The Journal of Laryngology & Otology
December 2000, Vol. **114,** pp. 925–929

Main Articles

Influence of human wet cerumen on the growth of common and pathogenic bacteria of the ear

A. Campos, L. Betancor*, A. Arias, C. Rodríguez, A. M. Hernández, D. López Aguado*, A. Sierra

Abstract

The available data on the effect of human wet cerumen on bacterial growth are not conclusive. Nevertheless it is widely accepted that cerumen has a bactericidal effect.

In this study the activity of human wet cerumen on bacterial growth was assessed by applying cerumen suspensions to bacterial cultures. Bacterial counts were performed before and after application of cerumen suspensions. A total of 383 assays was carried out with 73 pools of cerumen that were tested against cultures of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Corynebacterium* spp., *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Serratia marcescens*.

An increase in growth occurred much more frequently than a decrease in growth in almost every microrganism tested, with the mean increase percentage being much higher than the mean decrease percentage, except in the case of *S. aureus*.

The largest average growth increase was obtained with *E. coli*. The largest average decrease in bacterial growth was recorded with *S. marcescens*.

Our study does not support the conception of a decrease in bacterial growth produced by humen wet cerumen. *In vitro*, the most observable effect was in fact an increase in microbial growth.

Key words: Ear, External; Cerumen; Bacteria; Toxicity

Introduction

Cerumen secreted by both ceruminous and sebaceous glands in the external auditory canal (EAC) can be found dry or wet. These two forms are associated with race and are controlled by two autosomal alleles. Dry cerumen is brittle and yellowish grey, while wet cerumen is sticky and yellowish brown.[1,2]

The role human cerumen plays in protecting the external ear against infection has long been a subject of controversy. Some of its protective effects lie in the physical barrier provided by cerumen. Cerumen is water-resistant and as such protects the epithelium of the EAC.[1,3–6]

It has been suggested that cerumen is unable to prevent infection and that the rich nutrients of earwax support abundant growth of bacteria and fungi.[5,7–11]

On the contrary some studies suggest that cerumen might have antimicrobial and antifungal effect. The employed method for this study was first described by Chai and Chai and compared the count of microorganisms in buffer (control) with cerumen suspension. They claimed that cerumen is bactericidal revealing it as a decrease in growth of the microorganisms (*E. coli*, *S. marcescens*, *Haemophilus influenzae*, *P. aeruginosa*, *Streptococcus* sp, *Staphaureus*) when growing in the cerumen suspension.[12]

Further studies based on the same method gave similar results. Stone and Fulghum,[13] tested *Staph aureus*, *Staph epidermidis*, *Strep pyogenes*, *Streptococcus* sp., *P. acnes*, *Corynebacterium* sp. Megarry *et al.*[14] tested *Staph aureus*, *Candida albicans*. Campos *et al.*[15] however, tested *C. albicans* and reported no inhibition of microbial growth.

Using a different methodology Perry and Nichols[9] tested *Micrococcus aureus*, *Strep pyogenes, Corynebacterium* sp., *Micrococcus albus*, *Bacillus subtilis*, *E. coli* and *P. aeruginosa*. Jankowsky *et al.*[16] tested *Strep pyogenes*, *Strep viridans*, *Staph aureus*. Burtenshaw[8] using a different method from Chai and Chai tested *Strep viridans*, *Strep haemolyticus*, *Staph*

From the Department of Preventive Medicine and Public Health, University of La Laguna and the Department of Otolaryngology*, Hospital Universitario de Canarias, La Laguna, Tenerife, Spain.
Accepted for publication: 10 July 2000.

aureus, *Staph. pneumoniae*, *Corynebacterium diphtheriae*, and *Staph. epidermidis* achieved inconsistent results concerning the bactericidal activity of human cerumen.

The purpose of the present study was to test the effect of human wet cerumen on the growth of common and pathogenic bacteria of the ear (*Staph aureus*, *Staph epidermidis*, *Corynebacterium* spp., *E. coli*, *Proteus mirabilis*, *P. aeruginosa*, *S. marcescens*).

Materials and methods

Cerumen suspensions

Cerumen samples were collected from 380 subjects ranging in age from five to 45 years, who attended the Ear, Nose, and Throat out-patient clinic of the Hospital Universitario de Canarias, from 1993 to 1995.

A total of 72 cerumen pools were tested, each containing cerumen from four or five healthy subjects. None of these subjects presented a history of previous ear disease or were suffering from otitis at that moment.

The cerumen was removed with a sterile ear hook. It was stored in sterile jars and processed within 24 hours. All the cerumen samples were wet with a yellowish and sticky appearance.

