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*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**

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

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**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 37°C 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.



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

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

<sup>a</sup> 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	<i>Malassezia pachydermatis</i>	-	-	-	-	-	+	++	++
2	<i>Malassezia pachydermatis</i>	-	-	-	-	-	-	++	++
3	<i>Malassezia pachydermatis</i>	-	-	-	-	-	-	++	++
4	<i>Malassezia pachydermatis</i>	-	-	-	-	-	-	+	++
5	<i>Malassezia pachydermatis</i>	-	-	-	-	-	-	++	++
6	<i>Malassezia pachydermatis</i>	-	-	-	-	-	-	+	++
7	<i>Malassezia pachydermatis</i>	-	-	-	-	-	-	++	++
8	<i>Malassezia pachydermatis</i>	-	-	-	-	-	-	++	++
9	<i>Malassezia pachydermatis</i>	-	-	-	-	-	-	+	++

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



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