"BACTERIOLOGICAL STUDY OF CHRONIC SUPPURATIVE OTITIS MEDIA WITH REFERENCE TO AEROBES"

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ENDORSEMENT BY THE HOD, PRINCIPAL / HEAD OF THE INSTITUTION

This is to certify that the Dissertation entitled "**BACTERIOLOGICAL STUDY OF CHRONIC SUPPURATIVE OTITIS MEDIA WITH REFERENCE TO AEROBES**" is a bonafide research work done by the Candidate having Reg. No. BI0108002.

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LIST OF ABBREVATIONS USED

ARF	–	Acute renal failure
BIIS	–	Bacteriocin like inhibitory substance
CAP	–	Community acquired pneumonia
CLSI	–	Clinical and Laboratory Standard Institute
CSOM	–	Chronic Suppurative otitis media
dB	–	Decibels
DM	–	Diabetes Mellitus
DNS	–	Deviated Nasal Septum
ESBL	–	Extended Spectrum Beta Lactamase
GNB	–	Gram Negative Bacteria.
GPC	–	Gram Positive Cocci
HIV	–	Human Immuno Deficiency Virus
HTN	–	Hypertension
MIC	–	Minimum inhibitory concentration
MRSA	–	Methicillin Resistant Staphyloccus aureus
MSSA	–	Methicillin Sensitive Staphylococcus aureus
NOGC	–	No Organisms Grown in Culture
PCR	–	Polymerized Chain Reaction
PGE	–	Prostaglandin E
PDDT	–	Potentiated disc diffusion test
RFLP	–	Restriction Fragment Length Polymorphism
Spp.	–	Species
TM	–	Tympanic Membrane
URTI	–	Upper Respiratory Tract Infection

ABSTRACT

Background :

Chronic suppurative otitis media (CSOM) is a disease of multiple etiology and is well known for its persistence and recurrence in spite of treatment. Its importance lies in its refractoriness to treatment and chronicity leading to complications. CSOM is most likely a result of incomplete or unsuccessful treatment. Aerobic bacteria either in single or mixed culture and also resistant strains like MRSA and ESBL producers are responsible for most of CSOM cases. Indiscriminate use of antibiotics leads to resistance and poor follow up have resulted in persistent low grade infection, refractoriness to treatment, complications of CSOM and postoperative complications. Hence knowledge of microbacteria and sensitivity pattern is necessary in all CSOM cases for better outcome.

Objectives :

To isolate and identify the aerobic bacterial flora of CSOM and to study the antibiotic sensitivity pattern of isolates with special reference to MRSA and ESBL production and follow up of patients after antibiotics susceptibility testing and to record the response to therapy.

Methodology :

Clinically diagnosed new cases of CSOM (tubotympanic) of all age groups and both sexes attending ENT Out Patients Department at KLE's DR.Prabhakar Kore Hospital and MRC,Belgaum. Patients with otorrhoea more than two months duration are taken by random sampling technique. Two ear swabs collected from each patient studied with gram stain, culture and morphology of colony and identification of organism by standard methods. Antibiotic susceptibility done in all cases. MRSA and ESBL production detected by using standard methods.

Results :

Ninety nine CSOM patients and their isolates were studied for a period of one year. Majority of patients were in the age group of 11-20years,56.4% were males and 43.4% were females. Majority were from rural areas (72.7%) and unilateral infection (76.8%) was more common than bilateral.Right side (40.4%) was more commonly affected than left. Maximum number of cases were seen during the month of November to February (44.4%). Gram stain showed pus cells and organism (78.8%) of isolates.

Total number of gram negative (41.4%) isolates were more than gram positive (31.3%) bacteria. *S.aureus* (29.3%) was the common organism isolated followed *by P.aeruginosa* and *S.aureus + P aeruginosa* (21.4%) was the common mixed isolate. Among *S.aureus,* MRSA isolated in 51.7% and among gram negative bacteria 31.7% ESBL producers were isolated. *S.aureus* was sensitive to Gentamicin (72.4%), Amoxyclav (66.5%) and Ciprofloxacin(44.8%) and sensitivity to Ampicillin(27.6%) and Erythromycin(35.5%) was less. *P.aeruginosa* and other gram negative bacteria were sensitive to Amikacin(70-100%),Cefotaxime(50-100%),Amoxyclav(50-100%) and Levofloxacin(68-100%). Sensitive to Erythromycin (16-50%) was observed less but sensitive to Ciprofloxacin was moderate(50-60%). Sensitivity pattern of mixed cultures was varied (but most of them were sensitivity to Amikacin,Cefotaxime and Levofloxacine than Ampicillin and Erythromycin). MRSA showed moderately sensitivity to Gentamicin,Ciprofloxacin and Amoxyclav and resistant to Ampicillin,Erythromycin and Ceftazidime. Amomg ESBL producers were sensitive to Amikacin and Levofloxacin, but resistant to Erythromycin and Cefotaxime (moderatively sensitivity to Amoxyclav and Ciprofloxacin).

Combinations of MRSA + ESBL isolates were resistant to all drugs, MRSA+Non -ESBL isolates were variable in sensitivity.Only ESBL isolates shows resistant to third generation Cephalosporins (Ceftazidime and Cefotaxime) but sensitivity to Amikacin,Levofloxacin and Amoxyclav (for Ciprofloxacin and Erythromycin variable sensitivity were seen).

Conclusion :

Commonest isolate of CSOM was *S.aureus* followed by *P.aeruginosa*. Among *S.aureus* 50% of them were MRSA and mixed isolates were also seen significantly. Single isolates were resistant to routine antibiotics but susceptible to newer antibiotics like Amikacin, Levofloxacin and Cefotaxime. Majority of MRSA were resistant to most drugs and ESBL producers resistant to third generation Cephalosporins. Mixed cultures of MRSA and ESBL showed varied sensitivity pattern. So isolation of bacteria and study of sensitivity pattern is necessary in all the CSOM cases for better patient out come in both pre and postsurgical cases and also in preventing drug resistance.

