BACTERIA ASSOCIATED WITH THE HUMAN CERUMEN (EARWAX)

By

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Certification

This is to certify that this project work carried out by MOMOH AMINAT WITH MAT. NO. AST/2382070536 is adequate in scope and quality and is submitted to the Department of Biological Science Laboratory Technology, Auchi Polytechnic, Auchi in partial fulfilment of the requirements for the award of Higher National Diploma (HND) in Microbiology.

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DEDICATION

This work is dedicated to Allah Almighty for His infinite mercy, love and protection bestowed on me throughout my academic pursuits in the polytechnic.

Also, I dedicate this project work to my beloved parents (Mr and Mrs MOMOH), My guardians (Mr and Mrs Sa'eed), My dearest siblings, my darling husband (Engr. Sa'eed) and my friend (Ghaniyah Mrs) who stood by me firmly throughout my programme.

May Allah reward you all in folds

Acknowledgement

I wish to acknowledge the grace, love and mercies of Allah Almighty upon my life and realisation of these dreams.

I also wish to acknowledge the effort of my supervisor. Mr Audu O.J towards the success of this project work, my HOD, Mr Ohimai Anthony, and entire lecturers in Microbiology department. More so, in support of lovely and my caring parents.

Finally, to all those who contributed in one way or the other to the success of this work, I say may Allah Almighty make every mountains and crocked ground in your life become a levelled and smooth ground (Ameen)

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ABSTRACT

The purpose of this study is to understand the microbiology of human cerumen with the view to know the antimicrobial functions of cerumen and to understand the etiological agents responsible for ear infections. To do this, cerumen was collected from 10 volunteers using sterile earwax bud and microbial enumeration, and isolation was carried out on the cerumen specimens using selective and differential media.

The results of microbial enumeration in cerumen of volunteers showed that the total viable microorganisms (TVM) ranged from 8 to 324 cfu; total staphylococci count (TSC) ranged from 5 to 189 cfu; total coliform coun (TCC) ranged from zero to 37efu; and total fungal count (TFC) ranged from zero to 17cfu. TVM was generally higher than TSC, TCC and TFC in all the cerumen samples. Staphylococci were detected in all the 10 cerumen samples; coliforms were detected in 8 samples; and fungi were detected in only 4 samples.

The bacteria isolated from cerumen were identified as *Staphylococcus aureus*, *Bacillus*, Proteus, *Pseudomonas*, *Klebsiella* and *Staphylococcus species*.

The distribution of cerumen bacteria in the volunteers showed that *Staphylococcus aureus* was isolated from 5 persons; Staphylococcus species was recovered from 8 persons; Bacillus species was isolated from 7 persons. Proteus species was recovered from I person; Pseudomonas species was isolated from 3 persons; and Klebsiella species was recovered from 5 persons. At least one normal flora bacteria (Staphylococcus, Bacillus and Proteus species) was present in all the cerumen from the 10 persons. At least one pathogenic bacteria (*Staphylococcus aureus*. *Pseudomonas aeruginosa* and *Klebsiella pneumonia*) was present in the cerumen from 7 persons.

The findings of this work have shown that cerumen harbour microorganism of diverse taxonomic group including coliform bacteria, staphylococc fungi, Gram positive bacilli etc. They include pathogenic and normal flora organisms occurring as monobacterial and polybacterial cells. They occur in low numbers from <10 to 300, but the normal flora tend to be much more in healthy individuals than in otitis external patients, which suggest the protective role of normal flora against pathogens.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

The ear is the human organ that enables us to hear sounds around us. The main function of the ear is to maintain our sense of balance and to detect the various sounds. It helps us to convert the pressure variations into electronic signals that travel through the brain with the auditory nerve. The human ear has three parts which are the inner ear, middle ear, and outer ear. The outer car consists of the visible portion called the auricle, or pinna, which projects from the side of the head and the short external auditory canal, the inner end of which is closed by the tympanic membrane, commonly called the eardrum. The function of the outer ear is to collect sound waves and guide them to the tympanic membrane. The middle ear is a narrow air-filled cavity in the temporal bone. It is spanned by a chain of three tiny bones, the malleus (hammer), incus (anvil), and stapes (stirrup), collectively called the auditory ossicles. The inner ear consists of two functional units, the vestibular apparatus, consisting of the vestibule and semicircular canals, which contains the sensory organs of postural equilibrium, and the snail-shell-like cochlea, which contains the sensory organ of hearing.

1.2 Statement of the problem

Ears are delicate organs that can be damaged by physical injuries, bacteria or even changes in the environment. The car is not just the hearing organ. It is a complex system of parts that not only allows human to hear, but also makes it possible for human to walk.

Ear infections are the most common illness in babies and younger children, according to the NLM (National Library of Medicines). Common symptoms of ear infections are drainage from the car hearing loss, earache, fever, headache, pain in the ear and a feeling of fullness in the car according to the American Academic of Family Physicians.

Meniere's disease is a disease of the inner ear that may be the result of fluid problems inside the car. Symptoms include hearing loss, pressure or pain, dizziness and tinnitus. Tinnitus is a roaming in the ears. It can be caused by loud noises, medicines or a variety of other causes.

Cerumen, also called ear wax is made by the body in the outer ear canal as a way to protect and clean the car. It has antibacterial properties and also lubricates the ear.

The cerumen ear wax can build up and it symptoms includes feeling of blockage in the ear, coughing odor, discharge, itching and hearing loss.

