



HAL
open science

Evaluation of otoscope cone disinfection techniques on contamination by *Malassezia pachydermatis*

A.M. Boncea, F. Rethore, T. Brement, V. Bruet, L. Imparato, P. Bourdeau

► To cite this version:

A.M. Boncea, F. Rethore, T. Brement, V. Bruet, L. Imparato, et al.. Evaluation of otoscope cone disinfection techniques on contamination by *Malassezia pachydermatis*. *Revue Vétérinaire Clinique*, 2019, 54, pp.103 - 108. 10.1016/j.anicom.2019.04.002 . hal-03489249

HAL Id: hal-03489249

<https://hal.science/hal-03489249v1>

Submitted on 21 Jul 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Evaluation of otoscope cone disinfection

Evaluation of otoscope cone disinfection techniques on contamination by *Malassezia pachydermatis*

ANA M. BONCEA^{*,1}, FANNY RETHORE*, THOMAS BREMENT*, VINCENT BRUET* †, LAETITIA IMPARATO*, PATRICK BOURDEAU* †

* Unit Dermatology/Parasitology/Mycology. School of Veterinary Medicine-ONIRIS, Nantes 44307, France

†NP3 Research Unit. School of Veterinary Medicine-ONIRIS, CS 40706, Nantes UBL 44307, France

Corresponding author: Ana Maria Boncea* - Unit Dermatology/Parasitology/Mycology ONIRIS, Nantes 44307, France; bonceaana@gmail.com; phone: +40720772558

Sources of Funding: The study has been self-funded.

Conflicts of Interest: No conflict of interest have been declared.

1 Introduction

2 Speculum or otoscope cones (OCs) are used very frequently in veterinary practices to
3 examine healthy or infected ears in dogs and cats. Since OCs are reusable equipment, they can
4 be mechanical carriers of cross-contamination and cross infection between patients.¹ OCs are in
5 contact with the integument of the ear canal and thus may be easily become contaminated
6 with a variety of microorganisms.²

7 The human medical literature supports the concern for potential iatrogenic inoculation
8 of bacteria into a susceptible ear canal, if cleaning and disinfection are unappropriated.³⁻⁵

9 In veterinary medicine, a wide variety of bacteria and fungi may be isolated from the
10 canine ear canal and include for instance: *Staphylococcus pseudintermedius*, *Streptococcus spp.*
11 *Pseudomonas spp.*, *Proteus spp.* and *Malassezia*, mainly *Malassezia pachydermatis*.⁶⁻⁸ It has
12 been showed that *P. aeruginosa* can survive on otoscope cones cleaned and disinfected by
13 several commonly used methods.² *Malassezia* yeasts are lipophilic organisms, which have been
14 recognized for over a century as part of the normal human cutaneous flora, and as agents of
15 certain skin diseases.⁹

16 In small animals, apart from the body's skin, *M. pachydermatis* is usually found on
17 mucosae and in ear canals.^{2,6} It can be in very large number on the skin of healthy animals but
18 can be isolated in much higher numbers from diseased dogs. It often causes dermatitis together
19 with other pathogens (e.g. *Staphylococcus pseudintermedius*).¹² *Malassezia* yeasts can be very
20 easily carried by different fomites. Human hands bring also the question of a risk of
21 transmission of resistant/tolerant strains from infected to healthy dogs.¹³ Occasionally,
22 invasive infections by *M. pachydermatis* or lipid-dependent *Malassezia spp.* occur in neonates,
23 most often in those who are receiving parenteral lipid rich nutrition via a catheter.^{7,10,11} There is
24 still little knowledge about the possible acquisition of tolerance/resistance to antifungals in
25 *Malassezia* but in any case, the transplantation from dog to dog of any micro-organisms should
26 be avoided in good clinical practice. Although not described in veterinary medicine the
27 nosocomial infection with *Malassezia* by daily of otoscopes is a likely situation. Moreover, very
28 sparse information is available on sensitivity of *M. pachydermatis* to disinfection and common
29 methods used.¹⁴ There is a concern that OCs could serve as a carrier for the spread of
30 infection.²⁻⁵ The chemical composition of the host epidermis, including the ear canal, seems to
31 play a pivotal role in influencing the pathogenic or commensal phenotype of *Malassezia* yeasts
32 by selecting different genetic populations with specific physiological requirements, different cell
33 wall compositions, and different antifungal susceptibility profiles.¹⁵⁻¹⁷ There is still little
34 indication about the survival time of *M. pachydermatis*,¹ but the persistence of other yeasts,
35 such as *Candida glabrata*, was described to be around 5 months or may be shorter like in the
36 case of *Candida parapsilosis*, 14 days.¹⁸⁻²⁰