Keywords : Aerobic bacteria; MRSA; ESBL; Sensitivity

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INTRODUCTION

Chronic suppurative otitis media (CSOM) is one of the commonest ear disease in which there is a chronic inflammation of middle ear and mastoid, and in which the infection is present beyond six weeks because of non- intact tympanic membrane. It is a disease of multiple etiology and is well known for its persistence and recurrence inspite of treatment. Its importance lies in its refractoriness to treatment and chronicity, leading to complications. [1,2,41]

Incidence of CSOM is increasing during the past 10-20 years. The disease prevalence depends on race and socioeconomic factors like poor living conditions, over crowding, poor hygiene and nutrition.[1,3,4,56]

Chronic suppurative otitis media is most likely a result of incomplete or a unsuccessfully treated acute otitis media.

CSOM was found to be the single major cause for conductive deafness (66-3%) and it is also responsible for 1.5% of speech disorders.[4]

Hearing loss associated with CSOM leads on to educational backwardness in children that is well recognized by Otologists, Paediatricians and Educators. Development of speech, language and learning skills are severely hampered in these children making it difficult for them to achieve full academic potentials outdoor activities are also hampered.[5]

Aetiology of chronic suppurative otitis media is complex. Aerobic bacteria, anaerobic bacteria and fungus are the etiological agents responsible for CSOM. Aerobes are responsible for 71% of isolates. Mixed infections of aerobes, anaerobes and fungi are also seen. Among aerobes *Staphylococcus aureus* is most commonly isolated followed by *Pseudomonas spp.* and *Klebsiella spp.*[6,7]

Indiscriminate, haphazard and half hearted use of antibiotics and poor follow up of the patients have resulted in persistant low grade infection and development of bacterial resistance.[8,9]

It is well known fact that complications are the direct result of failure of body defence mechanism and giving disease, a chance to spread. With the advent of antibiotics and awareness regarding the disease and availability of technology, the incidence of complications has decreased from 2.3% to 0.04%.[10]

The study of microorganisms commonly associated with CSOM and their invitro antibiotic sensitivity pattern is very pertinent for the clinician to plan a general outline of treatment for the patient with a chronically discharging ear.

Identification and detection of MRSA and ESBL producers is also important before treatment of CSOM cases. As cases with these infection are resistant to routiene Beta-lactum antibiotics and Penicillin. They require new drugs like Vancomycin and Linazolid.

Isolation of these bacteria either in single or mixed infection require special techniques.In unusual resistant pattern suspicion of injection with MRSA and ESBL producers is essential.[39,103]

Knowledge of local microrganism pattern and their antibiotic sensitivity pattern is essential for effective low cost treatment. [10]

Changing flora of CSOM and emergence of strains resistant to the commonly employed antibiotics stimulated the study. The present work deals with the aerobic bacteriological study of CSOM to identify and categorise various organisms isolated and to evaluate their sensitivity pattern and to assess whether there has been any change in the causative organisms identified by the previous studies.

OBJECTIVES

1. To isolate and identify the aerobic bacterial flora responsible for chronic suppurative otitis media.
2. To study the antibiotic sensitivity pattern of isolates with special reference to Methicillin Resistant *Stophylococcus aureus* (MRSA) and Extended Spectrum β-lactamase (ESBL) producers for Gram negative isolates.
3. Follow up of patients after antibiotic susceptibility testing and record the response to therapy.

REVIEW OF LITERATURE

History :

Discharging ear and cholesteatomous destruction has been known to occur since 2600 years. Various mysterious beliefs fear about discharging ear which can be known by the statements of Hippocrates, "Acute pain in the ear, with continued strong fears is to be dreaded for there is danger that man may become delirious and die".

"Papyrus Ebears of Egyptian origin and written around 1550 BC, is the first medical writing in which prescription of aural discharge have been described. Olive oil had been recommended for use in aural discharge in the Egyptian mummies with perforations of tympanic membrane and mastoid destruction.

Writing in Athervana Veda (700 BC) and in the writing of the great Hindu Physician and Surgeon in his treatise "Sushruta samhita" written around 500 BC showed that our ancestors had knowledge of this ear disease.

The Greak Philosopher and the Father of Medicine, Hippocrates, was probably the first to inspect tympanic membrane.He thought that otorrhoea was secondary to cerebral abscess.Later Morogni showed that suppuration was the primary lesion and the brain abscess was secondary to it.

Galen showed that otitis was the sequence of some infections and as in his opinion the treatment was based on the nature of the disease. He advised promotion of drainage of otitis.

Adampolitzer in 1867 first proposed the "ex vacuo" theory of otitis. The theory postulates that chronic negative pressure secondary to eustachian tube malfunction results in the development of transudate into the middle ear space.[12]

Hoople (1950) described the features of middle ear effusions and emphasided the importance of diagnostic paracentesis so that many of these conditions especially in children would not escape detection.

Armstrong in 1954 advocated the use of indwelling polythene tube through the drum head in order to achieve ventilation and drainage.

Sentuna and collegues (1958, 1962) described the laboratory and animal studies, including chemical analysis of fluid. [13]

According to Bois (1959), chronic suppurative otitis media is a continued suppuration from the middle ear following an acute necrotic otitis media or primary suppuration in middle ear with hyperplastic or fibrotic mucosa. Anand et al (1971) found that in a study of 100 cases *Staphylococcus* species was the commonest followed by *Pseudomonas aeruginosa*.

Arnold Chanin isolated *E.Coli* in one case and later he came to know that the mother of the patient was putting urine in the discharging ear during patients child hood. This lead to the conclusion that urine was the cause for the origin of chronic *E.coli* infection in CSOM.

Anssi 1977 studies bacteriology of 70 consecutive cases of ative CSOM. He was able to isolate anaerobic organisms in his study.

