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ORIGINAL ARTICLE

Study on the ear microbes of school going children

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ABSTRACT

The mammalian ear is the organ of hearing and balance. The ear usually described and divided in to three parts- outer ear, middle ear and inner ear. The outer ear consists of the pinna and the ear canal. The middle ear consists of tympanic cavity and three ossicles The inner ear located in the bony labyrinth contains key structure to various senses. This very important sense organ more prone to several infections. The ear infections are caused by a wide variety of organisms including Staphylococcus, streptococcus, Candida albicans, Aspergillus etc. The study was carried out in the school going children because they are highly diversified with the ear microbial flora.
The present work involves the studies of ear microbes of the school going children. For this purpose the ear samples were collected from randomly selected 20 school going children of age group 10 to 15 years, with the help of sterile Johnson bud, samples were taken and diluted in a sterile 1ml of distilled water in sterile sample bottle. All the 20 samples were streaked over NA (Nutrient Agar) by four way streaking within two hours of collection of samples. Plates were incubated at 37^0 C for 24-48 hrs. from these 9 different isolates were selected. Microscopic Examination and Biochemical Characterization were carried out for these 9 selected samples. The results were found includes, more than 78% of the microflora consisted of Gram+ve organisms. Three out of 9 isolates of Gram +ve motile cocci. The major group being *Staphylococcus epidermidis*. The Lactose fermenting highly motile non E-coli Gram –ve organisms. All the 9 isolates were found to be sensitive to vancomycin.

Keywords- Mammal, Ear Microbes, Nutrient Agar, Johnson bud, Vancomycin.

1. INTRODUCTION

External ear canal and middle ear canal of school going children are more prone to infection. At the same, time the ear micro flora also exist

as normal flora which does not harm the normal healthy subjects. Increased cases of otitis have been reported in cancer patient Juan et al., [1]. Higher frequency of otitis is also common in critically illed immune condition Burkovski [2]. The ear infections are caused by a wide variety of organisms including Staphylococcus, streptococcus, Candida albicans, Aspergillus etc. Baron and Davis [3].

Since, school going children going age is almost common throughout India, with a tremendous heterogeneous background of school children, it is highly probable that their may be diversity of microbial flora of the ear in these children Martin and Clark [4].

Moreover higher rate of cross infection leading to increased rates of otitis among the children is possible Singer et al. [5].

In view of these observations we intended to go for the studies of a representative sample of ear micro flora of 20 school going children. In the age of 10-15years. A general survey pattern with oral recording was also conducted for general Parameters.

Aims and Objectives.

- To ramdomly screen school going children of the age 10-15 years and isolate indicative ear micro flora.
- To characterized the isolates morphologically and biochemically and antibiogram.
- To perform correlation studies with respect to ancilliary subjective parameter.

Ear microbiology is becoming an important study as many flourishing microorganisms have been reported to populated ear as a habitat Karin [6], Ryan and Ray [7].

Over the past decades the study of ear microbs as a normal microbial flora and also as a bonafide human payhogen and there role in head and neck cancer, due to observance of otitis externa in cancer patients have resulted in an explosion of scientific interest in ear microbiology Moller [8]. The study of ear is not new but it is necessary for study of ear microbiology.

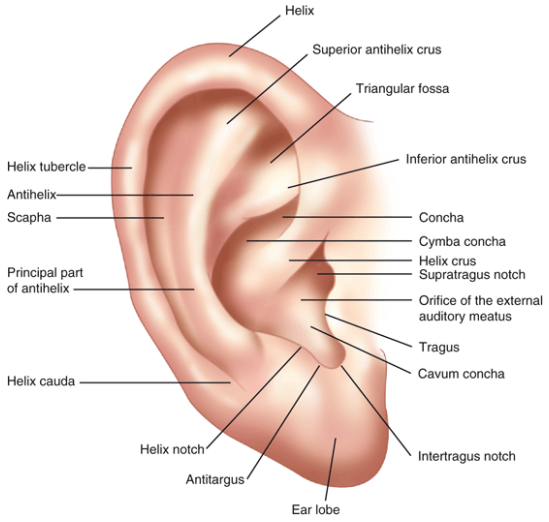

Figure 1: The physiology of ear

Ear

The human ear is the anatomical structure responsible for hearing and balance. The ear consists of three parts: the outer, middle, and inner ears.

