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The Antibacterial Effect of Earwax on Pathogenic Bacteria Isolated from Individual with Urinary Tract Infections

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Abstract

Background: The spread of bacterial resistance to antibiotics is a major clinical issue, so finding a new antibiotic with fewer side effects is a crucial step in microbiology. Earwax, known as cerumen auris, is a natural, protective secretion from outer cartilage for external auditory canal (. Dust, tiny particles, and other insects are kept from getting to the eardrum and harming it by earwax, which coats the external auditory canal. Additionally, it guards against microbial infections. **Aims:** The experimental study aims to investigate the role of earwax (cerumen auris) as an antimicrobial effect on some pathogenic bacteria. It was designed to detected the antibacterial properties of earwax and evaluate its protective action against pathogenic bacterial that isolated from UTI patients. **Methods:** A176 samples divided between urine and earwax. Earwax samples were taken from 88 healthy women after ensuring they were free of diseases of the outer and middle auditory canal from Hillah General Teaching Hospital, and Al-imam al-Sadiq hospital at the same time, 88 urines collected from UTI patients. Earwax They were dissolved in a solution containing 3% glycerol and 5% sodium bicarbonate. They were then cultured on nutrient agar to ensure their purity and freedom from microbes and to study their antibacterial effect. Earwax suspension was evaluated for its antimicrobial activity against bacterial isolated from urine culture: E. coli, P. mirabilis, P. aeruginosa, and S. aureus by agar diffusion. **Results:** Out of 88 bacterial samples, only 72 (81.8%) samples showed no bacterial growth, indicating the antibacterial effect of earwax. The results showed that the antimicrobial effect of the earwax suspension was exceptionally strong against inhibited the growth of all bacterial isolates by 100%. S. pyogenes (28.4%), P. aeruginosa (31.8%), E. coli (21.6%) and P. mirabilis (18.2%) were among the bacterial isolates from patients' urine (male and female) in varied percentage. **Conclusion:** It can be concluded that earwax, in addition to being a physical and chemical barrier, acts as a defensive cover over the external vocal tract due to its antibacterial action. The present study aligns with 3rd-Sustainable Development Goal (3rd-SDG): Good Health and Well-being, 9th-SDG: Industry, Innovation, and Infrastructure, and 12th: Responsible Consumption and Production, by enhancing the alternative antimicrobial solutions, minimizing the reliance on synthetic antibiotics, and reinforcing the scientific innovation of microbiology.

Keyword: Earwax, Antibacterial, UTI, Pathogenic bacteria.

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Introduction

Sebaceous, ceruminous, and apocrine glands located in the outer-third of the individual external auditory-canal wall secretes cerumen, known as earwax, which is an acidic coating that contributes to preventing microbial invasive of the external auditory canal [1]. Since earwax has an antibacterial function by physically shielding the ear canal, acidic reaction, and making the environment unfriendly for pathogens and antimicrobial agents like lysozyme, its absence might result in ear canals susceptible to infection [2]. Earwax creates a physiological wall between the deep external auditory canal and the outside world. The antibacterial properties of earwax, due to its chemical composition, have been the focus of attention of many [3]. Lipids and peptides are released separately by the sebaceous and ceruminous organs in the trench associated with sound. Additionally, glandular discharges produced by hairs in the outer third of the trench contribute to the organization of [4]. About 20% of dry wax is made up of lipid, compared to about 50% of wet cerumen. The two structures hardly exhibit any additional metabolic distinctions aside from this. Both structures are governed by two autosomal alleles and are associated with race [5]. There are disagreements on this topic even though it is acknowledged that human cerumen protects the external ear canal from illnesses. Several studies have suggested that cerumen cannot prevent diseases and that its rich supplements support the abundant growth of tiny organisms [6].

The yellow-brownish waxy substance known as cerumen (earwax), is consist by both sebaceous and ceruminous-glands and serves to shield the ear canal's surface from infection and moisture. By capturing germs, perspiration, debris, dead skin cells, oil, and hair, it keeps infections at bay. The cilia, which are hairs that line the outer ear canal, thrust the problematic particle in the direction of the ear opening so that the wax can be removed. Human cerumen comes in two varieties: dry and wet. These are determined by two autosomal alleles and are frequently linked to race [7]. There are multi-antithetical reports for the antibacterial manner of cerumen. Cerumen's antibacterial properties stem from its high nutritional content, which allows bacteria and fungi to proliferate before being destroyed by its acidic environment [8]. In order for cerumen to have a somewhat acidic composition, it inhibits the growth of germs and fungi in the external auditory canal's wet, dark environment. Without cerumen, it is nearly impossible to prevent infection in the ear canal. According to immunohistochemical research, the ear canal is protected against infectious microbes by antibody-mediated immune responses rather than earwax. Secretory IgA and IgG can trigger local immunity through piliary follicles, ceruminous and sebaceous glands, and the skin epidermis/dermis lining the external auditory-canal [3]. To ensure the type of host protection in this anatomical site, more research is necessary.

