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RESEARCH ARTICLE

COLONIZATION OF PATHOGENIC AEROBIC BACTERIA IN THE EXTERNAL EAR AND EFFECTS OF CERUMEN TYPES, AGE, AND GENDER ON COLONIZATION

Khaled Saad Abdulrahman Al-Khamesy[1], Khaled Abdul-Karim A Al-Moyed[2], Hassan Abdulwahab Al-Shamahy[2,3]

[1]*ENT Department, Faculty of Medicine and Health Sciences, Sana'a University, Republic of Yemen.*
[2]*Medical Microbiology and Clinical Immunology Department, Faculty of Medicine and Health Sciences, Sana'a University.*
[3]*Medical Microbiology department, Faculty of Medicine, Genius University for Sciences and Technology, Dhamar city, Republic of Yemen.*

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***Address for Correspondence:**
Dr. Hassan A. Al-Shamahy, Medical Microbiology and Clinical Immunology Department, Faculty of Medicine and Health Sciences, Sana'a University. Medical Microbiology department, Faculty of Medicine, Genius University for Sciences and Technology, Dhamar city, Republic of Yemen; Tel: +967-1-239551.
E-mail: *shmahe@yemen.net.ye*

Abstract

Aims: The study aimed to study the prevalence of pathogenic aerobic bacteria colonization in the outer ear and to determine the prevalence of cerumen types among different ages and sex of selected individuals in Sana'a city. Also determined the effects of cerumen types, age, and gender on the incidence of pathogenic aerobic bacterial colonization.
Methods: The present study was take place in the Department of Medical Microbiology and ENT Department, Faculty of Medicine and Health Sciences, Sana'a University. The study request was acceptable by the ethics committee of the Faculty. Written informed consent was taken from the chosen participants. Families of the study sample members residing in the various sectors of Sana'a city were visited at home in which participants were randomly selected from all age groups and sexes. Households were reviewed on time and clinical examination of the ear was done and results were recorded and ear swab samples were collected. The rapid visual identification of cerumen was performed. The culture samples were collected and transferred to the laboratory then culture and bacterial growth was identified by standard bacteriological methods.
Results: The study included 246 healthy persons, 33.3% of them male and 66.7% were females. The age ranged from 1 -80 years with mean age equal to 34.2±18.7 years**,** 96.3% of the study subjects have the wet type and only 3.7% have the dry type of cerumen, 2.03% had impacted cerumen. The most prevalent isolate from the outer ear was *Staphylococcus aureus* with 35 strains (14.2%), followed by *Psedumonas aureginosa* with 14 strains (5.7%). *Klebsiella* spp, *Streptococcus pyogenes*, and *Hemophilus influenzae* isolates were less frequent with 7 (2.8%), 6 (2.4%) and 5 (2.03%) isolates, respectively. Positive growth for bacterial pathogens was significantly associated with males (*OR*=1.8, *p*=0.03), ≤15 years group (*OR*=2.5, *p*=0.01), and dry-type cerumen (*OR*=8.9, *p*=0.001).
Conclusion: There are two distinct and specific types of cerumen, wet and dry, of which the wet type appears to be the most common in Yemen, and of the subspecies of the wet type, honey brown was dominant. The present study concluded that some pathogenic bacteria that can cause otitis externa are naturally present in the normal external auditory canal. The most prevalent isolate was *Staphylococcus aureus*, followed by *Psedumonas aureginosa*, *Klebsiella* spp, *Streptococcus pyogenes*, and *Hemophilus influenzae*. Hence clinicians must find a history of any predisposing factors before interpreting laboratory culture reports.
Keywords: aerobic bacteria, cerumen types, colonization, external ear.

INTRODUCTION

Cases of acute or chronic otitis externa are increasing globally, but despite this, there is no interest in conducting research on the causes and risk factors and their relationship to the types of cerumen, and other host factors as sex, and age. The most common cause of otitis externa is bacteria and this is mostly due to indigenous sources of the causative bacteria or from an external source. Risk factors for acute cases include

swimming, minor trauma from brushing, use of hearing aids and earplugs, and other skin problems, such as psoriasis and dermatitis. There is also a link between ear secretions and their accumulation inside the ear and the occurrence of inflammation[1,2]. Otitis externa affects 1-3% of people annually[1] and about 10% of people are affected at some point in their lives[2] while it occurs most commonly among children between the ages of seven and twelve and among the elderly[2,5]. Also, it occurs with approximately equal frequency in males and females, and people who live in warm, humid climates are most often affected[3].

