ANTIBIOGRAM OF BACTERIAL ISOLATES CAUSING OTITIS MEDIA FROM OTITIS MEDIA SYMPTOMATIC PERSON'S OF DHARAN

A

Project work submitted to

Department of Microbiology

Central campus of Technology, Tribhuvan University

In Partial Fulfillment for the Award of the Degree of

Bachelor of Science in Microbiology

Submitted by:

Yasoda Rai

Roll no.: 80020

TU Registration no.:5-2-8-46-2013

Department of Microbiology, CCT, Tribhuvan University

Hattisar, Dharan

2017

RECOMMENDATION

This is to certify that **Ms. Yasoda Rai** has completed this project work entitled **"ANTIBIOGRAM OF BACTERIAL ISOLATES CAUSING OTITIS MEDIA FROM OTITIS MEDIA SYMPTOMATIC PERSON'S OF DHARAN"** as a part of partial fulfillment of the requirement of Bachelor Degree in Microbiology under my supervision. To my knowledge this work has not been submitted for any other degree.

……..

Mr. Kalyan Rai

Supervisor

Department of Microbiology

Central Campus of Technology

Hattisar, Dharan, Nepal

Date: August, 2017

CERTIFICATE OF APPROVAL

On the recommendation of **Mr. Kalyan Rai**, this project of **Ms. Yasoda Rai** entitled **"ANTIBIOGRAM OF BACTERIAL ISOLATES CAUSING OTITIS MEDIA FROM OTITIS MEDIA SYMPTOMATIC PERSON'S OF DHARAN"** has been approved for the examination and is submitted to the Tribhuvan University in partial fulfilment of the requirements for Bachelor's degree in Microbiology.

……..

Mr. Shiv Nandan Sah

(Assistant professor)

Head of Department

Department of Microbiology

Central Campus of Technology

Hattisar, Dharan, Nepal

Date: August, 2017

BOARD OF EXAMINERS

Recommended by: ……..

Mr. Kalyan Rai

Supervisor

.....

Mr. Man Kumar Tamang

Co-supervisor

Approved by: ……..

Mr. Shiv Nandan Sah

Head of Department

Department of Microbiology

Examined by: ..……..

(Internal Examiner)

.....

(External Examiner)

Date:.....

ACKNOWLEDGEMENTS

I am highly grateful to my project work Supervisor, **Mr. Kalyan Rai** and Co-supervisor **Mr. Man Kumar Tamang** for giving me their valuable time and guidance. Their stimulating guidance throughout the study helped to get me its completion. I would like to thank my entire teaching and non-teaching staffs of Department of Microbiology of Central Campus of Technology.

With great respect and admiration, I express my deep gratitude to **Mr. Shiv Nandan Sah**, Head of Department of Microbiology and lecturer **Mr. Prince Subba** for their guidance and constant inspiration. I would also like to express my gratitude to **Mr. Aindra Bahadur Karki** and **Ms. Kamana Bantawa Rai**, Laboratory person of the Microbiology Laboratory of Central Campus of Technology.

My parents, **Mr. Hangjit Rai** and **Mrs. Manfulla Rai** are deeply acknowledged for their continuous motivation, encouragement and support. My very dear friends **Chunsaha Limbu, Sanju Magar, Chetan Maskey,Himal Kafle, Mahesh Karki, Santoshi Ghimire and Rojina Subedi** for their great support and encouragement during the study.

……

Yasoda Rai

Date: August, 2017

ABSTRACT

Otitis media is a common problem to all age group especially to the children, which causes inflammation of middle ear. This study aimed to isolate and identify the bacteria responsible for otitis media and perform the AST profile of identified isolates, from people having some sort of ear problems. This is a laboratory based cross- sectional study. The ear swab sample was taken from students of different schools, students of Central Campus of Technology, workers of Central Campus of Technology, complaining of ear problems. The samples taken were ear swab in the sterile cotton swab and all the collected samples were taken to the Laboratory and were processed as soon as possible in the Laboratory of Department of Microbiology. The total sample collected was 50. Out of 50 samples, 18 were from children, 16 were from adults and 16 were from old people. The 26 samples were form male and 24 samples were from female. Out of total, 92% showed the growth of bacteria and 8% did not showed growth. The bacteria isolated were *Staphylococcus aureus* (26%), *Staphylococcus epidermidis* (38%), *Bacillus* spp. (18%), *Pseudomonas aeruginosa* (6%), *Haemophilus* spp (2%). and *Moraxella* spp (2%) respectively. The highest number of isolates was seen in the children of age5-10. The least number of isolates was seen in the age group (21-50). The antibiotics used were Ciprofloxacin, Azithromycin, Gentamicin, Ceftazidime, Cotriomoxazole, Chloramphenicol, Imipenem and Ampicillin. Ciprofloxacin and Gentamicin were most effective which showed 100 % sensitivity. The least effective antibiotic was Ampicillin which showed 100 % resistivity.

Keywords: Antibiotics, Antimicrobial susceptibility pattern (AST), Otitis media

TABLE OF CONTENTS

LIST OF TABLES

LIST OF PHOTOGRAPHS

LIST OF FIGURE

LIST OF APPENDICES

ABBREVIATIONS

OM-Otitis Media

AOM-Acute Otitis Media

CSOM-Chronic Suppurative Otitis Media

OME-Otitis Media with Effusion

WHO-World Health Organization

MHA-Muller Hinton Agar

NA-Nutrient agar

AST-Antibiotic susceptibility test

CHAPTER –I
INTRODUCTION

1.1 Background

Otitis media (OM) is an inflammation of the middle ear cleft which is a common problem worldwide (Cripps, 2003) .The term otitis media consist a range of diseases, from acute to chronic and with or without symptoms (Stool et al , 1994). In defining the term of disease, acute otitis media (AOM)is defined as the rapid and short onset of sign and symptoms of inflammation in the middle ear. As a result of otitis media, fluid become trapped in the Eustachian tube. This liquid resulting from otitis media is referred to as middle ear effusion. The effusion may be classified as either serous-thin, water liquid; mucoid-thick, viscid, mucus-like liquid; or purulent- pus like liquid. Middle ear effusion that persists for longer than three months following an episode of acute otitis media is termed otitis media with effusion (OME) (Bluestone and klein,1995). Chronic suppurative otitis media is middle ear inflammation of greater than two weeks that results in episodes of discharge from ear. It may be complication of acute otitis media (Minovi and Dazert, 2014). All three may be associated with hearing loss (Qureshi et al, 2014).

OM most commonly results from dysfunction and poor drainage of the Eustachian tube. As a result of reflux of microorganisms present in the nasopharynx into the tubular lumen and their subsequent replication, fluid accumulates in the middle ear and subsequently may become infected(Adderson,1998).

Fluid accumulation and reflux of microorganisms into the middle ear may result from high negative middle ear pressure, high positive nasopharyngeal pressure, or abnormal patency of the nasopharyngeal opening of the Eustachian tube. In addition, high pressure caused by sneezing or crying may force secretion into the ear or abnormal patency of the tube can leave the tube open at rest or result in excessively low resistance to reflux(Adderson,1998).