Materials and method:

➢ Period of Samples Collection

The practical aspect of the experimental study was extended for the period between October 2024 and February 2025, which included 176 samples divided between urine and earwax. Earwax samples were taken from 88 healthy woman with age (18-50) year from General Hilah Teaching, Al-Emam al-Sadiq Hospital at the same time collected 88urine from UTI patients.

Bacteriological study:

Patients' urine specimen were taken by a sterile, disposable cotton swab and then inoculated on Nutrient media agar and incubated under aerobic conditions for period 24 hr. for bacterial activation [9]. Then cultured on selective and differentiation media (MacConkey agar, Mannitol salt agar and Blood agar) under aerobic conditions for isolation and identification of bacterial isolates.

The bacterial isolates were carefully examined macroscopically by comparing their morphological characteristics as described by McFadden et al., in addition to the biochemical tests to identify bacterial isolates were carried out using the appropriate biochemical tests [10]. Gram stain reaction was done on bacterial smear to examine under an oil immersion lens to identify genus type [11].

- **Collection And Preparation of Cerumen Samples**

The technique of Ambika and Lakshmi (30) was used to collect and prepare 88 cerumen samples by using sterile earwax buds and keeping them in sterile sealed tubes at 4°C in the refrigerator. The earwax items were dissolved in a solution containing 3% glycerol and 5% sodium bicarbonate to form a suspension. Microbiological tests were performed to ensure that it was free of bacteria. In order to discharge the cerumen into the tube, several buds were employed for each individual. The cerumen that appeared sterile from bacterial culture was then pooled and weighed to obtain 0.1g. This was suspended in 2.5 milliliters of sterile buffer (pH 8.2, 30% glycerol, 5% $NaHCO_3$). The mixture of cerumen and buffer was homogenized. This process produced a milky solution with 4% cerumen by breaking the cerumen up into tiny particles that were uniformly dispersed throughout the buffer.

- **Earwax inhibitory activity test:**

After ensuring earwax samples were free of microbs of the external and central auditory canal. They were dissolved in a solution containing 3% glycerol and 5% sodium bicarbonate. They were then cultured on nutrient agar to ensure their purity and freedom from microbes and antibacterial activity testing. All bacterial isolates that were activated in liquid nutritional broth. 0.1 ml of the produced inoculum was added to 0.9 ml of earwax suspension to create tubes with a 1:10 dilution of the earwax suspension and incubated for 24 hours at 37°C. In order to examine growth, 0.01 ml of the suspension was then moved from each tube to nutrient agar and cultured at the same condition.

Ethical Approval

Prior to the individuals of the study population being included in the study, valid consent was obtained from the hospital administration as well as from each patient and healthy individual. Before the samples were taken, each patient or their supporters were told of the procedure, ensuring that they fully knew what was going to be done. The subjects were aware that they could decline participation in the study at any time without suffering any negative consequences.

Results & Discussion

Incidence of Bacterial Pathogens

Eighty-eight bacterial samples were collected from males and females with UTIs to test the antibacterial efficacy of earwax, which included: *Escherichia coli (E. coli), Proteus mirabilis, Pseudomonas aeruginosa (P. aeruginosa), Staphylococcus aureus (S. aureus).* The estimation the prevalence of bacterial pathogens reveals distinct distribution patterns among the identified species as presented in Figure (1). *P. aeruginosa* showed a highest prevalence, accounting for 31.8% of total cases, *S. aureus* followed closely, with prevalence of 28.4%. The prevalence of *E. coli* and *P. mirabilis* found at 21.6% and 18.2%, respectively. These outcomes highlight the diverse dominance of bacterial isolates and providing crucial insights into pathogen distribution.