Outer Ear

The outer ear collects sounds from the environment and funnels them through the auditory system. The outer ear is composed of three parts, the pinna (or auricle), the external auditory canal (or external auditory meatus), and the tympanic membrane (or eardrum) Todar [9].

Pinna

The two flap-like structures on either side of the head commonly called ears are actually the pinnas of the outer ear. The pinnas of most humans cannot move, but these structures are very mobile in other mammals, such as cats and dogs Mestel [10].

External Auditory Canal

The external auditory canal is a passageway in the temporal lobe of the skull that begins at the ear and extends inward and slightly upwards. In the adult human it is lines with skin and hairs and is approximately 1 in (2.5 cm) long (Figure1) Dora et al.[11].

The outer one-third portion of the canal is lined with a membrane containing ceruminous (earwax producing) cells, and hair cells. The purpose of the cerumen and hairs is to protect the eardrum (which lies at the end of the canal) by trapping dirt and foreign bodies and keeping the

canal moist. In most individuals, cleaning of the external auditory canal (with Q-tips for example) is not needed. The inner two-thirds of the external auditory canal contains no glands or hair cells Stroman et al. [12].

Tympanic membrane/ eardrum

The human tympanic membrane or eardrum is a thin, concave membrane stretched across the inner end of the external auditory canal much like the skin covering the top of a drum. The eardrum marks the border between the outer ear and middle ear . The eardrum serves as a transmitter of sound by vibrating in response to sounds travelling down the external auditory canal, and beginning sound conduction in the middle ear.

Middle Ear

The middle ear transmits sound from the outer ear to the inner ear. It consists of an oval, air-filled space approximately 2 cubic cm in volume. There is a long hallway leading away from the side wall of middle ear room, known as the Eustachian tube. It is lined entirely with mucous membrane (similar to the nose) and is surrounded by the bones of the skull.

Eustachian tube

A passageway leading from the middle ear to the nasopharynx or throat.

Bones/ ossicles and muscles

Three tiny bones (the ossicles) in the middle ear form a chain which conducts sound waves from the tympanic membrane (outer ear) to the oval window (inner ear). The three bones are the hammer (malleus), the anvil (incus) and the stir. These bones are connected and move as a link chain might, causing pressure at the oval window and the transmission of energy from the middle ear to the inner ear. In addition to bones, the middle ear houses the two muscles, the stapedius and the tensor tympani, which respond reflexively, that is, without conscious control (Figure 2) Ogston [13].

Inner Ear

The inner ear is responsible for interpreting and transmitting sound (auditory) sensations to the brain. The inner ear is small (about the size of a pea) and complex in shape, where its series of winding interconnected chambers, has been compared to (and called) a labyrinth. The main components of the inner ear are the vestibule, semicircular canals, and the cochlea Alberti [14].

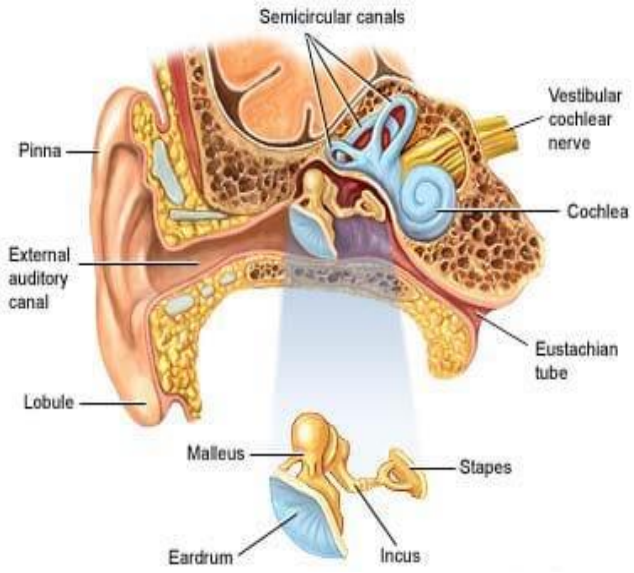

Figure 2: The anatomy of human ear

Vestibule

The vestibule, a round open space that various passageways, is the central structure within the inner ear. The vestibule contains two membranous sacs, the utricle and the saccule.

