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2 **Usage of antimicrobials and occurrence of antimicrobial resistance among**
3 **bacteria from mink**

4

5

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7

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24 **Abstract**

25 The usage of antimicrobials for treatment of mink and the occurrence of
26 antimicrobial resistance among the most important bacterial pathogens in mink was
27 investigated. The aim of the study was to provide data, which may serve as a basis for the
28 formulation of recommendations for prudent use of antimicrobials for mink. A total of 164
29 haemolytic staphylococci, 49 haemolytic streptococci, 39 *Pseudomonas aeruginosa*, 13
30 *Pasteurella multocida*, and 1093 *Escherichia coli* isolates from Danish mink were included in
31 the study. A high frequency of resistance among *S. intermedius* was found for tetracyclines
32 (54.7%), followed by penicillin (21.7%), lincosamides (20.4%), macrolides (19.1%), and
33 spectinomycin (18.5%). Very low frequencies of resistance were recorded for other
34 antimicrobials. The highest frequency among the *E. coli* isolates was recorded for ampicillin,
35 streptomycin, sulphonamides, and tetracyclines, whereas resistance to other antimicrobials
36 was rare. All *P. aeruginosa* were sensitive to gentamicin and colistin and sensitive or
37 intermediate to enrofloxacin, whereas most isolates were resistant to all other antimicrobials.
38 All *P. multocida* and haemolytic streptococci were sensitive to penicillin.
39 There was a steady increase in the use of antimicrobials during the period 2001 – 2006, the
40 majority of the prescribed amount being extended spectrum penicillins followed by
41 aminoglycosides, sulphonamides with trimethoprim, and macrolides.

42

43

44 **Key words:** fur animals, mink, antibiotics, antimicrobial resistance, treatment

45 **1. Introduction**

46 Mink may suffer from a number of infectious diseases, which demand therapy.
47 The most important bacterial infections include *Escherichia coli* (enteritis, pneumonia,
48 septicaemia), *Pseudomonas aeruginosa* (haemorrhagic pneumonia), haemolytic
49 staphylococci, most often *Staphylococcus intermedius* and occasionally *Staphylococcus*
50 *aureus* (mastitis, pneumonia, pleuritis, dermatitis, metritis, urinary tract infection,
51 septicaemia, and others), *Pasteurella multocida* (respiratory tract infection, pleuritis, wound
52 infection, and others), haemolytic streptococci, mostly Lancefield's group G and less
53 frequently group C (respiratory tract infection, pleuritis, dermatitis, wound infection,
54 septicaemia, and others). Other bacterial infections occur occasionally, such as infections
55 caused by *Salmonella*, *Plesiomonas shigelloides*, *Streptococcus bovis*, *Streptococcus*
56 *pneumoniae*, *Aeromonas* spp., and others. Although viral infections cannot be treated with
57 antibiotics, it is not unusual that such treatment is initiated in an attempt to reduce mortality
58 due to secondary bacterial infections to specific viral diseases, such as mink virus enteritis,
59 distemper, and "sticky kits". An increase in the prescription of antimicrobials for fur animals
60 has been noted from 659 kg active compound in 2001 to 1694 kg in 2006 (Anon. 2002, 2007),
61 but during the same period also an increase in production. So far, few investigations have
62 been carried out on antimicrobial resistance in bacteria from fur animals and the current
63 knowledge of antimicrobial resistance in important bacterial pathogens in mink is sparse. The
64 present investigation was undertaken in order to elucidate the occurrence of antimicrobial
65 resistance among important bacteria from infections in mink, and to present data on the
66 consumption of antimicrobials in mink in Denmark.

67

68 2. Material and methods

69 2.1. Bacterial isolates and culture conditions

70 Bacterial isolates were obtained from clinical samples submitted to the National
71 Veterinary Laboratory during the period 2000 – 2005. The isolates originated from more than
72 870 mink farms in Denmark and the number of isolates from each farm varied from 1 to 11,
73 the majority of farms being represented by 1 – 4 isolates of different bacterial species.
74 Therefore, the isolates were considered representative for the whole country.