1.3 Purpose of the study

- The major purpose of carrying out this research is to have a general understanding of the ear, when and how to care for it.
- This will enable us to know the importance of the human cerumen (ear wax), how it helps in preventing harmful bacterial and fungi from

penetrating through the ear, the diseases and infections associated with the ear.

- The research will also give a practical understanding on how to care for the ear, the bacterial associated with the ear, and human body in general and how to treat the infections of the ear.
- The research will give a practical understanding on the ear wax (cerumen), how it lubricates the ear and when there should be a cause for alarm.

1.4 Research questions

What is the role of cerumen in the inner ear?

- Cerumen lubricates the inner ear preventing it from bacterial infections that can cause several disorders to the ear as a whole.
- Cerumen prevents microbial infection of the inner ear.
- Is there any disease associated with the inner ear?

Meniere's disease is a disease of the inner ear that may be caused as a result of fluid problems inside the ear.

1.5 Research Hypothesis

H_0: pathogenic organisms are not found in the cerumen of persons without ear infections.

H_1: pathogenic organisms are found in the cerumen of persons without ear infections.

H_0: Microorganisms found in cerumen are not similar to those on human skin.

H_1: Microorganisms found in cerumen are t similar to those on human skin.

1.6 Scope of the study

- Collection of cerumen from ear of volunteers.
- Isolation of microorganisms in cerumen.
- Identification and characteristics of isolates.

1.7 Importance of the study

The importance of this study is to know the various bacterial associated with the human cerumen (ear wax), the human cerumen and how it helps keep the ear safe from several types of infections/diseases, how the cerumen lubricates the inner ear and keeps it safe from bacteria entry.

CHAPTER TWO

LITERATURE REVIEW

Earwax is a natural yellowish, waxy substance that is secreted in the ear canal. Earwax protects the ears and ear canals from external influences such as dust, bacteria, insects and others. It thus acts as a barrier to prevent foreign bodies from reaching the eardrum. Earwax is produced by ceruminous glands in the outer third of the cartilaginous portion of the ear canal. The skin inside the ear canal is constantly renewing itself and migrates from the depth of the ear canal outwards, carrying the wax with it. The earwax captures and removes epidermal waste (dirt and dead skin) as it passes and carries it to the outside.

The action of chewing and yawning encourages the movement of earwax outwards along the ear canal. When earwax accumulates in the ear canal, it can cause blockages and hearing problems.

Earwax is a necessary secretion of the outer third of the ear canal. The presence of earwax should not be thought of as dirty or not clean.

References to the "Clean ear" in the context of the removal and management of earwax are more about selling a service than helping patients understand their ears.

2.1 THE CERUMEN (EARWAX)

Cerumen is produced in the outer third of the ear canal. It is a mixture of secretion from oil (Sebaceous) glands and less-viscous ones from modified sweat (apocrine) glands. The primary components of earwax are 60% shed layers of skin (keratin) and 12 - 20% saturated and unsaturated long-chain fatty acid, alcohols and 6-9% is cholesterol.

There are two distinct genetically determined types of earwax which are:

- The wet type
- The dry type

East Asians and Native Americans are more likely to have the dry type of cerumen (Gray and flaky) 30 - 50% of south Asians, central Asians and pacific Islanders have the dry type of cerumen.
African and European people are more likely to have the wet type (honey-brown, dark orange to dark brown and moist).

2.2 FUNCTIONS OF EARWAX

- Cerumen (earwax) prevents the loss of water from the skin (desiccation) and so maintains the skins normal health balance and appearance. This is largely due to the oil content of cerumen and its fatty acids.
- Cerumen also has a bactericidal effect on some strains of bacteria. It is "bacteriostatic" as it may prevent some bacteria reaching deeper into the bony portion of the ear canal.
- Cerumen has been found to reduce the viability of a wide range of bacteria, including *influenza* and *staphylococcus aureus* .
- The growth of fungi commonly present in otomycosis is also significantly inhibited by human cerumen, however, cerumen is not an effective bactericidal against all pathogens .

- As well as providing moisture and acidity, the sticky texture of earwax also acts as barrier to foreign bodies entering the ear canal. Examples could be debris such as sand or even insect.

2.3 MIGRATION PROCESS; The ears self-cleaning mechanism

The skin from the eardrum (tympanic membrane) to the outer part of the ear canal, is created from the Centre of the eardrum called the Umbo New skin cells form at the umbo and move outwards to the edge of the eardrum before moving outward in the canal, towards the entrance. The skin is smooth in the inner most two thirds of the ear canal as it moves over bone. When the skin reaches the outer third of the meatus, it enters the fleshy (cartilaginous) portion. This contains the cells that produce cerumen (earwax) as well as hair follicle. The skin wrinkles and eventually breaks, forming dead skin cells that flake off or mix with the cerumen depending on the dry and wet type of earwax produced.

The process of skin cells migrating out of the ear canal is sometimes called the "Conveyor" if a black dot were placed just off Centre of the eardrum, it would take months for it to reach the outermost part of the ear canal. The movement (migration) of skin out of the ear canal is not fully understood and speed of migration is not affected by jaw movement.

If hearing aids or other objects routinely push earwax back into the ear canal, a build-up of excessive earwax results.

