## Main Articles

# Microbiology of cerumen in patients with recurrent otitis externa and cases with open mastoidectomy cavities

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## Abstract

This study investigated the common flora of human cerumen in patients with recurrent otitis externa, and subjects who had been operated on and had an open mastoidectomy cavity from chronic otitis media.

Cerumen samples were collected from three groups; group A (n = 20) consisted of patients with recurrent otitis externa, group B (n = 20) consisted of patients with an open cavity and group C (n = 30) consisted of healthy subjects.

The mean of the microbial count was  $3.4 \times 10^4$  in group A,  $3.08 \times 10^4$  in group B and  $2.48 \times 10^4$  in group C. The most commonly isolated microorganism from the three groups was *Staphylococcus epidermidis*. No growth was observed in five cases (25 per cent) in group A and in three cases (10 per cent) in group C. In group B antimicrobial growth was observed in all samples. In 46 (65 per cent) of the cerumen samples, the isolates were monomicrobial and 24 (35 per cent) of the cerumen samples were polymicrobial. The isolates were polymicrobial in 65 per cent of group A, 20 per cent in group B and 23.3 per cent in group C.

In the process of investigating the microbial flora of cerumen in all the three groups, microbial growth was observed from all the samples from patients with an open cavity, unlike the other groups, and it was determined that the group with recurrent external otitis had the most abundant microbial flora.

Key words: Cerumen; Otitis Externa

## Introduction

The skin of the cartilaginous part of the external auditory canal (EAC) has numerous sebaceous and ceruminous glands.<sup>1-4</sup> Ceruminous glands are modified apocrine sweat glands. There are approximately 1000 to 2000 ceruminous glands in a normally developed EAC.<sup>1-4</sup> In the normal human ear, infections of the EAC are relatively uncommon and this is thought to be due in part to the protective effect of human cerumen.<sup>5</sup> Some of the protective effect lies in the physical barrier afforded by cerumen and some of the protective effect may be bactericidal, but this is still doubtful.<sup>5,6</sup>

Acute diffuse otitis externa is an inflammatory and infectious process of the EAC.<sup>7</sup> The usual pH of the EAC is mildly acidic at a pH of 4 to 5 and is an important antimicrobial deterrent. The acidic pH changes with acute diffuse otitis externa.<sup>7</sup> This change may be brought on by an accumulation of moisture from a warm, humid climate, by retention

of water from swimming, bathing, snorkelling or scuba diving and by the removal of cerumen from the EAC. $^{7}$ 

After an open cavity operation, the anatomy of the EAC is altered and so the self-cleaning mechanism of the EAC epithelium is impaired. Although a dry ear is achieved in the post-operative period, there is a need for frequent cleaning of the cavity depending on the rate of formation of cerumen. Taking into account the existence of a wide cavity and the likely changes in the microbiological structure that might be caused by the alterations in the epithelial structure, the flora of the patients with an open cavity was also investigated.

In this study, the common flora of human cerumen in patients with recurrent otitis externa and subjects who had been operated on for chronic otitis media and had an open mastoidectomy cavity were investigated. Their results were compared with the flora of healthy adults. Finally the results of the groups were compared.

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TYPES OF MICROORGANISMS ISOLATED FROM THE CERUMEN IN RECURRENT OTITIS MEDIA, OPEN CAVITY AND HEALTHY POPULATION GROUPS

	Group A (recurrent otitis externa, n = 20)	Group B (open cavity, n = 20)	Group C (healthy subjects, n = 30)
No growth	5	_	3
Staphylococcus epidermidis	13 (1)*	15 (1)*	22
Staphylococcus aureus	(3)*	4 (3)*	4 (1)*
Diphtheroid	5 (2)*	3	4 (4)*
Candida albicans	5 (5)*	_	1 (1)*
Pseudomonas aeruginosa	2 (2)*	_	1 (1)*
Staphylococcus saprophyticus		_	2
Micrococcus spp.	_	2	_

\*The number in parentheses indicates the number of cerumen samples in which polymicrobial growth was observed.

#### Materials and methods

Cerumen samples were collected from three groups. Group A (n = 20) consisted of patients with recurrent otitis externa (two or more acute otitis externa attacks in the current year and symptom free for at least three months), Group B (n = 20) consisted of patients with an open cavity (at least one year since the operation) and Group C (n = 30) consisted of healthy subjects. Patients with diabetes mellitus or allergic diseases were excluded from the study.

Cerumen samples were collected from 70 subjects ranging in age from 15- to 56-years-old, who applied to our out-patient clinic. Cerumen suspension, quantification and identification of the common flora of the cerumen were performed according to Campos et al.<sup>2</sup> The cerumen was emulsified in a consistent buffer, in a solution of water and glycerol at a concentration of 30/70 (volume/volume) and five per cent sodium bicarbonate, a 3.5 per cent final concentration from the same being obtained (weight/ volume).<sup>8</sup> The cerumen suspension was mixed with a magnetic mixer until a homogenous milky solution was obtained. A sample of 0.1 ml of the cerumen suspension was taken and diluted in 9.9 ml of sterile saline serum. Then a series of decimal dilutions was made. From each of the decimal dilutions, and in duplicate, 1 ml was deposited on sterile Petri plates with a sterile pipette. Next previously liquefied culture medium was poured and adjusted to 45-47°C. The medium and the inoculum were mixed. After 24 hours of incubation at 37°C a microbial count was carried out. Tryptone soya agar (Difco) medium was used for the count.

