Update on antimicrobial susceptibilities of bacterial isolates from canine and feline otitis externa

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Abstract — The in vitro susceptibility of a total of 1819 bacterial isolates from canine and 103 isolates from feline otitis externa cases to 13 antimicrobial drugs over a 5-year period was evaluated. Among topically used drugs, 90% of isolates were susceptible to gentamicin. Susceptibility of *Pseudomonas aeruginosa* to gentamicin was 85%, and to polymyxin B 100%. For isolates other than the *Pseudomonas* sp., susceptibility was highest to amoxicillin-clavulanic acid.

Résumé — Mise à jour des antibiosensibilités d'isolats de bactéries provenant d'otites externes canines et félines. La sensibilité in vitro à 13 antimicrobiens d'isolats de bactéries provenant d'otites externes canine (1819) et félines (103) a été évaluée sur une période de 5 ans. Parmi les drogues d'usage topique, 90 % des isolats étaient sensibles à la gentamycine. La sensibilité de *Pseudomonas aeruginosa* était de 85 % à la gentamicine et de 100 % à la polymixyne B. Pour les isolats autres que *Pseudomonas* sp, la sensibilité était plus élevée à l'amoxicilline-acide clavulanique.

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Otitis externa has a multifactorial etiology, and bacteria play an important role in otic disease. Most of the bacteria incriminated in ear infections, including *Staphylococcus, Pseudomonas, Escherichia,* and *Proteus* species can be recovered on occasion, usually in small numbers from healthy ears. Clinical signs, such as an exudate in conjunction with the isolation of a particular bacterial species in large numbers, are of significance in most cases and may indicate the presence of a pathogen (1).

Uncomplicated cases of otitis externa are best treated with topical antimicrobials along with adjunct therapy, including antiinflammatory drugs and ear cleaning (2). Typically, much higher levels of drugs are used in topical application than are used for susceptibility testing by the Kirby-Bauer disk diffusion method. Nevertheless, results obtained by the Kirby-Bauer disk diffusion method can give an indication as to which drugs may be effective (1,3).

Many practitioners treat otitis externa on the basis of their clinical impressions and an examination of a stained smear of the exudate. If the smear shows only gram-positive cocci, they may use a drug that is likely to be effective against staphylococci, streptococci, and enterococci. If the smear shows gram-negative rods in large numbers, they need to consider that the rods represent multi-drug resistant strains of *Pseudomonas aeruginosa.* However, if data on culture and sensitivity are collected appropriately and continuously, they can serve as a basis for empirical therapy.

There have been no published surveys on the antimicrobial susceptibilities of various bacteria associated with otitis externa in dogs and cats in recent years in Canada, although the antimicrobial therapy of ear infections in small animals has been reviewed (2). To generate objective data for the prudent use of antimicrobial drugs, we conducted a retrospective analysis of bacterial isolates from clinical cases of otitis externa, including chronic cases, in dogs and cats during a 5-year period ending in 2003, by reviewing the records of the Bacteriology Diagnostic Laboratory of the Atlantic Veterinary College, which, receives specimens for routine culture and sensitivity testing from Prince Edward Island and the other provinces of Atlantic Canada.

Otitis externa specimens were cultured aerobically on blood agar (Columbia agar with 5% sheep blood) and MacConkey agar (Oxoid Canada, Nepean, Ontario); bacterial isolates were identified by the methods described by Quinn et al (4). Additional tests, using API bacterial identification strips (Analytab Products; BioMérieux Canada, St. Laurent, Québec) or an automated identification system (Sensititre; TREK Diagnostics Systems, Cleveland, Ohio, USA), were used when required. Susceptibility data on all otitis externa isolates were included in the study. The isolates were considered to be significant in causing the condition when there was heavy growth of a single bacterial species in mixed culture or moderate/light growth in pure culture. Antimicrobial susceptibility testing was performed by the Kirby-Bauer

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Table 1. Antimicrobial drug susceptibility of common bacterial isolates from dogs with otitis externa