37 Different techniques and disinfectants are used in daily practice in veterinary clinics to clean
38 OCs, without knowledge on their capacity in the prevention of the risk of transmission of
39 yeasts, like *M. pachydermatis*. The objective of this study is to assess the efficacy of some of
40 these techniques in laboratory conditions.

41 **Material and methods**

42 ***Otoscope cones – in vitro contamination with Malassezia***

43 We used metallic OCs, that were first sterilized (121⁰C, 20 minutes). *Malassezia* isolates were
44 obtained from fresh cultures provided by the Veterinary Teaching University Hospital (France).
45 *M. pachydermatis* clinical (ear) isolates from dogs were both identified morphologically and on
46 their ability to grow on media isolation without lipid supplementation (Sabouraud Dextrose
47 Agar).³¹ Colonies of *M. pachydermatis* were collected from 3 to 10 day old cultures with a
48 harvesting loop and vortexed with 2 mL of saline 0, 9% - Tween 20 (1%) solution, which was
49 used as *Malassezia* suspension. The final concentration of the suspension of each isolate was
50 adjusted to an optical density of 2.4 (+/- 0.1) McFarland (adjusted turbidity) using a turbid
51 meter (McFarland Densitometer, Grosseron) which was equivalent to 1 to 5 x 10⁶ colony
52 forming unit (CFU)/mL.¹⁰ *M. pachydermatis* suspension 2.4 Mc Farland concentration was
53 prepared and used for contamination of the OCs.

54 The entire conic part of the OCs was soaked for 10 minutes in 30 mL of the suspension of *M.*
55 *pachydermatis* (25mL NaCl 0,9%, 5 mL Tween 20%), then allowed to air dry for 10 minutes.

56

57 ***Otoscope cones – disinfection technique***

58 Seven different disinfectants ethanol 70% (Laboratoires Gilbert, Herouville Saint-Clair, France),
59 chlorhexidine 2% (F.M. Medical, Roubaix, France), hydroxide peroxide 60vol. (Savetis, Quevert,
60 France), peracetic acid disinfectant 0.03% (Phagogen, Vertou, France), and enilconazole
61 (Imaveral, Elanco, Neuilly sur Seine, France) diluted in sterile water (filtered 0.1 µm) to obtain a
62 final concentration of active ingredient of 0,2%, 0,4% and 0,8% concentration were used for the
63 disinfection of the contaminated cones. Cones were soaked for ten minutes in various
64 disinfectants. Water was used as a positive control. After soaking in the disinfectants, the cones
65 were immediately wiped by paper or just left to air dry for another 10 minutes duration. All the
66 manipulation were performed wearing latex sterile gloves.

67 ***Otoscope cones - fungal culture sampling***

68 Each cone was sampled with two swabs. One swab was used for inside surface and one swab
69 was used for outside surface. The swabs were rolled longitudinally 6 times in order to sample
70 by circling the entire surface (inside or outside). The samples were inoculated on modified
71 Dixon's agar (9 cm Petri dish) and incubated at 32°C for 3 days. Each test was repeated in
72 triplicate.

73 ***Reading of the results***

74 On day 3 the numbers of colonies of *Malassezia* (Colony Forming Unit = CFU) was precisely
75 counted up to 120 CFU. A stereo-microscope was used if necessary.