RamaRao MV and Jayakas PA (1980) studied bacteriology of CSOM and found *Staphylococcus aureus* was predominent along with *Streptococcus spp*., *Pseudomonas aeroginosa* and *Proteus* spp.

Ojala K et al (1981) compared pre and post operative bacteriology of chronic ears in 806 cases and found *Staphylococus* spp. (22%) was predominant followed by *Pseudomonas* spp. and *Proteus* spp.[14]

Sagita et al (1981) observed 62 isolates in that *Proteus* spp. was predominate.

Ibe Kwe and Ukafor (1983) studied pathogenic organisms in CSOM in Enugu Nigeria and found 62 isolates. Pseudomonas spp. was predominent in these isolates followed by *Staphylococcus* spp. They also isolated *Aspergillus* (4.3%) and *Candida* (1.3%).[15]

B. Narasinga Rao et al (1991) studied 120 cases of CSOM for bacterial and fungal isolates. They found *Staphylococcus aureus* (42.5%) was predominant followed by *Pseudomonas* spp.(18.33%) .

They also isolated *Candida* species in about 7.5% of cases and no growth was observed in 10% of ear swabs.[16]

Flirs et al (1992) worked on aerobic bacteriology of CSOM without cholesteatoma in children. Out of 170 isolates, 84% were *Pseudomonas aeruginosa* and 32% enteric gram negative bacilli.[17]

Dr.Gupta et al (1996) in their study of prevalence of complications of suppurative otitis media in rural area of Loni, observed complications of CSOM are common even in this era of antibiotics and technology. Though the incidence of complications has decreased in the western world and urban areas of India, in rural areas the rate is still high.

IbeKwe et al (1997) studied Microbiology of 102 cases of CSOM for aerobes, anaerobes and fungi. He found 44% pure cultures, 34% mixed and 19% no growth.In pure culture *Pseudomonas aeruginosa* was predominant followed by *Staphylococcus aureus* and fungus *Aspergillus* and he processed an anaerobe from 102 specimens.

Gulati et al in their study “Investigative profile in CSOM” isolated *Pseudomonas aeruginosa* 41.6%. *Candida* species was predominantly followed by *Aspergillus* and he says highest incidence in second and third decade. Males are predominantly affected than females. [8]

EMBRYOLOGY OF THE EAR :

Auricle :- First branchial cleft is the precursor of external auditory canal. Around 6th week of embroyonic life, six tubercles appear around bronchial cleft and progressively coalesce to form auricle. Tragus develops from the tubercle of the first arch, rest of pinna develops from remaining five tubercles of second arch. By 20th week pinna achieves adult shape.

External auditory meatus develops from first branchial cleft. By about 16th week, cells proliferate from the bottom of ectodermal cleft and forms a meatal plug. Recanalization of this plug forms the epithelial lining of bony meatus. Recanalization begins from deeper part near the tympanic membrane and progresses out wards. External canal is fully formed by the 28th week.

Tympanic membrane develops from all the three germinal layers. Outer epithelial layer is formed by the ectoderm, inner mucosal layer by the endoderm and the middle fibrous layer by the mesoderm.

Middle ear cleft, the eustachian tube, tympanic cavity, attic, antrum and mastoid air cells develop from the endoderm of tubo tympanic recess which arises from the first and partly from the second pharyngeal pouches.

Malleus and incus are derived from the mesoderm of first arch, while stapes from the second arch except foot plate and annular ligament which are derived from ottic capsule.

Inner ear – membraneous labyrinth develops from otocyst which is ectodermal in origin. Bony labyrinth develops from mesoderm around otocyst.[2]

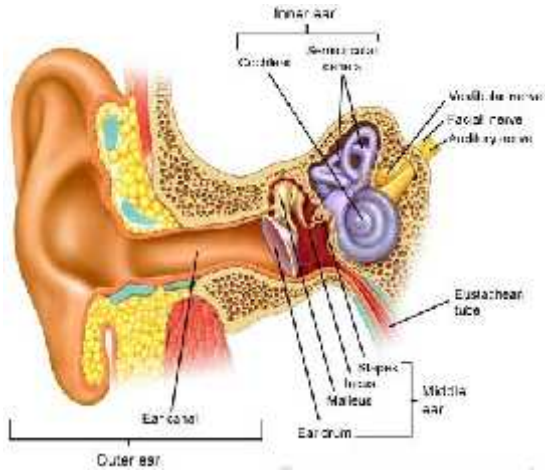


Fig.1 Anatomy of ear

Fig.2 : Central perforation in the tympanic membrane seen in CSOM

ANATOMY OF THE MIDDLE EAR :

Middle ear together with the eustachian tube : aditus, antrum and mastoid air cells is called middle ear cleft. It is lined by mucous membrane and filled with air.

It can be divided into 1) Mesotympanum. 2) Epitympanum 3) Hypotympanum 4) Protympanum. Middle ear can be likened to six sided box with a roof, medial, lateral anterior and posterior walls.

The roof is formed by a thin plate of bone called tegmen tympani. It also extends posteriorly to form the roof of the aditus and antrum. It seperates tympanic cavity from the middle cranial fossa. The floor is also thin plate of bone which separates, tympanic cavity from the jugular bulb. Some times, it is congenitally deficient and the jugular bulb may then project into the middle ear, separated from the cavity only by the mucosa.

Anterior wall has a thin plate of bone which separates the cavity from internal carotid artery. It also has two openings, the lower one for the eustachian tube and the upper one for the canal of tensor tympani muscle.

The posterior wall lies close to the mastoid air cells. It presents a bony projection called the pyramid through summit of which appears the tendon of the stapedius muscle to get attachment to the neck of stapes. Aditus, an opening through which attic communicates with the antrum, lies above the pyramid. Fascial nerve runs in the posterior wall just behind the pyramid. It is bounded medially by the vertical part of VIIth nerve, laterally by the chorda tympani and above by the fossa incudis.

