

## Main Article

Dr N Zebardast takes responsibility for the integrity of the content of the paper

**Cite this article:** Nemati S, Akbarpour M, Lotfali E, Nosrati R, Rafiee E, Zebardast N. The effects of peracetic acid solution on the treatment of otomycosis in an animal model: a new antifungal drug? *J Laryngol Otol* 2023;**137**: 799–803. <https://doi.org/10.1017/S002221512200264X>

Accepted: 6 December 2022  
First published online: 15 December 2022

### Key words:

Otomycosis; Peracetic Acid; Acetic Acid; Guinea Pigs

### Author for correspondence:

Dr N Zebardast, Cellular and Molecular Research Center, School of Medicine, Guilan University of Medical Sciences, 6th Kilometer of Rasht-Tehran Highway, Rasht 41937-1311, Iran  
E-mail: [nszebardast@gmail.com](mailto:nszebardast@gmail.com)

# The effects of peracetic acid solution on the treatment of otomycosis in an animal model: a new antifungal drug?

S Nemati<sup>1</sup>, M Akbarpour<sup>1</sup>, E Lotfali<sup>2</sup>, R Nosrati<sup>3</sup>, E Rafiee<sup>4</sup> and N Zebardast<sup>3</sup> 

<sup>1</sup>Otorhinolaryngology Research Center, Department of Otolaryngology and Head and Neck Surgery, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran, <sup>2</sup>Department of Medical Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran, <sup>3</sup>Cellular and Molecular Research Center, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran and <sup>4</sup>Razi Clinical Research Development Unit, Guilan University of Medical Sciences, Rasht, Iran

## Abstract

**Objective.** This study aimed to investigate the therapeutic effects of 1 per cent and 0.01 per cent peracetic acid as an antifungal agent in animal otomycosis.

**Method.** After creating a superficial scratch in the external auditory canal of guinea pigs, a suspension of *Aspergillus niger*, *Aspergillus fumigatus* and candida were inoculated into the ears of the animals. After otomycosis, the effect of 1 per cent or 0.01 per cent peracetic acid on otomycosis was evaluated by otomicroscopy and culture at 10 days post-treatment and compared with 2 per cent acetic acid as the control.

**Results.** A 10-day treatment with 1 per cent peracetic acid and 2 per cent acetic acid (control) showed normal otomicroscopy and negative cultures compared with 0.01 per cent peracetic acid. Drug sedimentation or other side effects in the external auditory canal or tympanic membrane were not observed during treatment with peracetic acid.

**Conclusion.** The findings of this study confirm that the treatment of otomycosis with 1 per cent peracetic acid in an animal model is beneficial and may be a novel therapeutic treatment for otomycosis.

## Introduction

Otomycosis refers to an acute, sub-acute or chronic fungal infectious disease of the outer ear and eardrum. It is a common superficial infection worldwide with a prevalence of 4 per 1000 people, ranging from 9 per cent in otitis externa to 30 per cent in patients with inflammatory conditions or symptoms of otitis.<sup>1–3</sup> The prevalence of infection is related to geographical location; it has higher prevalence rates in tropical and subtropical climates.<sup>4</sup>

Hot and humid climate, lack of hygiene, use of hearing aids and hands-free devices, ear inflammation because of bacterial infections, dermatomycosis, certain dermatological, systemic and immunological disorders, frequent showering and prolonged contact with water, manipulation of the ear, and traumatic injuries are some factors that contribute to the occurrence of otomycosis.<sup>4</sup> Middle-ear involvement and perforation of the tympanic membrane may occur after external ear infections and otomycosis.<sup>5</sup> Complaints such as itching and sometimes earache, ear discharge, tinnitus, hearing loss, inflammation, redness of the external auditory canal, and aural fullness (a feeling of fullness in the ear) are common in otomycosis.<sup>4,5</sup>

Mouldy saprophytic fungi such as *Aspergillus*, especially *Aspergillus niger*, and saprophytic yeasts, for instance *Candida albicans*, are the most common causes of otomycosis in humans.<sup>2,4,5</sup> However, other yeasts and dermatophytes can also cause the disease.<sup>3,6</sup> Treatment of fungal infections in the ear includes careful cleaning of the ear canal and using a topical medicine, such as a 2 per cent salicylic acid solution in alcohol and 1–2 per cent acetic acid because the acidic environment prevents fungal growth. Ointments containing nystatin or mycostatin are usually used to treat candida infections. Other drugs used to treat fungal ear infections include antifungal solutions such as clotrimazole, tolnaftate, amphotericin B, timolol solution and gentian violet solution.<sup>7</sup>