The most common bacteria involve in ear infection are *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pneumonia, Staphylococcus epidermidis, Haemophilus influenza and Moraxella catarrhalis*. However, *Bacillus* spp., *Proteus* spp. and *Escherichia coli* are also involved in infection (Bluestone and Klein, 2001). However, *Staphylococcus aureus, Staphylococcus epidermidis* and *Pseudomonas aeruginosa* are the normal flora but they can easily enter through perforated ear leading to otitis media (Abera and kibret, 2011)

An intregral symptom of OM is ear pain; other possible symptoms include fever, hearing loss and irritability. Since an episode of otitis media is usually precipitated by an upper respiratory tract infection there often are accompanying symptoms like a cough and nasal discharge (Lieberthal et al, 2013).

Risk factors of OM include exposure to smoke, use of pacifiers and attending day care and too short period of breasting feeding (Minovi and Dazert, 2014). Due to low socio-economic status, overcrowding, poor hygiene, inadequate health care, and recurrent upper respiratory tract infection, the burden is high in low and middle income countries(Kumar and Seth 2011; Akinpelu et al ,2008).

Globally, about 65-330 million people suffer from ear infection and 60% of them had significant hearing impairment(Woodfield and Dugdale, 2008).Worldwide AOM affect about 11% of people a year (about 325 to 710 million cases).Half the cases involve children less than 5 years of age and it is more common among males. Of those affected about 4.8% or 31 millions develop chronic suppurative otitis media(Monasta et al ,2012).Before the age of ten OME affects about 80% of children at some point(Minovi and Dazert,2014).OM resulted in 2,400 deaths in 2013-down from 4,900 deaths in 1990(GBD 2013).

Long term antibiotic decrease rates of infection during treatment however it has an unknown effect on long term outcomes such as hearing loss(Leach and Morris,2006).This method of prevention has been associated with emergence

of antibiotic-resistant otitic bacteria. They are thus not recommended (Lieberthal et al, 2013).

Pneumococcal conjugate vaccines when given during infancy decrease rates of acute otitis media by 6%-7% and if implemented broadly, would have a significant public health benefit (Jansen et al ,2009).Influenza vaccine is recommended annually (Lieberthal et al ,2013).

Risk factors such as season, allergy predisposition and presence of older siblings are known to be determinants of recurrent otitis media and persistent middle ear effusions (Rovers et al, 2004).

History of recurrence, environmental exposure to tobacco smoke, use of day care, and lack of breastfeeding all have been associated with increased risk of development, recurrence and persistent middle ear effusion (Etzel,1987).

Thus, cessation of smoking in the home should be avoided or day care attendance should be avoided or day care facilities with the fewest attendees should be recommended and breastfeeding should be promoted (Etzel, 1987).

It is a well known fact that microbial drug resistant is growing global problem. In Gram-negative bacteria, the most resistant pathogens are *Escherichia coli, klebsiella* spp. and *Pseudomonas aeruginosa* with increasing trends observed for all major anti-Gram agents (beta- lactams, fluoroquinolones and aminoglycosides) (Rossolini et al,2007).Serious infections caused by Gram-positive bacteria are increasingly difficult to treat because of pathogens such as methicillin-resistant *Staphylococcus aureus* and penicillin-resistant *Streptococcus pneumonia*.

Therefore, the objective of this study is to isolate and identify bacterial agents responsible for ear infection (otitis media) and their antibiotic susceptibility pattern among healthy person's ear of different age group.

In this project work, samples (ear wax) of 50 people of different aged group (5-60 years) were collected from different wards of Dharan in which 26 were male and 24 were female. Out of 50 people, *Staphylococcus aureus* were isolated from 13 sample i.e 26%(6 from children,3 from adult and 4from old people), *Staphylococcus epidermidis* were isolated from 19 sample i.e 38%(9

from children, 5 from adult and 5 from old),*Bacillus* spp. were isolated from 9 sample i.e 18%(3 from children,3 from adult and 3 from old), *Pseudomonas aeruginosa* were isolated from 3 sample i.e 6%(present only in children),*Haemophilus* spp. were isolated from one sample i.e 2%(present only in children), Moraxella spp. were isolated from one sample i.e 2%(present only in children) and no growth was seen in 4 sample i.e 8%(2 in old and 2 in adult).

Antibiotic susceptibility test were performed for all the bacteria that was isolated. The antibiotics used for antibiotic susceptibility test were Ciprofloxacin, Azithromycin, Gentamicin, Ceftazidime, Chloramphenicol, Cotriomoxazole, Imipenem and Ampicillin.

1.2 Objectives

General objective:

- To determine bacterial agent responsible for otitis media (ear infection) from ear swab and perform antibiotic susceptibility test of isolated bacteria.

Specific objectives:

- To isolate and identify bacteria responsible for otitis media from ear swab.
- To determine the antibiogram of isolated bacteria.

1.3 Rationale of the study

Otitis media (OM) is an inflammation of ear. If it is left untreated, OM leads to more complication including acute to chronic infection. The types of otitis media are acute otitis media (AOM), otitis media with effusion (OME) and chronic suppurative otitis media. The most bacteria associated with ear infection were *Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis, Haemophilus influenza, Moraxella catarrhalis, Bacillus* spp.*, Streptococcus pneumoniae, Proteus* spp., etc. OM causes pain in ear, fever and difficulty in hearing. Symptoms that may be associated with otitis media include pain in ear, sore throat (pharyngitis), neck pain, nasal congestion and discharge (rhinitis), headache and ringing (tinnitus), fever, buzzing or other noise in the ear and paralysis of facial part in severe case. Risk factors for otitis media include: Children, person attending day care, recent illness like cold, exposure to smoker, using pacifier, lack of breastfeeding, overcrowding, poor hygiene etc. So, it is expected that this study will provide basis for the control and prevention of otitis media. This study also helps to identify the antibiotics that are useful for treating otitis media and provide knowledge about otitis media and its impacts on our ear.

1.4 Limitations of the Study

1. This study was based only on certain area of Dharan. Therefore, the findings cannot be generalized for other area of Dharan.
2. The sample size used in this research is small due to the time limitation. Hence, the findings may be biased and non-conclusive.
3. Strict anaerobe and fungi which is also the causative agent of otitis media were not isolated in our study.

CHAPTER-II
LITERATURE REVIEW

2.1 Spectrum of Otitis Media (OM):

OM is a very common problem in general practice.OM is an umbrella term for a group of complex infective and inflammatory conditions affecting the middle ear. All OM involves pathology of the middle ear and middle ear mucosa. OM is a leading cause of healthcare visit worldwide and its complications are important causes of preventable hearing loss, particularly in the developing world (Monasta et al, 2012).

There are various subtypes of OM. These include acute otitis media (AOM), otitis media with effusion (OME) and chronic suppurative otitis media (CSOM). OM can be seen as a continuum/spectrum of diseases:

1. AOM is acute inflammation of the middle ear and may be caused by bacteria as well as viruses.
2. OME is chronic inflammation condition without acute inflammation, which often follows a slowly resolving AOM.
3. CSOM is long-standing suppurative middle ear inflammation, usually with persistently perforated tympanic membrane.