2.4 Composition Of The Cerumen

Cerumen or earwax is a mixture of desquamated keratinocyte from the outer part of the external auditory canal and secretion from sebaceous glands along with apocrine sweat glands. It creates a gray-brown-to-grayish-black-colored thick substance and deposited at the external auditory canal. Glandular secretions coming from the hair follicle of the external auditory canal also mix with the cerumen and make it a sticky substance which is known as cerumen.

Cerumen consists of amino acids, neurostearic acid, cerotic acid, triglycerides, cholesterol, hexone bases, lysozyme, immunoglobulin glycopeptides, copper and others.

2.5 EARWAX (CERUMEN) Impaction

What is earwax impaction?

Chewing and moving the jaw allows earwax to move toward the opening of the ear canal. The earwax will eventually dry, flake and fall out. But sometimes, earwax does not make it all the way out and builds up in the ear canal. This is called cerumen (earwax) impaction or blockage. Earwax blockage can occur from using headphones, hearing aids or inserting cotton swabs in the ears.

2.5.1 Causes of Cerumen (Earwax) Blockage

The following are the causes of cerumen blockage

- Cleaning methods: Cleaning methods with cotton swabs can push earwax farther into the ear canal.

- Earwax that becomes drier as you get older can more readily get stuck

- Extra hair in your ear canal can trap the earwax
- Hearing aids can block earwax
- Different shapes of the ear canal can trap earwax

2.5.2 Symptoms of Impacted Earwax

Often an earwax buildup causes no symptoms at all. But in some cases, impacted earwax can cause symptoms such as:

- Ear pain
- Ears that feel clogged or full
- Itching in the ear
- Drainage or bad smell from the ear
- Loss of hearing
- Ringing in the ears (tinnitus)

Keep in mind that other ear disorders can cause these symptoms too like an outer ear infection or Meniere's disease, a condition that causes ear pain, vertigo, tinnitus and hearing loss. That is why health care provides always start with an ear examination to look for earwax when a person has symptoms related to their ears or hearing .

2.5.3 Treatment of Impacted Earwax

Below are the treatment options for impacted earwax

- **Cerumenolytic agents (earwax softeners)**

Cerumenolytic agents are liquids or medications that can break up or dissolve earwax. Often, one needs to use these ear drops for several days in a row before the wax buildup is soft enough too removed.

Common earwax softening drops available over the counter includes:

- Almond oil
- Carbamide peroxide
- Docusate sodium
- Hydrogen peroxide, 3%
- Mineral oil
- Saline solution.

- **Irrigation**

Irrigation of the ears is usually done with warm water or a mixture of water and hydrogen peroxide. Different types of bulb and syringes are used to flush or irrigate the earwax, a Cerumenolytic agent can be used to help with removal.

- **Manual removal**

When irrigation and drops are not enough, a special instrument would be used by an health care provider to scoop or pull out the earwax. Because of the risk of injuring the ear canal and ear drum, only a health care professional should do this.

Long term risks of not treating impacted earwax

- ⇨ Temporary hearing loss
- ⇨ More permanent hearing loss can occur in children with earwax impaction
- ⇨ Difficulty looking into your ear, making it harder for your health care provider to diagnose other ear issues
- ⇨ Otitis externa: an outer ear infection

2.6 Ear Disease And Disorders

Below are the diseases and disorders of the ears

- ⇨ Ear infection
- ⇨ Fluid from the ear
- ⇨ Glue ear
- ⇨ Labyrinthitis
- ⇨ Meniere's disease
- ⇨ Otitis media

Ear infection:

There are several different types of ear infection, depending on which part of the ear is infected. Two common types are middle ear infections (otitis media) and outer ear infections (otitis externa). Ear infections can be caused by bacteria or viruses.

Fluid from the ear:

A discharge from the ear, also called otorrhea, is usually just the body getting rid of earwax, the oil and solid materials we produce naturally to

prevent dust and bacteria from getting into our ears. But sometimes sticky fluids build up in the middle ear, behind the ear drum. This mucus can lead to ear infections and loss of hearing and is very common in children
Fluid from the ear can be caused by

- A ruptured eardrum
- Damage to the ear
- Mastoiditis - an infection of the bone behind the ear, the mastoid can also cause fluid from the ear although this is rare

Glue ear:

Glue ear occur when the liquid inside the ear becomes thick like glue. Glue ear often follows repeated ear infections. It can affect hearing and lead to other problems. Glue ear is common in young children. It can last for weeks or months and can affect hearing, speech, learn learning and behavior.

Labyrinthitis:

Labyrinthitis is an infection of the balancing Centre in the inner ear (or labyrinth). The infection can affect the messages sent by the ear to the brain, causing changing in hearing and balance. It usually develops suddenly and if treated, clears up in a few weeks. It is an inflammation of the inner ear, usually caused by an infection. It can lead to mild or severe dizziness.

Meniere's disease:

Meniere's disease is a disorder of the inner ear that causes hearing and balance problems. There is no cure, but the symptoms can be managed. People with Meniere's disease experience attacks of vertigo that usually lasts between 2 and 4 hours. Some people have several attacks of Meniere's

disease in a short period of time, while other people only have it every few months or years. In between attacks, most people usually have mild or no symptoms. Most people with Meniere's disease have it in just one ear. It often leads to worsening hearing loss in that ear.

Otitis media (Middle ear infection):

Otitis media (another name for middle ear infections) is very common in children. It often disappears by itself, but sometimes it may need treatment. Otitis media can also affect adults. Otitis media usually starts with a cold or a sore throat caused by bacteria or a virus.