Peracetic acid (chemically known as peroxyacetic acid or PAA, ethaneperoxyacid, estosteril, acetic peroxide and Epsom AC) is an organic compound with the chemical formula CH<sub>3</sub>CO<sub>3</sub>H or C<sub>2</sub>H<sub>4</sub>O<sub>3</sub>. It is a mixture of acetic acid and hydrogen peroxide, and whenever these substances stabilise under certain conditions, they produce peracetic acid.<sup>8,9</sup> Peracetic acid (pKa 8) is a weaker acid than acetic acid. It is a colourless liquid with a strong and pungent odour, and its high oxidation potential makes it an ideal antimicrobial agent. The acid kills micro-organisms by denaturing their protein structure, disrupting cell wall function, and oxidising sulphur bonds in proteins, enzymes, and other metabolites. Peracetic acid is a powerful disinfectant and is used as an antibacterial and antifungal agent, especially in the food industry.<sup>8–10</sup> Studies on animal models have

also shown that this substance has no tissue-damaging effects on the kidney and liver. Other properties of peracetic acid are the absence of toxic residues or mutagenic stable or sub-mutant agents and low dependence on pH for effectiveness. The use of this substance is also cost-effective, and its antimicrobial effects on the tissue are fast and effective.<sup>11–13</sup>

In recent decades, antifungal drug resistance has become a worldwide problem. The topical application of many antifungal drugs to the ear, including 2 per cent acetic acid, is also painful. Another problem with 2 per cent acetic acid eardrops is the white deposits of the drug in the ear canal and on the tympanic membrane, which sometimes are difficult to differentiate from the fungal debris.

To the best of our knowledge and after searching all available references and databases, there are no other studies that have analysed the use of peracetic acid solution for the treatment of fungal infections, including otomycosis, in humans. Therefore, this study aimed to evaluate the therapeutic effects (improvement of infection and inflammation) and side effects of 1 per cent and 0.01 per cent peracetic acid solution compared with a traditional antifungal (acetic acid 2 per cent) in an animal model of otomycosis. We considered that drugs should have minimal side effects, should be affordable and the treatment of the disease should be completed in a short time.

## Materials and methods

### Animals

The male guinea pigs were obtained from Animal Resources Centre (Pasteur Institute, Iran). Eighteen healthy male and adult (3 to 4 years old) guinea pigs (weighing 700 to 1200 g) were used in this experimental study.<sup>14</sup> The guinea pigs were held in separate cages in a room with 12 hours of on/offlight cycles, according to the ordinary day and night rhythm, at constant temperature ( $20 \pm 2^\circ\text{C}$ ). The animals were provided with freely reachable food and water. The Biomedical Research Ethics Committee of Guilan University of Medical Sciences approved this study (registration number: IR.GUMS.REC.1398.285).

At first, each guinea pig was examined by otomicroscopy by an otolaryngologist (MA). All of them had a normal external auditory canal and tympanic membrane. Then they were randomly divided into three groups (A, B and C); in each

group, peracetic acid in two concentrations of 1 per cent and 0.01 per cent would be compared with 2 per cent acetic acid, with six animals included in each group.

