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Bacterial Contamination of Veterinary Ear Cleaners in Homes and Clinics

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Abstract Ear cleaning using ear cleaners is recommended for the prevention of canine otitis externa. This study aimed to investigate the prevalence of bacterial contamination in home- and clinical-use ear cleaners and identify characteristics associated with such a prevalence. To obtain data regarding home-use ear cleaners, 100 bottles of commercial ear cleaners were collected from clients who visited animal clinics with their dogs and completed a survey. Data for clinic-use ear cleaners were obtained by gathering 60 bottles of ear cleaners from private animal hospitals. Bacterial culture of the applicator tips and samples of the ear cleaners was performed. In addition, to determine the relationship between ear cleaner contamination and otitis externa (OE) in dogs, ear cultures were obtained from some of the dogs that visited the clinics. Approximately 5% and 4.91% of home- and clinic-use ear cleaner applicator tips, respectively, had bacterial contamination, although no contamination of the solution within the bottles was observed. Most of the contaminated ear cleaner tips were unclean on the outside given that the bottle was placed directly into the ear canal. The contamination rate was highest among owners who used ear cleaners once a week and among veterinarians who used the product for dogs with OE. We found no ingredients that affected the incidence of ear cleaner contamination.

Key words dog, ear cleaners, bacteria.

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Introduction

Canine otitis externa (OE) is one of the most frequent diagnoses in small animal practice. Ear cleaners are frequently used at home and in clinics as part of the treatment for OE and maintenance therapy to help prevent recurrence of otic infection in dogs (17,18). A wide range of cleaning products with various active ingredients, including cerumenolytics, surfactants, astringents, antimicrobials, and anti-inflammatories, have been available for use. Cerumenolytics and surfactants improve the efficacy of topical antimicrobials and anti-inflammatories by emulsifying and dissolving cerumen and debris (14). Astringents help prevent maceration by drying the surface of the ear canal. Anti-inflammatory agents are useful given that they can inhibit inflammation and pruritus (21). Finally, antimicrobials in ear cleaner stop and reduce the proliferation of microorganisms and help prevent contamination of the ear cleaner solution. One study showed that ear cleaners were effective in resolving infection and controlling clinical signs in dogs with OE (5).

Generally, animal hospital staff use ear cleaners for multiple patients with or without OE. Pathogenic bacteria could be transferred from one dog to another or to staff through contaminated fomites or surfaces (10). As such, proper use and management of ear cleaners are critical.

One previous study evaluated bacterial contamination of ear cleaners at home but failed to consider bacterial contamination of clinic-use ear cleaners (1). This study aimed to investigate the prevalence of bacterial contamination in ear cleaners used at home and in clinics according to use and management.

Materials and Methods

Ear cleaner and ear swab collection

Home-use cleaners

This study was conducted from January to March 2021 at two animal medical centers located in Busan and Ulsan. Clients were asked to bring used bottles of commercial ear cleaners to their clinicians. The clinician wore sanitary nitrile gloves and placed the ear cleaner into a plastic bag (Ziploc, SC Johnson, Thailand) that was then partly sealed to preclude contamination at the clinic but allow air flow into the bag. The bags were stored at room temperature until culture.

Clients' dogs were not required to have ear disease. Cleaner bottles and tips were collected in numbered plastic bags at each visit, and each client was given a questionnaire that asked about the patient's information, history of OE, and ear cleaner use (Table 1). Regardless of the diagnosis of OE, clinicians obtained an ear swab from the external surface of one ear from each dog with the owner's consent. Ear swabs were transferred into sterile transport media. Each cleaner and ear swab were assigned identification numbers, with the corresponding ear cleaner and ear swab being labeled with same numbers.

Clinic-use cleaner

Ear cleaner bottles were collected from December 2020 to March 2021 from animal clinics in South Korea. One person collected the ear cleaner bottles in the same manner that ear cleaner bottles from clients were collected. Animal hospitals were categorized according to the number of staff members working there: A, large hospitals with more than 10 staff members; B, clinics with 5 to 10 staff members; and C, clinics with <5 staff members. One staff member from each clinic was given a questionnaire that asked about the use of ear cleaners (Table 1).

Common records

Each bottle was examined prior to culture, and the cleanliness of the surface of both the bottle and tip was scored from 1 to 5 as follows: 1, the bottle or tip was clean; 2, one spot of debris was present on the surface; 3, decolored spot; 4, more than two spots of debris; 5, more than two spots of debris and discoloration.