Semicircular canals

Attached to the utricle within the vestibular portion of the inner ear are three loop-shaped, fluid filled tubes called the semicircular canals. The semicircular canals are a key part of the vestibular system and allow for maintenance of balance when the head or body rotates Brook [15], Perry [16].

Cochlea

A snail-shaped structure in the inner ear, which contains the anatomical structures responsible for hearing.

Ear Microbiology -

Human flora

The human flora are the microorganisms that constantly inhabit the human body. They include bacteria, fungi and archaea. Some of these organisms are known to perform that are useful for the human host, while the majority have no known beneficial or harmful effect Quek and Otto [17].

Those that are expected to be present, and that normal circumstances do not cause disease, are termed normal flora or studies on The Ear Microbes of school going children micro biota .an effort to better describe the micro flora of humans has been initiated Belkaid and Segre [18].
It is estimated that 3 to 4 species of bacteria live in human ear, and these are

- *Corynebacterium* spp
- *Staphylococcus aureus*
- *Staphylococcus epidermidis*

***Corynebacterium* sp.**
Corynebacterium is a genus of Gram-positive, aerobic or facultatively anaerobic, non –motile, non –sporulated, rod-sporulated, rod – shaped actinobacteria. Most do not cause disease, but are part of normal human skin flora. Some, such as *Corynebacterium* diphtheria, are important pathogens while others, such as *Corynebacterium* glutamicum, are of immense industrial importance kloos and Schleifer [19].

Staphylococcus aureus
S. aureus was discovered in Aberdeen, Scotland in 1880 by the surgeon Sir Alexander Ogston in pus from surgical abscesses Each year some 500,000 patients in American hospitals contract a staphylococcal infection [17,19].

S. aureus is a facultatively anaerobic, Gram – positive coccus, which appears as grape-like clusters when viewed through a microscope and has large, round, golden-yellow colonies, often with hemolysis, when grown on blood agar plates. The golden appearance is the etymological root of the bacteria's name: aureus means "golden" in latin [19].

Staphylococcus epidermidis
Staphylococcus epidermidis is a member of the bacterial genus Staphylococcus, consisting of Gram-positive cocci arranged in clusters. It is catalase-positive and coagulase-negative and occurs frequently on the skin of humans and animals and in mucous membranes. It is a facultative anaerobe that grows by aerobic respiration or by fermentation. It is not sensitive to the antibiotic Novobiocin, a feature that distinguishes it from the other common coagulase-negative organism S. saprophyticus. Due to contamination, S. epidermidis is probably the most common species found in laboratory tests *[19],* Vedamuthu and Nevile *[20].*

Micro Flora Study of Ear
The bacterial flora of external ear canal was investigated in a group of 64 patients with head and neck cancer. *Staphylococcus epidermidis* (48.5%), Pseudomonas sp. (18.8%), and Flavobacterium sp. (16.8%) were the prominent isolates from ear samples, and Gram-negative organisms, which rarely existed in normal canal, were found to be markedly increased (59.4%). Moreover, about half of all non-sterile ear canals were found to have more than one Gram-negative flora Write and Alexander [21].
The Susceptibility to penicillin and ceftriaxone of systematic isolates the prevalence of penicillin and ceftriaxone resistant S. pneumonia was more common in systematic isolates than the middle ear isolates. The incidence of staphylococcus coagulase negative and streptococci pneumoniae is higher in control group that than in cancer patients. The higher colonization of *S. aureus* is probably due to their resistance to antibiotics Mosaei et al. [22].

The colonization of nose and external ear canal in infants, 39% of the normal flora was shown to be potentially pathologic Gram positive microoraganisms with pre dominance of *staphylococcus epidermidis* from nostrils and 37% of the same organism from ear canal Ostfeld [23].