### Inducing ear-infection

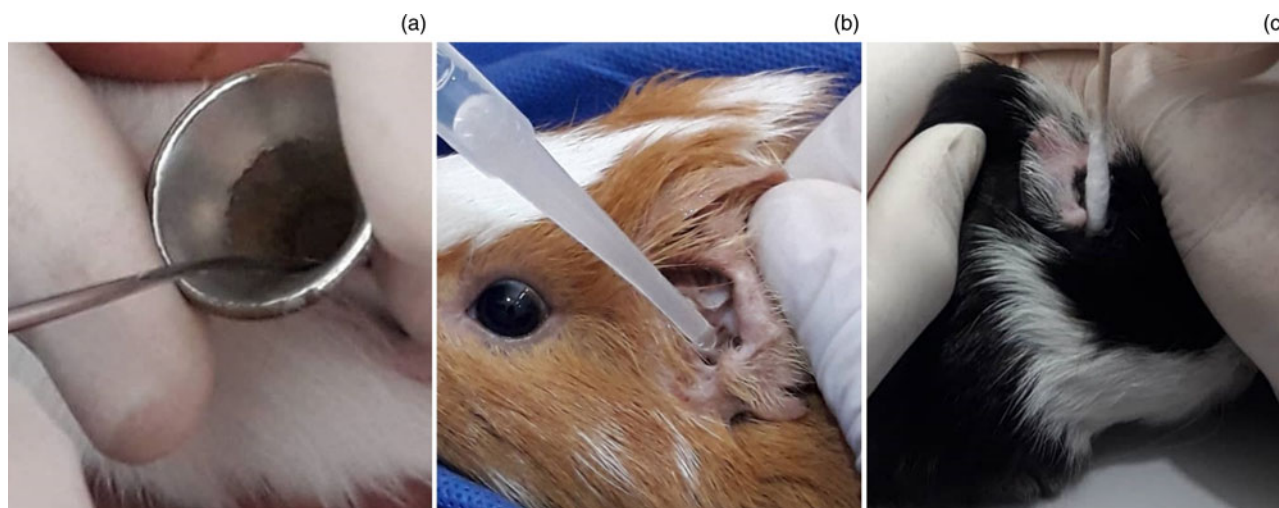
Initially, a suspension of *A niger*, *Aspergillus fumigatus*, *Candida glabrata* and *C albicans* (isolated from clinical specimens) was prepared with standard McFarland 0.5 opacity. In order to induce ear infection and otomycosis, a superficial scratch in the external auditory canal was created by the cotton port, and two drops of suspensions of *A niger* (group A), *A fumigatus* (group B) and candida (group C) were emptied in both ears of the guinea pigs (Figure 1).

In order to ensure otomycosis, 10 days after inoculation of micro-organisms, the ears were examined under an otomicroscope, and under sterile conditions, each ear was cleaned with a solution of 70 per cent alcohol, and then one sample from each external ear canal (ear secretion) was taken by sterile cotton swabs and placed in tubes containing sterile saline. All the initial and control otomicroscopic examinations were undertaken by the same otolaryngologist. During these examinations, the guinea pigs did not receive any drug for anaesthesia or sedation, and all the examinations and procedures were performed by using gentle physical restriction. In addition, all the cases and controls received the above-mentioned treatments in both ears after debridement of the fungal elements and debris of the external auditory canal, and no animals received any other systemic or topical antifungal drug during the research.

The swabbed samples were cultured on Sabouraud dextrose agar and incubated for one week at room temperature. The growth of fungi in the culture medium and microscopic assessment confirmed the development of otomycosis.

### Treatment and clinical examination

The animals of each group ( $n = 6$ ) were divided into two sub-groups, with each sub-group containing three animals. In sub-group 1, two drops of 1 per cent peracetic acid and 2 per cent acetic acid were poured into the right and left external auditory canal, respectively. In sub-group 2, the animals received two drops of 0.01 per cent peracetic acid and 2 per cent acetic



**Fig. 1.** (a) Creating a superficial scratch in the external ear canal, (b) inoculation of micro-organism suspensions in ear of guinea pig and (c) sampling after ear infection from guinea pigs.

acid in the right and left external auditory canal, respectively (Table 1).

The treatment was performed 3 times a day, with 2 drops every 8 hours during the 10-day period. At the end of the treatment (10 days), the effects of the peracetic acid on otomycosis, the infection in both ears, was checked again using the culture and otomicroscopic assessment, similar to the above-mentioned protocol. Assessment of treatment and evaluation of side-effects were made based on negative findings of otomicroscopy on days 3, 5, 7 and 10 and on the cultures.

Side effects, including inflammation and redness in the tympanic membrane and skin of the external auditory canal, oedema, any obstruction in external auditory canal, perforation of the tympanic membrane, and drug sedimentation on the tympanic membrane and external auditory canal, were examined by otomicroscopy. Any abnormal behaviour or movement disorder in the animals (because of pain, irritation or any other types of discomfort) were investigated during drug consumption and then up to one week after termination of the treatment.