Cerumen is produced in the cartilaginous part which is the outer third part of the ear canal. It is a combination of viscous secretions from the sebaceous glands and less viscous secretions from the modified apocrine sweat glands[4]. The primary constituents of earwax are layers of skin, with an average of 60% of earwax composed of keratin, 12–20% saturated and unsaturated long-chain fatty acids, alcohols, and squalene, and 6–9% cholesterol[5]. Despite the fact that studies up to the 1960 found insignificant facts to support the antibacterial activity of cerumen[6], some recent studies have found that cerumen has a bactericidal effect on some strains of bacteria in which cerumen inhibited the growth of a wide variety of bacteria in bacterial cultures, including Haemophilus influenzae, *S. aureus*, and *E. coli*, sometimes by up to 99% of bacterial growth[7,8]. In addition, the growth of two types of fungi commonly found in otomycosis were also significantly inhibited by human cerumen in vitro testing[9]. These antimicrobial properties are mainly due to the presence of saturated fatty acids, lysozyme, and in particular to the slight acidity in the waxy cerumen; usually a pH of about 6.1 in normal individuals[10]. On the contrary, research by Campos *et al.*, has found that waxy cerumen can support microbial growth and some cerumen samples have been found to have a bacterial count of up to 10^7/g of cerumen and mostly the bacteria were commensal normal flora[11]. The study aimed to study the prevalence of colonization of pathogenic aerobic bacteria in the outer ear among different ages and sexes of selected individuals in the city of Sana'a and to determine the effects of cerumen wax types, age and sex on the occurrence of colonization of pathogenic aerobic bacteria.

MATERIALS AND METHODS

Site of the study: The current study was carried out in the Department of Medical Microbiology and Department of ENT; Faculty of Medicine and Health Sciences, Sana'a University. The study proposal was permitted by the ethics committee of the Faculty. A written informed consent was taken from the chosen participants.

Study participants: The third author visited the families of the study sample members residing in the various sectors of Sana'a city. Individuals between 1 to 80 years of age were selected and the purpose of the study explained. The sample size requisite for the study was considered on the basis of the colonization prevalence of potentially pathogenic bacteria in the external ear of adult individuals obtained on the basis of a pilot study of 50 subjects and a statistical consultation.

Inclusion and exclusion criteria: The inclusion criteria were that the participants had no systemic debilitating disease, and had not taken or had taken antibiotics in the past three months. Individuals who underwent external ear whishing or treatment with ear drops were not included in the study.

The interested participants were randomly selected to form a study group of 246 subjects from all age groups and sexes. The selected individuals were instructed not to clean the external ear by cotton swabs or other tools or swimming, or smoke one day before their appointment. Households were reviewed by author (HAA) on time and clinical examination of ear was done and results were recorded and ear swab sample was collected. Prior to the commencement of the studies, the Registrar (HAA) was trained through frequent calibration sessions conducted in the faculty department for external ear investigation and determine the types of ear wax and their colored.

Cerumm color recording: All study individuals were examined by the same examiner. The rapid visual identification of color was performed. Ear wax chromaticity was calculated by rapid visual identification of ear wax color using a color atlas. Where the cerumen was collected with a white cotton swab, then the collected ear wax was spread on a transparent white paper, and then the colors were matched with the color chips printed in the atlas.

Microbiological procedure: The sample was collected and transferred to the laboratory immediately in thioglycolate culture medium and processed the same day. The sample was then rotated (15 s) and diluted 1:10 in isotonic saline before inoculation. One loop (1/10 ml of sample) was inoculated onto blood agar, one loop to McConkey agar and one loop to chocolate agar. Blood agar, MacConkey agar was incubated aerobically at 37°C for 24 hours. While the inoculated chocolate agar was incubated in a carbon dioxide-enriched atmosphere at 37°C for 24 hours. Then the growth was identified by standard bacteriological methods[12].