Medial wall is formed by the labyrinth, it presents a bulge called promontory, which is due to the basal coil of cochlea, oval window into which is fixed the foot plate of stapes, round window or the fenestra cochlea which is covered by the secondary tympanic membrane. Above the oval window is the canal for facial nerve. which is the prominence of lateral semi circular canal. Just anterior to the oval window medial wall presents a hook like projection called the processus cochleariformis. The tendon of tensor tympani takes a turn here to get attachment to the neck of malleus. Medial to the pyramid is a deep recess called sinus tympani, which is bounded by subiculum below and the ponticulus above.

Lateral wall is formed largely by the tympanic membrane and to lesser extent by bony outer attic wall called scutum.

Tympanic membrane :

It separates the external meatus form middle ear and functionally part of the middle ear, tympanic membrane consists of 3 layers.

- Outer layer of squamous epithelium continuous with external meatus.
- Middle layer of fibrous tissue which has radiating and circular fibres.

- Inner layer of mucous membrane continuous with the lining of the tympanic cavity.

Tympanic membrane is divided into two parts, pars flaccida in which fibrous layer is deficient and pars tense in which all the layers are present.

Contents of middle ear cavity:

- Air
- Bony ossicles – malleus, incus and stapes
- Tympanic plexus
- Chorda tympanic plexus
- Intra tympanic muscles, tensor tympani and stapedius.

Inner ear: It has two parts:

1) Bony labyrinth – vestibule, cochlea and semi circular canals.
2) Membranous labyrinth filled with endolymph and comprises of a) Saccule and utricle b)Membranous semi circular ducts within bony canals c) Ductus cochlearis in the bony cochlea.

Arterial supply of tympanic cavity:

1) Anterior tympanic branch of maxillary artery
2) Stylomastoid branch of posterior auricular artery
3) Stylomastoid branch of middle meningeal artery.
4) Superior tympanic branch of middle meningeal artery.
5) Inferior tympanic branch of ascending pharyngeal artery.
6) Carotico tympanic branch of internal carotid artery.

Nerve supply :

Branches of tympanic plexus of nerves supplies tympanic cavity and its contents Jacobsons nerve branch of glossopharygeal nerve contribute to plexus.

Sympathetic innervations to plexus by superior and inferior carotico tympanic nerves and para sympathetic fibres by the smaller superficial petrosal nerve.[2]

Epidemiology of CSOM[1,18,19,2]

CSOM had multifactorial causes including host factors, agent and environmental factors.

Host factors :

1) **Age:** Incidence and prevalence of CSOM peak in the preschool years and decreases as age increases. This may be due to increased risk of respiratory infection, decreased immunocompetence, and eustachian tube angle in infants and young children. An early, first otitis media episode may be the primary event that predisposes a child to recurrent and chronic otitis media by setting up an inflammatory process in middle ear and eustachian tube.[20,62,64]
2) **Race :** Some studies reported greater prevalence in whites than blacks, and Indians and Eskimos are more affected than whites. Difference in races may be due to differences in access to medical care, socio economic status, and anatomic and biologic susceptibility.
3) **Gender :** Many studies reported that males are affected more than females, but some studies showed no differences. Boys are affected more than girls, may be due to anatomical and growth difference.

4) **Socio economic status :** Many studies showed CSOM common in low socio-economic classes.

   It is related to education, low income and type of housing.

5) **Season :** Greater occurence is seen in winter and spring, and is closely followed that of upper respiratory disease.[1,19]

Other host factors :

1) Eustachian tube dysfunction :

a) Palatal problems – cleft palate and sub mucous cleft palate are associated with increased incidence of otitis media with effusion due to abnormal function of tensor palati muscle.

b) Altered mucociliary system:

   Infection – Inflammatory compounds originating in nasopharynx can cause the middle ear infection, is suggested by demonstration of same pathogens in both sites

- Bacterial adherence to nasopharygeal walls
- Surface endotoxin to *H.influenzae* may be responsible for the induction of otitis media.
- Complement changes and decreased levels of IgG antibodies against *Pneumococcus* spp.
- IgE antibodies and immune complexes responsible for secondary immune response. [18,63]

2) Allergy :

Increased susceptibility to respiratory infection found in the patients with allergy and is responsible for association.

3) Immunological factors :

- Mucosal immunity in the middle ear cavity provided by secretory IgA.

IgG mediated systemic immunity in man is the main defence system against bacterial infection of the middle ear. Delayed maturation of immunoglobulins and a low IgG_2 subclass level has been observed in children with recurrent otitis media compared with otitis free children.[21]

4) Surfactant deficiency :

Proteolytic enzyme activity from bacteria causes deficiency of surfactant in eustachian tube. So treatment of otitis media with effusion using the surfactant has produced equivocal results.

5) Ciliary abnormalitis :

Immotile cilia syndrome – (Kartagener's syndrome) and functional reduction in ciliary beat frequency as in tobacco smoking (passive smoking).

6) Hormonal factors :

Patient with high estrogen level and patient with hypothyroidism develop tubal dysfunction which leads to otitis media with effussion.

II Nasopharngeal disproportion :

a) Cranio facial abnormalities

Some syndromes with skull base or nasopharyngeal abnormalities are likely to have otitis media.

Eg:Down's syndrome, Hurlers syndrome, Hunters syndrome and Fragile X syndrome

b) Adenoids and nasopharynx :

Large adenoids causes morphological differences in skull base and nasopharynx, resulting in the condition of the eustachian tube causing ascending infection from nasopharynx leading to development of otitis media with effusion.

III. Blood Group :

Blood group A has been asscoiated with otitis media with effusion.