75 The haemolytic staphylococci (n=164), of which 157 were *S. intermedius* and 7
76 were *S. aureus* were derived from a variety of samples: skin, intestine or faeces, the urogenital
77 tract, lung, brain, abscesses, pleural cavity, mammary glands, spleen or liver. The *E. coli*
78 isolates included in this investigation (n=1093) were obtained from the intestinal tract,
79 including faeces, from the urogenital organs, mammary glands, lungs, liver, or spleen. When
80 *E. coli* was isolated from internal organs, they were assumed to be causative organisms,
81 whereas isolates from the intestine or from faeces could not with certainty be concluded to be
82 causative agents, as *E. coli* is also a natural inhabitant of the intestinal tract. The haemolytic
83 streptococci (n=49) belonged to Lancefield's group G (*Streptococcus canis*) (n=35) or C
84 (*Streptococcus dysgalactiae* subsp. *equisimilis* or *Streptococcus equi* subsp. *zooepidemicus*)
85 (n=14) were derived from a variety of sites including the respiratory tract and pleural cavity,
86 skin, mammary glands, urogenital system, or from liver or spleen in case of septicaemia. The
87 *Pseudomonas aeruginosa* isolates included in the study (n=39) were all derived from cases of
88 haemorrhagic pneumonia. The *Pasteurella multocida* isolates (n=13) were derived from a
89 variety of sites, but mainly from the respiratory tract. Primary cultures were made on blood
90 agar (blood agar base, OXOID, supplemented with 5% calf blood), Drigalski agar (Vulfson *et*
91 *al.* 2001) and Enteric medium (Statens Serum Institut, Copenhagen, Denmark) and
92 subcultured on blood agar. All media were incubated aerobically at 37°C for 18 – 24 h.

93 Bacteria were identified from their appearance on agar media, haemolysis, odour, cell
94 morphology, catalase and oxidase reaction and Gram properties. Rapid identification kits
95 were used as necessary (API ID 32E for *E. coli* and, API 20NE for *P. aeruginosa* and *P.*
96 *multocida*, API ID 32 STAPH for *S. intermedius*, and API rapid ID 32 STREP for
97 streptococci, bioMérieux, Marcy l'Étoile, France). Identification of haemolytic streptococci
98 was supplemented with a test for positive reaction with Lancefield's group G or C antiserum
99 (OXOID Diagnostic Reagents), and identification of *S. intermedius* and *S. aureus* with a
100 positive test for coagulase and a negative and positive test, respectively, for hyaluronidase.

101

102 2.2. Antimicrobial susceptibility testing

103 A semi-automated antimicrobial sensitivity testing system (Sensititre, Trek
104 Diagnostic Systems, East Grinstead, UK), based on the broth dilution method, was used
105 together with customised ready-to-use microtitre plates containing two-fold dilution amounts
106 of antimicrobials. Different panels were used for different bacterial species. MIC breakpoints
107 were as defined by CLSI (NCCLS) (2004), or, when not available here, Pedersen *et al.*
108 (2007).

109

110 2.3. Usage of antimicrobials

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

115

116 *2.4. Statistics*

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

120

121 **3. Results**122 *3.1. Antimicrobial susceptibility*

123 No *S. intermedius* isolate showed resistance to amoxicillin with clavulanic acid
124 or fluoroquinolones, and resistance was also low for fusidic acid, cephalothin, kanamycin,
125 potentiated sulphonamides, and chloramphenicol (Table 1). The far highest frequency of
126 resistance was recorded for tetracycline with 54.7% of the isolates, while resistance to
127 penicillin, macrolides, lincosamides and spectinomycin was around 20%. The *S. aureus*
128 isolates were significantly more often resistant to penicillin ($p = 0.009$) and spectinomycin ($p =$
129 0.030) compared to the *S. intermedius* isolates. Among isolates that were resistant to
130 tetracycline, a significantly higher proportion were also resistant to clindamycin than among
131 tetracycline sensitive isolates, and vice versa, ($p = 0.04$). A similar correlation was recorded for
132 clindamycin and penicillin ($p = 0.002$).