Drug[a]	Number of isolates tested and (percentage) resistant					
	S. intermedius	*P. aeruginosa*	*Streptococcus* spp.	*Proteus* spp.	*E. coli*	*Enterococcus* spp.
AK	660 (2)	319 (11)	219 (72)	184 (5)	178 (1)	44 (59)
AMC	660 (0)	306 (94)	220 (0)	184 (3)	178 (6)	44 (0)
AMP	660 (64)	306 (94)	219 (0)	184 (14)	178 (21)	44 (0)
CL	651 (1)	285 (100)	219 (3)	176 (19)	171 (13)	44 (91)
CHL[b]	651 (0)	289 (99)	217 (0)	175 (15)	170 (3)	44 (11)
CLM	659 (4)	290 (100)	219 (2)	176 (100)	171 (99)	44 (89)
DXC	651 (7)	285 (98)	219 (12)	176 (98)	171 (16)	44 (16)
ENR[b]	651 (1)	290 (38)	219 (6)	176 (2)	171 (2)	44 (11)
ERY	660 (5)	290 (100)	220 (2)	176 (99)	171 (97)	44 (18)
FUS[b]	651 (3)	285 (100)	219 (22)	176 (95)	171 (99)	44 (52)
GNT[b]	660 (2)	319 (15)	219 (29)	184 (13)	178 (1)	43 (30)
PEN[b]	651 (65)	290 (100)	219 (0)	176 (36)	171 (99)	44 (2)
TMS	660 (18)	309 (95)	219 (3)	184 (17)	178 (6)	44 (7)

[a]AK: amikacin; AMC: amoxicillin-clavulanic acid; AMP: ampicillin; CL: cephalexin; CHL: chloramphenicol; CLM: clindamycin; DXC: doxycycline; ENR: enrofloxacin; ERY: erythromycin; FUS: fusidic acid; GNT: gentamicin; PEN: penicillin; TMS: trimethoprim-sulfa
[b]Topical preparations are commercially available

disk diffusion method, as outlined by Quinn et al (4), on Mueller-Hinton agar. For bacteria, such as streptococci, that are unable to grow on Mueller-Hinton agar, laked horse blood was added to a final concentration of 5%. The disks used (Oxoid Canada) were amikacin (30 μg), amoxicillin-clavulanic acid (30 μg), ampicillin (10 μg), cephalexin (30 μg), chloramphenicol (30 μg), clindamycin (2 μg), doxycycline (30 μg), enrofloxacin (5 μg), erythromycin (15 μg), fusidic acid (10 μg), gentamicin (10 μg), penicillin (10 units), and trimethoprim-sulfa (TMS) (25 μg). The inhibition zones of all drugs, except fusidic acid, were interpreted according to the National Committee for Clinical Laboratory Standards (NCCLS), as summarized by Quinn et al (4). For fusidic acid, isolates with ≤ 4 mm zone diameter were considered resistant; 15 to 21 mm, intermediate; and ≥ 22 mm, sensitive. *Pseudomonas aeruginosa* isolates (n = 84) were also tested against polymyxin B (300 unit disks) (Oxoid Canada), and the zone sizes were interpreted according to the NCCLS guidelines, as summarized by Carter (5).

The most common species recovered from 1819 canine isolates was *Staphylococcus intermedius* (n = 660). *Pseudomonas aeruginosa* (n = 319) was the next most common, followed by *Streptococcus* spp. (n = 180 group G; 35 β hemolytic; 6 α hemolytic), *Proteus* spp. (n = 175 *P. mirabilis;* 9 *P. vulgaris*), *Escherichia coli* (n = 178), and *Enterococcus* spp. (n = 44, including 9 *E. faecalis*). The rates of resistance of these isolates to various drugs are summarized in Table 1. All of the gram-positive bacteria tested were susceptible to amoxicillin-clavulanic acid. In contrast, no single drug was effective against all gram-negative isolates. Ninety percent of gram-negative isolates showed susceptibility to gentamicin; however, none of the 84 isolates of *P. aeruginosa* tested were resistant to polymyxin B. While amikacin was most effective against the majority of *P. aeruginosa,* with only 11% resistant, enrofloxacin was effective against *E. coli* and *Proteus* spp., with only 2% resistant. Other bacterial species recovered from cases of canine otitis externa were *Pasteurella* spp. (n = 34, 30 *P. multocida*) and *Klebsiella* spp. (n = 18, 11 *K. pneumoniae*). The remaining 163 isolates were mostly gram-negative bacteria including *Citrobacter* spp. and *Enterobacter* spp. Most gram-negative bacteria other than *P. aeruginosa* and all *Pasteurella* spp. and *Klebsiella* spp. were susceptible to amoxicillin-clavulanic acid and gentamicin. Rarely recovered bacteria included a single *Stenotrophomonas maltophilia,* which was susceptible to amikacin, chloramphenicol, doxycycline, and enrofloxacin, while a lone isolate of *Arcanobacterium pyogenes* was susceptible to all drugs.

