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Main Article

Antibacterial and antifungal properties of human cerumen

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Abstract

Objective: To assess the antibacterial and antifungal properties of human cerumen by studying its effect on the growth of *Staphylococcus aureus, Esherichia coli, Pseudomonas aeruginosa* and *Candida albicans*.

Materials and methods: Cerumen samples were collected from 75 normal, healthy subjects aged from seven to 80 years, without ear pathology, who attended the ear, nose and throat out-patient clinic of the University Malaya Medical Center from May 2006 to October 2006. Of these 75 samples, 31 had no growth when cultured on nutrient agar. Inhibition studies on these 31 samples were performed for *Staphylococcus aureus* (American Type Culture Collection (ATCC) 25923), *Esherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Candida albicans*. Nutrient agar was used to conserve all three bacterial strains and Sabouraud dextrose agar was used for *Candida albicans*.

Results: A decrease in *Staphylococcus aureus* growth was observed for 27 of the 31 samples. All 31 samples induced decreased growth of *Pseudomonas aeruginosa*, while 29 induced decreased growth of *Candida albicans*. However, only four samples induced decreased growth of *Escherichia coli*.

Conclusions: Cerumen was demonstrated to have potential antimicrobial effects on strains of *Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans*.

Key words: External Auditory Canal; Cerumen; Antimicrobial; Otitis Externa

Introduction

Cerumen (ear wax) is produced by sebaceous glands, ceruminous glands and apocrine glands located in the lateral one-third of the human ear canal. Two forms of cerumen have been identified, 'wet' and 'dry'. These two forms are associated with race and are controlled by two autosomal alleles.[1] The wet phenotype is dominant and the dry recessive. Mongoloid populations possess the dry phenotype, while the wet phenotype is common among Caucasian and African populations.

Cerumen is believed to protect the external ear canal against infection. Besides providing a physical barrier against infection, it is believed that cerumen has antibacterial and antifungal properties. However, a review of the literature revealed conflicting results on this topic.

The aim of this study was to investigate the effect of human cerumen on the growth of *Staphylococcus aureus, Esherichia coli, Pseudomonas aeruginosa* and *Candida albicans*.

Materials and methods

Cerumen samples

Cerumen samples were collected from 75 normal, healthy subjects (age range seven to 80 years; 39 males and 36 females), without any ear pathology, who attended the ear, nose and throat out-patient clinic of the University Malaya Medical Center from May 2006 to October 2006. Subjects with a history of otitis externa or otitis media were excluded. Subjects with other concurrent disease, such as diabetes mellitus, immunocompromise and/or those receiving chemotherapy, were also excluded.

Cerumen samples were taken using a sterile ear hook, weighed and then emulsified in a solution containing 30 per cent glycerol with 5 per cent sodium bicarbonate, producing a cerumen suspension of 3.5 per cent (weight/volume). The mixture of cerumen and buffer was then emulsified by pumping it back and forth between two syringes connected by a three-way connector, as described by Stone and Fulghum.[10] The cerumen suspension was stored at -20°C until microbiological testing. The cerumen suspensions were not sterilised.

Of the 75 samples, 31 (16 male and 15 female) yielded no microbiological growth when cultured on nutrient agar, implying that they did not contain any bacteria or fungi. The other samples did yield microbiological growth; this may have been due to normal commensals. The 31 samples with no growth were then used to test the inhibitory nature of the cerumen on bacteria and fungi.

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Bacterial and fungal strains

The bacterial strains tested were *S. aureus* (American Type Culture Collection (ATCC) 25923), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) and *C. albicans*.

Antibacterial and antifungal assays

The three bacterial strains were cultured on nutrient agar and incubated overnight at 37°C. The candida strain was grown on Sabouroud's dextrose agar. A single colony of each culture was then transferred into 10 ml of Brain Heart Infusion (BHI) broth and incubated overnight at 37°C with shaking at 150 rpm. Absorbance values, as indicated by optical density at a wavelength of 600 nm, were taken at a range of 0.3–0.5.

Growth curves for the four microbial strains were determined. One hundred microlitres of each of the 24-hour microbial cultures was transferred into 10 ml of BHI broth and shaken at 150 rpm at 37°C. For each culture, an optical density reading at 600 nm was taken every 30 minutes for 10 hours and at the end of culture at 24 hours. Simultaneously, every 30 minutes, each microbial culture was serially diluted at a dilution of 10^{-1} to 10^{-15}. Ten microlitres of each dilution was then plated out in duplicate onto the appropriate agar and incubated overnight at 37°C, in order to obtain a viable colony count.