2.2 Pathophysiology

Dysfunction of Eustachian tube is responsible for OM. If Eustachian tube dysfunction is persistent, a negative pressure develops within the middle ear from the absorption and diffusion of nitrogen and oxygen into the middle ear mucosal cells. If it present for long enough and with appropriate magnitude, the negative pressure elicits a transudate from the mucosa, leading to the eventual accumulation of serous, essentially sterile effusion.

Because the Eustachian tube is dysfunctional, the effusion becomes a sessile medium ideal for the proliferation of bacteria and resultant OM (Thomas, 2017).

As dysfunction of Eustachian tube is responsible for OM, dysfunction of Eustachian tube can be caused by any number of circumstances from anatomic blockage to inflammation secondary to allergies, upper respiratory tract infection or trauma (Thomas, 2017).

The most common bacterial pathogen responsible for causing otitis media are *Staphylococcus aureus, Streptococcus pneumonia, Haemophilus influenza, Moraxella catarrhalis and Pseudomonas aeruginosa*. However, *Bacillus* spp., *Proteus* spp., *Escherichia coli* and *Staphylococcus epidermidis* are also involve in otitis media (Bluestone and Klein, 2001).

2.3 Epidemiology

Incidence of otitis media increases with occurrence of upper respiratory infection. Respiratory infection occurs most frequently in those with the least resistance (the very young and very old) and therefore the incidence of OM tends to increases as individual age (Ballenger, 1985). The incidence of OM in children peaks between 6 and 12 months of age with another lower peak occurring between 4 and 5 years of age. The incidence in adults is widely quoted as 0.25% per annum where smoking is a recognised risk factor.OM occurs more in the winter than in the summer months as it is usually associated with a cold (NICE CKS, 2015). According to WHO, estimates of 2015 over 5% of the world's population (328 million and 32 million children) have disabling hearing loss. 141 million people have mild hearing loss due to OM (2.1% of the population). Of 22 million visit annually to united state physician for OM, almost 4 million are by patients of 15 years old or older (Culppeper et al, 1993).

A 2012 literature review suggested that the annual global incidence of AOM is 10.85% comprising around 709 million cases per year about half in under-5s. CSOM develops in 4.76%. The authors estimated that 33 per 10 million die

due to complication of OM, mostly in developing countries and most less than 1 year of age (Qureishi et al, 2014).

2.4 Complications

While OM usually resolves uneventfully still there are a number of quite serious potential complications. Some of them are:

- **Mastoiditis**: Mastoiditis can develop if an infection spread out of the middle ear and into the area of bone underneath the ear (the mastoids). Symptoms like high fever, swelling behind the ear which pushes it forwards, creamy discharge from ear, hearing loss, etc are observed.

- **Cholesteatoma**: It is an abnormal collection of skin cells inside the ear that can sometimes develop as a result of recurring or persistent middle ear infection. If it is not treated, a cholesteatoma can eventually damage the delicate structures deep inside our ear, such as tiny bones that are essential for hearing. Weakness in half of our face, dizziness, tinnitus (hearing sound from inside their body rather than from an outside source) are the symptoms of cholesteatoma.

- **Labyrinthitis**: In some cases, an infection in the middle ear can spread into the inner ear and affect the delicate structure deep inside the ear called labyrinth. This is known as labyrinthitis.

- **Problems with speech and language development**: Frequent ear infections in Children lead to hearing loss.

- **Facial paralysis**: In very rare cases, the swelling associated with otitis media can cause the facial nerve to become compressed which lead to a person being unable to move some or all of their faces.

- **Meningitis and brain abscess**: Meningitis can occur if the infection spreads to brain or spinal cord while brain abscess is pus-filled swelling that develops inside the brain. Symptoms like severe headache, rapid breath, paralysis of one side of the body etc.

2.5 Laboratory Diagnosis

Laboratory diagnosis of Otitis media largely relies on culturing bacteria from clinical specimens. Ear swab is taken to the laboratory for testing. To detect pathogenic bacteria in clinical samples, the sample is most often cultured on Blood agar, MacConkey agar, Chocolate agar plate and Nutrient agar and then subculture on selective media. The suspicious colonies developed in media plate are further identified by biochemical test like catalase, oxidase, IMViC test etc (Fingole and Baron, 2002).

2.5.1 Cultural techniques:

Bacteria that are present in ear specimen that are responsible for OM (ear infection) should be cultured in Blood, Chocolate, Nutrient or MacConkey agar. The Blood, Nutrient and Macconkey agar plate should be incubated aerobically while Chocolate agar plate should be incubated under 5% carbondioxide atmosphere at 37°c for 24-48 hours. Then isolates are subculture on selective media. All positive cultured should be identified by their characteristic appearance on their respective media, Gram-staining reaction and confirmed by the pattern of biochemical reactions using the standard method. Gram- positive bacteria should be identified by coagulase, oxidase and catalase while Gram-negative should be indentify by IMViC test (Cheesbrough, 2006).

2.6 Prevention:

There are certain preventive measures that should be followed for controlling OM. Breast-feeding is one of the effective preventive measures against OM. Personal hygiene must be maintained and should avoid crowded area. Small-group day care for infants and toddlers and avoiding exposure to household tobacco and smoke are another preventive measure against OM. It is also useful to immunize children who have recurrent OM with influenza and pneumococcal vaccine. Antibiotic prophylaxis is the most effective method to reduce the frequency of new episodes of otitis in children with recurrent AOM, but it should be used with caution. Now, probably the best initial steps to take to prevent new episodes of otitis in children with recurrent AOM

episodes are antimicrobial treatment of each in individuals AOM episodes (Rodrigo, 1997).

2.7 Treatment

Oral and topical pain killer are effective to treat the pain caused by otitis media. Oral agents include ibuprofen, paracetamol and opiates. Topical agent shown to be effective includes antipyrine and benzocaline ear drops. It is important to weigh the benefits and harms before using antibiotics for acute otitis media. Antibiotics should be prescribed for severe bilateral or unilateral disease in all infants and children with severe sign and symptoms such as moderate to severe ear pain and high fever. The first line antibiotic treatment, if warranted is Amoxicillin. If there is resistance or use of Amoxicillin in the last 30 days then Amoxicillin- Clavulanate or another penicillin derivative plus beta lactamase inhibitor is recommended. Amoxicillin taken for twice or thrice a day is effective. While less than 7 days of antibiotics have less side effects, more than 7 days appear to be more effective. If there is no improvement after 2-3 days of treatment a change in therapy may be consider (Lieberthal et al, 2013).

A Cochrane review found that topical quinolone antibiotics can treat chronic suppurative otitis with discharge better than oral antibiotics (Macfadyen et al,2006).

Tympanostomy tube are recommended in those people who have 3 or more episodes of acute otitis media in 6 months or four or more in a year, with at least one episode or more attacks in the preceding 6 months. Complication of having a tympanostomy tube is otorrhea, which is discharge from ear. Oral antibiotics shouldn’t be used to treat uncomplicated acute tympanostomy tube otorrhea. Topical antibiotic ear drops can treat this condition (Lieberthal et al, 2013).

CHAPTER-III
Materials and Methodology

3.1 Study Design

The study is a laboratory based cross-sectional study. The samples were taken as per the convenience of the study.