Environmental factors:

a) **Exposure to passive tobacco smoke :** Passive smoking in infants and children increases the risk of CSOM. Passive smoke may damage nasopharygeal, middle ear, or eustachian tube mucosa, increasing susceptibility to viral and bacterial invasion or obstruction of the eustachian tube. Passive smoke may act indirectly by inflaming the small air ways of the respiratory tract, leaving a child more susceptible to respiratory infections and subsequent otitis media and CSOM.[1]

b) **Breast feeding :** It has been shown to protect against the development of otitis media. Cow milk, formula milk or bottle feeding is associated with high risk of otitis media. Factors present in breast milk like lysozyme, lactoperoxidase, lactoferrin, IgA Immunoglobulin, antistaphylococcal factors, neutrophils, etc. reduces the chance of getting otitis media in infants.[1]

c) **Genetic susceptibility :** Several studies reported familial clustering of otitis media and CSOM. It may suggest shared genes and environment or both. Studies also shown increased prevalence of CSOM among first degree relatives.Children who had

congenital conditions such as Downs syndrome and cleft palate have a high prevalence of CSOM. Absence of IgG_2 allotype G_2M may be markers for the hosts impaired ability to mount an immune response to polysaccharide antigens, which are common causative agents of CSOM.

d) **Exposure to other children (day care) :** Many researchers reported that otitis media and recurrent otitis media are significantly more common in day care attenders. Day care centres are efficient environments for the transmission of infectious agents that cause respiratory diseases including otitis media.[22]

e) **Education and socio economic status :** Majority of cases belonging to lower or middle socio economic status adn the unhygienic conditions. Poverty, illiteracy, overcrowding, malnutrition have been suggested, as a basis for wide spread prevalence of CSOM.

f) History of atopy in the family are also predisposes to CSOM.

g) Children with birth wt <2500gm and gestation of < 37 week delivery also more prone for CSOM.[1,19,20,23]

DEFINITION AND CLASSIFICATION

DEFINITION :

CSOM is a long standing infection of a part or whole of the middle ear cleft characterized by ear discharge and a permanent perforation. The condition is considered chronic if the tympanic membrane defect is present for a period greater than 3 months. Histologically, CSOM is defined as irreversible mucosal changes within the middle ear cleft.[2,12]

TYPES OF CSOM [2,12]:

Clinically it is classified into two types :

1) **Tubotympanic :** Also called the safe or benign type: it involves antero- inferior part of middle ear cleft and is associated with a central perforation. There minimal risk of serious complications.
2) **Attico antral :** Also called unsafe or dangerous type, it involves postero- superior part of the cleft (i.e. attic, antrum and mastoid) and is associated with an attic or a marginal perforation. The disease is often associated with a bone eroding process such as cholesteatoma, granulations or ostetis. Risk of complications is high in this variety.

TUBOTYMPANIC TYPE :

AETIOLOGY :

The disease starts in childhood and is therefore common in that age group.

1) It is the sequelae of acute otitis media usually following exanthematous fever and leaving behind a large central perforation.

The perforation becomes permanent and permits repeated infections from the external ear. Also the middle ear mucosa gets exposed to the environment and gets sensitized to dust pollen and other aero allergens causing persistent otorrhoea.

2) Ascending infections via the eustachian tube from tonsils, adenoids and infected sinuses may be responsible for persistent or recurring otorrhoea.
3) Persistent mucoid otorrhoea is sometimes the result of allergy to ingesants such as milk, eggs, fish etc.,

PATHOLOGY :

The tubotympanic disease remain localized to mucosa and mainly anterior part of the middle ear cleft. Like any of the chronic infection, the process of healing and destruction go hand in hand and either of them may take advantage over the other, depending on the virulence of organism and resistance of the patient. Thus, acute exacerbation is not uncommon. The pathological changes seen in this type of CSOM are.

1) **Perforation of pars tensa :** It is a central perforation and its size and position varies.
2) **Middle ear mucosa :** It may be normal when disease is quiescent or inactive, it is edematous and velvety when disease is active.
3) **Polyp :** A polyp is a smooth mass of edematous and inflamed mucosa which has protruded through a perforation and presents in the external canal. It is usually pale in contrast to pink,fleshy polyp seen in atticoantral disease..
4) **Ossicular chain :** It is usually intact and mobile but may show some degree of necrosis, particularly of the long process of incus
5) **Tympano sclerosis :** It is hyalinization and subsequent calcification of sub epithelial connective tissue. It is seen in remnants of tympanic membrane or under the mucosa of middle ear. It is seen as white chalky deposit on the promontory ossicles ,joints

tendons and oval and round window. Tympano sclerotic masses may interfere with the mobility of these structures and cause conductive deafness.

6) **Fibrosis and adhesions :** They are the result of healing process and may further impair mobility of ossicular chain or block the eustachain tube.

CLINICAL FEATURES :

1) **Ear discharge :** It is non offensive mucoid or mucopuralent, constant or intermittent. The discharge appears mostly at time of upper respiratory tract infection or on accidental entry water into the ear.

2) **Hearing loss :** It is conductive type, severity varies but rarely exceeds 50dB. Sometimes the patient reports of a paradoxical effect i.e. hears better in the presence of discharge than when the ear is dry.

   This is due to round window shielding effect produced by discharge which helps to maintain phase differential. In the dry ear with perforation, sound waves strike both the ovals and round windows simultaneously thus cancelling each others effect.

   In long standing cases, cochlea may suffer damage due to absorption of toxins from the oval and round windows and hearing loss becomes mixed type.

3) **Perforation :** Always central. It may lie anterior, posterior or inferior to the handle of malleus. It may be small, medium, or large or extending upto the annulus i.e. subtotal.

4) **Middle ear mucosa :** it is seen when the perforation is large. Normally it is pale pink and moist; when inflamed it looks red, oedematous and swollen. Occasionally, a polyp may be seen.

INVESTIGATIONS :

1) Examination under microscope is essential in every case and provides useful information regarding presence of granulations, in growth of squamous epithelium from the edges of perforation, status of ossicular chain, tympanosclerosis and adhesions. An ear which appears dry may show hidden discharge under the microscope. Rarely cholesteatoma may co exist with a central perforation and can be seen under a microscope.
2) Audiogram: It gives an assessment of degree of hearing loss and its type, usually the loss of conductive, but a sensoneural element may be present.
3) Culture and sensitivity of ear discharge : It helps to select proper antibiotic or ear drops.
4) Mastoid x-rays; Mastoid is usually sclerotic but may be pneumatised with clouding of antral cells. There is no evidence of destruction. Presence of bone destruction is a feature of attico antral disease

ATTICO ANTRAL TYPE :

It involves posterio superior part of middle ear cleft (attic, antrum and posterior tympanus and mastoid) and is associated with cholesteatoma, which because of its bone eroding properties, causes risk of serious complications. For this reason the disease is also called unsafe or dangerous type.