133 Resistance among *E. coli* isolates was highest for ampicillin, streptomycin,
134 sulphonamides, and tetracycline. (Table 2) whereas very low frequencies of resistance ($< 5\%$)
135 were recorded to fluoroquinolones, gentamicin, florfenicol, amoxicillin with clavulanic acid,
136 ceftiofur, chloramphenicol, colistin, nalidixic acid, and apramycin. Among isolates that were
137 resistant to ampicillin, a significantly higher proportion were also resistant to tetracycline than
138 among ampicillin sensitive isolates, and vice versa, ($p < 0.001$). Similar connections were
139 recorded for ampicillin and sulphonamides ($p < 0.001$), ampicillin and trimethoprim ($p < 0.001$),

140 tetracycline and sulphonamides ($p < 0.001$), tetracycline and trimethoprim ($p < 0.001$), and
141 sulphonamides and trimethoprim ($p < 0.001$). Haemolytic isolates were generally more often
142 resistant than non-haemolytic ones (data not shown), but the differences were only
143 statistically significant for ampicillin, streptomycin, sulphonamides, and tetracycline ($p < 0.001$
144 in all cases). Some differences in resistance patterns between isolates from different origins
145 were observed. Non-haemolytic isolates from the lungs were significantly more often resistant
146 to streptomycin ($p = 0.041$) and trimethoprim ($p = 0.009$) than isolates from faeces or
147 intestines, and were also significantly more often resistant to trimethoprim ($p = 0.002$) than
148 isolates from the spleen or liver. For haemolytic isolates, frequencies of resistance to
149 ampicillin ($p = 0.017$) were significantly higher in isolates from the urogenital tract or
150 mammary glands than in isolates from faeces or intestine.

151 All *P. aeruginosa* isolates were sensitive to gentamicin and colistin and only
152 5.1% of the isolates were resistant to enrofloxacin. In contrast, all isolates were resistant to
153 ampicillin, amoxicillin with clavulanic acid, cefalothin, chloramphenicol, lincosamides,
154 macrolides, and spectinomycin and most isolates were resistant to sulphonamides with
155 trimethoprim (92.3%), tetracycline (89.7%), and kanamycin (66.7%).

156 *P. multocida* isolates were invariably sensitive to penicillin and ampicillin,
157 chloramphenicol, gentamicin, colistin, spectinomycin, tetracycline, sulphonamides with
158 trimethoprim, cefalothin, enrofloxacin and kanamycin. In contrast, all isolates were resistant
159 or intermediately sensitive to erythromycin and clindamycin, the majority being resistant
160 (61.5% and 92.3%, respectively).

161 Isolates of haemolytic streptococci were all sensitive to penicillin, cephalothin,
162 and sulphonamides with trimethoprim. The highest frequency of resistance was recorded for
163 clindamycin and erythromycin, followed by tetracycline (Table 3). Although a difference was

164 noticed for tetracycline in resistance between group G (17.1%) and C (42.9%) isolates, this
165 difference was not statistically significant ($p=0.075$).

166

167 3.2. Usage of antimicrobials

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

172

173 4. Discussion

174 4.1. Antimicrobial susceptibility

175 *S. intermedius* is mostly associated with mink, dogs and other carnivores, and is
176 one of the most commonly isolated pathogenic bacteria in these animals. This bacterial
177 species is involved in a plethora of infectious conditions in mink, such as pneumonia and
178 pleuritis, dermatitis, urinary tract infections, metritis, and mastitis. Reliable data for
179 comparison only exist for dogs and to a lesser extent cats. There was considerable difference
180 between antimicrobial resistance of *S. intermedius* from mink compared to canine isolates
181 from the same period (Pedersen *et al.* 2007). The frequency of resistance was much lower to
182 penicillin, 21.7% of the mink isolates compared to 60.2% in dogs, and fusidic acid, 3.8% in
183 mink compared to 30.9% in dogs, whereas the frequency of resistance to tetracycline was
184 considerably higher, 54.7% in mink compared to 23.9% in dogs. The differences for fusidic
185 acid can be explained by the fact that this compound is never used for mink, but often in dogs
186 for treatment of skin-, ear-, and eye infections. Explanations for the differences observed for
187 penicillins and tetracyclines are less obvious but they may be due to usage patterns or to co-
188 selection as there seemed to be some correlation in resistance between certain antimicrobials.

189 Thus, there was a significantly higher proportion of tetracycline resistant isolates that were
190 also resistant to clindamycin than among tetracycline sensitive isolates, and vice versa, and
191 similar for clindamycin and penicillin. Any genetic background for these correlations is not
192 known but deserves to be investigated. A small number of *S. intermedius* and *S. aureus*
193 isolates were found to be resistant to cephalothin although they were sensitive to amoxycillin
194 with clavulanic acid. This may seem contradictory, but is likely to be a methodologic problem,
195 i.e. MIC values close to the breakpoints, or similar.