2.7 ANTIMICROBIAL AND ANTIBACTERIAL PROPERTIES OF THE CERUMEN

- **Antimicrobial Properties Of The Cerumen**

Cerumen is a hydrophobic protective barrier in the external auditory canal. It shields the skin of the ear canal from water damage, trauma, foreign bodies and infections. Cerumen also lubricates and clean the ear canal, traps dusts, and repels water from entering inside the canal (Shapiro j, et Al)

There are many contradictory reports on the antibacterial activity of cerumen. The antibacterial nature of cerumen is based on the consideration that the high nutrients of cerumen which enable bacteria and fungi to grow, which is against the antibacterial property which prevents infections at the external ear. (Gupta S *et al*).

Cerumen is slightly acidic in nature, which discourages the growth of bacteria and fungus in the moist and dark environment of the external

auditory canal. It is almost impossible to avoid infection at the ear canal without the presence of cerumen (Hyslop NE Jr. Earwax and host defense) It is expected that if cerumen provides immunity, its composition should alter in response to infection and exposure to bacteria and should induce antibacterial components of the cerumen at the ear canal.

- **Antibacterial Properties Of The Cerumen**

There is little known about the chemical composition of the human cerumen and its antimicrobial role. Several proteins are found in the cerumen such as antimicrobial peptides of human beta defensin (hBD) 1-3,lactoferrin, LL-37, bactericidal permeability-increasing (BPi),hSLPi, and HNP1-3 all have some role to prevent bacteria and fungi those causing infections at the external auditory canal. If this local defense system gets disturbed, infections of ear canal occur (Schwab M, Gurr A *et al*).

2.8 MICROORGANISMS ASSOCIATED WITH THE EAR

According to reports of many studies, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus mirabilis, Klebsiella pneumoniae and Escherichia coli are the common organisms isolated from cases of ear infection (Naqi, 2016 and Ambika *et al*, 2015)

2.8.1 OUTER EAR

The outer ear is exposed to the external environment and much like skin on other parts of the human body is in contact with microbial life. Both the auricle and the external auditory meatus house a variety of microbes under healthy conditions. The outer ear is exposed to the outside oxygen-filled environment, the majority of the bacterial flora on the auricle and in the

external auditory canal is made up of aerobic species. The outer ear is home to a diverse set of microbes including bacteria, viruses, and fungi (Kumar *et al*. 2017). The skin of the external auditory canal and auricle is predominantly occupied by Gram-positive over Gram-negative bacteria. The main Gram-positive bacteria are staphylococci, coryneforms, streptococci and enterococci, micrococci, and bacillus. Of the Gram-positive bacteria, the predominant species are Staphylococcus auricularis, S. capitis (both capitis and ureolyticus). S epidermidis, S. warnen, Turicella otitidis, Alloiococcus otitis, Micrococcus luteus, and E. coli. Gram-negative species inhabit the auricle and skin of the external auditory meatus to a much lesser extent with Pseudomonas aeruginosa and Moraxella osloensis in relative abundance. Some fungal microbes can be found in the skin of the outer car, but are less abundant than either Gram- positive or Gram-negative bacteria. Candida parapsilosis, C. albicans, and Penicillium species form the majority of fungal isolates of the ear integument (Campos *et al*, 2000).

The skin and the cerumen present different microbial populations in the external ear. Gram-positive bacteria still dominate the bacterial flora and the species distribution is relatively similar to that in the skin. However, the Gram-negative bacteria species are less common in the cerumen than the canal and almost non- existent in the cerumen with Pseudomonas present. Interestingly, fungal microbes across the board are more common in cerumen than the canal with similar species distribution (Stroman *et al*, 2001).

P. aeruginosa is a Gram-negative rod-shaped bacteria with a flagellum at one pole. P. aeruginosa is a facultative anaerobe, but prefers aerobic

respiration. This makes it well suited for life on the skin and the outer ear which is exposed to the oxygen-filled atmosphere. P. aeruginosa is able to utilize a wide variety of metabolites; it is also an opportunistic pathogen that causes multiple different diseases such as pneumonia, UTIs, and other skin diseases including acute diffuse otitis externa (Stroman *et al*, 2001).

S. epidermidis is a Gram-positive firmicute commonly found on the skin of humans. As the name suggests S. epidermidis is cocci shaped and unlike S. aureus is nonpathogenic for the most part. S. epidermidia has been known to infect immune compromised individuals. Naturally a part of the normal human skin flora it is no surprise that S epidermidis is found in the outer car due to the similarity in environments between the auricle and the rest of the human skin (Kumar *et al* 2017)

S auricularis is a Gram-positive firmicute also found in the outer ear. S auricularis is a cocci shaped microbe that is nonpathogenic and is a port of the normal ear flora (Stroman *et al*, 2001).

Penicillium chrysogenum is a fungal microbe that grows in damp environments and produces beta-lactam antibiotics, which is the active ingredient in penicillin. This microbe may contribute to the antibiotic properties of cerumen and help the human body keep ear infections at bay.

Aspergillus niger & Candida albicans are fungal microbes that causes symptoms of pain, hearing loss, aural fullness, and itching in extreme cases otomycosis can permanently damage the ear canal and the

tympanic membrane leading to hearing loss: C. albicans, like P. aeruginosa, is present in normal outer ear microbiota. C. albicans is also an opportunistic pathogen, attacking immune compromised individuals. Most of the C. ablicans in the human body resides in the human gut, but C. albicans is also found in the exterior of the human ear.