Statistical analysis: Epi Info software version 7 was used for data analysis. Difference in proportions, associated odds ratio, and significance test were calculated using 2X2 tables, an uncorrected chi-square statistical test, and two-tailed *p*-values for significance. The level of statistical significance was assumed at $p< 0.05$.

Ethical approval: The ethical approval of this study was No. 1728 dated April 2022 and it was taken from the Medical Ethics and Research Committee at Sana'a University, Faculty of Medicine and Health Sciences, and all procedures were in accordance with the ethical controls of the review committee. We required all participants to provide written informed consent prior to clinical examination, sample collection and information collection.

RESULTS

The study included 246 healthy persons, 33.3% of them male and 66.7% were females. The age ranged from 1-80 years with mean age equal to 34.2±18.7 years (Table 1). There are two distinct, determined types of earwax 96.3% of the study subjects have the wet type (dominant), and only 3.7% of the tested subjects have the dry type of cerumen (recessive).

Table 1: Age and Gender Distribution of individuals participated in the study.

Characters	Number (%)
Sex	
Male	82 (33.3)
Female	164 (66.7)
Age groups	
≤15 years	31 (12.6)
16-25 years	61 (24.8)
26-35 years	67 (27.2)
36-45 years	26 (10.6)
≥46 years	61 (24.8)
Total	246 (100)
Mean	34.2 years
SD	18.7 years
Min	1 years
Max	80 years
Median	30.5 years
Mode	23 years

Table 2: Types of external earwax (Cerumen) of healthy Yemenis.

Types	Number (%)
Wet type (Moist)	237 (96.3)
Honey-brown	134 (54.5)
Dark-orange	61 (24.8)
Dark-brown	42 (17.1)
Dry type	9 (3.7)
Gray	6 (2.4)
Flaky	3 (1.2)
Total	246 (100)

Considering subtypes of wet type, honey-brown was the predominant one counting 54.5% of the total, followed by dark-orange (24.8%), and dark-brown counting 17.1% of the total. The dry type of cerumen were rare counting only 3.7% of the total, 2.4% of the subject have gray dry type and 1.2% have flaky dry type (Table 2). When examining the walls of the external ear canal, 50.4% of subjects showed accumulation of cerumen on the canal walls and 46.7% showed accumulation of cerumen at the entrance of the canal while 2.03% had excess earwax or impacted cerumen (Table 3).

Considering the potential pathogenic aerobic bacteria isolated from the outer ear of 246 healthy Yemeni individuals, the most prevalent isolate from the outer ear was *S. aureus* with 35 strains (14.2%), followed by *P. aureginosa* with 14 strains (5.7%). *Klebsiella* spp, *Streptococcus pyogenes* and *Hemophilus influenzae* isolates were less frequent with 7 (2.8%), 6 (2.4%) and 5 (2.03%) isolates, respectively. Dual pathogens were also isolated in the outer ear in which 3 cases occurred from *S. aureus* + *Klebsiella* spp and 4 cases from *Hemophilus influenzea+klebsiella* spp. Considering the association of pathogen bacterial growth in the outer ear with age and sex of 246 healthy individuals, positive growth for bacterial pathogens was significantly associated with males as the positive growth rate for males was 39% (vs 25.6% for female), with an associated *odds ratio* of 1.8, the *CI* was −1.01. 3.2, X^2=4.3, p=0.03.

Table 3: Otoscopic examination of the outer ear of healthy individuals.

Characters	Number (%)
Cerumen accumulation at the canal walls	124 (50.4)
Cerumen accumulation at the entrance of the canal	115 (46.7)
Excessive earwax or impacted cerumen	5 (2.03)
Total	246 (100)

Considering the age groups, there was a significant association with positive growth for bacterial pathogens with ≤15 years group of positive growth rate being 48.4%, with an associated *odds ratio* of 2.5, *CI* was 1.2–5.3, X^2=5.7, p=0.01 (Table 5). Considering the association of potential pathogenic growth of bacteria in the outer ear with types of external earwax (cerumen), wet-type cerumen showed a protective association compared with dry-type cerumen where *OR* was 0.11, *CI*=0.02–0.56, X^2=10.1, p=0.001, Where the wet brown honey type showed a significant protective association with *OR* 0.34, *CI*=0.19-0.6, X^2=13.8, p=0.0002. Whereas cerumen from the wet dark orange type showed an odds ratio associated with positive growth with a positive growth rate of 42.6.4% (versus 20.1% for honey brown), with an associated odds ratio of 2.1, *CI* was 1.2–3.9, X^2=6.1, p=0.01.