The rates of resistance of the 75 most common of 103 feline isolates are summarized in Table 2. Thirty-one were coagulase-negative staphylococci (not identified to species level), 17 coagulase-positive staphylococci (*S. aureus* n = 10, *S. intermedius* n = 7), 15 *Pasteurella* (*P. multocida* n = 14, *P. dagmatis* n = 1), and 12 coliforms, including 9 *E. coli* isolates. The remainder consisted of a small number of isolates (≤ 5 each) including streptococci, enterococci, *P. aeruginosa, Proteus* spp., and *A. pyogenes.* All of the coagulase-negative staphylococci were sensitive to amikacin, amoxicillin-clavulanic acid, cephalexin, clindamycin, doxycycline, enrofloxacin, and fusidic acid. Resistance to the other drugs tested was ≤ 13%. All coagulase-positive staphylococci were sensitive to amoxicillin-clavulanic acid, cephalexin, chloramphenicol, doxycycline, enrofloxacin, and TMS. Resistance was most frequently detected to penicillin (69%), followed by ampicillin (65%). *Pasteurella* isolates were sensitive to all drugs except amikacin, clindamycin, and fusidic acid. Susceptibility of coliforms was limited to amoxicillin-clavulanic acid, chloramphenicol, and TMS. All other isolates, except *P. aeruginosa,* were susceptible to amoxicillin-clavulanic acid. *Pseudomonas aeruginosa* isolates (n = 4) were susceptible to amikacin and gentamicin.

Aminoglycosides, such as amikacin and gentamicin, have been suggested for topical application in otitis externa caused by gram-negative bacteria (6,7). Our current study shows that the majority of gram-negative bacteria, including *P. aeruginosa* (≥ 85%), are susceptible to amikacin and gentamicin. In a 1995 study of 113 isolates of *P. aeruginosa* of canine origin, the majority of which were from otitis externa cases from this region, ≥ 95% were susceptible to amikacin and gentamicin (8). Gram-negative bacteria other than *P. aeruginosa* were susceptible to amikacin, gentamicin, and enrofloxacin, with the maximum rate of resistance

Table 2. Antimicrobial drug susceptibility of common bacterial isolates from cats with otitis externa

Drug	Number of isolates tested and (percentage) resistant			
	Coagulase-negative staphylococci	Coagulase-positive staphylococci[a]	*Pasteurella* spp.[b]	Coliforms[c]
AK	31 (0)	17 (12)	15 (27)	12 (17)
AMC	31 (0)	17 (0)	15 (0)	12 (0)
AMP	31 (13)	17 (65)	15 (0)	12 (25)
CL	31 (0)	16 (0)	15 (0)	11 (45)
CHL	31 (3)	16 (0)	15 (0)	11 (0)
CLM	31 (0)	17 (6)	15 (93)	11 (100)
DXC	31 (0)	16 (0)	15 (0)	11 (18)
ENR	31 (0)	16 (0)	15 (0)	11 (9)
ERY	31 (3)	17 (6)	15 (0)	11 (91)
FUS	31 (0)	16 (13)	15 (80)	11 (100)
GNT	31 (3)	17 (6)	15 (0)	12 (17)
PEN	31 (13)	16 (69)	15 (0)	11 (100)
TMS	31 (3)	17 (0)	15 (0)	12 (0)

[a]*S. aureus* (n = 10) and *S. intermedius* (n = 7)
[b]*P. multocida* (n = 14) and *P. dagmatis* (n = 1)
[c]*E. coli* (n = 9) and *Klebsiella* spp. (n = 3)

for any single bacterial species being 2%. Enrofloxacin can be used systemically, as well as topically, in severe cases of otitis externa or chronic otitis externa with middle ear involvement (6,9,10). All gram-positive isolates were susceptible to amoxicillin-clavulanic acid, a drug that is used in the treatment of otitis media, followed by chloramphenicol (≥ 89%), a drug suggested for the treatment of otitis externa in both cats and dogs (7). It may be noted that more than half of the enterococci were resistant to fusidic acid, which is indicated for the treatment of otitis caused by *Staphylococcus* spp. (3). In general, our results agreed with those of a 1997 study by Kiss et al (11), who observed that the most common isolates from cases of otitis externa in dogs were *S. intermedius,* susceptible to amoxicillin-clavulanic acid, and *P. aeruginosa,* susceptible to gentamicin and polymyxin B.

In conclusion, among topically used drugs, the majority (≥ 70%) of all bacterial isolates of both canine and feline origin were most susceptible to gentamicin, followed by enrofloxacin. Susceptibility of *P. aeruginosa* was 100% to polymyxin B, and 85% to gentamicin. Excluding *Pseudomonas* sp. and *Stenotrophomonas* sp., the least resistance (≤ 6%) for all other isolates was seen against amoxicillin-clavulanic acid, a drug that can be used systemically. CVJ

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