for tetracycline in resistance between group G (17.1%) and C (42.9%) isolates, this difference was not statistically significant (*p* = 0.075).

Table 3
Antimicrobial resistance of haemolytic streptococci (*n* = 49) from Danish mink

Antimicrobial compound	% resistant	
	Group G (<i>n</i> = 35)	Group C (<i>n</i> = 14)
Clindamycin	68.6	57.1
Erythromycin	68.6	42.9
Tetracycline	17.1	42.9
Spectinomycin	8.6	14.3
Kanamycin	5.9	7.1
Enrofloxacin	2.9	0
Sulfamethoxazol with trimethoprim	0	0
Cephalothin	0	0
Penicillin	0	0

Table 4
Antimicrobials (kg active compound) sold for use in fur animals in Denmark during the period 2001–2006

Antimicrobial class	Active compound (kg)					
	2001	2002	2003	2004	2005	2006
Penicillins, extended spectrum	341	375	381	457	659	643
Aminoglycosides	166	167	206	285	304	369
Sulfonamides/trimethoprim	33	38	32	126	186	308
Macrolides	65	104	94	116	154	215
Lincosamides	34	45	44	43	63	63
Tetracyclines	19	36	14	39	53	89
Quinolones	0	0	0	0	1	0
Amphenicols	0	0	0	0	<1	0
Cephalosporins	0	0	0	<1	<1	<1
Penicillins, narrow spectrum	0	<1	0.2	<1	<1	<1
Pleuromutilins	0	0	0	<1	<1	5
Others	0	<1	0	<1	<1	<1
Fluoroquinolones	1	1	0.1	<1	0	<1
Total	659	766	771	1066	1420	1694

3.2. Usage of antimicrobials

The usage of antimicrobials for mink in Denmark during the period 2001–2006 is recorded in Table 4 (Anon., 2002, 2003, 2004, 2005, 2006, 2007). There has been a steady increase in usage every year during this period. The increase was noted for all antimicrobial classes.

4. Discussion

4.1. Antimicrobial susceptibility

S. intermedius is mostly associated with mink, dogs and other carnivores, and is one of the most commonly isolated pathogenic bacteria in these animals. This bacterial species is involved in a plethora of infectious conditions in mink, such as pneumonia and pleuritis, dermatitis, urinary tract infections, metritis, and mastitis. Reliable data for comparison only exist for dogs and to a lesser extent cats. There was considerable difference between antimicrobial resistance of *S. intermedius* from mink compared to canine isolates from the same period (Pedersen et al., 2007). The frequency of resistance was much lower to penicillin, 21.7% of the mink isolates compared to 60.2% in dogs, and fusidic acid, 3.8% in mink compared to 30.9% in dogs, whereas the frequency of

resistance to tetracycline was considerably higher, 54.7% in mink compared to 23.9% in dogs. The differences for fusidic acid can be explained by the fact that this compound is never used for mink, but often in dogs for treatment of skin-, ear-, and eye infections. Explanations for the differences observed for penicillins and tetracyclines are less obvious but they may be due to usage patterns or to co-selection as there seemed to be some correlation in resistance between certain antimicrobials. Thus, there was a significantly higher proportion of tetracycline resistant isolates that were also resistant to clindamycin than among tetracycline sensitive isolates, and vice versa, and similar for clindamycin and penicillin. Any genetic background for these correlations is not known but deserves to be investigated. A small number of *S. intermedius* and *S. aureus* isolates were found to be resistant to cephalothin although they were sensitive to amoxycillin with clavulanic acid. This may seem contradictory, but is likely to be a methodologic problem, i.e. MIC values close to the breakpoints, or similar.

The resistance patterns for the *S. aureus* isolates seemed different from those of *S. intermedius* from both mink and dogs (Table 1), although the differences were only statistically significant for few antimicrobials. This may indicate a different origin of the *S. aureus*. Thus, *S. intermedius* is closely connected with mink and other carnivores, and the infections are therefore possibly caused by strains from the mink

themselves, whereas the infections with *S. aureus* may have been acquired from other sources. *S. aureus* is often associated with cattle, poultry, and humans and although a certain host specificity of different *S. aureus* clones has been recorded, transmission between hosts occur (Van Leeuwen et al., 2005). However, clonality studies of staphylococci from mink have not been carried out, and thus, the origin and transmission of strains is not well elucidated.