Other pieces of information for each bottle, such as expiration date, were also recorded (Table 1). For statistical comparison of cleaning frequency, the following four groups were created: cleaning more than once weekly, cleaning once a week, cleaning twice monthly, and cleaning less than once monthly. Cleaning methods were also categorized into the following four groups: a, placing the bottle directly into the ear canal and squeezing; b, squeezing the bottle into the ear canal while being careful not to touch the ear with the bottle; c, wiping the ear with a tissue or cotton ball; d, no wiping of the ear.

Sample acquisition

Two people obtained samples for culture using the same protocol. All bottles submitted each week were sampled at the same time. To obtain samples, sterile cotton swabs (sterilized swab-wood-double, Poongsung, South Korea) soaked with 0.9% normal saline (JW Pharmaceutical, South Korea) were rubbed onto the tip of the ear cleaner bottle. Sterile cotton swabs were soaked in ear cleaner liquid.

Bacterial culture and isolation

Each cotton swab obtained from the bottle tip and ear cleaner liquid was cultured on sheep blood agar (Kisanbio, South Korea). All steps were performed in a laboratory clean bench. After incubation at 37°C for 24 hours, the culture results were determined. Culture negativity was then con-

Table 1. Survey of ear cleaners used at home and in clinics and common records of ear cleaners

Home-use cleaners		
Contents	Questions	Answers
Patient information	Species, age, sex	
OE history	Date of first diagnosis, number of treatments, last treatment	
	Diagnosis	Allergic, endocrine, immune-mediated, ectoparasitic, bacterial, fungal
	Clinical signs	Redness (color), ear wax, pruritus, odor
Ear cleaner use	Number of pets that use ear cleaner	Number of dogs (or cats)
	Last date of ear cleaner use	
	Frequency of ear cleaner use	More than once a week Once a week Twice a month Less than once a month
	Purchase of ear cleaner	Vet clinic, online, pet shop
	Method of use	Squeezed directly into the ear canal (a) Indirectly squeezed into the ear canal (b) Wiped with cotton balls or tissues (c) Was not wiped (d)
	Method of storage	Room temperature/refrigerated Closed/sealed
	Tip cleaning after use	Yes/no
Ear culture	Presence or absence	Positive/negative
Clinic-use cleaners		
Ear cleaner use	Number of staff members using ear cleaner daily	Only one person 2-5 6-10 Over 10
	Number of dogs using ear cleaner daily	1-4, 5-10, over 10
	Type of dogs using ear cleaner	Dogs with clinical signs of OE Only dogs diagnosed with OE Dogs with dirty ears All dogs
	Method of use	Squeezed directly into the ear canal (a) Indirectly squeezed into the ear canal (b) Wiped with cotton balls or tissues (c) Was not wiped (d)
	Replacement cycle	1-3 months 3-6 months 6 months-1 year Over 1 year
Common records		
Ear cleaner	Brand, ingredients, size	
	Amount remaining	
	Outer cleanliness	Score of 1-5*
	Expiration status	In date, expired, no date

*1, clean bottle or tip; 2, one spot of debris on the surface; 3, one decolored spot; 4, more than two spots of debris; 5, more than two spots of debris and discoloration.

firmed after 72 hours. Bacterial colonies were submitted to an analytical laboratory for identification (Solgent Co., South Korea). Identification of organisms was performed using the polymerase chain reaction band method.

Statistical analysis

The χ^2 test for multiple comparisons was used to determine whether tip cleanliness, bottle size, cleaning frequency, and

cleaning method differed significantly between samples with and without contaminated tips. Fisher's exact test was used to compared the OE history, clinical signs, and shared used of ear cleaners between samples with and without contaminated tips. A value of $p < 0.05$ was considered statistically significant. Both tests were performed with GraphPad Prism 9 (GraphPad Software, USA).

Results

A total of 160 ear cleaner bottles were collected for sampling. Home-use ear cleaners accounted for 100 bottles comprising 44 different products, whereas clinic-use ear cleaners accounted for 60 bottles comprising 21 different products.

Home-use ear cleaners

Bacteria were cultured in 5 (5%) home-use ear cleaner bottles that had bacterial growth from the applicator tips (Table 2). Moreover, 2 (2%) bottle tips were contaminated with *Staphylococcus pseudintermedius*. Other bacteria grown from the applicator tips included *Bacillus* spp., *Staphylococcus shleiferi*, and *Enterococcus* spp. None of the bottles had a contaminated solution within the bottle.

