ABSTRACT Background: Cerumen or ear wax is a mixture of sebaceous gland secretions (fat) and apocrine (ceruminous) glands with epithelial debris. This combination of compositions forms an acidic layer (normal pH of 6.8), which helps prevent infection of the ear canal. Cerumen contains various antimicrobial factors, which also prevent infection. This study aims to compare bactericidal activity on cerumen and ofloxacin in vitro in Staphylococcus aureus and Pseudomonas aeruginosa.

Method: The study used a type of in vitro laboratory experimental research to compare bactericidal activity in cerumen and ofloxacin drops against Staphylococcus aureus and Pseudomonas aeruginosa.

Result: Based on the research results for Staphylococcus aureus bacteria, the average inhibition capability of cerumen was 0.7 ± 2.1 mm. At the same time, the average inhibition capability of ofloxacin was 32.9 ± 0.9 mm. For the bacterium Pseudomonas aeruginosa, the average inhibition capability of cerumen is 0 ± 0 mm. At the same time, the average inhibition capability of ofloxacin was 31.3 ± 0.5 mm. Both variables show significant differences because both have a p-value lower than p < 0.05.

Conclusion: There are differences in inhibition capability of cerumen and ofloxacin eardrops against Staphylococcus aureus and Pseudomonas aeruginosa bacteria.

KEYWORDS Bactericidal, Cerumen, In-vitro, Pseudomonas aeruginosa, Staphylococcus aureus
common microorganisms found in the human ear are *Staphylococcus aureus* (24.6%), and *Pseudomonas* (9.0%). [2,3]

Research on an in-vitro comparison of cerumen bactericidal effect against *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria is still very limited, thus this study aims to determine the in vitro comparative effect of cerumen bactericidal and ofloxacin ear drops against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Methods**

This study uses in vitro experimental laboratory research to compare bactericidal activity in cerumen and ofloxacin ear drops against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This study was approved by the Research Ethics Commission of the Faculty of Medicine of Udayana University with No. 1950 / UN 14.2.2 VII.14 / LP / 2019 dated June 27, 2019. The samples were taken from visitors to the ENT-KL clinic at Sanglah Hospital, aged 5 to 42 years, including men and women. Cerumen was collected with sterile earwax hooks from individuals and stored in sterile bottles at 4 °C.

The well-collected samples were weighed and suspended in a buffer solution (5% of NaHCO₃, pH 8.2, containing 30% glycerol) at a concentration of 3.5% (weight/volume). The cerumen-buffer mixture is homogenized by repetition through a series of needles ranging from 19 to 23. This procedure breaks the cerumen into fine particles distributed evenly in the buffer and produces a milk-like suspension.

Tested bacteria are taken with a sterile needle, then implanted on the media by scraping to be tilted. Furthermore, it was incubated in an incubator at 37°C for 24 hours. The same treatment is carried out on each type of tested bacteria.[4] Observations were made after 24 hours of the incubation period. A clear area is a sign of bacterial sensitivity to antibiotics or other antibacterial agents used as test material expressed by the width of the inhibition zone diameter. Inhibition zone diameters are measured in millimetres (mm) using a scales bar using the overall diameter minus the disk diameter of 7 mm. [5,6]

**Results**

**Inhibitory zone against *Staphylococcus aureus***

From 18 cerumen samples, there were two samples, which gave inhibition against *Staphylococcus aureus*, with a diameter of 6 mm and 7 mm, respectively. Sixteen cerumen samples did not provide inhibition against the *Staphylococcus aureus*. The inhibition zone results can be seen in Table 1.

Based on the study results, the average inhibition capability of cerumen was 0.7 ± 2.1 mm. At the same time, the average inhibitory capability of the ofloxacin ear drop was 32.9 ± 0.9 mm. Both variables show significant differences because they have a p-value lower than 0.05. A comparison of inhibition capabilities between cerumen and ofloxacin ear drops can be seen below.

**Inhibitory zone against *Pseudomonas aeruginosa***

From 18 cerumen samples, all cerumen samples did not provide any inhibition capability against *Pseudomonas aeruginosa*. The inhibition zone results can be seen in Table 3.

Based on the study results, the average inhibition capability of cerumen was 0 ± 0 mm. At the same time, the average inhibition capability of ofloxacin was 31.3 ± 0.5 mm. Both variables show significant differences because they have a p-value lower than 0.05. A comparison of the inhibition capabilities of cerumen and ofloxacin eardrops can be seen in Table 4.