2.8.2 MIDDLE EAR

Although the middle ear is segregated from the external portion of the ear via the tympanic membrane, the middle ear is connected to the nasopharynx by the way of the Eustachian tube. In this way the middle ear is somewhat in contact with the external environment. However, bacteria would still need to travel through the nasal cavity and up the Eustachian tube which is no easy task. The mucous and cilia in the nasal cavity function to trap and expel foreign particles: including bacteria that may travel up to the middle. That being said microbes are present in the middle car. For instance alpha hemolytic streptococci are present in a healthy Alpha hemolytic streptococci are known to inhabit the middle ear. There is a possibility that the alpha hemolytic streptococci crowd out other bacteria and prevent middle ear infections. Lower incidence of alpha hemolytic streptococci has been observed in children with recurrent middle ear infections. This is of course a promising path to effective treatment of car infections. Middle ear infections are termed otitis media. Since the middle ear is not directly accessible by bacteria from the external environment, bacteria must either travel up the Eustachian tube or through a perforated tympanic membrane in order to access the middle ear. Some causes of otitis media are caused by

bacteria found in the outer ear. These bacteria are facultative anaerobes which makes them well-suited for the tympanic cavity environment. They access the middle ear via the Eustachian tube connecting to the nasopharynx.

S. pneumoniae are lancet-shaped, gram-positive, facultative anaerobic bacteria with more than 100 known serotypes. Most S. pneumoniae serotypes can cause disease, but only a minority of serotypes produces the majority of pneumococcal infections.

H. influenzae is a gram-negative, non-motile, coccobacillary, facultatively anaerobic, pathogenic bacterium of the family Pasteurellaceae. The bacteria are mesophilic and grow best at temperatures between 35 - 37ºC

Moraxella catarrhalis is a fastidious, nonmotile, Gram-negative, aerobic, oxidase-positive diplococcus that can cause infections of the respiratory system, middle ear, eye, central nervous system, and joints of humans. It causes the infection of the host cell by sticking to the host cell using trimeric autotransporter adhesins.

Allolococcus otitis and Turicella otitidis are commensal in the external auditory meatus yet possibly pathogenic in the middle ear. A. otitis is a specie of bacteria first isolated from human middle-ear fluid, the type species of its monotypic genus and 7. Otitidis is a non-fermenting Gram- positive bacillus isolated almost exclusively from car exudates. Its significance in acute or chronic otitis media is controversial (Stroman *et al*; 2001).

2.8.3 INNER EAR

Unlike the outer and middle ear, the inner ear is completely secluded from the outside environment by the bony labyrinth that encases the cochlea, semicircular canals, and vestibule. This makes it much more difficult for bacteria to translocate across the oval window. The immune system is more prevalent in the inner ear which also reduces the possibility of microbial entry into the inner ear (Alberti, 1988). There is no access to the external environment via the external auditory canal and Eustachian tube for the outer and middle ear respectively. In most cases the cause of inner ear infections are usually viral. The two main inner ear maladies are vestibular neuritis and Labyrinthitis. Both of these conditions affect the balance of an individual and cause vertigo and sometimes nausea. Both vestibular neuritis and Labyrinthitis onset are preceded by an upper respiratory infection such as the common cold, herpes simplex virus, or flu (Marill, 2011).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Materials

The materials used in this project work are Bunsen burner, autoclave, incubator, refrigerator, weighing balance, pippetes, petri dishes, test tubes, wire loop, bent glass rod. , hot air oven, and culture media: nutrient agar, manitol salt agar, MacConkey agar and Sabourand dextrose agar, distilled water, cotton wool, sterile earwax buds, sterile needles and syringes, aluminum foil, buffer (5% $NaHCO_3$, pH 8.2 containing 30% glycerol),cerumen(earwax)

3.2 STERILIZATION OF GLASSWARES AND APPARATUS

All glass wares were properly washed and rinsed with clean water. The method of sterilization adopted was the use of the autoclave at 121^{o}C for 15 minutes. The inoculating loop was sterilized using the flame from a Bunsen burner. The flaming of the wire loop was repeatedly done at the end of every inoculation. The work table top was disinfected using ethanol. Media used were sterilized using an autoclave.

3.3. MEDIA PREPARATION

3.3.1 Preparation of Nutrient Agar

This was done by weighing 14.0g of dehydrated nutrient agar powder into a conical flask containing 500ml of distilled water. The mixture was stirred with sterile spatula and placed over a Bunsen burner flame on a tripod stand

to fully dissolve the entire powdered agar. The dissolved mixture in the flask was covered with cotton wool and aluminum foil and placed in an autoclave and was sterilized at 121ºC for 15 minutes. Once the sterilization was complete the autoclave was allowed to cool and the molten agar was brought out and allowed to cool to about 45ºC.

3.3.2. Preparation of MacConkey Agar

This was done by weighing 12.0g of dehydrated MacConkey agar powder into a conical flask containing 250ml of distilled water. The mixture was stirred with sterile spatula and placed over a Bunsen burner flame on a tripod stand to fully dissolve the entire powdered agar. The dissolved mixture in the flask was covered with cotton wool and aluminum foil and placed in an autoclave and was sterilized at 121ºC for 15 minutes. Once the sterilization was complete the autoclave was allowed to cool and the molten agar was brought out and allowed to cool to about 45ºC.