AETIOLOGY :

Etiology is same as of cholesteatoma. It is seen in sclerotic mastoid, and whether the latter is the cause or effect of disease is not yet clear.

PATHOLOGY :

Attico antral disease is associated with the following pathological processes.

Cholesteatoma : Normally middle ear is not lined by squamous epithelium. It is the presence of latter type of epithelium in the middle ear or mastoid that constitutes a cholesteatoma. In other words, cholesteatoma is a "skin in wrong place". The term cholestetoma is misnomer because it neither contains cholesterol crystals nor is it a tumor to merit the suffix "oma" However, the term has been retained because of its wider usage. Essentially, cholesteatoma consists of two parts.

1) The matrix, which is made up of keratinising squamous epithelium resting on a thin stroma of fibrous tissues and
2) A central white mass, consisting of keratin debris produced by the matrix. For this reason, it has also been named epidermosis or keratoma.

ORIGIN OF CHOLESTEATOMA :

Genesis of cholestatoma is a matter of debate. Any theory of its genesis must explain how squamous epithelium appeared in the middle ear cleft. The various views expressed are:

1) Presence of congenital cell rests.
2) Invagination of tympanic membrane from the attic or posterio superior part of pars tensa in the form of retraction pockets (Witt maack theory). The outer surface of tympanic membrane is lined by stratified squamous epithelium which after invagination forms the matrix of cholesteatoma and lays down keratin in the pocket.

3) **Basal cell hyperplasia (Ruedis theory) :**The basal cells of germinal layer of skin proliferate under the influence of infection and lays down keratinizing sqamous epithelium.
4) **Epithelium invasion(Haberman'sTheory);** The epithelium from the meatus or outer drum surface grows into the middle ear through a pre existing perforation especially of the marginal type where part of annulus tympanis has already been destroyed.
5) **Metaplasia (Sades theory):** Middle ear mucosa, like respiratory mucosa else where, undergoes metaplasia due to repeated infections and transforms into squamous epithelium.

1) Expansion of cholesteatoma and destruction of bone :

Once cholesteatoma enters the middle ear cleft invades the surrounding structures, first by following the path of least resistance and then the enzymatic bone destruction. An attic cholesteatoma may extend backward into the aditus, antrum and mastoid, down wards into the mesotympanum. Medially it may surrounded by the incus and head of malleus.

Cholesteatoma has the property to destroy the bone. It may cause destruction of ear ossicles, erosion of bony labyrinth, canal of facial nerve, sinus plate, or tegmen tympani and thus cause several complications.

2) Osteitis and granulation tissue : Osteitis involves outer attic wall and posterio superior margin of the tympanic ring. A mass of granulation tissue surrounds the area of osteitis and may even fill the attic, antrum, posterior tympanus and mastoid. A fleshy red polyps may be seen filling the meatus.

3) **Ossicular necrosis :** It is common in attico antral disease. Destruction may be limited to the long process of incus or may also involve stapes super structure, handle of malleus or the entire ossicular chain. Therefore; hearing loss is always greater than in disease of tubotympanic type. Occasionally the cholesteatoma bridges the gap caused by the destroyed ossicles, and hearing loss is not apparent.
4) **Cholesterol granuloma :** It is a mass of granulation tissue with foriegn body giant cells surrounding the cholesterol crystals. It is a reaction to long standing retention of secretions or hemorrhage, and may or may not coexist with cholesteotoma when present in the mesotympanum, behind an intact drum, which appears blue.

SYMPTOMS :

1) **Ear discharge :** Usually scanty, but always foul smelling due to bone destruction. Discharge may be so scanty that the patient may not even be aware of it. Total cessation of discharge from an ear which has been active till recently should be viewed seriously, as perforation in these cases might be sealed by crusted discharge, inflammatory mucosa or a polyp obstructing the free flow of discharge. Pus in these cases, may find its way internally and cause complications.
2) **Hearing loss :** Hearing is normal when ossicular chain is intact or when cholesteatoma having destroyed the ossicles, bridges the gap caused by destroyed ossicles. Hearing loss is mostly conductive but sensori-neural element may be added.
3) **Bleeding :** It may occur from granulations or the polyp when clearing the ear.

SIGNS :

1) **Perforation :** It is either attic or posterio superior margin type. A small attic perforation may be missed due to presence of a small amount of crusted discharge. Sometimes the area of perforation is masked by a small granuloma.
2) **Retraction pocket :** An invagination of tympanic membrane is seen in the attic or posterio superior area of pars tensa. Degree of retraction and invagination varies. In early stages pocket is shallow and self cleansing but later when pocket is deep, it accumulates keratin mass and gets infected.
3) **Cholesteatoma :** Pearly white flakes of cholesteatoma can be sucked from the retraction pockets. Suction clearance and examination under operating microscope forms an important part of the clinical examination and assessment of any type of CSOM.

INVESTIGATIONS:

1) **Tunning fork tests and audiogram;** They are essential for pre operative assessment and to confirm the degree and type of hearing loss.
2) **X-ray mastoids** – They indicate extent of bone destruction and degree of mastoid pneumatisation. They are useful to indicate a low lying dura or an anteposed sigmoid sinus when operation is being contemplated on a selerotic mastoid. Cholesteatoma causes destruction of the area of attic and antrum better seen in lateral view. CT scan of temporal bone gives more information.
3) **Culture and sensitivity of ear discharge**; It helps to select proper antibiotic for local or systemic use.
4) Other investigations

CT Scan : (Computerised Tomogram)

- If cholesteatoma is suspected
- To rule out intracranial complications.
- Major role in pre operative evaluation and post operative follow up of patients with CSOM.

