icfBulletin
Scientific Investigation and Veterinary information
Results

Otodine®

A test on 150 clinical strains of canine origin indicates that Otodine® is active against all microorganisms associated with canine otitis in dogs

Luca Guardabassi
Associate Professor of Clinical Microbiology, University of Copenhagen (Denmark)

icf Bulletin
Scientific Investigation and Veterinary information
Results

A test on 150 clinical strains of canine origin indicates that Otodine® is active against all microorganisms associated with canine otitis in dogs

Luca Guardabassi
Associate Professor of Clinical Microbiology, University of Copenhagen (Denmark)

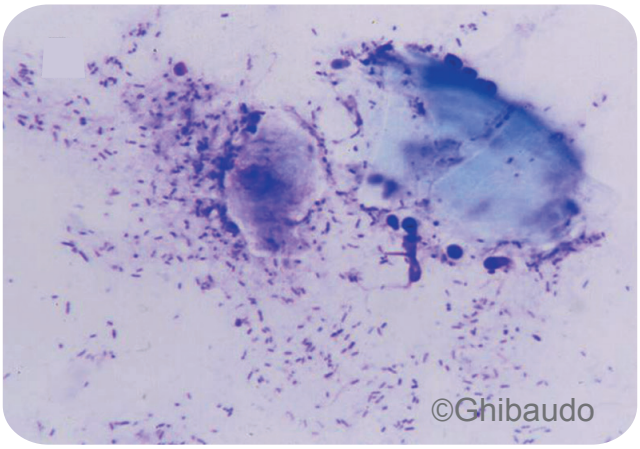
Otodine® is an ear antiseptic containing chlorhexidine digluconate and ethylene diamine tetra acetic acid-tromethamine (Tris-EDTA). Chlorhexidine digluconate exerts bactericidal activity by membrane disruption (1). Tris-EDTA increases permeability of the outer membrane in Gram-negative bacteria, thereby potentiating the effects of various antimicrobials, including antiseptics and systemic antibiotics (2-3). Combination of the two compounds results in a synergistic effect, allowing the use of low doses of chlorhexidine that are not ototoxic (4-6).
The antimicrobial activity of **Otodine®** was assessed by testing a comprehensive strain collection of pathogens associated with canine otitis.
The collection was composed by 150 clinical strains of canine origin belonging to *Corynebacterium auriscanis* (n=12), *Escherichia coli* (n=12), Malassezia pachydermatis (n=9), *Proteus mirabilis* (n=11), *Pseudomonas aeruginosa* (n=19), *Staphylococcus aureus* (n=22), *Staphylococcus pseudintermedius* (n=53) and *Streptococcus canis* (n=12).

Otodine® dilutions resulting in complete killing of the microorganisms tested were determined by the broth microdilution method (8) Briefly, each strain was incubated for 30 min at 37ºC in serial two-fold dilutions of **Otodine®** in Mueller-Hinton broth and strain survival was evaluated by transferring an aliquot of every dilution onto appropriate agar medium.

Activity on Gram-negative bacteria
All Gram-negative strains except one were completely killed at 1:8 dilutions of **Otodine®**. *P. mirabilis* was the most resistant species, whereas C. auriscanis was the most susceptible. *P. aeruginosa* and *E. coli* displayed intermediate levels of susceptibility. Strains belonging to these two species were generally eliminated by 1:16 dilution of the product (Table 1).

Activity on Gram-positive bacteria
Gram-positive strains were eliminated at 1:16 dilutions of the product. *S. pseudintermedius* and S. canis were more susceptible than *S. aureus* and 1:64 dilutions were generally sufficient to kill strains belonging to these two species. No significant difference was observed between meticillin-resistant and meticillin-susceptible staphylococci (Table 2).

Activity on yeasts
Surprisingly, in addition to the antibacterial activity against both Gram-negative and Gram-positive species, **Otodine®** showed an excellent fungicidal activity against *M. pachydermatis*. All strains tested were completely killed at 1:32 dilutions of the product (Table 3).