A previous study by Vulfson et al. (2001) demonstrated considerable differences in antimicrobial resistance of *E. coli* between different farms. The present study included isolates from a large number of farms but usually only one isolate from each farm, and the figures do therefore not allow for comparison between farms, nor do they allow for calculations as to whether a high frequency of resistance to certain antimicrobials on a farm in one bacterial species also leads to high frequency of resistance in other bacterial species.

The relatively high level of resistance to tetracyclines may seem surprising as tetracyclines are rarely used in the mink production, and has not been used to any significant extent during recent years. The investigation by Vulfson et al. (2001) revealed a connection between resistance to tetracyclines and sulphonamides and in the present investigation, we also demonstrated significant correlations between resistance factors. Thus, among isolates that were resistant to ampicillin, a significantly higher proportion were also resistant to tetracycline than among ampicillin sensitive isolates, and vice versa, and similar correlations were recorded between ampicillin and sulphonamides, ampicillin and trimethoprim, tetracycline and sulphonamides, tetracycline and trimethoprim, and sulphonamides and trimethoprim. The genetic background for these correlations is unknown but the phenomenon deserves further investigation as genetic linkages may very well lead to co-selection of resistance and thereby maintenance of a high frequency of resistance to certain antimicrobials, even if they are not used for treatment.

Investigations of antimicrobial resistance among *E. coli* isolates from Denmark have shown marked differences between animal species (Anon., 2006), and frequencies of resistance among indicator *E. coli* are considerably lower than those among pathogenic *E. coli* isolates. In the present investigation, we found

33.1% of the haemolytic isolates and 20.9% of the non-haemolytic isolates resistant to tetracycline. In an investigation of *E. coli* from clinical submissions, resistance frequencies among isolates from cattle and pigs were 91% and 72%, respectively, to tetracycline, 93% and 37% to ampicillin, 85% and 75% to sulphonamides, and 54% and 31% to trimethoprim (Anon., 2006). These proportions of resistant isolates are higher than those found in the present study.

We found no haemolytic streptococci resistant to penicillins, cephalosporins or sulphonamides with trimethoprim. A high frequency of resistance was recorded for tetracycline, in particular among the group C isolates, although tetracyclines, as previously mentioned, are not extensively used for mink. The highest resistance was recorded to lincosamides and macrolides, both with 68.6% resistance among the group G streptococci. This is much higher than recorded for group G streptococci from dogs isolated during the same period (15.5% and 10.8%, respectively (Pedersen et al., 2007)). The reason for this is not clear, but may be explained by the frequent use of tylosin or lincospectin for mink, whereas macrolides and lincosamides account for a smaller fraction of the total usage of antimicrobials for dogs (Pedersen et al., 2007). *S. canis* (group G) is almost restricted to carnivores and resistance data are only available from dogs – and now from mink – whereas group C streptococci cause infections in many animal species, including humans.

P. multocida is a common causative agent of infections of the respiratory tract, the urogenital tract and skin infections in several animal species. We found all isolates sensitive to all antimicrobials, except for macrolides and lincosamides, to which all isolates were resistant or intermediate sensitive. This is in accordance with a recent observation on isolates from dogs (Hirsh and Jang, 1994; Pedersen et al., 2007). In investigations on *P. multocida* from pigs (Lizarro et al., 2006; Gutiérrez Martín and Rodríguez Ferri, 1993; Fales et al., 1990), it has generally been reported that isolates were susceptible to penicillins, cephalosporins, aminoglycosides, tetracyclines, and fluoroquinolones, whereas results for macrolides and lincosamides have been contradictory. Lizarro et al. (2006) reported resistance to tylosin but sensitivity to erythromycin, something which is not very likely. This is probably a methodological problem.