A total of 59 ear swab samples were collected from the dogs, with bacteria having been cultured in 27 dogs (45%). Among these 27 dogs, 12 did not have history or clinical signs of OE history. Bacteria cultured from these ears included *S. pseudintermedius* in 16 dogs. Other bacteria included *Staphylococcus* spp., *Bacillus* spp., *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Klebsiella* spp., *Microbacterum* spp., *Brevibacterium* spp., *Paenarthrobacter* spp., and *Acinetobacter* spp. Among the five ear cleaners with contaminated tips, three matched the bacteria detected in the ear swab samples.

The owners were asked to choose a description that best described how they cleaned their dog's ears. Notably, four of the five owners whose ear cleaners had contaminated tips applied the ear cleaner directly into the ear and then wiped it with a cotton ball, whereas one applied the ear cleaner directly into the ear and did not wipe. Most owners whose home-use ear cleaners had a contaminated tip used a method in which the tip of the bottle touched the ear and was manually wiped using the hands. The cleaning method was found to be significantly associated with contamination rate in samples with contaminated applicator tips ($p = 0.0175$).

Regarding the cleaning of the tip of the bottle, 43% of the tips from home-use ear cleaners received a score of 1, 33% received a score of 2 and 3, and 24% received a score of 4 and 5. Considering that scores of 1, 2-3, and 4-5 indicate "clean," "normal," and "dirty," respectively, we found that home-use ear cleaner bottles with dirty tips were more easily contaminated by bacteria than did the others ($p = 0.0043$); however, it made no difference whether the bottle was visually dirty or clean at the time of sampling.

The frequency of ear cleaning ranged from daily to yearly. All five contaminated cases used ear cleaners once a week ($p = 0.0427$).

Neither the frequency of ear infection nor the presence of clinical signs associated with ear disease at the time of the survey and examination had a significant impact on the contamination rate.

Ear cleaners were most commonly purchased from a veterinarian (39 of 100). Other common sources of purchase were online (28 of 100) or at a pet shop (18 of 100). During statistical comparison, no significant difference in contamination rates was noted between these three groups.

Approximately 17% of owners routinely wiped or rinsed the applicator tip of the ear cleaner bottle after use. The method of cleaner bottle storage had no effect on the contamination rate.

Among the home-use cleaner bottles analyzed, 68 were known to be in date, whereas 23 were known to be expired. Eight bottles had no legible date printed on them.

Our data also showed that 17% of owners routinely wiped or rinsed the applicator tip after use. The method of cleaner storage had no effect on the contamination rate. Most clients stored the ear cleaner close to room temperature, 7% of the clients left the ear cleaner sealed at room temperature, and 2% of the clients stored the ear cleaner in the refrigerator.

Clinic-use ear cleaner

A total of 60 ear cleaner bottles were collected for sampling from animal clinics. Among the 60 clinics sampled, 39 were small clinics having between 1 and 4 veterinarians and staff members, 11 were medium-sized clinics employing between 5 and 10 staff members, and 10 were animal centers with over 10 people working in the clinic.

Bacteria were cultured from 3 (4.91%) clinic-use ear cleaner bottles with bacteria growth from the applicator tips (Table 3). Notably, 2 (3.2%) bottle tips were contaminated with *Bacillus* spp., whereas the other applicator tips were contaminated with *Staphylococcus* spp. None of the clinic-use ear cleaner bottles had a contaminated solution within the bottle.

Staff members from the clinics were asked to choose a description that best described how they clean the patients' ears. All clinics with contaminated tips applied ear cleaners directly into the ear and wiped the tip; thus, the tip of the bottle touched the ear and was manually wiped using the hands. Our finding showed that the cleaning method was associated with the contamination rate in samples with con-