Table 4: Potential pathogenic aerobic bacteria isolated from the external ear.

Bacteria	Male n=82 N (%)	Female n=164 N (%)	Total n=246 N (%)
S. aureus	16 (19.5)	19 (11.6)	35 (14.2)
P. aureginosa	7 (8.5)	7 (4.3)	14 (5.7)
Klebsiella spp	2 (2.4)	5 (3.1)	7 (2.8)
S. pyogenes	2 (2.4)	4 (2.4)	6 (2.4)
H. influenzea	1 (1.2)	4 (2.4)	5 (2.03)
S. aureus + Klebsiella spp	1 (1.2)	2 (1.2)	3 (1.2)
H. influenzea+klebsiella spp	2 (2.4)	2 (1.2)	4 (1.6)
Total	31/82	43/164	74/246

Table 5: Correlation of potential pathogenic bacterial growth in the outer ear with age and sex.

Characters	Positive growth No. (%)	*OR*	*CI 95%*	X^2	*p*
Sex					
Male n=82	32 (39)	1.8	1.01-3.2	4.3	0.03
Female n=164	42 (25.6)	0.55	0.31-0.97	4.3	0.03
Age groups					
≤15 years n=31	15 (48.4)	2.5	1.2-5.3	5.7	0.01
16-25 years n=61	21 (34.4)	1.3	0.7-2.4	0.72	0.39
26-35 years n=67	18 (26.9)	0.8	0.43-1.5	0.45	0.5
36-45 years n=26	7 (26.9)	0.84	0.33-2.1	0.13	0.71
≥46 years n=61	13 (21.3)	0.55	0.27-1.1	2.9	0.08
Total n=246	74 (30.1)				

Dry-type cerumen showed an odds ratio associated with positive growth as the positive growth rate was 77.8%, with an associated *odds ratio* of 8.9, *CI* was 1.8–43.8, X^2=10.1, *p*=0.001, this positive association increased to 83.3% of adjusted positive growth, with an associated *odds ratio* of 12.6, the *CI* was 1.4–108, X^2=8.2, *p*=0.003 with dry gray cerumen (Table 6).

DISCUSSION

The first objective of the current study was to determine the rates of different types of cerumen waxy in Yemen, it was found that 96.3% of the subjects in this study had the wet type, and only 3.7% of the tested subjects had the dry type of cerumen (Table 2). This result was consistent with the fact that there are two distinct, genetically determined types of cerumen: the wet type, which is dominant, and the dry type, which is recessive. East Asians, Southeast Asians, and Native Americans are more likely to have the dry type of earwax (gray and flaky), while Africans and Europeans are more likely to have the wet type of earwax, while Arab countries including Yemen have not been studied or reporting on the types of cerumen, and this study is one of the first studies that dealt with this subject[13]. The present study of the predominance of wet-type cerumen also differs from that reported in South Asia, Central Asia and the Pacific Islands where the wet-type is less frequent and about 30-50% of the population there has dry-type cerumen[14].

Table 6: Association of bacterial potential pathogenic growth in the external ear with types of external earwax (Cerumen).