The quality of water also exerts microbial variation in the ear canal. Wright has reported that the dominant Gram-positive flora of the ear is replaced by Gram-negative flora in swimmers and divers [21]. In the survey of microbial flora of the outer ear canal showed the pre dominance of Gram-positive bacteria. Staphylococus epidermidis was shown in 83% of the sample Dibb [24].

2. MATERIAL AND METHODS

The present work involves the studies of ear microbes of the school going children. For this purpose the ear samples were collected from randomly selected 20 school going children of age group 10 to 15 years. A general survey pattern with oral recordings was also conducted for general parameters.

Sample collection.
Samples were collected from ear of school going children of age group 10 to 15 years, for the test, 20 specimens were taken.

For the sample collection following procedure were follow with the help of sterile Johnson bud, samples were taken and diluted in a sterile 1ml of distilled water in sterile sample bottle.

Isolation of strain

All the 20 samples were streaked over NA (Nutrient Agar) by four way streaking within two hours of collection of samples. Plates were incubated at 37^0 C for 24-48 hrs. After incubation of plates in all 9 isolates were observed. Each of the 9 isolates were further of purified over NA plates colonies which were formed after 48 hrs incubation were further inoculated to nutrient agar medium to get isolated colonies. Two slants were prepared from each isolate for further analysis.

Microscopic Examination

Two major microscopic examination namely Gram staining and motility by hanging drop method were selected. Crystal violet was used as a primary stain while saffranin as counter stain and 70% alcohol was used as a decolorizing agent for performing Gram staining. Single cavity hanging drop method was used for assessing the motility of the organisms. All these methodologies were used as per the protocol prescribed in the standard methods Gorems et al.[25], Mane and Basawraju [26].

Cultural characterization on TSI slant

All isolates were obtained on nutrient agar and inoculated on TSI agar for acid and gas production.

Biochemical characterization

Biochemical characterization were done on selected isolates, IMViC test and sugar fermentation were carried out for these 9 isolates.

IMViC test

Four metabolise test namely Indole production test, methyl red test, voges proskaur test and citric acid test collectively known as IMViC test. The test was perform according to standard procedure.

Sugar Fermentation

All 9 isolates were used for testing sugar fermentation. The sugar which
were used for this purpose-

1) Dextrose (Simple sugar, monosaccharide)
2) Lactose (Compound sugar, diasaccharide)
3) Mannitol (Alcohol group containing sugar)

The test were perform according to standard procedure.

Colony characterization

Most of the primary isolates were typically isolated on nutrient agar medium with repeated sub culturing. All the isolates were typically characterized based on colony characterization.

1) Colony characterization
2) Margine characterization
3) Type character
4) Uniformity

Each of these colonies was assigned respective nomenclature as per standard nomenclature prescribed Benson [27].

Test for that Organism can grow anaerobically or not. - WRIGHTs Tube method

- Slant culture of the selective microorganism
- Pyragallol
- Sodium hydroxide
- Cork

Tests for Obligate Halophiles Requiring 7.5 % NaCl for Growth.

Lysine Decarboxylase Test

Dispend 5ml sterile medium in screw-capped test tube sterile by autoclaving at 15 lbs pressure for 15 min. cool the medium in upright position. Inoculate the tube and overlay with 2-3 ml of mineral oil.

Catalase Test

Perform catalase test in 3% hydrogen peroxide.

Cytochrome Oxidase Test

Dissolve 0.2 gm of tetramethylene-p Phynylenediamine dihydrochloride in 20 ml of distilled water.

To perform Antibiotic Sensitivity test of Vancomycine.

3. RESULTS

The present work was sub divided into following categories.

- To randomly screen school going children of the age 10-15 years and observe representative micro flora.

- To characterized the isolates morphologically and biochemically.
- To perform correlation studies with respect to ancillary subjective parameter.

Isolation of Organism

Samples were taken from 20 different students and inoculated on NA plates. From these 9 different isolates were selected and these isolates then inoculated on slant. The isolate obtain are shown in table (Table1).

Microscopic Examination

Microscopic examination of all 9 selected isolates were done including Gram staining and motility. The results are shown in table (Table-2).