196 The resistance patterns for the *S. aureus* isolates seemed different from those of
197 *S. intermedius* from both mink and dogs (Table 1), although the differences were only
198 statistically significant for few antimicrobials. This may indicate a different origin of the *S.*
199 *aureus*. Thus, *S. intermedius* is closely connected with mink and other carnivores, and the
200 infections are therefore possibly caused by strains from the mink themselves, whereas the
201 infections with *S. aureus* may have been acquired from other sources. *S. aureus* is often
202 associated with cattle, poultry, and humans and although a certain host specificity of different
203 *S. aureus* clones has been recorded, transmission between hosts occur (van Leeuwen *et al.*
204 2005). However, clonality studies of staphylococci from mink have not been carried out, and
205 thus, the origin and transmission of strains is not well elucidated.

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

212 The relatively high level of resistance to tetracyclines may seem surprising as
213 tetracyclines are rarely used in the mink production, and has not been used to any significant

214 extent during recent years. The investigation by Vulfson *et al.* (2001) revealed a connection
215 between resistance to tetracyclines and sulphonamides and in the present investigation, we
216 also demonstrated significant correlations between resistance factors. Thus, among isolates
217 that were resistant to ampicillin, a significantly higher proportion were also resistant to
218 tetracycline than among ampicillin sensitive isolates, and vice versa, and similar correlations
219 were recorded between ampicillin and sulphonamides, ampicillin and trimethoprim,
220 tetracycline and sulphonamides, tetracycline and trimethoprim, and sulphonamides and
221 trimethoprim. The genetic background for these correlations is unknown but the phenomenon
222 deserves further investigation as genetic linkages may very well lead to co-selection of
223 resistance and thereby maintenance of a high frequency of resistance to certain antimicrobials,
224 even if they are not used for treatment.

225 Investigations of antimicrobial resistance among *E. coli* isolates from Denmark
226 have shown marked differences between animal species (Anon. 2006), and frequencies of
227 resistance among indicator *E. coli* are considerably lower than those among pathogenic *E. coli*
228 isolates. In the present investigation, we found 33.1% of the haemolytic isolates and 20.9% of
229 the non-haemolytic isolates resistant to tetracycline. In an investigation of *E. coli* from
230 clinical submissions, resistance frequencies among isolates from cattle and pigs were 91%
231 and 72%, respectively, to tetracycline, 93% and 37% to ampicillin, 85% and 75% to
232 sulphonamides, and 54% and 31% to trimethoprim (Anon. 2006). These proportions of
233 resistant isolates are higher than those found in the present study.

234 We found no haemolytic streptococci resistant to penicillins, cephalosporins or
235 sulphonamides with trimethoprim. A high frequency of resistance was recorded for
236 tetracycline, in particular among the group C isolates, although tetracyclines, as previously
237 mentioned, are not extensively used for mink. The highest resistance was recorded to
238 lincosamides and macrolides, both with 68.6% resistance among the group G streptococci.

239 This is much higher than recorded for group G streptococci from dogs isolated during the
240 same period (15.5% and 10.8%, respectively (Pedersen *et al.* 2007). The reason for this is not
241 clear, but may be explained by the frequent use of tylosin or lincospectin for mink, whereas
242 macrolides and lincosamides account for a smaller fraction of the total usage of antimicrobials
243 for dogs (Pedersen *et al.* 2007). *S. canis* (group G) is almost restricted to carnivores and
244 resistance data are only available from dogs – and now from mink -, whereas group C
245 streptococci cause infections in many animal species, including humans.

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

257 From a veterinary point of view, *P. aeruginosa* is often involved in chronic otitis
258 externa in dog and it is the causative agent of haemorrhagic pneumonia in mink. Apart from
259 these conditions, this bacterial agent is usually only a sporadic pathogen in animals, such as in
260 septicaemia in chickens or bovine mastitis. *P. aeruginosa* is reputed for its innate resistance to
261 most antimicrobials (Murray *et al.* 1999). Complete sensitivity was only found for two
262 antimicrobials in this investigation: colistin and gentamicin. For enrofloxacin, only 5.1% of
263 the isolates were resistant, but for most other antimicrobials, most or all isolates were

264 resistant. It should be mentioned, though, that 18 of 39 isolated showed intermediate
265 susceptibility to enrofloxacin, indicating that the MIC values of this bacterium lie close to the
266 breakpoints. In a study of *P. aeruginosa* from Danish dogs, 35.9% were found to be resistant
267 to enrofloxacin, and low levels of resistance were also noted for colistin (2.56%) and
268 gentamicin (15.4%). These differences may be related to the use of gentamicin and
269 polymyxins for treatment of ear infections in dogs. Considering the results of the sensitivity
270 testing it is surprising that practicing veterinarians often prescribe vaccination in combination
271 with treatment with potentiated sulphonamides during outbreaks of haemorrhagic pneumonia.
272 In spite of the lack of *in vitro* susceptibility, these antimicrobials are reported clinically to
273 limit the course of an outbreak.