Concluding remarks
To my knowledge, this was the first study investigating the antimicrobial activity of a commercial ear antiseptic against a large collection of clinical strains isolated from dogs, including multi-resistant bacteria such as meticillin-resistant staphylococci. **Otodine®** was shown to be active *in vitro* against any pathogenic microorganisms involved in canine otitis, including multi-resistant Gram-positive and Gram-negative bacteria as well as *M. pachydermatis*. Such a broad spectrum of antimicrobial activity is particularly advantageous in the treatment of otitis externa as this disease condition of the dog often involves different microorganisms. Each of the 150 strains tested was completely killed after 30 min exposure to 1:4 or higher dilutions of **Otodine®** and comparable results were obtained by reducing the exposure time to 10 min. As similar or even higher concentrations are likely achieved in vivo following application of **Otodine®** in the infected ear canal, it is reasonable to conclude that this product can be successfully employed to treat any cases of ear infections by following the application time recommended by the manufacturer (i.e. at least 10 min).
Interestingly, meticillin-resistant and meticillin-susceptible staphylococci were equally affected by **Otodine®**. Combination of chlorhexidine digluconate and Tris-EDTA is efficacious against meticillin-resistant staphylococci, which notoriously are resistant to multiple antibiotics and represent a serious therapeutic problem(8), and does not seem to facilitate selection and spread of these harmful bacteria. The results of this study indicate that the formulation of **Otodine®** may be a valuable therapeutic tool to combat the recent emergence of meticillin-resistant staphylococci in veterinary dermatology.

Results

References

1. Kuyyakanond T, Quesnel LB. The mechanism of action of chlorhexidine. FEMS Microbiology Letters 1992; 79: 211–215.

2. Lambert RJW, Hanlon GW, Denyer SP. The synergistic effect of EDTA/antimicrobial combinations on Pseudomonas aeruginosa. Journal of Applied Microbiology 2004; 96: 244-253.

3. Farca AM, Piromalli G, Maffei F, Re G. Potentiating effect of EDTA-Tris on the activity of antibiotics against resistant bacteria associated with otitis, dermatitis and cystitis. Journal of Small Animal Practice 1997; 38: 243-245.

4. Mills PC, Ahlstrom L, Wilson WJ. Ototoxicity and tolerance assessment of a TrisEDTA and polyhexamethylene biguanide ear flush formulation in dogs. Journal of Veterinary Pharmacology and Therapeutics 2005; 28: 391–397.

5. Ghibaudo G, Cornegliani L, Martino P. Evaluation of the in vivo effects of Tris-EDTA and chlorhexidine digluconate 0.15% solution in chronic bacterial otitis externa: 11 cases. Veterinary Dermatology 2004; 15: 65.

6. Merchant SR, Neer TM, Tedford BL, et al. Ototoxicity assessment of a chlorhexidine otic preparation in dogs. Prog Vet Neurol 1993; 4:72-75.

7. Swinney A, Fazakerley J, McEwan N, Nuttall T. Comparative in vitro antimicrobial efficacy of commercial ear cleaners. Journal of Veterinary Dermatology 2008; 19: 373-379.

8. Loeffler A, Linek M, Moodley A, Guardabassi L, Sung JML, Winkler M, Weiss R, Lloyd DH.. First report of multiresistant, mecA-positive Staphylococcus intermedius in Europe: 12 cases from a veterinary dermatology referral clinic in Germany. Veterinary Dermatology 2007; 18: 412-421.

Table

Table 1. Otodine® dilutions resulting in complete killing of Gram-negative bacteria