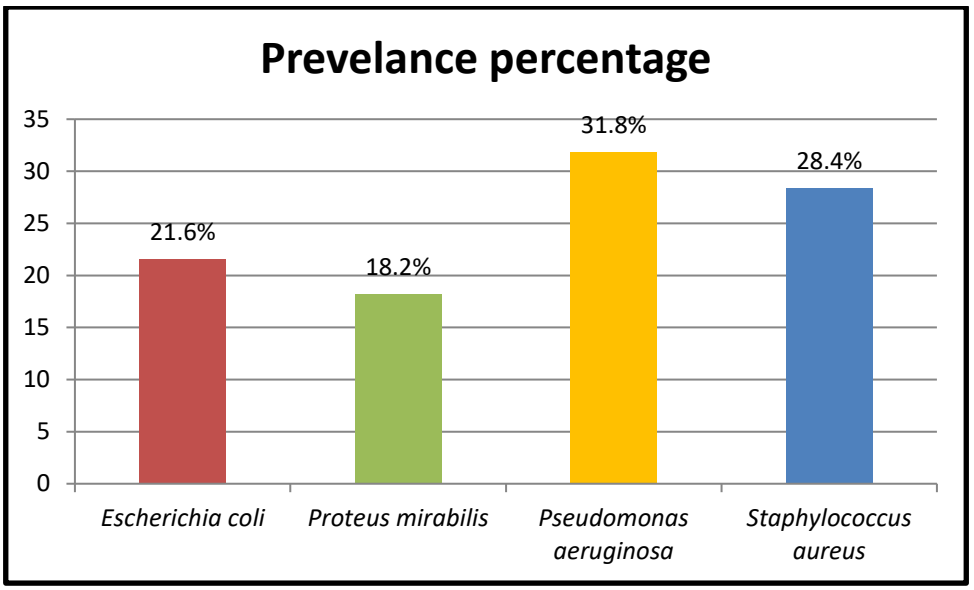


Figure (1): Incidence of Bacterial Pathogens in UTIs patients

The current study identified P. aeruginosa as the most prevalent pathogen, followed by S. aureus, E. coli, and P. mirabilis. Several previous researches support our findings, and indicated that P. aeruginosa has considerable roles in complicated and hospital-acquired urinary infections, especially due to it possesses several resistance mechanisms and biofilm-formation capabilities [12] . World Health Organization (WHO) has been stated S. aureus as an emerging cause in UTI, despite it has been usually associated with skin infection [13] . However, another previous investigation contradicts our finding, which reported E. coli as a dominant uropathogen in about 80% of cases [14]. The varying prevalence rate may be attributed to differences in patient demographics, antibiotics exposure, and regional variations. Mancuso et al. [15] emphasized that emerging pathogens as well as geographical differences could significantly shift the distribution of pathogens in UTIs. Considering microbial's landscape in UTI patients is critical to optimize therapeutic strategies, so further research is required to evaluate the effect of antibiotic resistance and environmental factors on bacterial prevalence.

Sex-based variations in The Incidence of Bacterial Pathogens in UTIs Patients

Table (1) displays the distribution of bacterial pathogens among UTIs cases, which showed distinct sex-based variation. Overall, total UTI cases number was 88, with a higher percent in female (63.6%) compared to male (36.4%). *E. coli* was detected with higher prevalence in male (52.6%) compared to female (37.4%). *P. mirabilis* showing a stronger presence in female (68.75%) compared to men (31.25%). *P. aeruginosa* distributed almost evenly between female (57.1%) and male (42.8%). *S. aureus* has a significantly higher occurrence in female (80%) compared to male (20%).

Table (1) Bacteria isolated from males and females with urinary tract infections

Bacteria	Sex				Total
	Male		Female		
	No.	%	No.	%	
Escherichia coli	10	52.6%	9	37.4%	19 (21.6%)
Proteus mirabilis	5	31.25%	11	68.75%	16 (18.2%)
Pseudomonas aeruginosa	12	42.8%	16	57.1%	28 (31.8%)
Staphylococcus aureus	5	20%	20	80%	25 (28.4%)
Ttotal	32	36.4%	56	63.6%	88

Earwax Impact on Bacterial Isolates

Table (2) and Figure (2) show the results of earwax sample culture on culture medium; where 81.8% of cultured earwax was sterile (free from bacteria), while only 18.2% was unsterile. The impact of earwax on studied bacterial isolates has been estimated, where the present study revealed that using the earwax leads to inhibit all studied bacterial isolates at percent of 100% as shown in Figure (3).