## Results

### Investigation of culture-based fungal infection

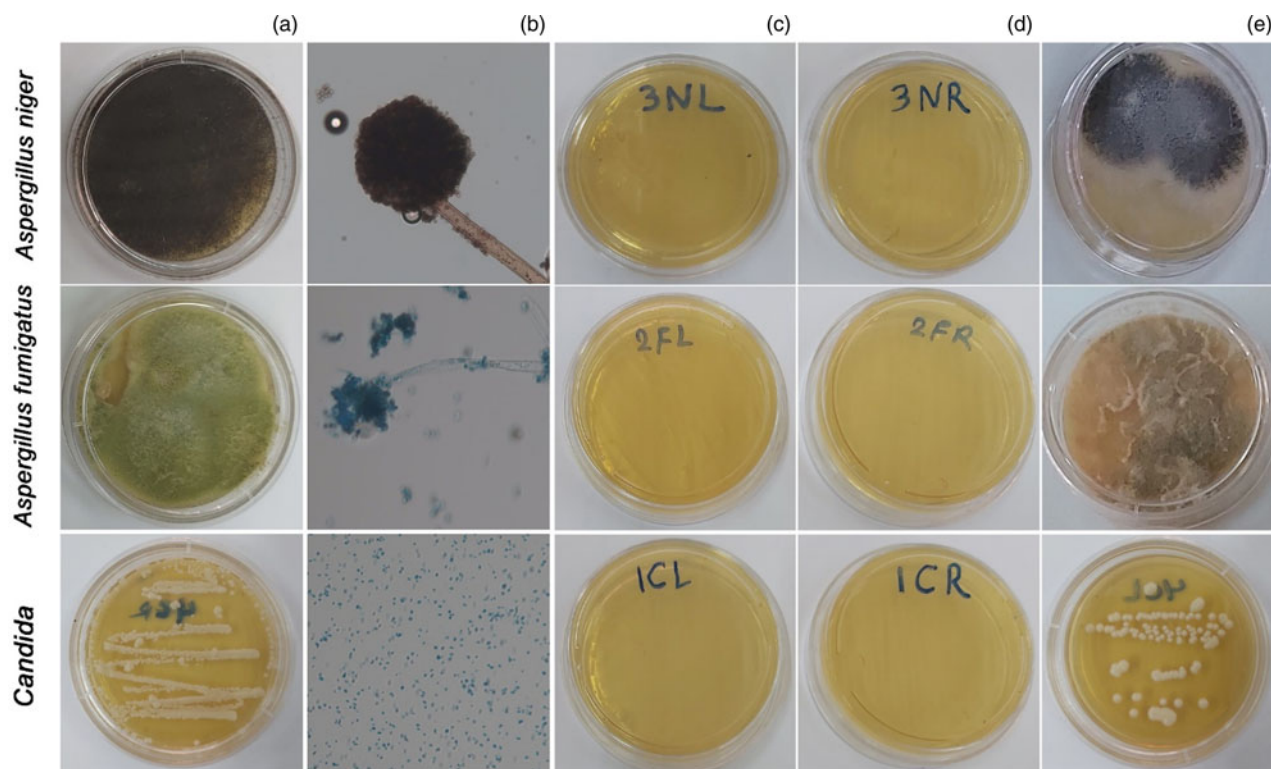
Ten days after inoculation of fungi in external auditory canals, otomycosis symptoms including redness, oedema, and white-grey debridement on the external auditory canal and tympanic membrane were observed in all guinea pigs by otomicroscopy. In addition, fungal infection was confirmed in all samples based on a smear and culture test. As shown in Figure 2a and b, in the animals infected by *A niger* (group A), a black filamentous morphology was observed on the Sabouraud dextrose agar medium after a few days, and conidia, vesicles and conidiophores were seen in a microscopic view. In animals of group B, the green-coloured colonies (spiked conidia) with a flat surface were observed on the culture, which confirmed the ear infection by *A fumigatus*. Microscopic observation also showed the rounded conidia morphology and conidiophores. The cultured fungi from group C was confirmed to be candida, with creamy white and smooth colonies forming after 72 hours. The budding yeast cells of candida were also observed by a light microscope. Repeated cultures of the ear samples yielded the same organism. No bacterial growth was detected in the culture (Figure 2a and b). No death or any adverse effect was observed in guinea pigs during the treatment course.

