

In vitro efficacy of Otic Domeboro against *Pseudomonas aeruginosa*

W. L. DIBB

Department of Microbiology, Haukeland Hospital, Bergen, Norway

Dibb WL. In vitro efficacy of Otic Domeboro against *Pseudomonas aeruginosa*. Undersea Biomed Res 1985; 12(3):307-313.—The in vitro activity of prophylactic ear drops (Otic Domeboro) which are widely used by saturation divers against external otitis has been assessed. Four strains of *Pseudomonas aeruginosa*, 1 reference strain and 3 clinical isolates from cases of external otitis were employed. Two of the clinical isolates were from saturation divers who were using Otic Domeboro. Otic Domeboro solution was bactericidal even when diluted 1:2 in serum or broth. There were no significant differences between strains. There was no measurable inoculum effect. Survival kinetic analysis showed 60% killing at 5 min and 99.9% killing at about 2 h. Otic Domeboro is an effective anti-*P. aeruginosa* agent. There was no evidence of increased resistance among strains isolated from divers. The rate of killing in relation to usual prophylactic recommendations suggests that frequent dripping may be more effective. The therapeutic use of Otic Domeboro in saturation diving should be evaluated to avoid unnecessary use of antibiotic drops.

external otitis
diving
acetic acid

prophylaxis
Pseudomonas aeruginosa
antibacterial activity

External otitis, an infection of the outer ear canal, can be an extremely serious disease in the hyperbaric environment. It is common (1) and can often, particularly in saturation divers (2), be severe. The problem has therefore consequences both for the individual diver and economically in commercial diving.

Little research on the reasons for the high incidence and severity of external otitis in saturation divers has been performed. Correspondingly, only a few studies have examined possible prophylactic measures (1, 2) and treatment regimens in this group. Prophylactic measures which are widely carried out but lack sufficient scientific documentation include regular chamber disinfection and use of preventive ear drops during saturation diving. The purpose of both these measures is to depress the potentially pathogenic bacterial flora, particularly the Gram-negative rod *Pseudomonas aeruginosa* ("pyo") (3), in the chamber and in the diver's ears, respectively. In this study, in an attempt to document a prophylactic regimen, the antibacterial

activity of a widely used commercial preparation of ear drops, Otic Domeboro, has been evaluated against *P. aeruginosa*.

MATERIALS AND METHODS

Bacterial strains

Four strains of *P. aeruginosa* were employed in the study. These were:

1. A reference strain from the American Type Culture Collection (ATCC 27853) hereafter designated strain *A*.
2. A clinical isolate from our laboratory from the outer ear canal of a patient with external otitis who was not a diver and had not been using prophylactic ear drops, designated as strain *B*.
3. Two isolates from the ears of different saturation divers who had been using Otic Domeboro and had developed symptoms and signs of external otitis, designated as strains *C* and *D*.

The strains were identified by standard bacteriological methods (colonial and microscopic morphology, the oxidase reaction, growth on cetrinide agar) (4) and by the N/F System (Flow Laboratories, New York). They were stored in nutrient agar stab cultures until used.

Prophylactic ear drops

Otic Domeboro solution (Batch BFH3) (Miles Pharmaceuticals, West Haven, CT) was employed. This contains 2% acetic acid in aluminium acetate (modified Burow's) solution. It was stored at room temperature until used.

Culture media

Sensitivity determination was carried out in Mueller-Hinton broth (Difco Laboratories, Detroit, MI) at pH 7.3 which had been autoclaved at 120°C for 20 min. For some experiments, sterile-filtered pooled human serum which was inactivated at 56°C for 30 min and had a pH of 7.3 was used. Subcultures from these media and initial culture were performed on human blood agar. All incubation of bacteria was at 37°C.

Determination of bacteriostatic activity

The bacterial strains were cultured in Mueller-Hinton broth (2 ml) and incubated overnight. This suspension was then diluted in the broth by the use of 10 µl calibrated loops so that the final inoculum concentration (expressed as colony forming units, CFU) tested in the ear drop dilutions was approximately 10^3 , 5×10^5 , and 10^7 /ml in respective experiments. The Otic Domeboro drops were diluted in twofold steps with Mueller-Hinton broth and, in some experiments, human serum. These inoculated dilutions were incubated in sterile, tightly closed 8-ml disposable plastic tubes. They were incubated for 18 h and then for a further 24 h. The highest dilution that inhibited macroscopic growth of *P. aeruginosa* was regarded as the maximum inhibitory dilution.

Determination of bactericidal activity

After overnight incubation, subculture was carried out from the plastic tubes with 10 μ l calibrated loops. The subculture was performed on human blood agar plates (9 cm) and the whole surface of the plate was covered so that the Otic Domeboro was maximally diluted and was then incubated overnight. The highest dilution of Otic Domeboro that resulted in at least 99.9% killing as compared to the original inoculum was defined as the maximum bactericidal dilution.