**Discussion**

Cerumen forms an acidic coating that helps prevent infection in the ear canal. Cerumen functions as an antimicrobial by physically protecting the external auditory canal, building low pH and producing antimicrobial compounds such as lysozyme. Cerumen plays a clinical role in host defence, which appears to be relatively weak. If the cerumen plays a role in strengthening the host defence system, its composition will change in response to infection. Exposure to bacteria will trigger the regulation of the antibacterial component of cerumen. However, the patient’s cerumen with otitis externa do not appear to contain more antibacterial unsaturated fatty acids than those that are not in a state of infection. According to Davis and Stout, the inhibition of cerumen is relatively weak, and the inhibition of ofloxacin is classified as very strong against *Staphylococcus aureus*. These results follow Chai’s study, which states that newly collected (dry form) samples were suspended at a concentration of 3% in the glycerol-sodium bicarbonate buffer showing bactericidal activity against several bacterial strains tested. This suspension reduces the viability of Haemophilus influenzae, *Escherichia coli* K-12, and Serratia marcescens by more than 99%. In contrast, the viability of two isolates of *Pseudomonas aeruginosa*, *E. coli* K-1, *Streptococcus*, and two *Staphylococcus aureus* isolates originating from humans was reduced by 30 to 80%. The results support the hypothesis that cerumen kills certain foreign organisms, which enter the ear canal.[6,7]
Table 2 Comparisons of Inhibition Capabilities Between Cerumen and Ofloxacin Ear Drops.

| Variable (mm) Mean ± SD | Cerumen (n=18) | Ofloxacin Ear Drop (n=18) | p Value |
|-------------------------|----------------|---------------------------|---------|
| 0.7 ± 2.1               |                | 32.9 ± 0.9                | 0.000   |

Table 3 Inhibitory Zone Against *Pseudomonas aeruginosa* (in mm).

| Sample’s code | Cerumen | Ofloxacin Ear Drop |
|---------------|---------|-------------------|
| 1             | 0       | 31                |
| 2             | 0       | 31                |
| 3             | 0       | 31                |
| 4             | 0       | 32                |
| 5             | 0       | 32                |
| 6             | 0       | 31                |
| 7             | 0       | 31                |
| 8             | 0       | 31                |
| 9             | 0       | 32                |
| 10            | 0       | 32                |
| 11            | 0       | 31                |
| 12            | 0       | 31                |
| 13            | 0       | 31                |
| 14            | 0       | 32                |
| 15            | 0       | 32                |
| 16            | 0       | 31                |
| 17            | 0       | 31                |
| 18            | 0       | 31                |

*Staphylococcus aureus* is a Gram-positive coccus shaped germ and lived as individual organisms, in pairs and groups. *Staphylococcus* is a non-motile, nonporous, catalase-positive bacterium and is part of normal human flora found in the axillary, inguinal, perineal and interior nares. These microorganisms produce toxins that can cause specific diseases or syndromes and can cause the pathogenesis of *Staphylococcus* infections.[10]

There were reports of bactericidal activity of cerumen. The report is based on the consideration that nutrient-rich ear wax allows bacteria and fungi to grow. On the other hand, several reports describe the antimicrobial effects of ear wax on various bacteria, including *Haemophilus influenzae*, *Staphylococcus aureus*, *Escherichia coli* and fungi. Stoeckelhuber et al. reported detecting antimicrobial proteins β-defensin-1, β-defensin-2, cathelicidin, lysozyme, lactoferrin, MUC1 and the secretory component of IgA in glandular cells obtained in the histochemical analysis. Not only the glands but also the skin of the ear canal can produce antimicrobial peptides such as human β-defensin 1 (hBD1) and human β-defensin 2 (hBD2). Yong et al. has isolated hBD1 and hBD2 in human cerumen in 2008.[3]

Several studies have proven that each antimicrobial peptide has an antimicrobial effect. Singh et al. showed that the combination of lactoferrin and SLPI was synergistic, and the combination involving human β-defensins, LL-37 and tobramycin had an additive effect. Chen et al. analysed the individual and synergistic activity of hBD1-3, LL-37 and lysozyme in different environments. They found that this AMP showed antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* in a dependent way on normal pH temperature and acidic pH temperature. The antibacterial activity of hBD1-3 was significantly enhanced in an acidic environment (pH 4.6). The synergistic effect of hBD, LL-37 and lysozyme on *Staphylococcus aureus* is significantly enhanced in an acidic environment. Protein has been shown to have an unsynergetic additive effect on the antibacterial activity in an acidic environment against *Escherichia coli*. [11]

*Pseudomonas aeruginosa* produces several exotoxins and extracellular products that support local invasion and microorganisms spreading. These extracellular toxins and products include extracellular proteases, cytotoxins, haemolysins, and pyocyanin. For systemic diseases, products that support invasion include antiphagocytic capsules, endotoxins, exotoxins A, and exotoxins S.[8]