76

77 **Results**

78 A total of 96 test/cultures were performed. Results are summarized in Table 1. From 96
 79 cultures, the results showed the growth of *Malassezia* in all cultures from positive
 80 controls/water (over 200 CFU in 6 from 12 tests) with or without wiping. Similar results were
 81 observed when enilconazole was used at 0,2% concentration (over 200 CFU in 3 from 12 tests).
 82 All the cultures from ethanol, chlorhexidine, hydrogen peroxide and peracetic acid treated
 83 cones (with or without wiping) were negative (Table 1). Enilconazole 0,4% and 0,8% were
 84 perfectly efficient and gave negative results also. These results demonstrate that disinfection by
 85 soaking for 10 minutes the OCs in common disinfectants (with or without paper wiping) can be
 86 efficient against *M. pachydermatis* for all products tested, except enilconazole 0,2%.

87 Table 1. Number of colonies forming unit (CFU) obtain after disinfection

N°	time soaki ng (min)	Disinfectant used and method	Inside A	Inside B	Inside C	Outside A	Outside B	Outside C
1	10	Water (no wiping)	30 CFU	42 CFU	13 CFU	>200 CFU	>200 CFU	>200 CFU
2	10	Water + wiping	1 CFU	4 CFU	80 CFU	>200 CFU	>200 CFU	>200 CFU
3	10	Ethanol 70% (no wiping)	0	0	0	0	0	0
4	10	Ethanol 70 % + wiping	0	0	0	0	0	0
5	10	Chlorhexidine 2% (no wiping)	0	0	0	0	0	0
6	10	Chlorhexidine 2% + wiping	0	0	0	0	0	0
7	10	Hydrogen peroxide (no wiping)	0	0	0	0	0	0
8	10	Hydrogen peroxide + wiping	0	0	0	0	0	0
9	10	Peracetic acid	0	0	0	0	0	0

		disinfectant 0.03% (no wiping)							
10	10	Peracetic acid disinfectant 0.03% +wiping	0	0	0	0	0	0	0
11	10	Enilconazole 2% (no wiping)	>120 CFU	>150 CFU	>130 CFU	>200 CFU	>200 CFU	>200 CFU	>200 CFU
12	10	Enilconazole 2% + wiping	3 CFU	6 CFU	7 CFU	1 CFU	>150 CFU	>150 CFU	>150 CFU
13	10	Enilconazole 4% (no wiping)	0	0	0	0	0	0	0
14	10	Enilconazole 4% +wiping	0	0	0	0	0	0	0
15	10	Enilconazole 8% (no wiping)	0	0	0	0	0	0	0
16	10	Enilconazole 8% +wiping	0	0	0	0	0	0	0

88

89

90

Discussion

91 *M. pachydermatis* is of importance in both veterinary and human medicine, with
 92 potential effect on humans in some circumstances (e.g. nosocomial infection if
 93 immunosuppressed). Skin and ear disease associated with this lipophilic yeast is commonly
 94 recognized, especially in dogs. However, further studies are required to elucidate the
 95 mechanism which allow this yeast to proliferate and induce disease.²¹⁻²³

96 OCs are instruments with irregular inner surface that may harbor pathogenic
 97 microorganisms.⁵ According to manufacturer's instructions, OCs can be cleaned externally with
 98 a damp cloth and they can be disinfected with aldehydes, surfactants, and alcohols.^{5, 24,25} In
 99 human medicine, a previous work evaluated 53 OCs by sampling for microbiological evaluation
 100 and 22 were found to be contaminated with bacteria and/or fungi.⁵ Eleven of them were
 101 colonized by one organism, 11 were colonized by more than one organism.⁵ With the same
 102 purpose in another publication it was shown that from 42 of OCs microbiological evaluated,
 103 more than 80% were contaminated with *Staphylococcus spp.*, showing an ineffective