3.2 Study Area/ site

The samples were taken from individuals of different ages and were students from schools, campus and workers of CCT of Dharan. The study was conducted in the Laboratory of Department of Microbiology, Central campus of Technology, Dharan.

3.3 Study population

The study population included were individuals of different age groups and were students and workers of schools and campus respectively.

3.4 Sample size

Altogether 50 ear swabs were collected from different age group of population.

3.5 Sample collection

Ear swab of 50 people were collected using a sterile swab stick from the ear of people complaining of ear problems. During sample collection, precautions were taken by wearing gloves, laboratory coat and masks. Then, the sterile cotton swab was inserted carefully in the ear and the ear wax was collected on the swab. Each sample was labelled with code number and various other information including age, sex, address, person's name etc were also recorded.

3.6 Transportation of the sample

The aseptically collected sample was kept in the sterile nutrient broth and was transported to the laboratory for further processing as fast as possible.

3.7 Sample processing

The specimens were processed by culturing the sample on selective media for possible pathogens and by performing antibiotic susceptibility test. The samples were inoculated on nutrient agar plate for 24 hours at 37°C. Isolated bacteria were further sub-culture on Blood agar plate at 37ºC for 24 hours. Then, the isolated bacteria was identified by colonial appearance, gram reaction, cultural characteristics and biochemical test like Catalase, Oxidase, IMVIC test and Coagulase test. Discrete colonies of isolates were gram stained on the slides and were examined for detecting whether the particular bacteria were gram-positive or gram-negative. Gram positive bacteria were further identified by performing biochemical test: Coagulase test, Catalase test and Oxidase test whereas gram-negative were identified by IMViC test. Procedure of above mention tests are present in appendix iv.

3.8 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of the isolates was performed by Kirby Bauer Disc diffusion method according to Clinical Laboratory Standard Institute (CLSI)**.**

From a culture, colonies of bacteria was taken and transferred to a tube containing 5 ml of nutrient broth and mixed gently until a homogenous suspension was formed and incubated at 37°C for 24 hours. With a sterile swab the bacterial suspension was evenly spreaded over the entire surface of Muller-Hinton agar plate. The inoculated plates were left at room temperature to dry for 3-5 minutes and discs of following antibiotics such as Ciprofloxacin, Azithromycin, Gentamicin, Ceftazidime, Chloramphenicol, Cotriomoxazole, Imipenem and Ampicillin were placed on the surface of the inoculated MHA plate. After overnight incubation at 37ºC, the diameter of the zone of inhibition was measured around each disc. By referring to the standard, a qualitative report of susceptibility or resistance against antibiotics was found.

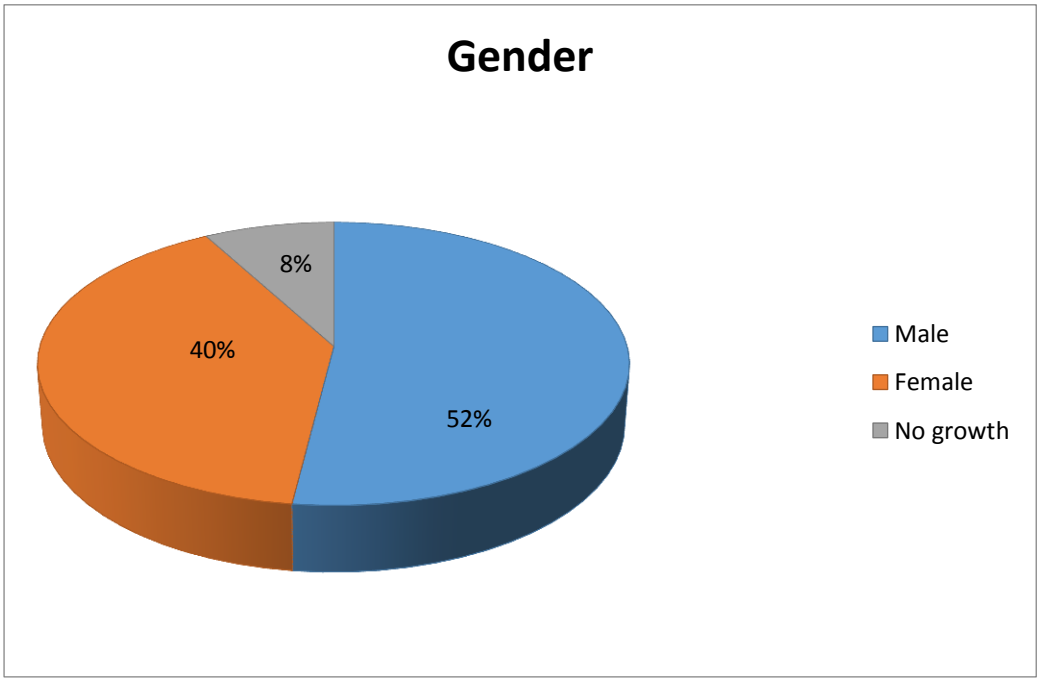
CHAPTER-IV

RESULT

4.1 Gender wise study population

Out of 50 ear swab samples, 26(52%) male samples showed the growth and only 20(40%) female samples showed the growth whereas 4 samples (8%) of female didn't show the growth.

Figure 1: Sex wise study population



4.2 Different isolated bacteria

Out of 50 samples, pathogenic bacteria were isolated from 46(92%) samples and 4 samples didn't show growth.

Table 1: Distribution of isolated organisms from ear swab samples

Organisms	No. of organisms	Frequency (%)
Staphylococcus epidermidis	19	38
Staphylococcus aureus	13	26
Bacillus spp.	9	18
Pseudomonas aeruginosa	3	6
Haemophilus spp.	1	2
Moraxella spp.	1	2

4.3 Culture positive and culture negative

Out of 50 samples, 46 showed growth and 4 samples were sterile.

Table 2: Distribution of culture positive and culture negative

Total sample	No. of positive culture	No. of negative culture
50	46	4

4.4 Distribution of bacteria in different ages of individuals.

Staphylococcus epidermidis was the highest bacteria isolated in all three aged group i.e children, adults and old people and the bacteria isolated in least number was *Haemophilus* spp. and *Moraxella* spp.

Table 3: Bacteria isolated in different aged group

Organisms	In children(no.)	In adults(no.)	In old people(no.)
Staphylococcus epidermidis	9	5	5
Staphylococcus aureus	6	3	4
Bacillus spp.	3	3	3
Pseudomonas aeruginosa	3	-	-
Haemophilus spp.	1	-	-
Moraxella spp	1	-	-

4.5 Age wise distribution of samples

Large number of bacteria was observed in children of aged group (5-10) comparing to adults and old people.

Table 4: Age wise distribution of the study population

Age group	No. of sample	No. of female	No. of male	No. of positive culture	No. of negative culture
<15	18	9	9	18	0
15-30	16	7	9	14	2
>30	16	8	8	14	2

Out of 50 samples, colonies were observed only in 46 samples in nutrient agar plate after incubation for 24 hours which was further subcultured on Blood agar. Gram-staining was performed from the colonies obtained in the plate. On Gram-staining, 41samples were Gram-positive however 32 were cocci shaped and 9 were rod shaped. Whereas, 5 were Gram-negative (4 were rod whereas 1 was cocci shaped).Further confirmation was made by performing biochemical test like catalase, oxidase and IMViC test. Thus, the most pathogenic bacteria were isolated in large number from the children of age 5-10 compared to other age group.