Table 2. Comparison of contaminated and non-contaminated home-use ear cleaners

	Ear culture	Tip culture	OE history	Clinical sign	Frequency of use	No. of animal	Method of use	Cleanliness of tip	Expiration status
Contaminated bottles									
P8	*Brevundimonas aurantiaca*, *Staphylococcus pseudintermedius*	*Staphylococcus pseudintermedius*	Recurrent	Redness, brown ear wax, odor	Once a week	3 dogs	Direct to ear, wiped	5	In date
P38	*Pseudomonas aeruginosa*, *Staphylococcus pseudintermedius*	*Bacillus licheniformis*	No	No	Once a week	1 dog	Direct to ear, wiped	1	In date
P57	*Bacillus safenis*, *Enterococcus faecium* *Bacillus pumilus*	*Enterococcus durans*, *Enterococcus faecium*, *Bacillus safenis*	No	No	Once a week	1 dog	Direct to ear, wiped	1	Expired
P61	*[Brevibacterium] frigoritolerans strains*	*Staphylococcus schleiferi*	Recurrent	Pruritus, redness, brown ear wax	Once a week	1 dog	Direct to ear, wiped	3	In date
P67	*Staphylococcus pseudintermedius*, *Corynebacterium auriscanis*	*Staphylococcus pseudintermedius*	Once	Ear wax, odor	Once a week	2 dogs	Direct to ear, wiped	3	In date
	Ear culture	**Tip culture**	**OE history**	**Clinical signs**	**Frequency of use**	**No. of animal**	**Method of use**	**Cleanliness of tip**	**Expiration status**
Noncontaminated bottles									
P2	*Klebsiella pneumonia*, *Staphylococcus schleiferi*	Negative	Recurrent	Yellow ear wax, odor	Once a week	1 dog	Direct to ear, not wiped	4	In date
P25	*Acinetobacter septicus*, *Staphylococcus schleiferi*	Negative	Recurrent	Pruritus, redness, ear wax, odor	Once a week	2 dogs	Indirect to ear, not wiped	3	In date
P39	*Enterococcus faecalis*, *Enterococcus rivorum*, *Enterococcus wangshamyuanii*	Negative	No	No	Once a week	1 dog	Direct to ear, not wiped	5	In date
P68	*Staphylococcus pseudintermedius*	Negative	No	No	Twice a week	1 dog, 1 cat	Indirect to ear, not wiped	5	Expired
P86	*Microbacterium oxydans*	Negative	No	No	Twice a week	1 dog	Direct to ear, not wiped	4	In date

Method of use: direct to ear: contact with ear canal and squeeze, wipe: clean the debris with tissue or cotton after solutions into the ear, cleanliness: clean (1) to dirty (5).

P, patient; OE, otitis externa; No., number.

taminated applicator tips ($p = 0.0226$).

Staff members were asked about the type of patients for whom ear cleaners were used in the clinics. Notably, 11 clinics used ear cleaners for all patients visiting the clinic, 12 used ear cleaners for patients with clinical symptoms of OE, 18 used ear cleaners for patients whose ears were dirty, and

Table 3. Characteristics of the contaminated ear cleaners used in animal clinics

	AH44	AH51	AH60
Bottle tip culture	Bacillus subtilis, Bacillus wiedmannii, Bacillus vallismortis	Bacillus subtilis, Bacillus proteolyticus, Bacillus cereus	Staphylococcus epidermidis, Staphylococcus capitis, Staphylococcus saccharolyticus
Bottle size	300 mL	120 mL	500 mL
Cleanliness of the tip	4	4	2
Expiration	No date	In date	In date
No. of people	2 veterinarians	2 veterinarians	2 veterinarians & 2 technicians
No. of patients (/a day)	3-4	5	5-7
To whom	OE patients	OE patients	OE patients
How to clean	Direct to ear, wipe	Direct to ear, wipe	Direct to ear, wipe
Replacement cycle	Over 1 year	Over 1 year	1 year
Clinic type*	B	B	C
Ingredient	Boric acid Propanediol Glycerin USP Sodium hydroxide Octylphenol ethtoxylate Polysorbate	Propylene glycol Glycerin Ethanol B-glucan Disodium EDTA Salicylic acid Lactic acid Propylparaben Methylparaben Phenoxyethanol	Propylene glycol Ethanol Cocamidopropyl betaine Methyl p-hydroxybenzoate Disodium EDTA Salicylic acid Chlorhexidine digluconate Ethylhexyglycerin

AH, animal hospital; OE, otitis externa; EDTA, ethylenediaminetetraacetic acid.

*Clinic type: A, animal center which have over 10 staff; B, medium-sized with between 5 and 10 staff; C, small-sized clinics having between 1 and 4 staff.

16 used ear cleaners for patients diagnosed with OE. All contaminated tips were from clinics that used cleaners only for patients with OE ($p = 0.0239$).

Among the clinic-use cleaners analyzed, 47 (77%) bottles were known to be in date, whereas 9 (14%) bottles were known to be expired. Five bottles had no legible date printed on them. Expiration was not a significant factor associated with contamination in both groups.

The replacement cycle for ear cleaners in clinics varied from monthly to over yearly. Although two of the three cleaners in the contaminated group had been replaced after more than a year, no significant relationship was observed between the replacement cycle and contamination rate. In addition, the number of patients treated daily, the number of people who treated patients with an ear cleaner in a clinic, and the ingredients of the cleaners were not associated with ear cleaner contamination.