### Drug efficacy

The results of treatments in all groups showed that all guinea pigs receiving 1 per cent peracetic acid and 2 per cent acetic acid (control) recovered at the end of treatment (10 days) based on negative findings at otomicroscopy and culture (Figure 2c and d). The mean clinical recovery period was 7 ± 1 days in the 1 per cent peracetic acid group and 6 ± 1 days in the acetic acid group. There was no difference between the groups receiving 1 per cent peracetic acid and 2 per cent acetic acid regarding the time to complete recovery. Treatment with 0.01 per cent peracetic acid was not successful at the end of treatment, and even one week after that, according to otomicroscopy findings that showed fungal remnants and mild redness and inflammation in external auditory canal. In addition, in this group, the culture results were not

Table 1. Clinical examination of guinea pigs with otomycosis treated with peracetic acid 0.01 and 1 per cent and acetic acid 2 per cent solutions: clinical findings at 10 days after beginning of treatment

Group (6 guinea pigs each)	Sub-group (3 guinea pigs each)	Inoculation site	Treatment (drug)	Negative culture & smear	Tympanic membrane perforation	Erythema of canal	White drug sedimentation	External auditory canal oedema	Specific behavioural disorders
Group A	Sub-group 1	Right ear	1% peracetic acid	Yes	No	No	No	No	No
		Left ear	2% acetic acid	Yes	No	No	Yes	No	No
Group B	Sub-group 2	Right ear	0.01% peracetic acid	No	No	Yes	Fungal infection	Yes	No
		Left ear	2% acetic acid	Yes	No	No	Yes	No	No
	Right ear	1% peracetic acid	Yes	No	No	No	No	No	
	Left ear	2% acetic acid	Yes	No	No	Yes	No	No	
Group C	Sub-group 2	Right ear	0.01% peracetic acid	No	No	Yes	Fungal infection	Yes	No
		Left ear	2% acetic acid	Yes	No	No	Yes	No	No
	Right ear	1% peracetic acid	Yes	No	No	No	No	No	
	Left ear	2% acetic acid	Yes	No	No	Yes	No	No	
	Right ear	0.01% peracetic acid	No	No	Yes	Fungal infection	Yes	No	
	Left ear	2% acetic acid	Yes	No	No	Yes	No	No	



**Fig. 2.** (a) Culture after creating infection, (b) microscopic view of fungi stained with lactophenol cotton blue  $\times 400$ , (c) culture after treatment by 1 per cent peracetic acid, (d) 2 per cent acetic acid and (e) 0.01 per cent peracetic acid.

negative at the end of treatment, although colonies after treatment were visually less than before treatment (Figure 2e).

### Clinical examination

The visual examination of animals' ears by otoscopy confirmed no drug sedimentation or any other side effects, such as tympanic membrane perforation or external auditory canal and/or tympanic membrane irritation during treatment with peracetic acid. However, white drug sedimentation was observed in the external auditory canal of the acetic acid group (Table 1). Moreover, there were no signs of fungal infection in the treated ears, especially in the acetic acid and peracetic acid 1 per cent groups. Moreover, we did not see any disequilibrium, imbalance, or any other specific behavioural reactions in all guinea pigs receiving 1 per cent peracetic acid and 2 per cent acetic acid during and at least one week after the end of treatment.

### Discussion

Although fungal infections of the outer ear are rarely life-threatening, because of long-term treatment and the possibility of recurrence, these infections can be a serious and sometimes frustrating challenge for patients, otolaryngologists and general practitioners.<sup>7</sup>

There are four main classes of drugs for the treatment of fungal infections, which include polyenes, triazoles, nucleoside analogues and echinocandins.<sup>2</sup> In this regard, the main issue is the antifungal resistance of various pathogens.<sup>1,15</sup> Early detection of the type of fungal pathogen and provision of the appropriate specific treatment (formulation, dose, course of treatment) of the infectious agents can address this problem.<sup>3</sup> Studies show that the widespread and sometimes unnecessary

use of antibiotic eardrops to treat otitis media and otitis externa may be associated with a significant increase in the prevalence of otomycosis. Secondary fungal overgrowth is a known consequence of the use of broad-spectrum antibiotics such as quinolones.<sup>2,16</sup>