Antibiotic treatment that can effectively fight *Pseudomonas aeruginosa* is quite difficult to find, and this is due to the antibiotic-resistant mechanism *Pseudomonas aeruginosa*. How to deal with this bacterial infection is with aggressive antimicrobial therapy, such as combining two bactericidal antibiotics such as aminoglycoside, β-lactam antibiotics, anti-pseudomonal, or quinolones.[10]

*Pseudomonas* are gram-negative, aerobic, positive catalase, positive oxidase, unable to ferment, can oxidize carbohydrates, are not sporous, have no sheath and have monotonic flagella that are always moving.[9] Factors that cause these germs to fight the body’s defence system and causing manifestations are phylly. In contrast, the factor that determines the pathogenic power of these germs is exotoxin A, an ADP-ribose transferase that functions to inhibit protein synthesis of eukaryotes. Symptoms of sepsis and septic shock are the results of the presence of *Pseudomonas aeruginosa* endotoxins. The occurrence of chromosome mutations in bacterial genes that encode drug target enzyme subunits, DNA gyrase, and topoisomerase IV, disrupting the target enzymes to work in affinity with ofloxacin. Another thing that can cause resistance is the inhibition of the drugs diffusion process into the cytoplasm of bacteria. The activity of extracellular enzymes can create a nest that is protected against the normal defence of the mucosa as well as against antibiotics. The growth of *Pseudomonas aeruginosa* in its nest is wrapped in exopolysaccharide (biofilm), thus protecting it against the penetration of antibiotics, antibodies, complement and phagocytic cells. These properties give rise to resistance to antibiotics and difficult to eradicate the disease.
Table 4 Comparison of inhibition capability between cerumen and ofloxacin eardrop

| Variable                      | Material Tested                  | p-value |
|-------------------------------|----------------------------------|---------|
| Inhibition Capability (mm)    | Cerumen (n=18)                   | 0 ± 0   |
|                               | Ofloxacin Eardrop (n=18)         | 31.3 ± 0.5 | 0.000 |

Conclusion

This study concludes that there was an inhibition capability of cerumen against the growth of Staphylococcus aureus bacteria. There was no inhibition capability of cerumen against the growth of *Pseudomonas aeruginosa* bacteria. There were differences in the inhibition capabilities of cerumen and ofloxacin ear drops against Staphylococcus aureus and *Pseudomonas aeruginosa* bacteria.

Funding

This study was funded by ORL-HNS Department Udayana University / Sanglah Hospital Denpasar.

Conflict of interest

There are no conflicts of interest to declare by any of the authors of this study.

References

1. Arfandy RB. Patogenesis dan Etiologi Rinosinusitis. Dalam: Kursus, Diseksi dan Demo Bedah sinus Endoskopik Fungional II. 2003. Makassar. 1-4.
2. Rao JJ, et al. I. Classification Septum Nasal Deviations- Relation The Sinonasal Pathology. Indian Journal of Otolaryngology and Head and Neck Surgery. 2005. July-September. 3.
3. Zinreich SJ, Gotwald T. (2001). Radiographic Anatomy of the Sinuses. In: Kennedy DW, Bolger WE, Zinreich SJ, editor. Diseases. Hamilton BC Decker Inc. 13-26.
4. Erhan E, Vural F, Ersem G. Radiologic imaging in chronic sinusitis. In: Erhan E, Vural F, Ersem G. Different Aspects of Rhinosinusitis. Turkey: SMGE Books; 2016. p1-13.
5. Jenny KH, James DE, Christopher LT, Christine MG. Multiplanar Sinus CT: A Systematic Approach to Imaging Before Functional Endoscopic Sinus Surgery. American Journal of Roentgenology. 2010; 194:527-536.
6. Bestari JB, Surya A. Rinosinusitis Kronis Dengan Variasi Anatomi Kavum Nasi. Bagian THT-KL FK Unand Padang. 2010. h.1-7.
7. Dua K, Chopra H, Khurana A, Munjal M. CT Scan Variations in Chronic Sinusitis. Ind J Radiol Imag. 2005;15(3):315-320.
8. Aramani A, Karadi RN, Kumar S. A Study of Anatomical Variations of Osteomeatal Complex in Chronic Rhinosinusitis Patients-CT Findings. Journal of Clinical and Diagnostic Research. 2014; 8(10):1-4.
9. Delfitri M. Variasi Anatomi pada Rinosinusitis Kronis di RS H. Adam Malik Medan. Majalah Kedokteran Nusantara Volume 39 No. 3, 2006. h.225-229.
10. Shephali S Pawar, Saksham Bansal. CT anatomy of paranasal sinuses – corelation with clinical sinusitis. International Journal of Contemporary Medical Research. 2018;5(4): D1-D3.
11. Dua K, Chopra H, Khurana A, Munjal M. CT Scan Variations in Chronic Sinusitis. Ind J Radiol Imag. 2005;15(3):315-320.