MRI: (Magnetic Resonance Imaging)

- It can give lot of mucosal detail
- It can differentiate among tumour, blood and inflammation.
- Safe and non invasive method of evaluating the middle ear and mastoid mucosa.

Features indicating complications in CSOM :

1) Pain : Pain is uncommon in uncomplicated CSOM. Its presence is considered serious as may indicate extra dural, perisinus or brain abscess. Sometimes it is due to otitis externa associated with discharging ear.
2) Vertigo : It indicates erosion of lateral semi circular canal which may progress to labyrinthitis or meningitis. Fistula test may be performed in all cases.
3) Persistent head ache is suggestive of an intra cranial complication.
4) Facial weakness: Indicates erosion of facial canal.
5) A restless child refuses to take feed and easily goes to sleep (extra dural abscess)
6) Irritability and neck rigidity (meningitis)
7) Diplopia (Gardinigo syndrome)
8) Ataxia (Labyrinthitis or cerebellar abscess)
9) Abscess around the ear (mastoiditis)

It is not uncommon for patient present first time with complications.

Complications of suppurative otitis media :

Though there is a general decline in the incidence of complications, they are still frequently seen in our country. The causes are poor socio economic conditions, lack of education and awareness about health care and lack of availability of trained specialist in rural areas.

Factors causing complications :

Development of complications also depends on factors such as:

1) High virulence of organisms.
2) Poor resistance of patient
3) Inadequate antibiotic treatment of acute middle ear and mastoid infections.
4) Presence of chronic systemic diseases eg. Diabetes mellitus, Tuberculosis, Nephritis, Leukemia etc.,
5) Resistance of organisms to antibiotics which are becoming common these days.
6) Microbial films in cholesteatoma (when surgical eradication is the only treatment)[24,25,26,27,28]

Pathways of spread of infections :

1) **Direct bone erosion :** In acute infections it is the process of hyperaemic decalcification. In chronic infection it may be osteitis, erosion by cholesteatoma or granulation tissue.
2) **Venous thrombophlebitis :** Veins of Haversion canals are connected with dural veins which in turn connect with dural venous sinuses and superficial veins of brain. Thus, infection from the mastoid bone can cause thrombophlebitis of venous sinuses and even cortical vein thrombosis. This mode of spread is common in acute infections.

3) **Preformed pathways :** 1) Congenital dehiscences, eg. in bony facial canal, floor of middle ear over the jugular bulb.
4) **Previous skull fractures :** The fracture sites heal only by fibrous scan which permits infections.
5) **Surgical defects** : Eg- Stapedectomy, fenestration and mastoidectomy with exposure of dura.
6) Oval and round windows.
7) Infection from labyrinth can travel along internal acoustic meatus,aqueduct of the vestibule and that of the cochlea to the meninges.

Classification :

Complications of otitis media are classified into two main groups.

a) Intra temporal (within the confines of temporal bone)
    1) Mastoiditis
    2) Petrositis
    3) Facial paralysis
    4) Labyrinthitis.
b) Intra cranial
    1) Extra dural abscess
    2) Sub dural abscess
    3) Meningitis
    4) Brain abscess
    5) Lateral sinus thrombophlebitis
    6) Ottic hydrocephalus [2,12,29,30,31]

TREATMENT:

Goals of management :

1) Relief of symptoms
2) Elimination of infection
3) Ventilation of the middle ear and mastoid
4) Avoidance of complications
5) Prevention of further infection.
6) Reconstruction of conducting mechanism to correct hearing loss

Treatment of tubotympanic type:

1) **Aural toilet :** Remove all discharge and debris from the ear. It can be done by dry moping with absorbent cotton buds. Suction clearance under microscope or irrigation with sterile normal saline. Ear must be dried after irrigation.
2) **Ear drops :** Antibiotic ear drops containing Neomycin, Polymyxin, Chlormycetine or Gentamicin are used. They are combined with steroids which have local anti inflammatory effect. To use ear drops, the patient lies down with the diseased ear up. Antibiotic drops are installed and then intermittent pressure applied on the tragus for antibiotic solution to reach the middle ear. This should be done three or four times a day. Acid pH helps to eliminate *Pseudomonas* infection and irrigation with 1.5% Acetic acid are useful.

   Care should be taken as ear drops are likely to cause maceration of canal skin, local allergy, growth of fungus or resistance of organisms. Some ear drops are potentially ototoxic. [55]

3) **Systemic antibiotics :** They are useful in acute exaceberation of chronically infected ear, other wise role of systemic antibiotics in the treatment of CSOM is limited.
4) **Precautions :** Patients are instructed to keep water out of the ear during bathing, swimming and hair wash. Rubber inserts can be used. Hard nose blowing can also push the infection from nasopharynx to middle ear and should be avoided.
5) **Treatment of contributory causes :** Attention should be paid to treat concomittently infected tonsils, adenoids, maxillary antra and nasal allergy.
6) **Surgical treatment :** Aural polyp or granulations, if present should be removed before local treatment with antibiotics. It will facilitate ear toilet and permit ear drops to be used effectively. An aural polyp should never be avulsed as it may be arising from the stapes facial nerve or horizontal canal and thus lead to facial paralysis of labyrinthitis.
7) **Reconstructive surgery :** Once ear is dry myringoplasty with or without ossicular reconstruction can be done to restore hearing. Closure of perforation will also check repeated infection from the external canal.