104 disinfection method.²⁴ Relatively little attention has been paid to the effectiveness of
105 disinfectants on pathogenic fungi. In order to eliminate the probability of nosocomial
106 infections, appropriate disinfection measures should be undertaken.⁵ How often an OCs must
107 be cleaned to limit contamination is not well established.^{5,25} For example, a study showed that
108 alcohol-swabbing alone is sufficient for sterilizing OCs, at least for bacteria; but still, the
109 recommendation was that further studies are required to establish the most appropriate
110 disinfection protocol to prevent bacterial transfer.²⁵

111 The results of this study demonstrate that *M. pachydermatis* on OCs can survive and
112 could be easily transferred when cones are cleaned only by water. If OCs are not adequately
113 cleaned, there is a concern that they could act as vector for infection. Wiping is important to be
114 used to decrease numbers of *Malassezia*. The present study is in accordance with the results of
115 another study performed in different conditions, were all the three preparations (H1: two-
116 component preparation, based on hydrogen peroxide, at concentrations 100%, 10% and 5%;
117 Pedox: multi-component preparation based on peracetic acid, at concentrations 3%, 1% and
118 0.5% and Savo hypochlorite preparation, at concentrations 100%, 50% and 10%), showed 100%
119 antifungal activity against 10 clinical isolates of *M. pachydermatis*, the standard strain of *M.*
120 *pachydermatis* and *Candida albicans* at each concentration tested.²⁶

121 In this study, all the cultures from ethanol, chlorhexidine, hydrogen peroxide, peracetic
122 acid or enilconazole 0,4% and 0,8% soaked OCs (with or without wiping) were negative, leading
123 to the conclusion that the usual disinfectants are efficient against *M. pachydermatis*. These
124 results demonstrate that disinfection by soaking the OCs for 10 minutes in the disinfectant bath
125 should be enough. Unexpectedly, enilconazole when used at 0,2% concentration gave positive
126 results (with or without paper wiping. Basing on these results, when enilconazole is elected as
127 disinfectant, it should be used at minimal concentration of 0,4%. It is interesting to consider
128 that enilconazole 0,2% (the therapeutic concentration on skin) has an incomplete effect. This
129 could be due to a slow (metabolic) activity *in vitro* as compared to rapid chemical toxicity of
130 disinfectants. *In vivo* most of these disinfectants cannot be used or lose rapidly their activity
131 which is not the case for enilconazole. Thus, the concentration of enilconazole if used on
132 material should be at least 0,4% to have the same immediate efficacy as not antifungal
133 disinfectants. This active concentration remains lower than the one recommended by the
134 manufacturer for fungal disinfection in the environment (0.6%). As showed in the Table 1, the
135 wiping procedure decreased the numbers of CFU. Numbers of CFU on positive cones were most
136 often > 200 in the absence of wiping, whereas in case of wiping average numbers are 47.5 CFU
137 (1 to 150).

138 At the beginning of the study, the experiment was performed once with dry and once
139 with humidified swabs. Because of a much better sensibility of dry swabs, the experiment was
140 performed by using only dry swabs (Table 2). The results from this study showed that the
141 collection of *Malassezia* presents a higher sensitivity by using dry rather than humidified swabs
142 (respectively by using dry swabs over 200 CFU were obtained versus 3 up to only 18 CFU when
143 using humidified swabs) as showed in Table 2.

144 Table 2. Sensitivity of the sampling technique

Sampling technique	Outside OCs (CFU)	Inside OCs (CFU)
Dry swab	> 200	> 200
Humidified swab	13	8

145 CFU – colony forming unit

146

147 Several parameters may limit the interpretation of the study results. First, only seven
 148 disinfectants were employed, even if each method implied also mechanical cleaning part done
 149 with paper wiping. Besides, there was only a small number of cones used for each method. In
 150 this study, only metallic sterilized cones were used. In contrast, OCs in clinical practice are
 151 intensively used and often acquire surface scratches and other defects, especially the plastic
 152 ones. This may enhance bacterial and fungal survival and therefore increase the risk of
 153 transmission of agents from one patient to another one. As for every disinfection procedure, an
 154 optimal method for the clinical setting will require thorough physical cleaning to remove any
 155 organic debris prior to disinfection.¹⁶