Strain	Species	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
1	*Pseudomonas aeruginosa*	-	-	-	-	+	++	++	++
2	*Pseudomonas aeruginosa*	-	-	-	-	-	++	++	++
3	*Pseudomonas aeruginosa*	-	-	-	-	-	++	++	++
4	*Pseudomonas aeruginosa*	-	-	-	-	+	++	++	++
5	*Pseudomonas aeruginosa*	-	-	-	-	+	++	++	++
6	*Pseudomonas aeruginosa*	-	-	-	-	-	+	++	++
7	*Pseudomonas aeruginosa*	-	-	-	-	+	++	++	++
8	*Pseudomonas aeruginosa*	-	-	-	-	-	++	++	++
9	*Pseudomonas aeruginosa*	-	-	-	-	-	++	++	++
10	*Pseudomonas aeruginosa*	-	-	-	-	-	++	++	++
11	*Pseudomonas aeruginosa*	-	-	-	-	+	++	++	++
12	*Pseudomonas aeruginosa*	-	-	-	-	-	++	++	++
13	*Pseudomonas aeruginosa*	-	-	-	-	-	++	++	++
14	*Pseudomonas aeruginosa*	-	-	-	-	+	++	++	++
15	*Pseudomonas aeruginosa*	-	-	-	-	-	++	++	++
16	*Pseudomonas aeruginosa*	-	-	-	-	+	++	++	++
17	*Pseudomonas aeruginosa*	-	-	-	-	-	++	++	++
18	*Pseudomonas aeruginosa*	-	-	-	-	-	++	++	++
19	*Pseudomonas aeruginosa*	-	-	-	++	++	++	++	++
20	*Escherichia coli*	-	-	-	-	-	++	++	++
21	*Escherichia coli*	-	-	-	-	-	++	++	++
22	*Escherichia coli*	-	-	-	-	+	++	++	++
23	*Escherichia coli*	-	-	-	-	+	++	++	++
24	*Escherichia coli*	-	-	-	-	+	++	++	++
25	*Escherichia coli*	-	-	-	-	++	++	++	++
26	*Escherichia coli*	-	-	-	-	+	++	++	++
27	*Escherichia coli*	-	-	-	-	-	++	++	++
28	*Escherichia coli*	-	-	-	-	+	++	++	++
29	*Escherichia coli*	-	-	-	-	+	++	++	++
30	*Escherichia coli*	-	-	-	++	++	++	++	++
31	*Escherichia coli*	-	-	-	-	+	++	++	++
32	*Proteus mirabilis*	-	-	-	++	++	++	++	++
33	*Proteus mirabilis*	-	-	-	+	++	++	++	++
34	*Proteus mirabilis*	-	-	-	-	-	17	++	++
35	*Proteus mirabilis*	-	-	-	++	++	++	++	++
36	*Proteus mirabilis*	-	-	++	++	++	++	++	++
37	*Proteus mirabilis*	-	-	-	++	++	++	++	++
38	*Proteus mirabilis*	-	-	-	-	++	++	++	++
39	*Proteus mirabilis*	-	-	-	++	++	++	++	++
40	*Proteus mirabilis*	-	-	-	++	++	++	++	++
41	*Proteus mirabilis*	-	-	-	+	++	++	++	++
42	*Proteus mirabilis*	-	-	-	+	++	++	++	++
43	*Corynebacterium auriscanis*	-	-	-	-	-	-	-	-
44	*Corynebacterium auriscanis*	-	-	-	-	-	-	-	-
45	*Corynebacterium auriscanis*	-	-	-	-	-	-	-	-
46	*Corynebacterium auriscanis*	-	-	-	-	-	-	-	-
47	*Corynebacterium auriscanis*	-	-	-	-	-	-	-	-
48	*Corynebacterium auriscanis*	-	-	-	-	-	-	-	-
49	*Corynebacterium auriscanis*	-	-	-	-	-	-	-	-
50	*Corynebacterium auriscanis*	-	-	-	-	-	-	-	-
51	*Corynebacterium auriscanis*	-	-	-	-	-	-	+	+
52	*Corynebacterium auriscanis*	-	-	-	-	-	-	-	-
53	*Corynebacterium auriscanis*	-	-	-	-	-	-	-	-
54	*Corynebacterium auriscanis*	-	-	-	-	-	-	+	++

- complete killing (no growth)	+ partial inhibition (1-100 colonies)	++ no effect (confluent growth)

Table

Table 2. Otodine® dilutions resulting in complete killing of Gram-positive bacteria