Today, in addition to commercially accessible drugs, such as steroids and antibiotics, oto-topical compounds are often used for the treatment of ear infection-related diseases, with disinfectant and acidic drops often being used to treat inflammation and infection of the middle ear along with outer ear infections and perforation of the tympanic membrane. Topical application of these substances increases the concentration of the drug in the used areas, has a lower risk of causing bacterial resistance and does not cause systemic side effects.<sup>14</sup>

Several studies have reported otomycosis treatment by weak acids, which have an inhibition effect on fungal growth. Than *et al.* showed a successful treatment and 100 per cent recovery from otomycosis after topical cleansing and using 10 per cent 5-fluorocytosine ointment once a week in the external ear canal.<sup>17</sup> Anwar and Gohar described 2 per cent salicylic acid in alcohol along with thorough cleaning of the ear canal as being effective in otomycosis treatment.<sup>18</sup> In a report, a 4 per cent boric acid solution in 70 per cent alcohol was suggested as an effective and inexpensive treatment choice for otomycosis.<sup>19</sup> Erkan *et al.* analysed 1–2 per cent acetic acid with hydrocortisone and alcohol-saturated boric acid for fungal infection treatment. They found that topical application of 2 per cent acetic acid plus hydrocortisone twice daily for three weeks was very effective and all patients recovered.<sup>20</sup> In Iran, Yeganeh-Moghadam *et al.* used acetic acid (2 per cent) every eight hours for three weeks for otomycosis treatment. Most patients (78.8 per cent) were completely cured after treatment with a mixture of alcohol and acetic acid.<sup>21</sup>

- Otomycosis is a worldwide ear infection caused by fungus
- Peracetic acid as an antifungal agent has potential for otomycosis therapy
- The therapeutic effects of 1 and 0.01 per cent peracetic acid were investigated in the animal model of otomycosis
- Findings confirm efficient treatment of otomycosis by 1 per cent peracetic acid in an animal model

Here, we used peracetic acid as a new treatment for fungal external auditory canal infections, albeit in an animal model (guinea pig). Our study showed that the use of peracetic acid in concentrations of 1 per cent in the treatment of otomycosis in the animal model was successful. No side effects were observed during the treatment period until one week later (Figure 1). Although using 0.01 per cent dilution of peracetic acid was not able to eliminate otomycosis, the fungi population grown after treatment was less than before (Figure 2).

To the best of our knowledge, after searching the English medical literature and databases, this is the first study analysing peracetic acid solution for treatment of otomycosis. This study used an animal model for otomycosis, and its strengths were the use of otomicroscopy and the investigation of treatment effects by fungal culture. One of our study's weaknesses was not assessing the hearing and balance function of the treated animals by objective tests. Also, we did not test the drug in animals with a perforated tympanic membrane. Regarding this, the lack of specific behavioural disorders or imbalances in the tested guinea pigs, and the health of their eardrums before, during, and after treatment, as well as the non-toxicity of acetic acid (the past version of peracetic acid that was used clinically for many years) can all potentially preclude the ototoxic effect of this new drug, and it must be tested more objectively in future studies.

## Conclusion

Our findings confirm the efficient treatment of otomycosis by 1 per cent peracetic acid in an animal model (guinea pig). Because of the therapeutic effect of peracetic acid for otomycosis in the animal model and the absence of side effects, we recommend researching the use of this weak acidic solution as a new drug for the treatment of otomycosis and investigating its efficiency and possible side effects in human patients and patients with fungal otitis externa. In addition, it is suggested that the ototoxicity of peracetic acid should be investigated more objectively in future studies, for example, in animal models with perforated tympanic membrane or in chronic otitis media patients.

**Acknowledgements.** This work has been funded by the Guilan University of Medical Sciences, Guilan, Iran (grant number: IR.GUMS.REC.1398.285). The authors acknowledge the financial support from Guilan University of Medical Sciences, Guilan, Iran.