4.6 Biochemical test of isolated bacteria

Table 5: Biochemical test of Gram-positive bacteria

Bacillus spp.		
Catalase	**Oxidase**	**Starch hydrolysis test**
+	+	+

Staphylococcus aureus			***Staphylococcus epidermidis***		
Catalase	**Oxidase**	**Coagulase**	**Catalase**	**Oxidase**	**Coagulase**
+	-	+	+	-	-

Table 6: Biochemical test of Gram-negative bacteria

Moraxella spp				***Pseudomonas aeruginosa***			
Indole	**MR**	**VP**	**Citrate**	**Indole**	**MR**	**VP**	**Citrate**
-	-	-	-	-	-	-	+

Haemophilus spp.				
Indole	**MR**	**VP**	**Citrate**	**Urease**
-	**-**	**-**	**-**	**-**

4.7 Antibiotic Susceptibility Pattern:

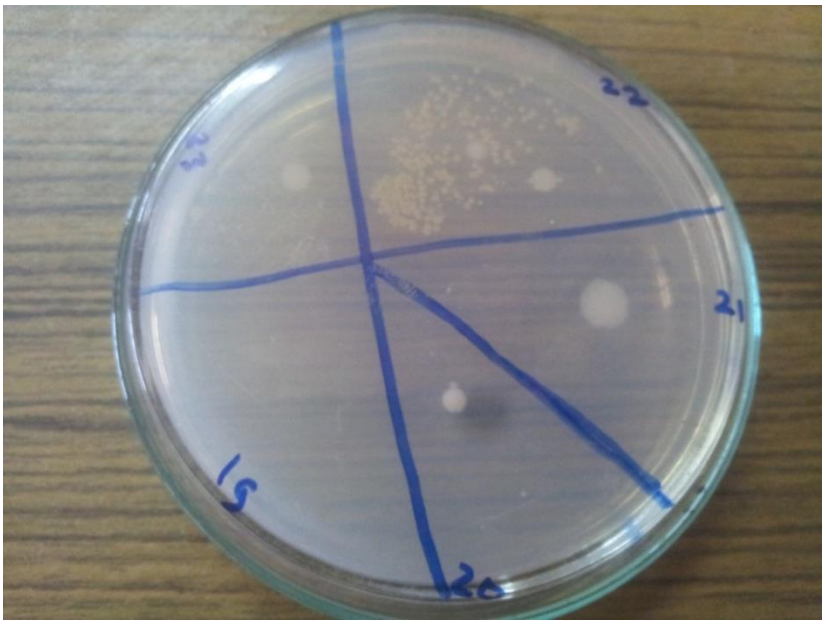
Both Gram-positive and Gram-negative bacteria were resistant to Ampicillin whereas most of the bacteria showed sensitivity towards antibiotics like Gentamicin, Chloramphenicol, Imipenem and Ciprofloxacin.

Table 7: AST of Gram-positive bacteria

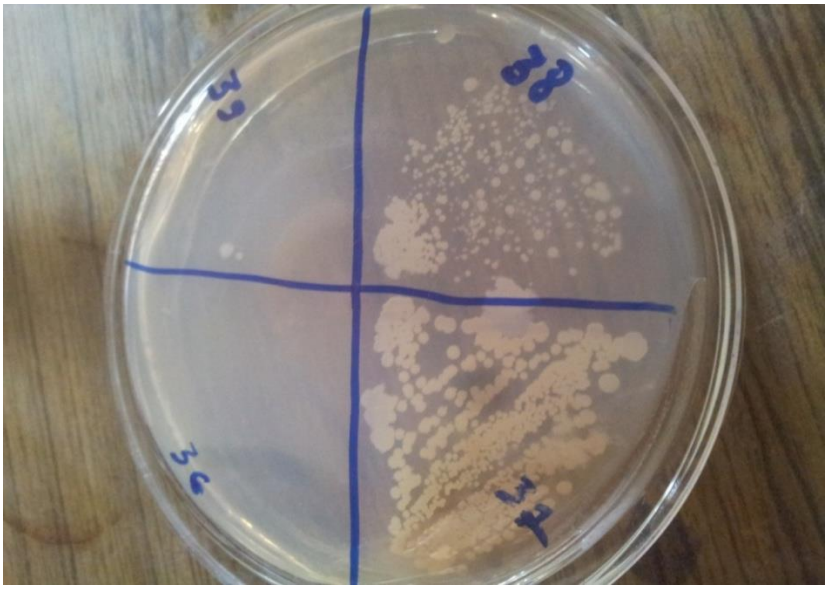
Antibiotics	***Staphylococcus aureus* (no=13)**		***Staphylococcus epidermidis* (no=19)**		***Bacillus* spp. (no=9)**	
	S%	R%	S%	R%	S%	R%
Azithromycin	13(100%)		8(47.62%)	11(52.38%)	9(100%)	
Gentamicin	13(100%)		19(100%)		9(100%)	
Ceftazidime		13(100%)		19(100%)		9(100%)
Chloramphenicol	13(100%)		19(100%)		9(100%)	
Cotriomoxazole	13(100%)		19(100%)		9(100%)	
Ampicillin		13(100%)	3(23.81%)	16(76.19%)		9(100%)
Imipenem	13(100%)		19(100%)		9(100%)	
Ciprofloxacin	13(100%)		19(100%)		9(100%)	

Table 8: AST of Gram-negative bacteria

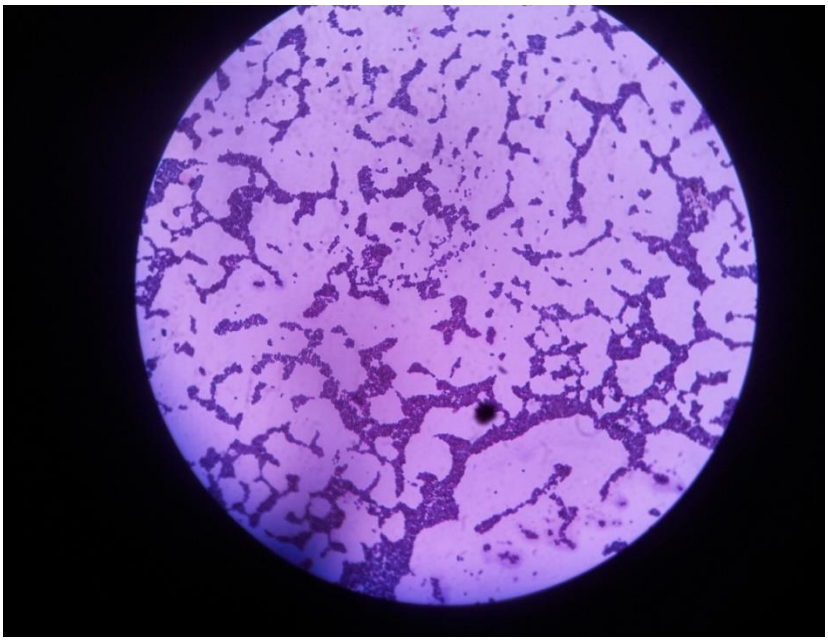
Antibiotics	***Pseudomonas aeruginosa* (no=3)**		***Haemophilus* spp. (no=1)**		***Moraxella* spp. (no=1)**	
	S%	R%	S%	R%	S%	R%
Azithromycin	3(100%)		1(100%)		1(100%)	
Gentamicin	3(100%)		1(100%)		1(100%)	
Ceftazidime	3(100%)		1(100%)			1(100%)
Chloramphenicol	3(100%)		1(100%)		1(100%)	
Cotriomoxazole		3(100%)	1(100%)		1(100%)	
Ampicillin		3(100%)		1(100%)		1(100%)
Imipenem	3(100%)		1(100%)		1(100%)	
Ciprofloxacin	3(100%)		1(100%)		1(100%)	