All four microbial strains were then grown up to logarithmic phase and 100 μl of the culture was mixed with 150 μl of 3.5 per cent cerumen suspension from each of the 31 no-growth samples; inoculated suspensions were then incubated at room temperature for 20 minutes. A 10-fold dilution ranging from 10^{-1} to 10^{-9} was performed by adding 100 μl of the incubated suspension to 900 μl of BHI broth. Ten microlitres of each dilution was then plated out in triplicate onto the appropriate agar. A colony count was performed after 24 hours' incubation at 37°C. A suspension prepared with cerumen buffer alone was used as a negative control.

Statistical analysis

A Wilcoxon sign rank test was performed to determine whether the cerumen inhibited microbial growth or not.[2,3] If the cerumen had inhibitory properties, then the sum of ranks assigned to positive differences would be expected to be large. If, on the other hand, the cerumen favoured microbial growth, then this sum should be small. A similar argument can be applied to the sum of ranks assigned to negative differences. If the cerumen acted as a bactericide and fungicide, the sum of ranks assigned to negative differences would be small. However, if microbial growth were favoured, then the sum of ranks assigned to negative differences would be large.[4] In our study, the p value was set at 0.05.

Results

Results for the range and mean of the viable colony counts obtained from the cerumen suspensions and the control (i.e. buffer) are presented in Table I.

Table II indicates the effect of the cerumen suspensions on microbial growth, showing the number of agar plates with increased and decreased viable colony counts (compared with the growth curves established earlier), and the average percentage increase or decrease in growth, for each micro-organism.

Results for *S. aureus* indicated that 27 of 31 (87.1 per cent) samples showed a decrease in growth, while four of 31 (12.9 per cent) samples showed an increase in growth. However, the average growth increase (184.1 per cent) was far greater than the average growth decrease (96.7 per cent).

Results for *P. aeruginosa* indicated that all 31 samples (100 per cent) showed a decrease in growth. The average growth decrease was 85.8 per cent.

Results for *E. coli* indicated that only four of 31 (12.9 per cent) samples showed a decrease in growth, while 26 out of 31 (83.8 per cent) samples showed an increase in growth. The largest average increase obtained for *E. coli* was 299 900 per cent. However, one sample registered the same number of counts as the control.

Results for *C. albicans* indicated that 29 out of 31 (93.5 per cent) samples showed a decrease in growth, while one sample (1/31; 3.2 per cent) showed an increase in growth and one sample showed no difference in growth compared with the control. However, the average increase in growth (900 per cent) was far greater than the average decrease (60 per cent).

Statistical analysis of our data (Table III) indicated that cerumen inhibited the growth of *S. aureus* (the sum of ranks assigned to positive differences was large, while the sum of ranks assigned to negative differences was also small; $p < 0.0001$). Statistical analysis confirmed that cerumen had a significant bactericidal effect on *P. aeruginosa* ($p < 0.05$). Statistical analysis enabled us to conclude that cerumen

TABLE I

VIABLE MICROBIAL COLONY COUNTS OBTAINED FROM CERUMEN SUSPENSIONS AND BUFFER

Microbe	Range (cfu/ml)		Mean (cfu/ml)	
	Cerumen	Buffer	Cerumen	Buffer
S. aureus	$4.2 \times 10^6 - 5.7 \times 10^{10}$	$(8.3–9.3) \times 10^9$	3.5×10^9	8.8×10^9
P. aeruginosa	$4.6 \times 10^5 - 4.0 \times 10^8$	$(5.0–5.1) \times 10^8$	7.1×10^7	5.0×10^8
E. coli	$1.1 \times 10^6 - 3.3 \times 10^{12}$	2.0×10^8	5.0×10^{11}	2.0×10^8
C. albicans	$0.0 - 3.0 \times 10^9$	3.0×10^8	1.2×10^8	3.0×10^8

Cfu = colony-forming units

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TABLE II

EFFECT OF CERUMEN SUSPENSIONS ON MICROBIAL GROWTH

Microbe	Decreased growth*		Increased growth*	
	Samples (*n* (%))	Mean change* (%)	Samples (*n* (%))	Mean change* (%)
S. aureus	27/31 (87.1)	96.7	4/31 (12.1)	184.1
P. aeruginosa	31/31 (100)	85.8	0/31 (0)	NA
E. coli†	4/31 (12.9)	79.0	26/31 (83.87)	299 900
C. albicans†	29/31 (93.5)	60	1/31 (3.2)	900

*Measured as colony-forming units per ml. †One subject showed no difference in counts. NA = not applicable

had no bactericidal effect on *E. coli* and in fact favoured its growth (the sum of ranks assigned to positive differences was small, at 30, while the sum of ranks assigned to negative differences was large, at 435; $p < 0.0001$). Statistical analysis also indicated that cerumen inhibited the growth of *C. albicans* (the sum of ranks assigned to positive differences was large, at 435, while the sum of ranks assigned to negative differences was small, at 30; $p < 0.001$).