Table (2): Results of earwax sample culture on culture medium

Ear wax	No.	%
Sterile	72	81.8%
Unsterile	16	18.2%
total	88	%100

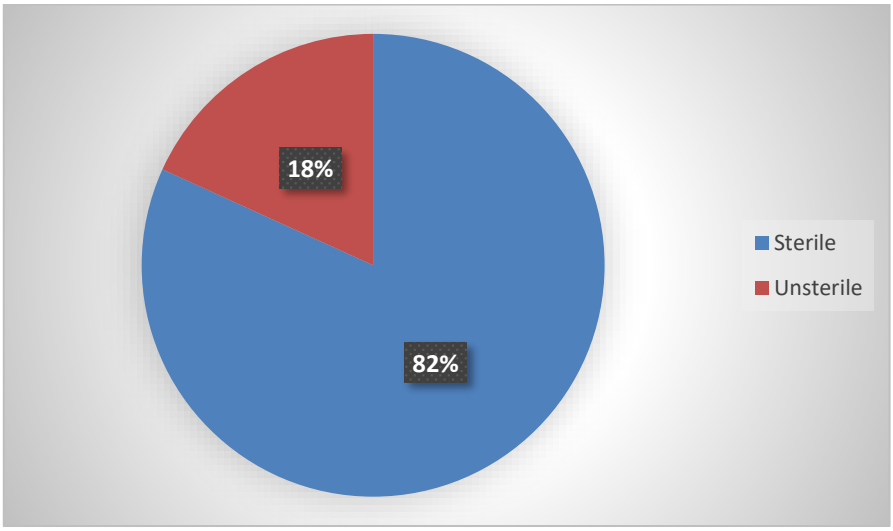


Figure (2): Results of earwax sample culture on culture medium

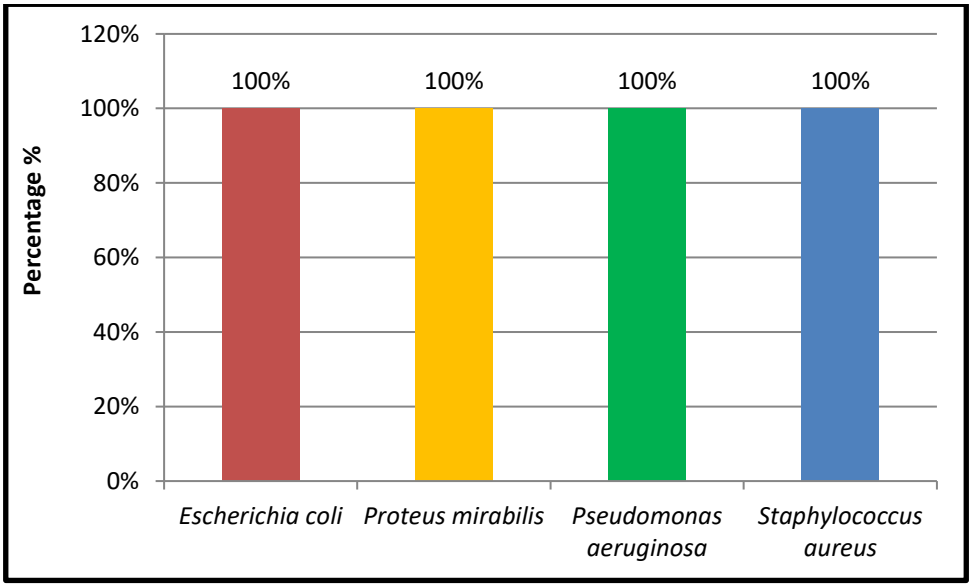


Figure (3): Earwax effect on bacterial isolates as antibacterial

These results indicate the effective role of earwax in inhibiting the growth of pathogenic bacteria.

The current study reveals that human earwax showed 100% inhibition rate against *E. coli*, *P. mirabilis*, *P.*

aeruginosa, and *S. aureus*, indicating that it has powerful antimicrobial properties. Earwax or cerumen plays important biological and clinical roles for host vital defense although it is probably not strong, it could create an acid nature barrier inside external environment of auditory canal, which prevents infections [11]. This strong impact of earwax seems to be due to it possesses fatty acids, lysozyme, and antimicrobial peptides that could inhibit bacterial growth [16].

The present finding is consistent with previous study conducted by Sirigu et al., who supported the idea that earwax has a considerable role in local immune defense mechanism, including its potential effect against microbial threat, the study found that IgA, IgM, and IgG were actively engaged with protecting the external auditory canal [17]. Similarly, our finding agrees with another previous studies, which confirmed the protective properties of earwax and demonstrated its ability to reduce bacterial growth of *P. aeruginosa* [18].

The current finding also aligns with the study of Naz [19], who demonstrated that cerumen had significant antibacterial activities, particularly against *P. aeruginosa* and *S. aureus*. However, some investigations challenge these findings and suggested that cerumen's efficacy varied among bacterial strains.

Chai and Chai showed that earwax lessened the viability of *E coli K-12* strain, and S. marcescens by 99%, while the viability of two P. aeruginosa strains, *E. coli K-1*, *Streptococcus*, and two S. aureus strains of human origin was inhibited by 30-80% [20]. As well, another study revealed that while earwax has an antimicrobial activity, but its effect differed, with *E. coli* and *P. mirabilis* showed lower susceptibility compared to *P. aeruginosa* and *S. aureus* [19]. Such differences may be assigned to variations in cerumen composition among populations or variations in bacterial resistance mechanisms.

Conclusions

The human earwax showed more antibacterial activity against P. aeruginosa, *S. aureus*, *P. mirabilis* and *E. coli*. The study came to the conclusion that cerumen's antibacterial qualities serve as a protective layer over the external auditory canal. As a result, it is suggested as a natural antibacterial for certain animal infections. Thus, regular ear cleaning and wax removal are not required until impacted wax is causing conductive hearing loss or ear pain.

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