156 The laboratory steps followed in this study were necessary to demonstrate the real
 157 activity of the different disinfectants on cones of otoscopes used in defined conditions
 158 potentially close to the daily practice. For this purpose, a very rich suspension of *Malassezia*
 159 was used. One difficult factor to reproduce in laboratory was the effect of the presence of
 160 variable amount of organic debris (cerumen, pus) that is likely to modify the efficacy of
 161 disinfection of the OCs. In practice, infected ears usually contain excessive amounts of organic
 162 material and this present material has major impact on the efficacy of most disinfectants.¹⁶
 163 However in practical conditions OCs are mechanically cleaned from cerumen before any use of
 164 different disinfectants. Literature is still limited on the variation of sensitivity of *M.*
 165 *pachydermatis* to antifungals. Another important point is to consider that the frequent use of
 166 disinfectants agents topically or systemically (specifically or not against *Malassezia*) could
 167 enhance by pressure selection the progressive tolerance of this yeast. A study showed that 3 to
 168 7 isolates from 62 isolates of *M. pachydermatis* exposed to different antifungals, 4,8 to 11,1%
 169 could be classified resistant.²⁷

170 In the present study, metallic OCs were used *in vitro* conditions, thus further studies would be
 171 useful to evaluate the efficacy of different methods of disinfection of plastic OCs in clinical
 172 situations against persistent contamination with *M. pachydermatis*.

173

174 In conclusion, the accidental transfer of such tolerant strains of *M. pachydermatis* via
175 OCs should be avoided by appropriate disinfection. For a good practice, OCs should be cleaned
176 after each examination using disinfectant followed by wiping. The results of this study indicate
177 that with the exception of enilconazole 0,2%, the commonly used antiseptic solutions are
178 adequate to prevent the transfer of *Malassezia pachydermatis*.

179

180 References

181

- 182 1 Hakan Korkmaz, Yeliz Cetinkol, Mukadder Korkmaz. Cross-contamination and cross-
183 infection risk of otoscope heads. *Eur Arch Otorhinolaryngol* (2013) 270:3183-3186.
- 184 2 Newton HM, Rosenkrantz WS, Muse R et al. Evaluation of otoscope cone cleaning and
185 disinfection procedures commonly used in veterinary medical practices: a pilot study.
186 *Veterinary dermatology*, 2006, 17; 147-150
- 187 3 Rampling A, Wiseman S, Davis L et al. Evidence that hospital hygiene is important in the
188 control of methilicin resistant *Staphylococcus aureus*. *J Hosp Infect* 2001; 49:109-16.
- 189 4 Farnell S. Are tympanic thermometers a source of cross-infections? *Nurs Times* 2005 10-
190 16; 101(19):62-3.
- 191 5 Hakan Korkmaz, Yeliz Cetinkol, Mukadder Korkmaz. Cross-contamination and cross-
192 infection risk of otoscope heads. *Eur Arch Otorhinolaryngol* (2013) 270:3183-3186.
- 193 6 Kirby AL, Rosenkrantz WS, Ghubash RM et al. Evaluation of otoscope cone disinfection
194 technique and contamination level in small animal private practice. *Veterinary*
195 *Dermatology*, 2010; 21, 175-183
- 196 7 Chang HJ, Miller HL, Watkins N et al. An epidemic of *Malassezia pachydermatis* in an
197 intensive care nursery associated with colonization of heath care workers pet dogs. *N*
198 *Engl J Med*. 1998; 338(11):706-11.
- 199 8 Muller&Kircks. Small animal dermatology 7th Ed. 2013, p 741-766.
- 200 9 Teun Boekhout, Eveline G, Peter M et al. *Malassezia* and the skin. *Science and Clinical*
201 *practice*. Springer 2010, p.2.
- 202 10 Long JG, Keiserling HL. Catheter-related infection in infants due to an unusual lipophilic
203 yeast-*Malassezia furfur*. *Pediatrics* 1985; 76(6):896-900.
- 204 11 Powell DA, Aungst J, Snedden S et al. Broviac catheter-related *Malassezia furfur* sepsis in
205 five infants receiving intravenous fat emulsions. *J Pediatr*. 1984; 105(6):987-90.
- 206 12 Dorogi J. Pathological and clinical aspects of the diseases caused by *Malassezia spp*. *Acta*
207 *Microbiol Immunol Hung*. 2002; 49(2-3):363
- 208 13 Bourdeau P, Travers F, Bruet V et al, Hand carriage of *Malassezia pachydermatis* and
209 other fungal pathogens detection with carpet method: comparison of contamination
210 from dermatological cases or apparently healthy pets. *Veterinary dermatology*, 2008, 19
211 (Suppl.1), p. 29, FC-6