Biochemical Characterization

Biochemical characterizations of the isolates were done as per the standard methodology books. It includes Indole, methyl red, Voges Proskerues and citrate utilization test. In second phase of biochemical characterization the different sugar fermentation capacity of the organism were observed. The results are as shown in the table (table-3).

Other Ancillary Tests

Few other selective test for presumptive indentification of isolates such as Lysine decarboxylase test, TSI test, cytochrome oxidase test, catalase test, test for growth of isolates in anaerobic condition and obligate halophiles growth in NaCl medium, and the antibiogram of the isolates for vancomycine. The results are shown in the table (Table-: 4,5,6,7,8).

Table 1: Colony Characterization of Primary Isolates

Isolate No.	Colony Characteristics On Nutrient Agar.
1	Yellow coloured, pinpoint, circular colonies.
2	Creamy white, circular, pinpointed colonies.
3	Lawn like colony with irregular margine.
4	Red pinpointed colony
5	Transparent colony having irregular margine
6	Zone having pinpointed colony
7	Reddish orange circular colony
8	Circular white colony
9	Pinkish coloured colony

Table 2: Microscopic Examination

Isolate No.	Gram Staining	Motility
1	Gram + ve	Non motile
2	Gram-ve	Highly motile
3	Gram -ve	Highly motile
4	Gram +ve	Non motile
5	Gram +ve	motile
6	Gram +ve	Non motile
7	Gram +ve	Highly motile
8	Gram +ve	Highly motile
9	Gram +ve	Highly motile

Table 3: Biochemical Characterization

Isolate no.	Dextrose		Mannitol		Lactose	
	Acid	Gas	Acid	Gas	Acid	Gas
1	+	-	+	-	+	-
2	+	-	+	-	+	-
3	+	-	+	-	+	+
4	+	-	+	+	-	-
5	+	-	-	-	-	-
6	-	-	-	-	-	-
7	-	-	-	-	-	-
8	+	-	+	-	-	-
9	-	-	+	-	+	-

Table 4: IMViC Characterization of Isolates

Isolate No	Indole	MR	VP	Citrate
1	-	+	-	-
2	-	-	-	-
3	-	+	-	-
4	-	+	+	+
5	-	-	-	+
6	-	-	-	+
7	-	-	-	+
8	-	-	-	-
9	-	-	-	+

Table 5: Catalase Cytochrome Oxidase Lysine Decarboxylase test of isolates

Isolate No	Catalase	Cytochrome Oxidase	Lysine Decarboxylase
1	+	+	-
2	-	+	+
3	+	+	-
4	+	+	-
5	+	+	-
6	+	-	+
7	+	+	-
8	+	+	-
9	+	+	-

Table 6: TSI profile Of Isolates

Isolate No	Butt	Gas	H2s
1	Acidic	+	-
2	Acidic	-	-
3	Acidic	+	-
4	Acidic	+	-
5	Acidic	+	-
6	Acidic	-	-
7	Acidic	-	-
8	Acidic	-	-
9	Acidic	-	-

+Positive Test , - Negative Test

Table 7: Observe the Growth of Microorganism under the Anaerobic Condition and For Obligate Halophiles Requiring 7.5 % of NaCl for Growth

Isolates No	Observations Of Anaerobic Condition	Observations Of Obligate Halophiles
1	Growth	Growth
2	No Growth	Growth
3	No Growth	Growth
4	No Growth	Growth
5	Growth	Growth
6	No Growth	Growth
7	Growth	Growth
8	Growth	No Growth
9	Growth	Growth

Table 8: Antibiotic Sensitivity Test FOR Vancomycin.