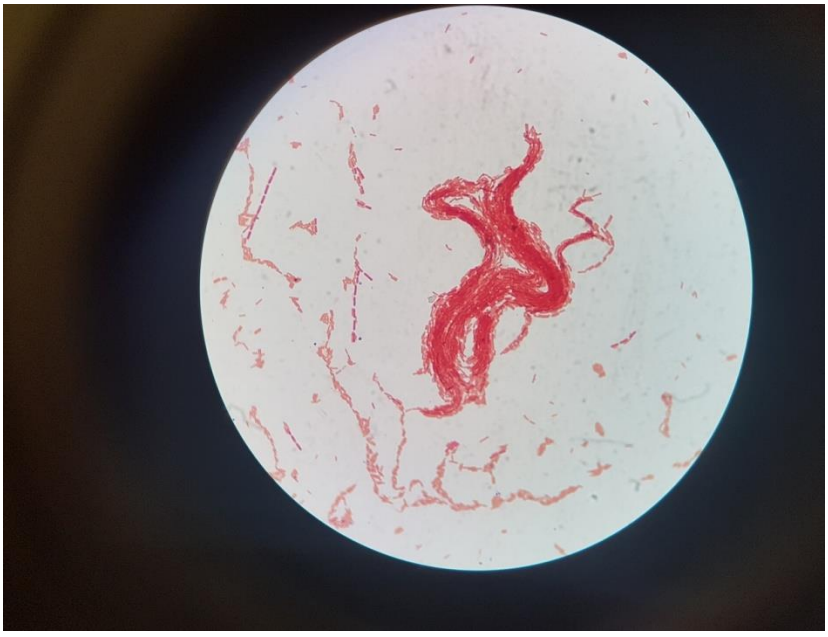
Photograph 1: Growth of bacteria from the sample of adult's ear on NA



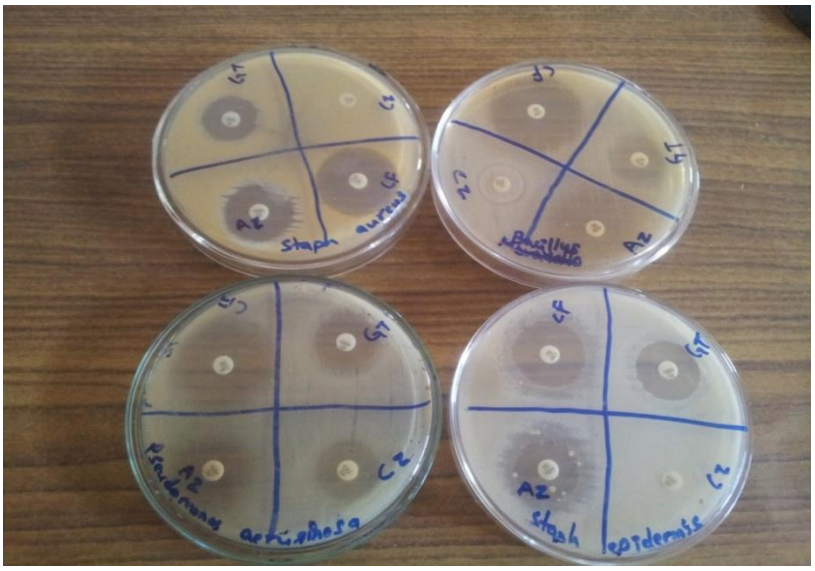
Photograph 2: Growth of bacteria from the sample of children ear on NA



Photograph 3: Gram-positive bacteria (coccus) under microscope

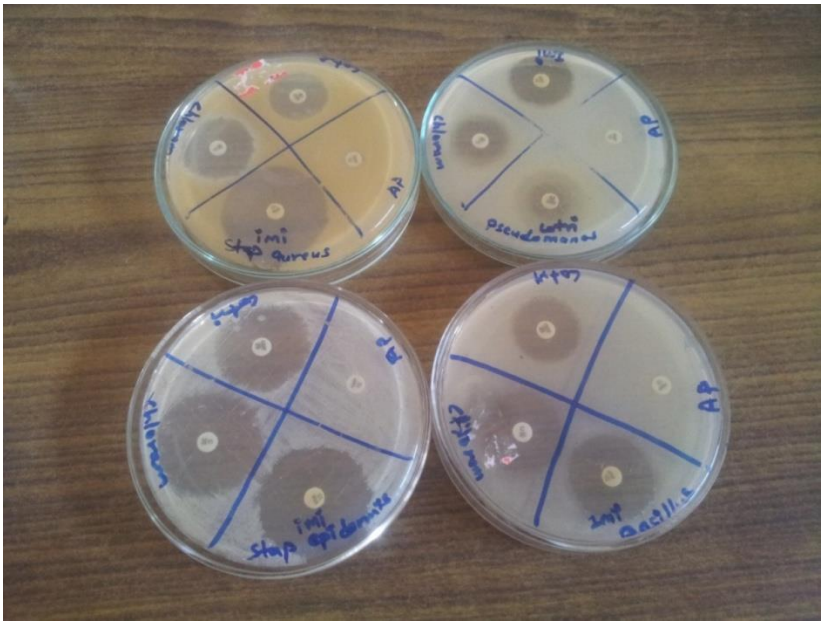


Photograph 4: Gram-negative bacteria (rod) under microscope



Photograph 5: AST of isolated bacteria

Note: GT- Gentamicin, CZ- Ceftazidime, CF- Ciprofloxacin, AZ- Azithromycin



Photograph 6: AST of isolated bacteria

Note: AP-Ampicillin, Imi- Imipenem, Cotri- Cotriomoxazole, Chloram- Chloramphenicol

CHAPTER-V

DISCUSSION

This study was conducted to identify different bacteria from the ear swab of individuals in the laboratory of microbiology department, Central Campus of Technology, Dharan. Altogether, 50 ear swab samples were collected and were subjected to standard microbiological procedure. The identified bacteria were tested for their sensitivity and resistivity towards a group of individual's antibiotics in a standard MHA plates.