3.3.3. Preparation of Sabourand Dextrose Agar

This was done by weighing 35.5 g of dehydrated Sabourand Dextrose agar powder into a conical flask containing 500ml of distilled water. The mixture was stirred with sterile spatula and placed over a Bunsen burner flame on a tripod stand to fully dissolve the entire powdered agar. The dissolved mixture in the flask was covered with cotton wool and aluminum foil and placed in an autoclave and was sterilized at 121ºC for 15 minutes. Once the sterilization was complete the autoclave was allowed to cool and the molten agar was brought out and allowed to cool to about 45ºC.

3.3.4 Manitol salt agar

This was done by weighing 57.5g of manitol salt agar into a conical flask containing 500ml of distilled water. The mixture was stirred with sterile spatula and placed over a Bunsen burner flame on a tripod stand to fully dissolve the entire powdered agar. The dissolved mixture in the flask was covered with cotton wool and aluminum foil and placed in an autoclave and was sterilized at 121^{o}C for 15 minutes. Once the sterilization was complete the autoclave was allowed to cool and the molten agar was brought out and allowed to cool to about 45^{o}C.

3.4 COLLECTION AND PREPARATION OF CERUMEN SAMPLES

Collection and preparation of cerumen samples was carried out by the method of Ambika *et al* (2015). Cerumen was collected with sterile ear wax bud from 10 persons and stored in sterile capped tubes at 4^{o}C in the refrigerator. For each person, multiple buds were used and scrapped on the mouth of the tube to discharge the cerumen into it, pooled and weighed to obtain 0.1g. This was suspended in 2.5ml of sterile buffer (5% $NaHCO_3$, pH 8.2 containing 30% glycerol). The cerumen-buffer mixture was homogenized by repeated passage through a series of needles ranging from 19 to 23guage with sterile syringes. This procedure broke the cerumen into fine particles distributed evenly in buffer and resulted in a milky suspension containing 4% cerumen.

3.5 ENUMERATION OF MICROORGANISMS

Enumeration of bacteria was carried out using the modified method of Ambika *et al* (2015). Plate counts were performed on the milky suspension

using spread plate method. With the aid of sterile pipettes, 0.1ml is transferred onto the surface of sterile dry plates of the culture media and spread evenly with the aid of a sterile bent glass rod. Enumeration of total viable microorganisms (TVM) was carried out on nutrient agar, and incubated at 30˚C for 24 hours. Enumeration of coliform bacteria (TCB) was carried out on MacConkey agar and incubated at 30˚C for 24 hours; enumeration of fungi (TFC) was carried out on Sabourand Dextrose agar and incubated at 30˚C for 48 hours; and enumeration of Staphylococci (TSC) was carried out on manitol salt agar and incubated at 30˚C for 24 hours. At the end of incubation, each plate was observed for growth and colonies appearing on each medium were counted with the aid of magnifying lens and recorded as colony-forming units. Enumeration in all the samples was in triplicates with the mean values calculated and recorded.

3.6 ISOLATION AND IDENTIFICATION OF BACTERIA

Bacterial colonies from the enumeration agar plates were purified by repeated sub culturing on nutrient agar plates using streak method. Using sterile loop, the purified isolates were transferred onto agar slants for storage in the refrigerator until required. Purified isolates from overnight broth culture were characterized by cultural characteristics, Gram's stain, motility test and other biochemical tests. The isolates were identified by comparing their characteristics with those of known groups using the scheme of Buchanan and Gibbons (1974).

3.6.1 Gram Staining

Test organisms were heat-fixed on slides and flooded with crystal violet for about 60 seconds and rinsed with water for about 5 seconds. The slides were then flooded with iodine solution for about 60 seconds and rinsed with water. Ethanol was then added as a decolourizer and rinsed with water afterwards. Finally, the slides were flooded with saffranin for about 60 seconds, rinsed with water, blotted dry, and viewed under a microscope. Gram-positive organisms appeared blue/purple under the microscope while Gram-negative organisms appeared red/pink. The cell shapes were also viewed under this procedure.

3.6.2 Capsule stain

Make a smear of the bacterial isolates, air dry, place the slide on a beaker of boiling water and cover the smear with 1.0% aqueous solution of crystal violet for one minute. Wash with 20% copper sulphate solution, blot dry and view under oil immersion of microscope. Capsules appear as faint blue-violet zone surrounding purple bacterial cell.

3.6.3 Spore stain

Make smears of the isolates culture, air dry and heat fixed properly. Flood the slide with malachite green and heat over a beaker of boiling water for 5 minutes. Wash with clean water and counterstain with safranin for about 30 seconds. Wash with water, blot dry and examine the slide under oil immersion objective of the microscope. The presence of endospores is indicated by green spot in a red background.

3.6.4 Motility test

A wire loop was used to inoculate a motility medium by making a stab to the bottom of the tube and afterwards incubated for 24-48 hours. If the organism is motile, the tube will appear cloudy and the organisms will spread out of the stab line. Non-motile organisms will grow along the streak line only and the media will not be cloudy.

3.6.5 Oxidase Test

Fresh growth is removed from the agar plate using a non-metallic instrument such as a sterile plastic inoculating loop or a sterile swab or wooden splint. The oxidase test strip is moistened slightly with oxidase reagent and the growth is rubbed into the moistened paper of the strip. If the microbe has cytochrome oxidase, it will add electrons to the reagent, changing it from its colourless appearance to a deep indigo blue in a matter of 10-20 seconds. Waiting any long than this increases the likelihood that the reagent turns blue due to natural chemical changes caused by exposure to air. If the colour does not turn blue within 20 seconds, the test is negative for the presence of oxidase.