274

275 4.2. Usage of antimicrobials

276 Use of antimicrobials in mink (and all other animals) in Denmark is based on
277 veterinary prescription only. This would prevent misuse or overuse of antimicrobials, as it
278 must be assumed that all antimicrobial use in mink is based on needs deemed by a veterinary
279 practitioner. The usage of antimicrobials for fur animals during the period 2001 – 2006 is
280 listed in Table 4. There has been a steady increase in consumption during this period, which
281 may be explained by an increase in production and increased problems with specific disease
282 problems, in particular haemorrhagic pneumonia caused by *P. aeruginosa*, distemper, mink
283 virus enteritis, and “sticky kits”. The increase in usage was recorded for all major classes of
284 antimicrobials. From 2001 to 2006 the total increase was 1035 kg corresponding to 157%.
285 The highest increase in terms of kg active compound was penicillins with 302 kg, but in terms
286 of percent it was potentiated sulphonamides with 275 kg or 833%. The increase has been
287 highest on compounds for which the dosage per animal is high, and this makes the increase
288 seem more dramatic. The most used antimicrobials were extended spectrum penicillins,

289 mainly amoxicillin. An unofficial survey among Danish veterinarians working with fur
290 animals, indicated that amoxicillin was used for treatment of a number of infectious diseases,
291 i.e. “sticky kits”, diarrhoea, urinary tract infections, pneumonia, pleuritis, and abscesses. The
292 second most used antimicrobials were aminoglycosides; this seemed to be mostly apramycin
293 and neomycin, which was used for treatment of diarrhoea. Sulphonamides with trimethoprim
294 came up third. Potentiated sulphonamides, which was sulphadiazine with trimethoprim
295 available from various companies, both for oral and parenteral application, were used against
296 a broad spectrum of diseases, e.g. diarrhoea, urinary tract infections, and pneumonia (*P.*
297 *aeruginosa*-associated). Sulphonamides with trimethoprim are the drug of choice for
298 treatment of haemorrhagic pneumonia caused by *P. aeruginosa*. The fourth most used
299 antimicrobials were macrolides, almost exclusively tylosin, which was mostly used for sticky
300 kits. Lincosamides and tetracyclines were less often used, but in similar amounts.
301 Lincosamides were mainly used in the form of lincospectin. This was used for a number of
302 infectious diseases, e.g. “sticky kits”, diarrhoea, pneumonia, and pleuritis. Tetracyclines were
303 used for treatment of “sticky kits”, pneumonia (not *P. aeruginosa*-associated), and maybe
304 other infections. Other antimicrobials were used only in negligible amounts.

305

306 4.3. Final remarks

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

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

320

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325 **References**

- 326 Anonymous, 2002. DANMAP 2001 – Use of antimicrobial agents and occurrence of
327 antimicrobial resistance in bacteria from food animals, foods and humans in Denmark.
328 Copenhagen, Denmark, ISSN 1600-2032.
- 329 Anonymous, 2003. DANMAP 2002 – Use of antimicrobial agents and occurrence of
330 antimicrobial resistance in bacteria from food animals, foods and humans in Denmark.
331 Copenhagen, Denmark, ISSN 1600-2032.
- 332 Anonymous, 2004. DANMAP 2003 – Use of antimicrobial agents and occurrence of
333 antimicrobial resistance in bacteria from food animals, foods and humans in Denmark.
334 Copenhagen, Denmark, ISSN 1600-2032.
- 335 Anonymous, 2005. DANMAP 2004 – Use of antimicrobial agents and occurrence of
336 antimicrobial resistance in bacteria from food animals, foods and humans in Denmark.
337 Copenhagen, Denmark, ISSN 1600-2032.
- 338 Anonymous, 2006. DANMAP 2005 – Use of antimicrobial agents and occurrence of
339 antimicrobial resistance in bacteria from food animals, foods and humans in Denmark.
340 Copenhagen, Denmark, ISSN 1600-2032.
- 341 Anonymous, 2007. DANMAP 2006 – Use of antimicrobial agents and occurrence of
342 antimicrobial resistance in bacteria from food animals, foods and humans in Denmark.
343 Copenhagen, Denmark, ISSN 1600-2032.
- 344 Fales, W.H., Turk, J.R., Miller, M.A., Bean-Knudsen, C., Nelson, S.L., Morehouse, L.G.,
345 Gosser, H.S., 1990. Antimicrobial susceptibility of *Pasteurella multocida* type D from
346 Missouri swine. J. Vet. Diagn. Invest. 2, 80-81.
- 347 Gutiérrez Martín, C.B., Rodríguez Ferri, E.F., 1993. *In vitro* susceptibility of *Pasteurella*
348 *multocida* subsp. *multocida* strains isolated from swine to 42 antimicrobial agents.
349 Zentralbl. Bakteriologie. 279, 387-393.