In this study, out of 50 ear swab sample, 46 (92%) showed growth and 4 (8%) of the ear swab sample did not show growth. The percentage of growth was very high in the ear swab sample. This indicates that there are many normal flora in our ear and many of them are risk factors and causative agent of otitis media. These same isolated bacteria can cause severe problems related to ear in many individuals. Out of 50 individuals, 26 were the male individuals and 24 were female individuals. The culture showed growth (100%) from every male ear samples whereas only 20 (83.33%) showed growth and 4 (16.66%) did not show growth. This study shows high growth in male and low growth in female, which may be due to different reasons like different lifestyle of male and female. Also male individuals are exposed frequently in the different working environment compared to female individuals which obviously has the high chance of bacterial occurrence in the male. Although, the findings doesn't match with the CDC report which states that there is no any statistical significance between male and female with regards to otitis media. (Schappert, 1992) but matched with the finding of Klein which states that male gender are at high risk factor for otitis media (Klein et al,1994).

In our study, 46(92%) samples showed the growth in which different bacteria were isolated like *Staphylococcus epidermidis, Staphylococcus aureus, Bacillus* spp., *Pseudomonas aeruginosa, Moraxella* spp. *and Haemophilus* spp. The highest isolated bacteria was *Staphylococcus epidermidis* (38%) and the least isolated bacteria was *Haemophilus* spp. and *Moraxella* spp. (2%

each).Other isolated bacteria were *Staphylococcus aureus* (26%),*Bacillus* spp.(18%) and *Pseudomonas aeruginosa*(6%). Most of the bacteria isolated in our study were normal flora of our body which indicated that normal flora can also causes problem regarding ear if proper hygiene isn't maintained, this finding matched with the finding of Abera and Kibert which states that normal flora of the skin can easily enter through perforated ear that lead to ear infection (Abera and Kibert, 2011). Since *Staphylococcus epidermidis* was in large number comparing others in our finding which matched with the finding of Sunder et al which states that *Staphylococcus epidermidis* was the most prevalence bacteriological agent in otitis media (Sunder et al, 2009). But according to Lieberthal et al, the most predominant bacteria were *Haemophilus influenza* and *Moraxella* catarrhalis which did not matched with the study and this may be due to the differences of bacteria in different individuals according to their habit and habitat (Lieberthal et al, 2013).

Our study showed that large number of bacteria were isolated from children (36%) as compare to adult and old people (32% each) which indicates that children were more prone to otitis media which may be due to their shorter Eustachian tube, lack of breastfeeding and use of pacifier. According to Bluestone and Klein, Otitis media is more severe in children due to their shorter Eustachian tube and this tube is made up of more cartilage which can impair its opening and infecting organisms reached the middle ear from the nasopharynx (Bluestone and Klein, 2001) and this particular finding correlate with our study, in which all the samples taken from the children showed the growth of bacteria responsible for otitis media.

Antibiotics susceptibility pattern of bacteria isolated during our study were carried out in which the susceptibility and resistivity of isolated bacteria were checked according to Clinical Laboratory Standard Institute. According to our study, almost all bacteria showed the resistant towards Ampicillin (100%) which indicated that this antibiotic didn't contain antibacterial properties toward the isolated bacteria. However, antibiotics like Ciprofloxacin, Gentamicin, Chloramphenicol and Imipenem showed 100% sensitivity towards isolated bacteria which indicated that these antibiotics contain antibacterial properties towards isolated bacteria and our study correlate with

the study of Araya and Yilikal which state that Ciprofloxacin and Gentamicin showed sensitivity towards most bacteria that are responsible for otitis media.

Otitis media is not spread from person to person. Sign and symptoms like pain in ear, fever, difficulty in hearing, irritability, sore throat, neck pain, nasal congestion and discharge, headache,or other noise in the ear (Lieberthal et al, 2013).

Predisposing factor of otitis media include lack of breastfeeding, attendant of day care, exposure to tobacco and smoke, use of pacifier, craniofacial abnormalities example: Down's syndrome, cleft palate, overcrowding, poor hygiene, inadequate healthcare, etc (Minovi and Dazert, 2014).

Hence, otitis media should be cured as soon as possible as untreated condition leads to complications like meningitis, brain abscess, hearing impairment, etc.

CHAPTER-VI

CONCLUSION AND RECOMMENDATION

6.1 Conclusions

Different bacterial spp. were isolated and identified from the ear swab sample from individuals suspected from Otitis Media i.e. middle ear infection. Different bacteria identified are the proof that these sorts of bacteria are responsible for causing middle ear infection in many individuals. Otitis media thus can be prevented by simple routine inspection. Many reports of ear problems go unchecked and thus leads to great loss of health related to ear. The bacteria identified showed resistance to many antibiotics used and those sorts of antibiotics should not be used in the clinical treatment purpose. Only the correct diagnosis and correct treatment of otitis media is a safer practice to get rid of these problems and routine screening of asymptomatic as well as symptomatic ear problems is needed.

6.2 Recommendation

- Different awareness related to ear complication will be useful to the individuals, thereby preventing the likelihood of ear problems and infection.
- People must follow personal hygiene of the ear as most of the bacteria isolated were from unhygienic ears.
- Routine general ear inspection of the individuals should be followed to prevent the infections of different bacteria associated with the ear.
- Routine screening of ear related problems must be done by all individuals to minimize the risk of infection of ear.

REFERNCES

Abera B, Kibert M(2011).Bacteriology and antimicrobial susceptibility of otitis media at desire regional health research laboratory, Ethiopia, Ethiopian J Health Develop.25(2):161-167

Adderson EE (1998).Preventing otitis media: Medical Approaches.Pediatric Annals, Mekelle University, Northern Ethiopia 27:101-107

Akinpelu OV, Amusa YB, Komolafe EO, Adeolu AA, Oladele AO, Ameye SA (2008).Challenges in management of chronic suppurative otitis media in a developing country.Laryngol otol.122(1):16-20

Araya Gebereyesus Warihun and Yilikal Zemene(2015).Bacterial profile and Antibiotics susceptibility Patterns of Otitis media in Ayder Teaching and Referral Hospital.4:701

Ballenger, J.J (1985).Diseases of Nose, Throat, Ear, Head and Neck.Philadelphin:Lea and Febiger.13:98-99

Bluestone CD, Klein JO(2001).Otitis media in infants and children.Philadelphia.58-78

Cheesbrough M (2006).District Laboratory practice in tropical countries part II .2.New York: Cambridge University Press.