3.6.6 Coagulase Test

A drop of sterile distilled water was placed on each end of a sterile slide. A culture of the test organism was emulsified on each spot to make two thick suspensions. A loop-full of plasma was added to one of the suspensions and mixed gently. The slide was examined for clumping or clotting of the organisms within 10 seconds. Plasma was not added to the second suspension, which served as control.

3.6.7 Citrate utilization test

Aseptically prepare slants of Simmons's citrate agar in capped tubes, inoculate with each isolate and incubate at 37^0C for 3-4 days. A positive result is indicated by change in medium colour from green to blue.

3.6.8 Indole Test

Tryptophan broth was inoculated with test organism and incubated for 24 hours. Drops of Kovacs Reagent were then added to the broth. Formation of a red ring at the surface of the broth signified a positive result.

3.6.9 Catalase test

Two drops of hydrogen peroxide was place on the surface of clean grease free glass slide A and B (control).Using a clean glass rod the test organism was transferred to A; and the gas bubbling or effervescence indicates a positive reaction.

3.6.10 Sugar fermentation test (glucose, lactose sucrose and manitol)

The basal medium used was sterilized peptone water with a drop of phenol red added as p^H indicator. Also added is 1.0% of sugar prepared in 99ml of peptone water and sterilized in the autoclave at 121^0C for 15minutes. A loopful of pure culture of the test organism was inoculated into the sterile solution of sugar and incubated at 37^0c for 48 hours. At the end of the incubation, the formation of yellow colour due to acid production and gas production in the Durham tube immersed in the medium indicates a positive result.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

The results of microbial enumeration in cerumen of volunteers showed that the total viable microorganisms (TVM) ranged from 8 to 324 cfu; total staphylococci count (TSC) ranged from 5 to 189 cfu; total coliform count (TCC) ranged from zero to 37cfu; and total fungal count (TFC) ranged from zero to 17cfu. TVM was generally higher than TSC, TCC and TFC in all the cerumen samples. Staphylococci were detected in all the 10 cerumen samples; coliforms were detected in 8 samples; and fungi were detected in only 4 samples (Table 1).

The bacteria isolated from cerumen were identified as *Staphylococcus aureus*, *Bacillus*, *Proteus*, *Pseudomonas*, *Klebsiella* and *Staphylococcus* species (Table 2).

The distribution of cerumen bacteria in the volunteers showed that *Staphylococcus aureus* was isolated from 5 persons; *Staphylococcus* species was recovered from 8 persons; *Bacillus* species was isolated from 7 persons; *Proteus* species was recovered from 1 person; *Pseudomonas* species was isolated from 3 persons; and *Klebsiella* species was recovered from 5 persons. At least one normal flora bacteria (*Staphylococcus*, *Bacillus* and *Proteus* species) was present in all the cerumen from the 10 persons. At least one pathogenic bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*) was present in the cerumen from 7 persons.

Polybacteria was obtained from 9 persons, while monobacteria was obtained from only one person (Table 3).

Table1: Enumeration of microorganisms in cerumen from volunteers

Volunteers	**TVM**	**TSC**	**TCC**	**TFC**
1	37	12	6	0
2	106	49	13	7
3	65	38	10	0
4	324	189	23	0
5	212	78	19	11
6	8	5	0	0
7	24	15	4	0
8	83	34	14	5
9	269	103	37	17
10	31	12	0	0

Table 2: Characterization of bacteria isolated from cerumen

Tests	***Pseudomonas* sp,**	***Klebsiella* sp**	***Proteus* .sp**	***Staphylococcus aureus***	***Staphylococcus* sp**	***Bacillus* sp.**
Gram stain	***-ve*** rod	***- ve*** rod	-ve rod	+ve cocci	+ve cocci	+ve rod
Capsule stain	-	+	-	-	-	-
Spore stain	NT	NT	NT	NT	NT	+
Oxidase	+	-	+	-	-	+
Catalase	+	+	+	+	+	+
Coagulase	NT	NT	NT	+	-	NT
Urease	-	-	+	+	-	-
Citrate	+	+	+	+	-	+
Motility	+	-	+	-	+	+
Indole	+	-	-	+	-	-
Glucose	+	+	+	+	+	+
Lactose	-	+	-	-	-	NT
Manitol	NT	NT	NT	+	+	NT

NT = Not tested + = Positive - = negative

Table 3: Distribution of bacteria isolates in cerumen of volunteers

Volunteers	**Bacteria isolated from cerumen**	**Pathogenic**	**Normal flora**
1	***Staphylococcus aureus, Bacillus* sp**; ***Staphylococcus* sp**	**present**	**present**
2	***Staphylococcus aureus, Bacillus* sp**; ***Staphylococcus* sp; *Klebsiella* sp**	**present**	**present**
3	***Bacillus* sp**; ***Staphylococcus aureus,***	**present**	**present**