Cerumen	Positive growth No. (%)	*OR*	*CI 95%*	X^2	*p*
Wet type (Moist) n=237	67(28.3)	0.11	0.02-0.56	10.1	0.001
Honey-brown n=134	27 (20.1)	0.34	0.19-0.6	13.8	0.0002
Dark-orange n=61	26 (42.6)	2.1	1.2-3.9	6.1	0.01
Dark-brown n=42	14 (33.3)	1.2	0.6-2.4	0.25	0.6
Dry type n=9	7 (77.8)	8.9	1.8-43.8	10.1	0.001
Gray n=6	5 (83.3)	12.6	1.4-108	8.2	0.003
Flaky n=3	2 (66.7)	4.7	0.4-53	1.9	0.16
Total n=246	74 (30.1)				

The type of cerumen has been used by anthropologists to track human migration patterns, such as that of the Inuit[15] in Japan, wet earwax is more prevalent among the Ainu, in contrast to the majority of the Yamato[16]. Wet-type earwax differs biochemically from dry-type mainly because of its higher concentration of lipid granules and pigment; for example, the wet type is 50% fat while the dry type is only 20%[5]. In the current study subtypes of wet type, honey-brown was the predominant one counting 54.5% of the total, followed by dark-orange (24.8%), and dark-brown counting 17.1% of the total (Table 2). This result is roughly similar to that reported among Africans and Europeans[13]. A specific gene that determines whether people have wet or dry earwax has been identified[17]. The difference in the type of cerumen was traced to a single base change (single nucleotide polymorphism) in a gene known as the "ATP-binding C11 cassette gene," specifically rs17822931[18]. Dry-type individuals are homozygous for adenine while wet-type individuals require at least one guanine.

Wet earwax is associated with underarm odor, which increases by increasing sweating[19,20]. In the current study, examination of the walls of the external ear canal showed that 50.4% of subjects showed normal level accumulation of cerumen on the canal walls and 46.7% showed wax accumulation at the entrance of the canal (Table 3). This result indicated that the external ear cleaning process is done well in 50.4%, while 46.7% (accumulation of wax at the entrance to the canal) may suffer from obstruction of the entrance to the canal over time. It is known that cleaning of the ear canal takes place due to the result of the 'conveyor belt' process of epithelial migration assisted by jaw movement. Cells created in the center of the tympanic membrane transfer towards the outside from the ear opening to the walls of the ear canal, moving toward the entrance to the ear canal. The cerumen in the ear canal is also flushed out, taking away any particulate matter that may have collected in the canal. The movement of the jaw aids this process by removing

debris attached to the walls of the ear canal, making it more likely to be expelled[21].

In the current study, 2.03% of the tested individuals had excess earwax or impacted cerumen (Table 3). Normally, earwax moves toward the ear opening and falls out or is washed away, but some people's ears secrete a lot of wax; This is referred to as excessive earwax or impacted cerumen[5,22]. Excessive of earwax may obstruct the passage of sound in the ear canal, causing mild hearing loss, ear pain, itching, or dizziness. Left untreated, impacted wax can lead to hearing loss, social withdrawal, impaired work function, and even mild paranoia. People with impacted wax may also develop a perforated eardrum; this is usually self-induced because compressed earwax alone cannot perforate the eardrum[5,23]. The 2.03% of impacted cerumen in the current study is similar to that reported elsewhere[5,24-26]. Most reports indicate that this condition is the most associated factor for hearing aids (60-80%)[24] because hearing aids prevents the removal of earwax from the ear canal, which leads to its blockage[5]. Earwax can also enter the openings and receivers of the aids[25]. Excessive earwax production can also cause tinnitus, constant ringing in the ears[26], fullness of the ear leading to hearing loss, and persistent ear pain[5,26].

Otits externa is one of the most common conditions with which patients go to ENT clinics worldwide. The external auditory canal has the potential to harbor potential pathogenic bacteria. Of note, there is no appropriate literature regarding the presence of potential pathogenic bacteria in the external auditory canal. Thus clinicians have difficulty interpreting the in vitro diagnostic report[27]. The external auditory canal can harbor some microorganisms[28]. Coagulase-negative *S. aureus* and *S. pneumoniae* are the most common bacteria isolated from the external ear canals of healthy subjects[29]. In the current study, the most prevalent outer ear isolate was *S. aureus* with 35 strains (14.2%), followed by *P. aureginosa* with 14 strains (5.7%). *Klebsiella* spp, *S. pyogenes* and *H. influenzae* isolates were less frequent with 7 (2.8%), 6 (2.4%) and 5 (2.03%) isolates, respectively (Table 4). Also, this assertion can be supported by the finding that *Klebsiella* bacteria and Gram-negative bacteria can be found in the outer, middle, and inner ears, and can be associated with chronic otitis media[30].