Cripps AW, Kyd J (2003). Bacterial otitis media:current vaccine development strategies.Immunol Cell Biol,81:46-51

Culpepper L, Froom J, Bartelds AL, Bowers P, Bridges-webbc, Grob P, Grava-Gubins C,Green L, Lion J, Rosser W, et al(1993).Otitis media in adults.9:55-56

Etzel RA (1987). Smoke and ear effusions.Pediatrics.79 (2):309-311

Fingole SM, Baron EJ, Baily S (2002).diagnostic microbiology. Tornto, St-Louis:m Moby Company, 10:150-170

GBD 2013 Mortality and causes of Death, Collaborators (17 Dec 2014). Global, regional and national age-sex specific all cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease study 2013 .Lancet. 385(9963):117-71

Jansen AG,HK E. Veenhoven RH, Damoiseaux RA, Schilder AG, Sanders EA (2009). Jansen AG, ed.Pneumococcal conjugates vaccines for preventing otitis media. Cochrane Database system Rev, 2:48-49

Klein JO, Tos M,Casselbrant ML, Daly KA, Karma P et al.(1994).Epidemiology and Natural History.Annuals of Otolaryngology, Rhinology and Laryngology.103(Suppl 164):9-12

Kumar H, Seth S (2011).Bacterial and fungal study of 100 cases of chronic suppurative otitis media. J Clin Diagn Res, 5:1224-1227

Leach AJ, Morris PS (2006).Leach AJ.ed. Antibiotics for the prevention of acute and chronic supporative otitis media in children. Cochrane Database system Rev, 4:44-45

Lieberthal, AS; Carroll, AE;Chonmaitree, T;Ganiatis,TG; Hoberman, A; Jackson, MA; Joffe, MD; Miller, DT; Rosenfeld, RM; Sevilla, XD; Schwartz, RH; Thomas, PA; Tunkel, DE(Feb 25,2013).The Diagnosis and Management of Acute Otitis Media.Pediatrics.131(3): 64-99.

Macfadyen CA, Acuin JM, Gamble C(2006)Systemic antibiotics versus topical treatment for chronically discharging ears with underlying eardrum perforation [review] Conchrane Database Syst Rev,1:56

Minovi, A; Dazert, S(2014). Disease of the middle ear in childhood.GMS current topics in otorhinolaryngology head and neck surgery, 13:123-124

Monasta, L: Ronfani, L; Marchetti, F; Montiw,M; Vecchi Brumatti, L; Bavcar, A; Grasso, D; Barbiero, C; Tamburlini, G(2012). Burden of disease caused by otitis media: systematic review and global estimates.PLOS ONE.7(4):36

NICE CKS (March 2015).OM-acute.28:33

Qureishi, A; Lee, Y; Belfield, K; Birchall, JP; Daniel, M (10 Jan 2014).Update on otitis media-Prevention and treatment. Infection and drug resistance, 7:15-24

Rodrigo C. Prevention of acute otitis media. Clin microbial infection (online) 1997 Jun: S55-S58 [viewed 02 November 2014] Available from http://www.ncbi.nim.nih.gov/pubmed/11869231

Rossolini GM, Mantengoli E, Docquier JD, et al (2007).Epidemiology of infections caused by multiresistant gram-negative: ESBLs, MBLs, panresistant strains. New Microbiol,30:332-339

Rovers MM, Schilder AG, Zielhuis GA, Rosenfeld RM(2004). Otitis media. Lancet. 363(9407):564-573

Saunder JE, Raju RP, Boone J, Berryhill W(2009), Current Bacteriology of Suppurative Otitis: Resistant Patterns and Outcomes Analysis, Otology and Neurology,30(3):339-343

Schappert,SM(1992). Office visits for Otitis media: United States 1975-1990.Advance Data from vital Health Statistics of the CDC/ National Center for Health Statistics ,Washington DC, US Department of Health and Human Services,214:3-18

Survey on prevalence of deafness and ear diseases (1992). Tribhuvan University Teaching Hospital and British Nepal otology society.1:26-27

Stool SE, Berg AO, Bermans, Carney CJ, Cooley JR et al(July 1994). Managing Otitis Media with Effusion in Young Children . Quick Reference Guide for clinicians. AHCPR Publication No.94-0623, Rockville MD: Agency for the Health Care Policy and Research, Public Health Services, US Department of Health and Human Services,1:1-13

Thomas S Higgins, JR, MD, MSPH (April 25, 2017). Rhinologist and Associate in Otolaryngology- Head and Neck Surgery, Kentuckiana Ear, Nose and Throat,1:17

Woodfield G, Dugdale A(2008). Evidence behind the WHO guidelines: hospital care for children.What is the most effective antibiotic regime for chronic suppurative otitis media in children?J Tropical Pediatric 54(3):151-156

APPENDICES

Appendix-i

Material used

Equipments

1. Refrigerator
2. Incubator
3. Hot air oven
4. Autoclave
5. Microscope
6. Burner

Glasswares and others

1. Test tubes
2. Conical flask
3. Beakers
4. Petriplates
5. Glass rods
6. Slides
7. Disposal Gloves

Appendix-ii

Media compositions and preparation techniques

1. Nutrient agar media

Ingredient	Gm/l
Peptone	5gm
Sodium chloride	5gm
Beef extract	1.5gm
Yeast extract	1.5gm
Agar	20gm
PH	7.2

2. Muller Hinton agar media

Ingredients	Gm/l
Beef extract	2gm
Acid hydrolysis of casein	17.5gm
Starch	1.5gm
Agar	17gm
PH	7.3gm

3. Blood agar media

Ingredients	Gm/l
Pancreatic Digest of Casein	15gm
Peptic Digest of Soybean Meal	5gm
Sodium chloride	5 gm
Sheep Blood	50 ml
Agar	12 gm

Appendix-iii

Antibiotic susceptibility test (AST):

Standardized inoculums of bacteria were swabbed on the surface of a Muller Hinton agar (MHA) plate. Filter paper disc impregnated with antimicrobial agents such as Ciprofloxacin, Ceftazidime, Azithromycin, Gentamicin, Chloramphenicol, Imipenem, Ampicillin and Cotriomoxazole were placed on the agar. After overnight incubation, the diameter of the zone of inhibition was measured around each disc. By referring to the standard, a qualitative report of susceptible or resistance was obtained.

Appendix-iv

Biochemical tests

Catalase test

1. A small amount of bacterial colony was transferred to a surface of clean, dry glass slide using a sterile loop.
2. A drop of 3%$H_2O_2$was placed on the slide and mixed and small bubbles were observed.

Oxidase test

1. Filter paper soaked with tetramethyl-p-phenylenediamine dihydrochloride was taken.
2. The paper was moistened with sterile distilled water.
3. The colony to be tested was picked with the platinum loop and was smeared in the filter paper.
4. Inoculated area of paper was observed for a colour change to deep blue or purple within 10-30 second.

Coagulase test

1. A drop of plasma was placed in the clean slide and the colony to be tested was mixed with plasma with sterile needle.
2. Clumping of cocci within 5-10 seconds was observed which indicated the coagulase positive bacteria.

Indole test

1. Tryptone broth was prepared.
2. One of the test tube containing tryptone broth was inoculated with the suspected colony and another test tube containing tryptone broth was uninoculated which was kept as control.
3. Both tubes were incubated at 35°C for 48 hours.
4. After 48 hours of incubation, 1ml of kovac's reagent was added.
5. Red layer at the top of the tube was observed.

MRVP test

1. MRVP broth was prepared.
2. Two test tube containing MRVP broth was inoculated with the bacterial colony and incubated for 48 hours at 35°C.
3. Methyl red indicator was added in one of the test tube and Barritt's A and Barritt's B was added in another test tube.
4. Change of colour to red was observed.

Citrate test

1. Simmon's citrate agar was prepared.
2. Slant was prepared in the test tube using Simmon's citrate agar media.
3. The desire colony was inoculated in the slant and incubated for 48 hours at 37°C.
4. Change of green media to blue was observed for positive result.