Isolate No	Sensitivity In mm
1	14mm
2	14mm
3	17mm
4	16mm
5	14mm
6	17mm
7	15mm
8	16mm
9	18mm

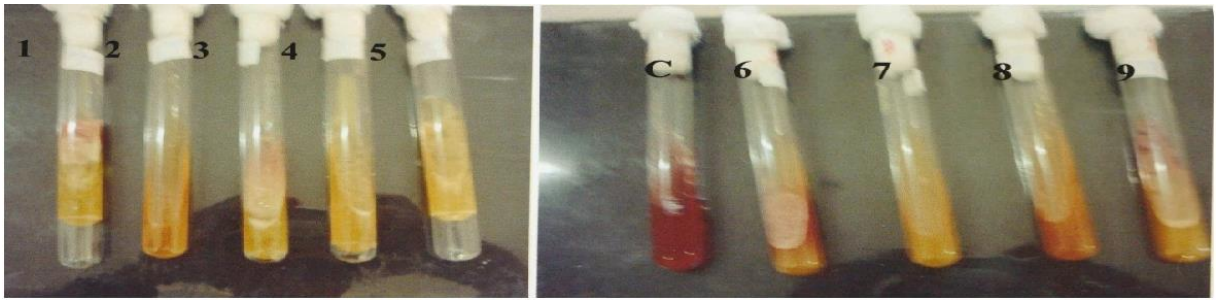

Figure 3: Growth on TSI Agar

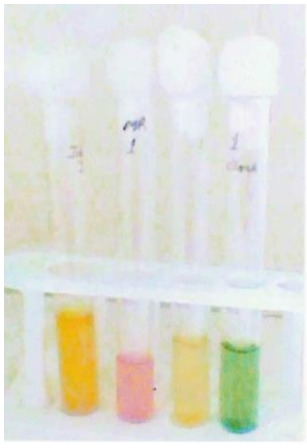

Figure 4: IMVIC Test

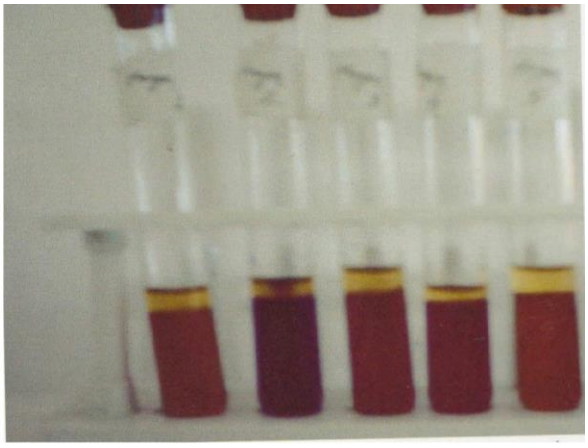

Figure 5: Lysine Decarboxylase Test

4. DISCUSSION

The present study on the micro flora of school going children revealed that the common micro flora of the ear canal in Gram +ve cocci. Only 22% samples should highly motile Gram–ve organisms. Most of the Gram –ve profiles were lactose fermenters not belonging to Escherichia group which can be confirm from the IMViC test. All the Gram +ve test were catalase +ve and grew at 7.5% Nacl concentration. Three strain of the Gram +ve cocci were motile and produce acids from carbohydrates. One of these isolate that is isolate no.8 is a member of malinococcus.

The isolate with a high motility and heavy growth may be member of salinococcus. Rest all of them with Gram + ve cocci and non-motility belong to *Staphylococcus epidermidis* group.

The Pathogenic strains were not located as no typical *S.aureus* would be identified. All through primary isolates suggested 11 out of 20 samples giving yellow colonies on NA but many of them are found to be motile their by rejecting the possibility of being *S.aureus*.

All the 9 isolates were found to be sensitive to Vancomycin as can be seen.

The identification carried out here is nearly tentative. Since some of the distinguishing test like major menaquinone, peptidoglycan structure could not be done.

CONCLUSION

The present study on the school children and their micro flora of the external ear provided many information's, Some of the common conclusions that could be made from this studies include-

- More than 78% of the micro flora consisted of Gram +ve organisms
- Three out of 9 isolates of Gram +ve motile cocci.
- One isolate was clearly indicating of being a member of malinococcus and another salinococcus.
- The major group being *Staphylococcus epidermidis*.
- Lactose fermenting highly motile non *E. coli* Gram –ve organisms was also found. The pathogenic properties of these isolates could not be confirmed but they were found to susceptible to Vancomycin.

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Conflict of interest
No conflict of interest influenced in this research.

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