Cerumen was weighed and then emulsified in a 30/70 v/v water and glycerol buffer, containing five per cent of sodium bicarbonate. This procedure yielded a homogenous, milky solution with a final concentration of 3.5 per cent (w/v).[12] The cerumen suspension was not sterilized.

Micro-organism cultures

The bacteria to be tested were those known as the most frequently indigenous and pathogenic in the ear (Table I).[17,18] *S. marcescens* was included because in the previous studies by Chai and Chai[12] and Stone and Fulghum[13] it proved to be the most susceptible bacteria to the effects of cerumen.

TABLE I
STRAIN SOURCE

Micro-organisms	Strain source
Staphylococcus aureus	Wound ATCC 9144
Staphylococcus epidermidis	Blood culture ATCC 12228
Corynebacterium spp.	Wound ATCC 14665
Escherichia coli	Urine ATCC 10536
Proteus mirabilis	Sputum ATCC 9484
Pseudomonas aeruginosa	Sputum ATCC 27853
Serratia marcescens	Bronchial aspirate ATCC 14756

Micro-organisms growth media

Tryptic soy agar (DIFCO) was used to conserve strains and tryptic soy broth (DIFCO) to revivify them. Once revivified, micro-organisms were placed on tryptic soy agar plaques and then incubated at 37°C for 24 hours. The inoculate was prepared from the 24-hour culture.

From the 24 hours tryptic soy agar culture, one or two colonies were transferred, by means of a platinum loop, into test tubes containing 3 ml of tryptic soy broth. These tubes were homogenized by means of vortex and then incubated at 37°C for two hours, until they became visibly opaque (0.5 on the McFarland scale, 10^7–10^8 UFC/ml).

Treatment procedures

Chai and Chai[12] described the method used. From the cerumen suspension 0.15 ml were taken and mixed with 0.025 ml of microbial culture, which gave a three per cent cerumen concentration. The mixture was left at room temperature for 20 minutes. Using a sterile pipette, a 0.1 ml sample of the mixture was then obtained and it was diluted in 9.9 ml of sterile saline solution. This procedure was repeated to give a series of decimal dilutions.

Duplicate 0.1 ml samples from each decimal dilution were deposited in sterile Petri plates. Fifteen ml were liquidated and heated up to 45–47°C, agar was then added to each plate. The medium and the inoculate were well mixed; after solidification, they were covered with five ml of the same medium, and allowed to solidify again. The plates were incubated at 37°C for 24 hours. The outside layer was applied in order to prevent excessive growth and spread of the colonies over the surface, thus facilitating counting.

Simultaneously, controls were established by taking only 0.15 ml of buffer solution and 0.025 ml of each microbial culture, and repeating the described procedure.

Statistical method for data analysis

A Wilcoxon sign rank test (a non-parametric sample comparison method used for detecting differences between distributions of paired samples) was carried out in order to determine whether cerumen acts as a bactericide or, on the contrary, favours bacterial growth.[19,20]

If cerumen has bactericidal properties, it would be expected that the sum of the ranks assigned to positive differences (R^+) to be large. If, on the other hand, it favours bacterial growth, this sum would be small.

A similar argument can be applied to the sum of the ranks assigned to negative differences (R^-). If cerumen acted as bactericide, R^- would be small, becoming larger if growth were favoured.

In order to draw any conclusions the confidence interval (alpha) must be established to ascertain whether the *p* value is greater or less than alpha. In our study alpha = 0.05, or alpha = 0.01.

https://doi.org/10.1258/0022215001904635 Published online by Cambridge University Press

TABLE II
RANGES AND AVERAGE OF THE VIABLE CELL COUNTS/ML IN THE BUFFER (CONTROL) AND AFTER CERUMEN APPLICATION

Micro-organisms	Range		Mean	
	Buffer	Cerumen	Buffer	Cerumen
Staph. aureus	$5.1 \times 10^5 - 2.6 \times 10^8$	$6.5 \times 10^4 - 8.9 \times 10^8$	4.1×10^7	5.0×10^7
Staph. epidermidis	$1.1 \times 10^4 - 3.7 \times 10^7$	$1.3 \times 10^5 - 2.2 \times 10^8$	1.4×10^7	2.5×10^7
Corynebacterium spp.	$2.6 \times 10^4 - 9.7 \times 10^7$	$5.8 \times 10^3 - 3.8 \times 10^7$	5.8×10^6	8.7×10^6
E. coli	$2.0 \times 10^4 - 2.8 \times 10^8$	$1.1 \times 10^4 - 3.8 \times 10^8$	5.4×10^7	1.0×10^8
P. mirabilis	$2.7 \times 10^5 - 5.7 \times 10^8$	$7.0 \times 10^4 - 5.0 \times 10^8$	5.5×10^7	6.0×10^7
P. aeruginosa	$1.8 \times 10^4 - 3.9 \times 10^7$	$2.5 \times 10^5 - 1.1 \times 10^8$	4.8×10^7	7.1×10^7
S. marcescens	$1.2 \times 10^5 - 5.8 \times 10^8$	$3.0 \times 10^4 - 6.7 \times 10^8$	1.1×10^8	7.6×10^7

Results

Table II shows the range and average of the viable cell counts/ml obtained in the buffer (control) and after cerumen application.