Strain	Species[a]	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
1	*Streptococcus canis*	-	-	-	-	-	-	+	++
2	*Streptococcus canis*	-	-	-	-	-	-	+	++
3	*Streptococcus canis*	-	-	-	-	-	-	+	++
4	*Streptococcus canis*	-	-	-	-	-	-	+	++
5	*Streptococcus canis*	-	-	-	-	-	-	++	++
6	*Streptococcus canis*	-	-	-	-	-	-	+	++
7	*Streptococcus canis*	-	-	-	-	-	+	++	++
8	*Streptococcus canis*	-	-	-	-	-	-	+	++
9	*Streptococcus canis*	-	-	-	-	-	-	+	++
10	*Streptococcus canis*	-	-	-	-	-	-	+	++
11	*Streptococcus canis*	-	-	-	-	-	-	+	++
12	*Streptococcus canis*	-	-	-	-	-	-	+	++
13	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	+	++
14	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	+	++
15	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	-	+
16	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	+	++
17	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	+	++
18	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	+	++
19	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	+	++
20	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	-	+
21	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	+	++
22	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	+	++
23	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	+	++
24	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	+	++
25	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	+	++
26	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	+	++
27	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	+	++
28	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	+	++
29	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	+	+
30	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	+	++
31	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	+	++
32	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	+	++
33	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	+	++
34	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	+	++
35	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	+	+
36	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	+	++
37	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	+	++
38	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	+	++
39	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	+	++
40	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	-	++
41	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	-	++
42	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	+	+
43	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	-	++
44	*Staphylococcus pseudintermedius* – MS	-	-	-	-	-	-	-	++
45	*Staphylococcus pseudintermedius* – MR	-	-	-	-	-	-	+	++
46	*Staphylococcus pseudintermedius* – MR	-	-	-	-	-	-	+	++
47	*Staphylococcus pseudintermedius* – MR	-	-	-	-	-	-	+	++
48	*Staphylococcus pseudintermedius* – MR	-	-	-	-	-	-	+	++
49	*Staphylococcus pseudintermedius* – MR	-	-	-	-	-	-	+	++
50	*Staphylococcus pseudintermedius* – MR	-	-	-	-	-	-	+	++
51	*Staphylococcus pseudintermedius* – MR	-	-	-	-	-	-	+	++
52	*Staphylococcus pseudintermedius* – MR	-	-	-	-	-	-	+	++
53	*Staphylococcus pseudintermedius* – MR	-	-	-	-	-	-	+	++
54	*Staphylococcus pseudintermedius* – MR	-	-	-	-	-	-	+	++
55	*Staphylococcus pseudintermedius* – MR	-	-	-	-	-	-	+	++
56	*Staphylococcus pseudintermedius* – MR	-	-	-	-	-	-	+	++
57	*Staphylococcus pseudintermedius* – MR	-	-	-	-	-	-	+	++
58	*Staphylococcus pseudintermedius* – MR	-	-	-	-	-	-	+	++
59	*Staphylococcus pseudintermedius* – MR	-	-	-	-	-	-	+	++
60	*Staphylococcus pseudintermedius* – MR	-	-	-	-	-	-	+	++
61	*Staphylococcus pseudintermedius* – MR	-	-	-	-	-	-	+	++
62	*Staphylococcus pseudintermedius* – MR	-	-	-	-	-	-	+	++
63	*Staphylococcus pseudintermedius* – MR	-	-	-	-	-	-	+	++
64	*Staphylococcus pseudintermedius* – MR	-	-	-	-	-	-	+	++
65	*Staphylococcus pseudintermedius* – MR	-	-	-	-	-	-	+	++
66	*Staphylococcus aureus* - MS	-	-	-	-	-	-	+	++
67	*Staphylococcus aureus* - MS	-	-	-	-	-	+	++	++
68	*Staphylococcus aureus* - MS	-	-	-	-	-	-	+	++
69	*Staphylococcus aureus* - MS	-	-	-	-	-	+	+	++
70	*Staphylococcus aureus* - MS	-	-	-	-	+	+	++	++
71	*Staphylococcus aureus* - MS	-	-	-	-	+	+	++	++
72	*Staphylococcus aureus* - MS	-	-	-	-	-	+	++	++
73	*Staphylococcus aureus* - MS	-	-	-	-	+	+	++	++
74	*Staphylococcus aureus* - MS	-	-	-	-	++	++	++	++
75	*Staphylococcus aureus* - MS	-	-	-	-	++	++	++	++
76	*Staphylococcus aureus* - MS	-	-	-	-	-	+	++	++
77	*Staphylococcus aureus* - MS	-	-	-	-	-	+	++	++
78	*Staphylococcus aureus* - MS	-	-	-	-	+	+	++	++
79	*Staphylococcus aureus* - MR	-	-	-	-	-	-	+	+
80	*Staphylococcus aureus* - MR	-	-	-	-	-	+	++	++
81	*Staphylococcus aureus* - MR	-	-	-	-	+	++	++	++
82	*Staphylococcus aureus* - MR	-	-	-	-	+	++	++	++
83	*Staphylococcus aureus* - MR	-	-	-	-	+	++	++	++
84	*Staphylococcus aureus* - MR	-	-	-	-	++	++	++	++
85	*Staphylococcus aureus* - MR	-	-	-	-	+	++	++	++
86	*Staphylococcus aureus* - MR	-	-	-	-	+	++	++	++
87	*Staphylococcus aureus* - MR	-	-	-	-	+	++	++	++

[a] MS, meticillin-susceptible; MR, meticillin-resistant.

- complete killing (no growth)	+ partial inhibition (1-100 colonies)	++ no effect (confluent growth)

Table

Table 3. Otodine® dilutions resulting in complete killing of yeasts

Strain	Species	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
1	*Malassezia pachydermatis*	-	-	-	-	-	+	++	++
2	*Malassezia pachydermatis*	-	-	-	-	-	-	++	++
3	*Malassezia pachydermatis*	-	-	-	-	-	-	++	++
4	*Malassezia pachydermatis*	-	-	-	-	-	-	+	++
5	*Malassezia pachydermatis*	-	-	-	-	-	-	++	++
6	*Malassezia pachydermatis*	-	-	-	-	-	-	+	++
7	*Malassezia pachydermatis*	-	-	-	-	-	-	+	++
8	*Malassezia pachydermatis*	-	-	-	-	-	-	++	++
9	*Malassezia pachydermatis*	-	-	-	-	-	-	+	++

- complete killing (no growth)	+ partial inhibition (1-100 colonies)	++ no effect (confluent growth)

Luca Guardabassi
Associate Professor of Clinical Microbiology, University of Copenhagen (Denmark)

DP0073/01

Via G.B. Benzoni, 50
Palazzo Pignano (CR) - 26020, Italy
Tel. +39.0373.982024
Fax +39.0373.982025

www.icfpet.com