	***Staphylococcus* sp; *Klebsiella* sp**		
4	***Pseudomonas* sp; *Klebsiella* sp; *Staphylococcus* sp**	**present**	**present**
5	***Bacillus* sp; *Staphylococcus* sp; *Klebsiella* sp; *Staphylococcus aureus***	**present**	**present**
6	***Staphylococcus* sp**	**Absent**	**present**
7	***Bacillus* sp; *Staphylococcus* sp;**	**Absent**	**present**
8	***Pseudomonas* sp; *Proteus* .sp; *Staphylococcus aureus***	**present**	**present**
9	***Bacillus* sp; *Staphylococcus* sp; *Klebsiella* sp; *Pseudomonas* sp.**	**present**	**present**
10	***Bacillus* sp; *Staphylococcus* sp;**	**Absent**	**present**

4.2 DISCUSSION

The detection of various groups of bacteria and fungi in cerumen samples is an indication that it supports the growth of diverse organisms. This is supported by the report of Ambika *et al* (2015) that earwax protects the tissues of the ear, and helps to prevent infection by trapping irritants such as microorganisms, dead skin cells, sweat, oil, hair and dirt from the atmosphere. Part of the ear canal is lined with fine hairs called cilia that help to catch particles and microorganisms that enter the ear and tie them down in cerumen. It is also rich nutrients that support luxuriant growth of bacteria and fungi (Singer *et al*; 1952). According to Bovo *et al* (2012) diverse forms of organisms were found in cerumen of patients of otitis externa and healthy persons.

The low numbers of fungi and their absence in many samples is supported by the findings of Naqi (2016), that non- bacterial organisms constitute 6.0% and 2.8% in ear patients and healthy persons respectively.

The comparatively low numbers of microorganism detected in cerumen (8-324cfu) could be as a result of antimicrobial effects of cerumen on trapped microbes. Nevertheless, there is little evidence to support this concept but according to Hyslon (1971), earwax is slightly acidic, which discourages bacterial or fungal growth in the moist and dark environment of the ear canal and without earwax it would be almost impossible to avoid ear infections. The organisms *Staphylococcus aureus*, *Bacillus*, *Proteus*, *Pseudomonas*, *Klebsiella* and *Staphylococcus* species isolated in this study are similar to the ones isolated in previous studies (Bovo *et al* ;2012; Ambika *et al*;2015; and Naqi, 2016).

TVM was generally higher than TSC, TCC and TFC in all the cerumen samples because there may be some other bacteria that were not captured in the enumeration study. This was confirmed by the isolation of *Bacillus*, *Proteus* and *Pseudomonas* species from cerumen samples. At least one normal flora bacteria (*Staphylococcus*, *Bacillus* and *Proteus* species) was present in all the cerumen from the 10 persons. At least one pathogenic bacteria (*Staphylococcus aureus*, *Pseudomonas* and *Klebsiella* species) were present in the cerumen from 7 persons. This was based on the classification of Naqi (2016) that *Klebsiella pneumonia*, *Staphylococcus aureus*, *Haemophillus influenza*, *Streptococcus pneumonia* and *Pseudomonas* species are considered as pathogenic bacteria of the external ear, and other bacteria were categorized as normal flora. *Staphylococcus* and *Bacillus*

species were the most abundant normal flora bacteria isolated in this study. This supports the findings of Naqi (2016) that coagulase -*Staphylococcus* species constitute 41.5% of isolates from healthy persons; and *Bacillus* species constitute 21.2% of isolates from otitis externa patients. *Staphylococcus aureus* and *Klebsiella* species were the most abundant pathogenic bacteria isolated in this study. This also supports the findings of Naqi (2016) that *Staphylococcus aureus* constitute 24.6% of isolates from otitis externa patients; and *Klebsiella* species were the most common Gram negative bacteria isolated from ear infections.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

The findings of this work have shown that cerumen harbour microorganisms of diverse taxonomic group including coliform bacteria, staphylococci, fungi, Gram positive bacilli etc. They include pathogenic and normal flora organisms occurring as monobacterial and polybacterial cells. They occur in low numbers from <10 to >300, but the normal flora tend to be much more in healthy individuals than in otitis external patients, which suggest the protective role of normal flora against pathogens.

5.2 RECOMMENDATIONS

It has been suggested that cerumen is unable to prevent infection and that the rich nutrients of earwax support luxuriant growth of bacteria and fungi (Singer *et al*; 1952). On the other hand, it has been suggested that cerumen might have antimicrobial activity although little evidence has been presented to support this contention. In the light of this I recommend that;

1. More research should be carried out to validate the antimicrobial activity of cerumen.

2. Further studies should be conducted to ascertain the interactions between normal flora and pathogenic organisms in cerumen.

3. Routine wax removal and ear cleaning should be avoided in order not to destroy the physical barrier of cerumen against pathogenic organisms.

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APPENDIX

Composition of Media

Nutrient agar

Peptone……...5.0g

Beef extract…….3.0

Agar…… 15.0g

Distilled water……1 litre

Mackonkey agar

Pancreatic digest of gelatin…… 17.0g

Proteose Peptone……..3.0g

Lactose monohydrate…… 10.0g

Sodium chloride…….. 5.0g

Bile salts…….....1.5g

Neutral red……...0.03g

Crystal violet……0.001g

Agar……...13.5g

Distilled water……1.0 litre

Mannitol salt agar

Pancreatic digest of casein……9.0g

Peptic digest of animal tissue……5.0g

Beef extract……1.0g

Sodium chloride…….. 75.0g

D-mannitol……... 10.0g

Phenol……0.025g

Agar……..15.0g

Sabourand dextrose agar

Peptone……...10.0g

Dextrose…….40.0g

Agar…… 20.0g

Distilled water……1.0 litre

pH……...5.6