**Competing interests.** None declared

## References

- 1 Nemati S, Hassanzadeh R, Jahromi SK, Abadi ADN. Otomycosis in the north of Iran: common pathogens and resistance to antifungal agents. *Eur Arch Otorhinolaryngol* 2014;**271**:953–7
- 2 Munguia R, Daniel SJ. Otological antifungals and otomycosis: a review. *Int J Pediatr Otorhinolaryngol* 2008;**72**:453–9
- 3 Vennewald I, Klemm E. Otomycosis: diagnosis and treatment. *Clin Dermatol* 2010;**28**:202–11
- 4 Gharaghani M, Seifi Z, Mahmoudabadi AZ. Otomycosis in Iran: a review. *Mycopathologia* 2015;**179**:415–24
- 5 Jia X, Liang Q, Chi F, Cao W. Otomycosis in Shanghai: aetiology, clinical features and therapy. *Mycoses* 2012;**55**:404–9
- 6 Bineshian F, Irajian GR, Fredonian M. A study on the frequency of fungal agents in otitis externa in Semnan. *Iran J Pathol* 2006;**1**:141–4
- 7 Viswanatha B, Naseeruddin K. Fungal infections of the ear in immunocompromised host: a review. *Mediterr J Hematol Infect Dis* 2011;**3**:e2011003
- 8 Kitis M. Disinfection of wastewater with peracetic acid: a review. *Environ Int* 2004;**30**:47–55
- 9 Kim J, Huang C-H. Reactivity of peracetic acid with organic compounds: a critical review. *ACS ES&T Water* 2021;**1**:15–33
- 10 Shahbaz B, Norouzi M, Tabatabai H. Mechanism of action and application of virocidis in health care associated viral infections. *Tehran Univ Med J* 2016;**73**:837–55
- 11 Costa SAdS, Paula OFPd, Leão MVP, Santos SSFD. Stability of antimicrobial activity of peracetic acid solutions used in the final disinfection process. *Braz Oral Res* 2015;**29**:1–6
- 12 Yoganarasimha S, Trahan WR, Best AM, Bowlin GL, Kitten TO, Moon PC *et al.* Peracetic acid: a practical agent for sterilizing heat-labile polymeric tissue-engineering scaffolds. *Tissue Eng Part C Methods* 2014;**20**:714–23
- 13 Hushangi R, Hosseini Shekarabi SP. Effect of a peracetic acid-based disinfectant on growth, hematology and histology of juvenile rainbow trout (*Oncorhynchus mykiss*). *Fishes* 2018;**3**:1–8
- 14 Salihoglu M, Dogru S, Cesmeci E, Caliskan H, Kurt O, Kuçukodaci Z *et al.* Ototoxicity of boric acid powder in a rat animal model. *Braz J Otorhinolaryngol* 2018;**84**:332–7
- 15 Nemati S, Gerami H, Habibi AF, Kazemnejad E, Shabani N, Aghsaghloo V *et al.* Sertaconazole versus clotrimazole and miconazole creams in the treatment of otomycosis: a placebo-controlled clinical trial. *Iran J Otorhinolaryngol* 2022;**34**:27–34
- 16 Jackman A, Ward R, April M, Bent J. Topical antibiotic induced otomycosis. *Int J Pediatr Otorhinolaryngol* 2005;**69**:857–60
- 17 Than KM, Naing KS, Min M. Otomycosis in Burma, and its treatment. *Am J Trop Med Hyg* 1980;**29**:620–3
- 18 Anwar K, Gohar MS. Otomycosis; clinical features, predisposing factors and treatment implications. *Pak J Med Sci Q* 2014;**30**:564
- 19 Ozcan KM, Ozcan M, Karaarslan A, Karaarslan F. Otomycosis in Turkey: predisposing factors, aetiology and therapy. *J Laryngol Otol* 2003;**117**:39–42
- 20 Erkan M, Asian T, Soyuer O. Treatment of otomycosis with acetic acid and boric acid. *Erciyes Tip Dergisi* 1992;**14**:1–30
- 21 Yeganeh-Moghadam A, Asadi MA, Dehghani R, Mahmoudabadi AZ, Raygan F, Hooshyar H *et al.* Evaluating the effect of a mixture of alcohol and acetic acid for otomycosis therapy. *Jundishapur J Microbiol* 2010;**3**:66–70