- 212 14 Madrid IM, Teles AJ, Santin R et al. Efficacy of disinfectant solutions in the elimination of
213 fungi of medical and veterinary importance. *Archives of Veterinary Science*, 2013, 18 (1),
214 65-70.
- 215 15 Aristeia Velegraki, Claudia Cafarchia, Georgios Gaitanis et al. *Malassezia* infections in
216 Humans and Animals: Pathophysiology, Detection, and Treatment. *PLOS Pathol* 2015; e
217 1004523
- 218 16 Saunders CW, Scheynius A, Heitman J (2012). *Malassezia* fungi are specialized to live on
219 skin and associated with dandruff, eczema, and other skin diseases. *Plos Pathog* 8:
220 e1002701.
- 221 17 Sei Y. *Malassezia* infections. *Med Mycol J*. 2012;53(1):7-11
- 222 18 Axel Kramer, Ingeborg Schwebke, Gunter Kampf. How long do nosocomial pathogens
223 persist on inanimate surfaces? A systematic review. *BMC Infect Dis*. 2006; 6:130.
- 224 19 Neely AN, Maley MP. Survival of enterococci and staphylococci on hospital fabric and
225 plastic. *Journal of Clinical Microbiology*. 2000; 38:724-726.
- 226 20 Hirai Y. Survival of bacteria under dry conditions from a viewpoint of nosocomial
227 infection. *Journal of Hospital Infection*. 1991;19:191-200.
- 228 21 Guillot J, Bond R. *Malassezia pachydermatis*: a review. *Med Mycol* 1999; 37(5):295-306.
- 229 22 Rie Nagata, Hiroshi N, Daiki O et al. Transmission of the major skin microbiota,
230 *Malassezia*, from mother to neonate. *Pediatrics International*/vol.5, Issue 3; 2012.
- 231 23 Kathleen O, Fances S. S., Shelley Rankin. *Malassezia pachydermatis* Carriage in Dog
232 Owners. Centers for Disease Control and Prevention. *Emerging Infectious Disease*
233 *journal* ISSN: 1080-6059. Vol11, nr. 1; 2005.
- 234 24 Cohen HA, Amir J, Matalon A et al. Stethoscopes and otoscopes-a potential vector of
235 infection? *Fam Pract*. 1997; 14(6):446-9.
- 236 25 Cheol Hyo Ku, Young Sub Lee, Young Joon Seo et al. Is Alcohol Swabbing Sufficient to
237 clean Oscopes in Hospitals? *Otology. Korean J Otorhinolaryngol-Head Neck Surg* 2016;
238 59(8):578-82.
- 239 26 Vyrostkova J, Conkova E, Laciakova A et al. Effectiveness of selected disinfectants on
240 *Malassezia pachydermatis*, *Polish journal of veterinary sciences*, 2012, 15 (3), 567-568
- 241 27 Cafarchia C, Figueredo L, Iatta R et al. *In vitro* evaluation of *Malassezia pachydermatis*
242 susceptibility to azole compounds using Etest® and CLSI microdilution methods. *Medical*
243 *Mycology* 2012; 50, 795-801.

244
245
246
247
248
249
250
251