Table III shows the number of assays carried out (n^0) average percentage (Avg.) and standard deviation (SD) of the increase and decrease in growth of the micro-organisms after cerumen is applied.

In our study, 68.96 per cent of the assays showed an increase in microbial growth. Decrease in growth was obtained in 31.03 per cent. The average growth increase was much higher than the average decreased in growth.

Results of *Staph aureus* assays showed a similar proportion between increase and decrease in growth, although the average growth increase was far greater than the average growth decrease.

The largest average decrease in bacterial growth was obtained with *S. marcescens* (61.1 per cent).

The lowest average decrease in bacterial growth was obtained with *P. aeruginosa* (28.8 per cent).

The largest average increase in growth was obtained with *E. coli* (8854.9 per cent).

Table IV shows the analysis of the results after applying a Wilcoxon sign rank test.

Discussion

The microbial counts obtained were high (10^6). This could be related to climatic conditions and to the length of time the cerumen had remained in the external auditory canal before collection.[18] This leads us to believe it may have repercussions on the final test results in spite of confirmation from Chai and Chai[12] that the count of cerumen micro-organisms does not influence the viability of the test cultures. However, as our counts of cerumen micro-organisms were higher (10^6) than those obtained by Chai and Chai[12] (10^4) and Stone and Fulghum[13] (3.5×10^3), we have subtracted these from the final count in order to avoid any distortion these might cause. This subtraction was done to be rigorous from a mathematical point of view.

We did not sterilize the cerumen suspension. If we had done so we would have eliminated the possibility of growth interference between the flora of the cerumen and the micro-organisms tested. Burtenshaw[8] verified reduction or abolition of sterilization power of saline extract from skin, hair, nail and cerumen when heated at 75°C. Sterilization of cerumen by means of heat causes alteration of protein and fatty acids these being supposedly responsible for the bactericidal activity.[3–5] The use of ethylene oxide for sterilization was rejected because of the 15-day aeration period needed for elimination of the entire residue.[21]

In the 65 assays carried out on *Staph aureus*, cerumen caused a decrease in growth in 32 samples, and favoured growth in the remaining 33. Although the average increase in growth was far higher than the average decrease, after statistical processing of the data, no significant difference was demonstrated. In other words, the cerumen neither inhibited, nor favoured its growth. The average percentage decrease obtained in our study (56.0 per cent) is very close to the figures reported by Chai and Chai[12] (58.1 per cent) and Stone and Fulghum[13] (59.24 per cent).

Burthenshaw[8] reported inconsistent bactericidal activity of cerumen against this micro-organism. Megarry *et al.*[14] reported a constant bactericidal activity. Other authors found no bactericidal effect of cerumen on this micro-organism.[9,16]

TABLE III
NUMBER OF ASSAYS, AVERAGE PERCENTAGE AND STANDARD DEVIATION OF THE INCREASE AND DECREASE IN GROWTH OF MICRO-ORGANISMS AFTER APPLYING CERUMEN SUSPENSION

Micro-organism	Decrease in growth			Increase in growth		
	n	avg.	SD	*n*	avg.	SD
Staph. aureus	32	56.00%	0.33	33	505.9%	13.70
Staph. epidermidis	19	42.32%	0.23	50	1630.0%	45.75
Corynebacterium spp.	15	46.32%	0.18	37	354.5%	4.97
E.coli	10	53.85%	0.27	29	8854.9%	294.57
P. aeruginosa	15	28.58%	0.25	34	354.5%	129.81
P. mirabilis	23	55.26%	0.30	42	1044.3%	2255
S. marcescens	23	61.10%	0.33	41	1206.4%	20.06

avg. = average percentage; SD = standard deviation.

https://doi.org/10.1258/0022215001904635 Published online by Cambridge University Press

TABLE IV
ANALYSIS OF THE RESULTS AFTER APPLYING WILCOXON SIGN RANK TEST

Micro-organisms	*p* value	R+	R–	Significance (*p*)
Staphylococcus aureus	0.82	32.45	33.53	NS
Staphylococcus epidermidis	3.04×10^{-5}	510.49	1904.50	0.01
Corynebacterium spp.	1.66×10^{-4}	234.49	990.50	0.01
Escherichia coli	8.52×10^{-3}	212.00	577.99	0.01
Proteus mirabilis	0.01	535.50	1175.99	0.01
Pseudomonas aeruginosa	0.02	237.99	581.99	0.05
Serratia marcescens	0.03	724.00	1356.00	0.05

R+ = positive differences; R– = negative differences; NS = not significant.