Discussion

Our study investigated the prevalence of bacterial contamination of home- and clinic-use ear cleaners. Notably, we found that the prevalence rate of contamination was 5% and 4.91% for home- and clinic-use ear cleaners, respectively, with no significant differences in prevalence rate between them. To the best of our knowledge, only one study has ever investigated veterinary ear cleaner contamination. Moreover, this particular study investigated only home-use ear cleaners and not clinic-use ones. Compared to the mentioned study, our study observed a lower incidence rate of contamination, with all instances of contamination occurring on the applicator tip and not the ear cleaner solution.

The lack of contamination in the ear cleaner solutions may be attributed to several ingredients within the solution, including antibacterial agents and surfactants, that inhibit the survival and growth of microbials. Many different ear cleaning solutions have been shown to possess antibacterial activity (2-5,7,8,11,12,15,17,19,21,22). The most important mechanism by which topical agents exert their antimicrobial activity may be through to the pH of the solution. Organic acids, such as acetic acid, citric acid, lactic acid, and salicylic acid, in ear cleaning decrease the pH of solution, likely providing them with good antimicrobial properties (21). Cerumenolytics soften and dissolve cerumen to facilitate cleaning. Surfactants emulsify debris, breaking it up and keeping it in the solution. Astringents dry the ear canal surface, prevent-

ing maceration. All of these agents work together to inhibit microbial proliferation (14).

One study showed that the presence of Tris-EDTA in ear cleaners contributed to the bacterial contamination of the ear cleaners (7). However, our study found that no single ingredient significantly affected the incidence of contamination. Tris-EDTA is a buffering agent that has a mild to moderate alkalizing effect and is an emulsifier that can damage bacterial cell walls. This mild alkalizing effect may favor bacterial proliferation (9,20) but may also provide a suitable environment for some antibiotics (5,18).

According to our findings, the significant factors that contributed to contamination were the method of cleaning and visual cleanness. It is presumed that direct contact between the tip of the cleaner and bacteria in the ear canal can facilitate the transfer of bacteria to the tip of the bottle and that manual wiping off of residue using the hands can increase exposure to bacteria. In the case of home-use cleaners, the frequency of use increased the opportunity of contamination, whereas in clinics, the use of cleaners for patients with OE was more likely to cause bacterial contamination.

The current study identified several bacteria from the tip of the bottles, including *Staphylococcus* spp. and *Bacillus* spp., and the bacterial species identified from the bottle tips showed little to no correlation with the bacteria found in the ear cultures from dogs. In addition, among these cases, some patients did not show OE history and related clinical signs. Some bacterial genera, such as *Staphylococcus*, can be considered as normal microflora of the ear canal in healthy dogs (13,16). However, several previous studies have shown that bacterial interchange events between the environment and hands or intrapersonal hand to hand transmission is possible (10). In addition, these microbials can function as perpetuating factors in unhealthy ear canals at any time (6,23). Based on our research, some owners used the same ear cleaner for multiple animals, allowing the possible transfer of pathogenic bacteria from one dog to another. Thus, it is important to maintain hygiene at all times even if the current the dog is not showing signs of OE.

Our study showed that expired ear cleaner tips were more likely to be contaminated than in-date cleaners. Contamination was observed on expired ear cleaner tips; however, no statistically significant relationship was found between expiration status and contamination rate. This could be attributed to the gradual loss of preservative activity in the ingredients, which may not disappear suddenly when the cleaner expires. Hence, owners and clinicians should periodically check the expiration date of the ear solutions

One limitation of our study is that some experimental results were drawn from surveys of owners and clinicians and that the specific pathways of contamination could not be determined.

Based on our findings, the bacterial contamination rate of ear cleaners, particularly with pathogenic bacteria, was lower than expected. Moreover, bacterial contamination only occurred on the tip of ear cleaners and not in the solution. The incidence of contamination increased when there was frequent direct contact between the ear cleaner tip and the ear canal followed by hand manipulation. Thus, we recommend that owners and clinicians apply the ear cleaner indirectly into the ear, rinsing or washing the applicator tip after use and checking the expiration date. Finally, given that owners commonly purchased ear cleaners from veterinarians, education on the correct use of ear cleaners to owners can help reduce the contamination rate of ear cleaners.

Conflicts of Interest

The authors have no conflicting interests.

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