Survival kinetics

One strain (*D*) was cultured overnight in broth. This suspension was then diluted in broth. A calibrated loop was used to transfer the diluted bacterial suspension to undiluted Otic Domeboro (2 ml) so that the final inoculum was 1.6×10^7 CFU/ml. Samples were then taken from the Otic Domeboro at various time intervals with calibrated loops. These were inoculated onto blood agar, and colony counts were performed after incubation.

Controls

The ATCC 27853 strain was included as a control. All strains were cultured in broth that did not contain Otic Domeboro in each experiment so that growth viability could be confirmed.

RESULTS

At an inoculum density of 5×10^5 CFU/ml strains *A*, *B*, *C*, and *D* were all inhibited by Otic Domeboro at a maximal dilution of 1:8 with serum or broth (Table 1). This result was not altered by further incubation after the overnight reading.

Strains *A* and *D* were examined at high and low inoculum values of 10^7 and 10^9 CFU/ml in broth. The maximal inhibitory dilutions were the same as above within the standard, accepted variation of this method (plus or minus one dilution step).

Bactericidal activity is shown in Table 2. There was no significant difference in bactericidal activity between experiments performed in serum and in broth. Bactericidal dilutions were one or two twofold dilutions lower than the corresponding

TABLE 1
INHIBITORY ACTIVITY OF OTIC DOMEBORO WHEN DILUTED IN BROTH AND SERUM

Strain of <i>P. aeruginosa</i>	Maximum Inhibitory Dilution	
	In Broth	In Serum
<i>A</i>	1:8	1:8
<i>B</i>	1:8	1:8
<i>C</i>	1:8	1:8
<i>D</i>	1:8	1:8

TABLE 2
 BACTERICIDAL ACTIVITY OF OTIC DOMEBORO WHEN DILUTED IN BROTH AND SERUM

Strain of <i>P. aeruginosa</i>	Maximum Bactericidal Dilution	
	In Broth	In Serum
<i>A</i>	1:4	1:2
<i>B</i>	1:4	1:2
<i>C</i>	1:2	1:2
<i>D</i>	1:2	1:2

LOG. NO. OF SURVIVING BACTERIA

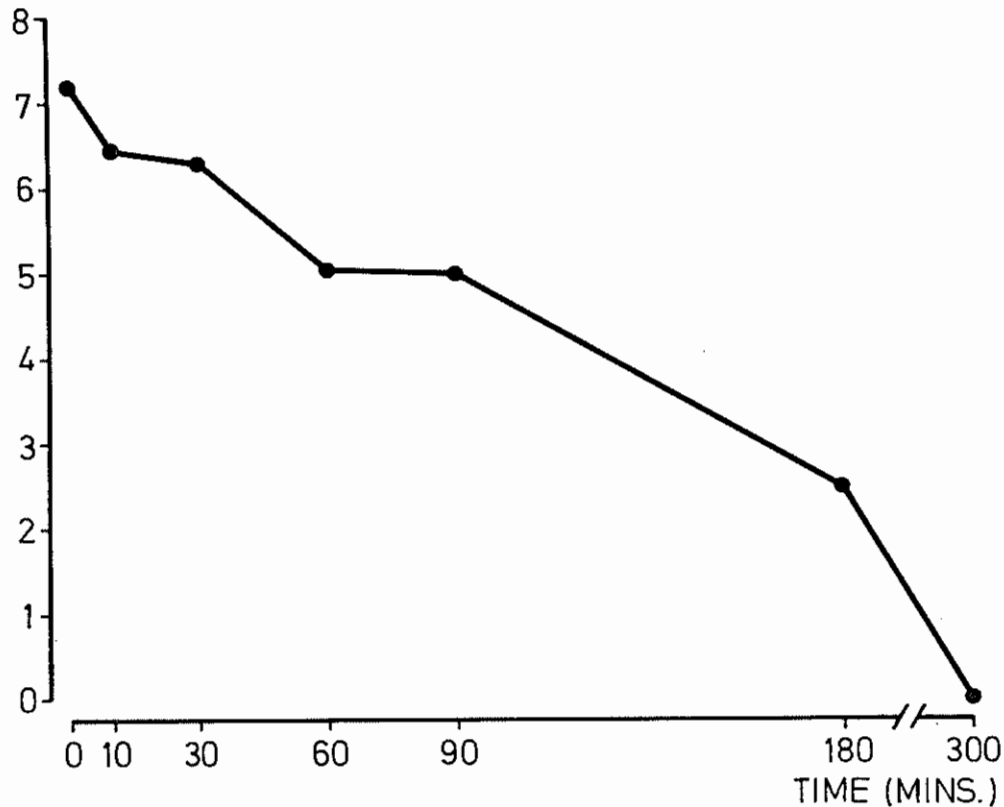


Fig. 1. Survival curve for one strain of *P. aeruginosa* (*D*) in undiluted Otic Domeboro

bacteriostatic concentrations. Bactericidal dilutions with high and low inocula were not significantly different from those performed at an inoculum of 5×10^5 .