A sample of the cerumen suspension was retrieved with a plantinum loop, and seeded in plates of blood agar, MacConkey agar and chocolate agar for the identification of microorganisms. Blood and Mac-Conkey agars were incubated at 37°C for 24 or 48 hours, chocolate agar was incubated in an atmosphere of five to 10 per cent  $CO_2$  at 37°C. In the plates where growth occurred, Gram staining was carried out on each one of the different colonies. Identification was performed by the API Scan (Biomeriux). The catalase, oxidase test and API 32 Staphylococcus panel and API IP 32 Streptococcus panel were used for Gram positive organisms. The API ID32E panel was used for Gram negative organisms. The filamentation test was used for Candida sp.

#### Statistical method for data analysis

The ANOVA test and post hoc Tukey test were used to compare the changes in counts of microbial growth in the three groups. A p<0.05 value was accepted as significant.

## **Results and analysis**

The mean of the microbial counts was  $3.41 \times 10^4 \pm$  $3.36 \times 10^4$  in Group A,  $3.08 \times 10^4 \pm 2.25 \times 10^4$  in Group B and  $2.48 \times 10^4 \pm 1.86 \times 10^4$  in Group C. According to the mean microbial count (cases with no microbial growth were excluded), there was only a statistically significant difference between Group A and C (p = 0.04). In 46 of the cerumen (65 per cent) samples, the isolates were monomicrobial. In 24 of the cerumen (35 per cent) samples, the isolates were polymicrobial. In group A, the isolates were 35 per cent monomicrobial and 65 per cent polymicrobial. But the isolates in Group B and Group C were 80 per cent and 76.7 per cent monomicrobial and 20 per cent and 23.3 per cent polymicrobial respectively. The type of the microorganism having grown in each group is shown in Table I. The most commonly isolated microorganism from the three groups was Staphylococcus epidermidis. The most abundant microbial flora was in Group A. While the most frequently seen bacterium was Staphylococcus epidermidis, the others were diphtheroids and Candida albicans respectively in Group A. The most fre-

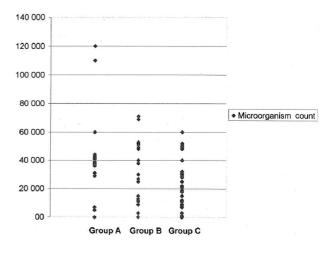


FIG. 1 Count of microbial growth in groups.

quently isolated bacterium was Staphylococcus epidermidis, the others were Staphylococcus aureus, and diphtheroids respectively in Group B and Group C (Figure 1).

- The objective of this paper is to investigate the common flora of human cerumen in patients with recurrent otitis externa, open mastoidectomy cavities and in healthy subjects
- · Microbial growth was observed in all the patients with an open cavity and those with recurrent external otitis had the most abundant microbial flora

### Discussion

In our investigation the mean count of microorganisms was 10<sup>4</sup> per ml in Group C. Chai and Chai found 10<sup>4</sup> microorganisms/ml of cerumen suspension.<sup>8</sup> Stone and Fulghum reported that the count of microorganisms was 10<sup>3</sup> microorganism/ml.<sup>3</sup> Campos et al. found 10<sup>6</sup> microorganisms/ml in the suspension of cerumen.<sup>2</sup> Campos et al. attributed this difference to the fact that the study was conducted in the Canary Islands, a subtropical region. They said that another factor that may justify their high count finding is fresh cerumen.

We observed no growth in five cases (25 per cent) in Group A and in three cases (10 per cent) in Group C. There was microbial growth in all samples in Group B. This result may be explained by the fact that the open mastoid cavity in Group B retains more cerumen.

The predominant microorganism was Staphylococcus epidermidis (40 per cent in Group A, 62.5 per cent in Group B, 59 per cent in group C) in this study. Chai and Chai<sup>8</sup>, Stone and Fulghum<sup>3</sup> and Campos et al.<sup>2</sup> also isolated mainly Staphylococcus epidermidis. However, Stroman et al. mainly isolated Staphylococcus auricularis.<sup>9</sup> Staphylococcus epidermidis was the second most common bacterium isolated from cerumen in that study.

In this study cerumen samples were taken from the external auditory canal using a sterile ear hook. These samples were taken with difficulty in some cases because of lack of cerumen. Previous studies had also demonstrated a low amount of cerumen in patients with recurrent external otitis.7,10 Lack of cerumen may be due to an alteration in the glandular activity.<sup>4</sup> Absence of cerumen may lead to infection for two reasons. Firstly, the act of cerumen removal may be traumatic and lead to breaks in the fragile external auditory canal skin. Secondly, the cerumen has an antimicrobial role through physically protecting the external auditory canal skin, establishing a low-pH, inhospitable environment for pathogens, and producing antimicrobial compounds such as lysozyme, so that its absence leaves the canal vulnerable to infection. Cerumen forms an acidic coat that aids in the prevention of external auditory

canal infection, thus, the absence of cerumen may be a predisposing factor for otitis externa.<sup>7</sup> However further studies need to clarify whether the reduction of cerumen is the result of the inflamed meatal skin or the cause of it. In addition to the explanation of lack of cerumen, some local and systemic predisposing factors and previous local medications may affect the external auditory canal milieu. It may be the reason for the increase in the mean count of microorganism and polymicrobial flora in patients with external otitis. The results of this study indicate that patients with recurrent external otitis had a statistically high microbial count compared to normal ears. It could be speculated that there are some subtle physiological changes, which lead to this increased bacterial flora, which may be triggered at some point to an active infection.

#### Conclusion

As a result, in the process of investigating the microbial flora of cerumen in all the three groups, growth was observed in all the patients with an open cavity, and it was determined that the group with recurrent external otitis had the most abundant microbial flora.

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Y. S. Pata takes responsibility for the integrity of the content of the paper.

Competing interests: None declared