Sixty-nine assays were carried out on *Staph epidermidis*. A decrease in growth was obtained in assays and an increase was found in 50. Our average decrease in growth is 42.32 per cent. Burthenshaw[8] reported an inconsistent bactericidal effect of cerumen on *Staph epidermidis*. Stone and Fulghum[13] with only two assays, reported a decrease in growth (41.11 per cent). Statistical analysis of our data indicates that cerumen favours growth of *Staph epidermidis*.

Of the 49 assays carried out on *Corynebacterium* spp. 15 showed a decrease in growth and 34 an increase. The average decrease was among the lowest figures obtained (28.58 per cent), similar to Stone & Fulghum's result (29.11 per cent).[13] Burthenshaw[8] found a strong and constant bactericidal effect, similar to Stone and Fulghum's[13] second assay (95.65 per cent). Perry and Nichols[9] reported no bactericidal effect. After the data analysis, our study shows that cerumen favours *Corynebacterium* spp. growth.

Of the 39 assays carried out on *E. coli*, a decrease in growth was obtained in 10 cases. Burthenshaw[8] did not detect any bactericidal effect of cerumen on this micro-organism. Perry and Nichols[9] found no evidence of a decrease in growth due to cerumen action. Bauman *et al.*[10] reported bactericidal activity on some strains of *E. coli*. Chai and Chai[12] when testing two standard *E. coli* strains, obtained mortality rates of 72.9 per cent and 99.8 per cent, the highest in the series. Stone and Fulghum[13] achieved mortality rates of 95.69 per cent and 90.24 per cent from their cultures. Analysis of our data indicates that cerumen favours growth of *E. coli*.

Fifty-eight assays were carried out with *P. mirabilis*, a decrease in growth was shown in 19 samples. Only Bauman *et al.*[10] have tested the effect of cerumen on this micro-organism, reporting a bactericidal effect on some strains only. Once analysed, our data leads us to state that cerumen favours *P. mirabilis* growth.

For *P. aeruginosa*, 40 assays were carried out and 12 showed a decrease in growth. The mean percentage of growth increase was the lowest in our study (28.58 per cent). Bauman *et al.*[10] and Perry and Nichols[9] reported a lack of bactericidal effect of cerumen on this bacteria. Stone and Fulghum[13] failed in drawing conclusions because of difficulties in counting, indeed, the counts appeared to be higher after treatment with cerumen, in comparison with the use of buffer alone. Chai and Chai[12] found mortality rates of 52.7 per cent and 29.4 per cent, which indicates a low percentage of their series. Statistical analysis results allow us to state that cerumen favours *P. aeruginosa* growth.

Sixty-four assays were carried out on *S. marcescens*. A decrease in growth was noted in 23 instances and an increase in 41. The mean percentage of growth decrease (61.10 per cent) was the highest of our study as it was for Chai and Chai,[12] and Stone and Fulghum[13] who obtained high mortalities of, 99.7 per cent and 76.47 per cent respectively. The mean percentage of increase in growth (1206.4 per cent) also was the highest for this bacteria. From the analysis of our data cerumen favours *S. marcescens* growth.

In spite of coincidences in some results with Chai and Chai[12] and Stone and Fulghum,[13] we cannot share their conclusion on the bactericidal effect of human wet cerumen. These authors processed just one and two samples respectively and an inconstant result of cerumen's bactericidal effect will be achieved with a small sample size.

From this study and as far as statistical data process concern we come to the conclusion that there is no evidence supporting the bactericidal effect of human wet cerumen on the micro-organisms tested. In fact, we can state that, *in vitro*, the most observable effect of humen wet cerumen is that it favours bacterial growth.

The so widely extended conception of cerumen's bactericidal effect perhaps could be considered as an aphorism. Further studies using a different methodology capable of providing more stable results are needed in order to elucidate it.

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Address for correspondence:
M. Antonia Campos Domínguez,
Cátedra de Medicina Preventiva y Salud Pública,
Facultad de Medicina, Universidad de La Laguna,
La Laguna 38071,
Tenerife, Canary Islands, Spain.

A. Campos takes responsibility for the integrity of the content of the paper.
Competing interests: None declared