In the current study, positive growth for bacterial pathogens was significantly associated with males as the positive growth rate for males was 39% (vs 25.6% for female), with an associated odds ratio of 1.8, the *CI* was −1.01. 3.2, X^2=4.3, p=0.03 (Table 5). This result can be explained by the fact that males are more exposed to infection sources such as increased moisture in the external auditory canal causing edema and a more suitable environment for bacterial overgrowth. Cerumen has an acidic pH, and helps prevent bacterial growth in EAC. Lack of cerumen and exposure to moisture leads to infection and cleaning with a cotton applicator can traumatize the delicate epithelial lining of the bony layer of the ear, which then leads to colonization of pathogenic bacteria. All of the above factors can create an ideal environment for the growth of bacteria, especially *Pseudomonas* and *Staphylococcus* species[32]. Considering the age groups, there was a significant association with positive growth for bacterial pathogens as the positive growth rate for the less than15-year group was 48.4%, with an associated odds ratio of 2.5, and the *CI* was 1.2–5.3, X^2=5.7, p=0.01 (Table 5). This increased risk with younger ages could contribute to increased exposure to infection sources and reduced immune system efficacy in children compared to adults[32].

Considering the association of potential pathogenic growth of bacteria in the outer ear with types of external earwax (cerumen), wet-type cerumen showed a protective association compared with dry-type cerumen where *OR* was 0.11, *CI*=0.02–0.56, X^2=10.1, p=0.001, and for dry-type cerumen showed an odds ratio associated with positive growth as the positive growth rate was 77.8%, with an associated odds ratio of 8.9, *CI* was 1.8–43.8, X^2=10.1, p=0.001(Table 6). In contrast, studies have found that cerumen in wet or dry types in general have a bactericidal effect on some strains of bacteria. Cerumen has been found to reduce the viability of a wide variety of bacteria, including *H. influenzae*, *S. aureus*, and several variants of *E. coli*[7,8]. These antimicrobial properties are mainly due to the presence of saturated fatty acids, lysozyme, and, in particular, to the slight acidity in cerumen (usually a pH of about 6.1 in normal individuals)[10].

Yemen reviews showed that there are no studies discussing the types of cerumen, otitis and recent studies in Yemen are mostly concentrated in eye infections, general infections, autoimmune diseases, liver diseases, gastrointestinal infections, tuberculosis, and antibiotic resistance[33-47], the study shows the importance of prospective studies of extensive ear infections and their risk factors, and antibacterial effects of cerumen etc. This study provided consistent evidence indicating the effect of earwax types on the presence of pathogenic bacteria in the outer ear and the risk factors for their reproduction in the outer ear.

Limitation of the study

The study should include the colonization of aerobic and anaerobic bacteria and fungi that may affect the external ears, and the risk factors and causative agents of otitis externa should be studied with a larger sample size, the detection of antibiotics and antifungal sensitivity of isolates.

CONCLUSIONS

There are two distinct and specific types of cerumen, wet and dry, of which the wet type appears to be the most common in Yemen, and of the subspecies of the wet type, honey brown was dominant in Yemen. The present study concluded that some pathogenic bacteria that can cause otitis externa are naturally present in the normal external auditory canal. The most prevalent isolate was *S. aureus*, followed by *P. aureginosa*, *Klebsiella* spp, *Streptococcus pyogenes*, and *H. influenzae*. Hence clinicians must find a history of any predisposing factors before interpreting laboratory culture reports. Positive growth of bacterial pathogens in external auditory canal was significantly associated

with males, age group <15 years, and with dry type cerumen.

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AUTHOR'S CONTRIBUTIONS

Al-Khamesy KSA: Conceived idea, data collection, data analysis. **Al-Moyed KAKA:** methodology, investigation. **Al-Shamahy HA:** review, supervision. All the authors approved the finished version of the manuscript.

DATA AVAILABILITY

The data supporting the findings of this study are not currently available in a public repository but can be made available upon request to the corresponding author.

CONFLICT OF INTEREST

None to declare.

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