Discussion

In our study, we used the 31 cerumen samples (out of a total of 75 samples collected) shown to have no growth of any organism (on agar culture; this culture was performed before subjecting these cerumen suspensions to further microbial testing). This was done in order to eliminate any cerumen samples contaminated during collection and transportation, and also to eliminate samples containing micro-organisms at source.

We did not sterilise the cerumen suspensions, in order to avoid alteration of protein and fatty acids (thought to be responsible for bactericidal activity).[5–7] The use of ethylene oxide for sterilisation was not considered because of the large amount of time needed for this technique. Testing of the various micro-organisms was done simultaneously in order to eliminate bias due to environmental factors.

In the *S. aureus* assays, cerumen impaired growth in 27 samples and favoured growth in the remaining four. However, the average increase in growth was much greater than the average decrease in growth. Statistical analysis showed that cerumen inhibited the growth of *S. aureus* (the sum of ranks assigned to positive differences was large, while the sum of ranks assigned to negative differences was small; $p < 0.0001$).

TABLE III

STATISTICAL ANALYSIS OF RESULTS*

Microbe	SR +ve	SR −ve	*p*
S. aureus	431.00	65.00	<0.0001
P. aeruginosa	346.00	150.00	<0.05
E. coli	30.00	435.00	<0.0001
C. albicans	435.00	30.00	<0.0001

*Wilcoxon sign rank test. SR +ve = sum of ranks assigned to positive differences; SR −ve = sum of ranks assigned to negative differences

Burtenshaw reported inconsistent bactericidal activity of cerumen against *S. aureus*.[8] Megarry *et al.* and Stone and Fulghum reported a significant decrease in counts.[9,10] Other authors found no significant bactericidal effect of cerumen against *S. aureus*.[4,11,12]

All 31 no-growth samples caused decreased growth of *P. aeruginosa*, the average decrease being 85.8 per cent. Statistical analysis confirmed that cerumen had a significant bactericidal effect against *P. aeruginosa*.

Chai and Chai reported mortality rates of 52.7 and 29.4 per cent, regarding the bactericidal effect of cerumen on two strains of *P. aeruginosa*; these rates are lower than our results.[13] Other authors reported a lack of bactericidal effect of cerumen on *P. aeruginosa*.[4,11,14]

- **This study aimed to assess the antibacterial and antifungal properties of human cerumen by studying its effect on microbial growth**
- **Cerumen showed potential inhibitory effects on the growth of strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans***
- **Human cerumen has both bactericidal and fungicidal effects, which may play an important role in the prevention or eradication of otitis externa**

In our *E. coli* assays, 26 samples showed increased growth, one sample showed no difference in growth compared with the control, and the remaining four samples showed decreased growth. The average increase was 299 900 per cent, whereas the average decrease was 79.0 per cent. Statistical analysis allowed us to conclude that cerumen had no bactericidal effect on *E. coli* and in fact favoured its growth (the sum of ranks assigned to positive differences was small, at 30, while the sum of ranks assigned to negative differences was large, at 435; $p < 0.0001$).

Our findings on *E. coli* agree with those of Campos *et al.*[4] However, Stone and Fulghum, Chai and Chai, and Bauman *et al.* all reported a bactericidal effect of cerumen on *E. coli*.[10,13,14]

In our *C. albicans* assays, 16 samples showed decreased growth, 13 samples showed no growth at all, one sample showed no difference in growth

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compared with the control and one sample showed increased growth. These assays were repeated and the same outcome was obtained. We conclude that cerumen inhibited candida growth in 29 samples (including the 13 samples showing no viable colony count). Statistical analysis indicated that cerumen inhibited the growth of *C. albicans*.

Megarry *et al.* (the only other group to study the mycocidal effect of cerumen) reported that seven of nine samples showed decreased growth of this micro-organism.[9]

The conflicting results reported in the literature may be explained by differences in individual differences, culture media, micro-organism virulence and methodology. *Staphylococcus aureus, P. aeruginosa* and *C. albicans* are common pathogens which cause otitis externa, and the presence of cerumen in the ear canal may reduce the likelihood of infection by such micro-organisms. Interestingly, Pata *et al.*[15] noted that patients with otitis externa had a reduced amount of cerumen. *Escherichia coli* is not a normal commensal of the ear canal, and thus may not be recognised by the immune system of the ear canal.

Conclusion

Human cerumen has both bactericidal and fungicidal effects. These may play important roles in the prevention or eradication of otitis externa, in addition to the physical barrier created by cerumen.

The findings of this study should be treated with caution as the number of microbial strains investigated was small. A more extensive study should be undertaken in order to verify the bactericidal and fungicidal roles of cerumen. In addition, the differences between the reported results of various studies may be due to the fact that cerumen from different patients varies in its composition and content of inhibitory factors, due to genetic differences.

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