The survival curve for strain *D* is shown in Fig. 1. At approximately 2 h, 99.9% killing was obtained. After 5 min (the time recommended by some for saturation

divers to lie down and keep the drops in the ears by closing the tragus) approximately 60% of the bacteria were killed.

All experiments shown in the tables were reproduced twice without changes in the results. The Otic Domeboro solution was sterile when cultured before inoculation. It had a pH value of 4.7.

DISCUSSION

The etiology of infectious external otitis has not been fully determined. There is general agreement, however, that exposure to moisture and a warm environment are important predisposing factors (5), and these are, of course, usually present in saturation diving. These factors lead to superhydration of the skin and favorable conditions for colonization with *P. aeruginosa* (6). It has been shown that saturation chambers can be reservoirs of the Gram-negative rods that potentially cause external otitis (7, 8). The saturation diver is therefore exposed to both predisposing factors for external otitis and a reservoir of the causative organisms.

Development of external otitis is preceded by a shift in the microbiological flora of the ear canal from the normal Gram-positive bacteria to dominance of Gram-negative rods (9). Otic Domeboro prophylaxis aims to prevent this shift. This study shows that these drops have potent bactericidal activity in vitro against *P. aeruginosa* even when diluted, as might well be the case in divers with water in the ear canals. The findings of Ochs (10), who stated that acetic acid at a concentration as low as 0.06% inhibited growth of *P. aeruginosa*, could not be confirmed however. The lack of inoculum effect is encouraging; this finding suggests that Otic Domeboro will probably be effective even in the presence of high concentrations of *P. aeruginosa*.

The results show no signs of in vitro development of resistance to Otic Domeboro among isolates from divers using this as a prophylactic. It is therefore probable that cases of external otitis that develop while using Otic Domeboro are the result of faulty prophylactic regimens. Otic Domeboro has been shown to be effective in the prevention of external otitis in a controlled naval study (1).

A relatively high initial inoculum was chosen for the survival study. This was an attempt to mimic the massive contamination of the external ear canal that we often find in ear samples from divers. The curve shows a relatively slow decline in relation to recommended contact time, which is usually 5 min (1), and the usual frequency of prophylaxis, which is twice a day and after contact with water. If such a regimen functions well with a negligible incidence of external otitis among divers, there seems to be no reason to change this. If, however, external otitis becomes a problem, increased contact time and more frequent dripping should be more effective, provided no irritant effect develops.

Treatment of external otitis in the hyperbaric environment is based mainly on use of antimicrobial ear drops. Some of these contain potentially toxic agents that are used in the parenteral therapy of serious systemic infection, e.g., gentamicin. Infections caused by *P. aeruginosa* and related organisms are a special problem because these organisms are usually multiresistant (11). There is a danger of induction and selection of resistant Gram-negative bacteria among divers. Treatment of external otitis is the normal use of Otic Domeboro and this solution has been reported to be effective (12). Lambert (13) demonstrated that potent antibiotic ear drops were no

more effective than hygroscopic treatment with aluminum acetate solution. It would be of interest to evaluate therapeutic use of Otic Domeboro in saturation divers to avoid use of antibiotics. More frequent dosage than that in common use prophylactically would of course be necessary.

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Dibb WL. L'efficacité in vitro de l'Otic Domeboro contre *Pseudomonas aeruginosa*. Undersea Biomed Res 1985; 12(3): 307-313.—L'activité des gouttes prophylactiques pour les oreilles (Otic Domeboro), fréquemment utilisées par les plongeurs à saturation contre l'otite externe, a été évaluée in vitro. Quatre souches de microbes de *Pseudomonas aeruginosa* furent employées: une souche de référence et trois souches d'isolements cliniques de cas d'otites externes. Deux des souches d'isolements cliniques furent obtenues de plongeurs à saturation qui utilisaient Otic Domeboro, une solution qui était bactéricide même lorsque diluée 1:2 dans un sérum ou un bouillon. Aucune différence significative ne fut trouvée entre les souches, ainsi qu'aucun effet mesurable d'inoculum. L'analyse cinétique de survie montra un taux d'élimination de 60% à 5 min et de 99.9% au bout d'environ 2 h. Otic Domeboro est un agent anti-*P. aeruginosa* efficace. Il n'y avait aucune évidence de résistance accrue parmi les souches isolées des plongeurs. Le taux d'élimination relatif aux recommandations prophylactiques habituelles suggère que l'utilisation plus fréquente des gouttes pourrait être plus efficace. L'emploi thérapeutique de l'Otic Domeboro lors des plongées à saturation devrait aussi être évalué afin d'éviter l'usage inutile des gouttes antibiotiques.

otite externe
plongée
acide acétique

prophylaxie
Pseudomonas aeruginosa
activité antibactérienne

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