- 350 Hirsh, D.C., Jang, S.S., 1994. Antimicrobial susceptibility of selected infectious bacterial
351 agents obtained from dogs. *J. Am. Anim. Hosp. Assoc.* 30, 487-494.
- 352 Lizarro, Y.A., Ferri, E.F., de la Fuente, A.J., Martín, C.B., 2006. Evaluation of changes in
353 antimicrobial susceptibility patterns of *Pasteurella multocida* subsp. *multocida* isolates
354 from pigs in Spain in 1987, 1988 and 2003 - 2004. *Am. J. Vet. Res.* 67, 663-668.
- 355 Martine, P., Stanachi, N. 1997. Susceptibility of microorganisms recovered from dead mink
356 kits (*Mustela vison*) to fourteen antimicrobial agents. *Scientifur* 21, 225-227.
- 357 Murray, P.R., Baron, E.J., Tenover, F.C., Tenover, F.C., Tenover, F.C., Tenover, F.C., Tenover, F.C.,
358 Murray, P.R., Baron, E.J., Tenover, F.C., Tenover, F.C., Tenover, F.C., Tenover, F.C., Tenover, F.C.,
359 National Committee for Clinical Laboratory Standards, 2004. Performance standards for
360 antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals;
361 informational supplement. M31-S1, NCCLS, Wayne, Pennsylvania, USA.
- 362 Pedersen, K., Pedersen, K., Jensen, H., Finster, K., Jensen, V.F., Heuer, O.E., 2007.
363 Occurrence of antimicrobial resistance in bacteria from diagnostic samples from dogs. *J.*
364 *Antimicrob. Chemother.* 60, 775-781.
- 365 Van Leeuwen, W.B., Melles, D.C., Alaidan, A., Al-Ahdal, M., Boelens, H.A.M., Snijders,
366 S.V., Wertheim, H., van Duijkeren, E., Peeters, J.K., van der Spek, P.J., Gorkink, R.,
367 Simons, G., Verbrugh, H.A., van Belkum, A., 2005. Host- and tissue-specific pathogenic
368 traits of *Staphylococcus aureus*. *J. Bacteriol.* 187, 4584-4591.
- 369 Vulfson, L., Pedersen, K., Chriél, M., Frydendahl, K., Holmen Andersen, T., Madsen, M.,
370 Dietz, H.H., 2001. Serogroups and antimicrobial susceptibility among *Escherichia coli*
371 isolated from farmed mink (*Mustela vison* Schreiber) in Denmark. *Vet. Microbiol.* 79,
372 143-153.

Table 1. Antimicrobial resistance among haemolytic staphylococci from Danish mink

Antimicrobial compound	% resistant isolates	
	<i>S. intermedius</i> (n=157)	<i>S. aureus</i> (n=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 [□]	1.7	0
Chloramphenicol	0.6	0
Sulfamethoxazole with trimethoprim	0.6	0
Enrofloxacin	0	0
Amoxicillin with clavulanic acid	0	0

[□] Kanamycin: for *S. intermedius* n=57 and for *S. aureus* n=3.

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

Antimicrobial compound	% resistant isolates				
	Faeces or intestine (n=700)	Lung (n=195)	Spleen or liver (n=173)	Urogenital tract or mammary glands (n=25)	Total (n=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*	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

* 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.

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

Antimicrobial compound	% resistant	
	Group G (n=35)	Group C (n=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	Kg active compound					
	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