From a veterinary point of view, *P. aeruginosa* is often involved in chronic otitis externa in dog and it is the causative agent of haemorrhagic pneumonia in mink. Apart from these conditions, this bacterial agent is usually only a sporadic pathogen in animals, such as in septicaemia in chickens or bovine mastitis. *P. aeruginosa* is reputed for its innate resistance to most antimicrobials (Murray et al., 1999). Complete sensitivity was only found for two antimicrobials in this investigation: colistin and gentamicin. For enrofloxacin, only 5.1% of the isolates were resistant, but for most other antimicrobials, most or all isolates were resistant. It should be mentioned, though, that 18 of 39 isolated showed intermediate susceptibility to enrofloxacin, indicating that the MIC values of this bacterium lie close to the breakpoints. In a study of *P. aeruginosa* from Danish dogs, 35.9% were found to be resistant to enrofloxacin, and low levels of resistance were also noted for colistin (2.56%) and gentamicin (15.4%). These differences may be related to the use of gentamicin and polymyxins for treatment of ear infections in dogs. Considering the results of the sensitivity testing it is surprising that practicing veterinarians often prescribe vaccination in combination with treatment with potentiated sulphonamides during outbreaks of haemorrhagic pneumonia. In spite of the lack of *in vitro* susceptibility, these antimicrobials are reported clinically to limit the course of an outbreak.

4.2. Usage of antimicrobials

Use of antimicrobials in mink (and all other animals) in Denmark is based on veterinary prescription only. This would prevent misuse or overuse of antimicrobials, as it must be assumed that all antimicrobial use in mink is based on needs deemed by a veterinary practitioner. The usage of antimicrobials for fur animals during the period 2001–2006 is listed in Table 4. There has been a steady increase in consumption during this period, which may be explained by an increase in production and increased problems with specific disease problems, in particular haemorrhagic pneumonia caused by *P. aeruginosa*, distemper, mink virus enteritis, and “sticky kits”. The increase in usage was recorded for all major classes of antimicrobials. From 2001 to 2006 the total increase was 1035 kg corresponding to 157%. The highest increase in terms of kg active compound was

penicillins with 302 kg, but in terms of percent it was potentiated sulphonamides with 275 kg or 833%. The increase has been highest on compounds for which the dosage per animal is high, and this makes the increase seem more dramatic. The most used antimicrobials were extended spectrum penicillins, mainly amoxicillin. An unofficial survey among Danish veterinarians working with fur animals, indicated that amoxicillin was used for treatment of a number of infectious diseases, i.e. “sticky kits”, diarrhoea, urinary tract infections, pneumonia, pleuritis, and abscesses. The second most used antimicrobials were aminoglycosides; this seemed to be mostly apramycin and neomycin, which was used for treatment of diarrhoea. Sulphonamides with trimethoprim came up third. Potentiated sulphonamides, which was sulphadiazine with trimethoprim available from various companies, both for oral and parenteral application, were used against a broad spectrum of diseases, e.g. diarrhoea, urinary tract infections, and pneumonia (*P. aeruginosa*-associated). Sulphonamides with trimethoprim are the drug of choice for treatment of haemorrhagic pneumonia caused by *P. aeruginosa*. The fourth most used antimicrobials were macrolides, almost exclusively tylosin, which was mostly used for sticky kits. Lincosamides and tetracyclines were less often used, but in similar amounts. Lincosamides were mainly used in the form of lincospectin. This was used for a number of infectious diseases, e.g. “sticky kits”, diarrhoea, pneumonia, and pleuritis. Tetracyclines were used for treatment of “sticky kits”, pneumonia (not *P. aeruginosa*-associated), and maybe other infections. Other antimicrobials were used only in negligible amounts.

4.3. Final remarks

Very few investigations on antimicrobial resistance among bacteria from mink have been carried out. A study by Martine and Stanachi (1997) comprised too few isolates of each bacterial species to draw meaningful conclusions, and included a number of bacterial species that were irrelevant from a therapeutic point of view. Thus, the present study is, to our knowledge, the first thorough investigation of the occurrence of antimicrobial resistance among important bacterial pathogens from mink and the usage of antimicrobials for fur animals. Valid comparison of

the amounts of antimicrobials used in mink in Denmark and other countries is difficult, as few countries have comparable production regimes and consumption data is sparse. Our data on antimicrobial resistance and usage of antimicrobials are therefore mainly descriptive, and do hardly allow conclusions to be drawn as to the appropriateness of resistance and usage levels. However, the present data constitute a baseline, which is applicable for development of recommendations to veterinarians on prudent use of antimicrobials in fur animal production.

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