Treatment of attico antral type :

1) **Surgical :** It is the main stay of treatment. Primary aim is to remove the disease and render the ear safe, and second in priority is to present or reconstruct the hearing but never, at the primary aim. Two types of surgical procedures are done to deal with cholesteatoma.
    a) **Canal wall down procedures :** They leave the mastoid cavity; open in to the external auditory canal so that the diseased area is fully exteriorized. The commonly performed operations for attico antral disease are atticotomy, modified radical mastoidectomy and rarely, the radical mastoidectomy.

b) **Canal wall up procedures :** Here disease is removed by combined approach through the meatus and mastoid but retaining the posterior bony metal wall intact, there by avoiding an open mastoid cavity. It gives dry ear and permits easy reconstruction of hearing mechanism. However there is danger of leaving some cholesteatoma behind. Incidence of residual or recurrent cholesteatoma in these cases is very high and therefore long term follow up is essential. Some even advise routine re-exploration in all cases after 6 months or so. Canal wall up procedures are advised only in selected cases. In combined approach or intact canal wall mastoidectomy, disease is removed both permanently and through cortical mastoidectomy and posterior tympanotomy in which a window is created between the mastoid and middle ear through the facial recess, to reach sinus tympani

2) **Reconstructive surgery :** Hearing can be restored by myringoplasty or tympanoplasty. It can be done at the time of primary surgery or as a second stage procedure.

Conservative treatment :

It has a limited role in the management of cholesteatoma, but can be tried in selected cases, when cholesteatoma is small and easily accessible to suction clearance under operating microscope. Repeated suction clearance and periodic checkups are essential. It can also be tried oral in elderly patients and above 65, and those who are unfit for general anesthesia or those refusing surgery. Polyps and granulations can also be surgically removed by cup forceps or cauterised by chemical agents like silver nitrate or trichloro acetic acid. Other measures, like aural toilet and dry ear precautions are also essential.[2]

MICROBIOLOGY OF CSOM [3,6,7,24,32,33,35,36,37,38,39,40,59,60]

Many different bacteria may be associated with chronic suppurative otitis media. Some are virulent and others are saprophytes. The common saprophytes of the ear canal are *Staphyloccocus epidermidis, Diptheroids,* except in the relatively unusual cause of traumatic. Introduction of bacteria into the middle ear is through a ruptured tympanic membrane. Most cases of otitis media arise from the nasopharynx and affect the middle ear secondarily. This impairment of middle ear ventilation is accompanied by exudative and transudative fluid collection in the middle ear. This results in extreme vulnerability of the middle ear, to bacterial assault by direct expansion from infected tissue of the nasopharynx.

Diseases of the ear in infancy and early childhood may arrest the normal pneumatization of the mastoid. It is possible that the some process alters the mucosa of the middle ear, making it more susceptible to recurrent infections than the normal ear.

The auditory tube is responsible for protecting the middle ear into the nasopharynx and ventilating the middle ear, so that air pressure is equilibrated, with that in the external ear canal. If any of these functions becomes compromised and fluid develops in the middle ear, infection my occur. This in turn compromises auditory tubes ventilating function thereby resulting in a negative, rather than a positive pressure in the middle ear. This change in pressure then allows for potential pathogenic bacteria present in the nasopharynx to enter the middle ear.

The factors of host resistance and bacterial virulence will then determine the progress of the disease from this point.[41]

Pseudomonas aeruginosa : *Pseudomonas aeruginosa* is infrequently found in the normal ear, but often inhabits the external auditory canal in association with injury, maceration, inflammation or simply wet or humid conditions.

A quantitative study of aerobic microbes in active CSOM showed rather exceptionally high counts of *Pseudomonas.spp.*of 10^6 bacilli / ml compared with the counts of other main aerobic species.[42] The problem is the increasing incidence of *Pseudomonas spp.*and Pyocyaneous infections, particularly in those individuals who have received prolonged antibiotic therapy. This is undoubtedly the result of over growth after the gram positive organisms have been eliminated by the therapy. These infections can be most difficult to treat. *Pseudomonas. spp.* and *Proteus* species do not normally inhabit the upper respiratory tract and their emergence in the chronic middle ear infection cannot be discribed to the primary deviation from the nasopharynx through the eusthacian tube. These organisms were considered mostly secondary invaders from the external canal entering the middle ear via the perforated tympanic membrane resulting from an acute episode of otitis media [42]. *Pseudomonas aeruginosa* dominated in those patients presenting seven days-two months after the onset of symptoms .

Pseudomonas aeruginosa is the most common bacterial pathogen isolated from the middle and external ear of children and adults with CSOM. Isolation rates as high as 72% have been reported. Although Microbiology of chronic middle ear infection is complex, *Pseudomonas aeruginosa* was identified in 67% of the specimens obtained from middle ear of children and was the only organism grown in 31%.

Occasionally, *Pseudomonas* infection of the external auditory canal becomes locally invasive by penetrating epithelium and invading underlying soft tissue. This

process is usually chronic and indolent, also destructive and ultimately life threatening, if not promptly and appropriately treated.

The occurence of *Pseudomonas aeruginosa* as predominate offending organism could be attributed to several factors. Pollock M stated that the ability of these organism to survive in competition with other organisms could be due to minimum nutritional requirement, its relative resistance to antibiotic and its armamentarium of antibacterial product via Pyocyanian and Bacteriocin. Apart from the above said reasons *Pseudomonas aeruginosa* use pili to attach to necrotic or diseased epithelium of middle ear. Once attached the organisms produce enzymes like proteases, lipopolysaccharides etc., to elude from normal defense mechanism required for fighting infections *Pseudomonas aeruginosa* pose a serious challenge to maintain the dry ear in CSOM, by facilitating the growth of fungi in the middle ear. Failures after surgical treatment were most common in *Pseudomonas* ears .[43,60]

Staphylococcus aureus : About 35-50% of normal adults carry *Staphylococcus aureus* in the anterior nares. Other sites of colonization include skin folds, perineum, axillae and vagina. Skin carriage rates of 10-20% are found in most areas of the body, where upto 40% swabs may yield *S.aureus*. It is shed by patients and carriers contaminate fomites such as hand kerchiefs, bedlinens, blankets and may persists on them for days or week. *Staphylococcus* disease may follow endogenous or exogenous infection. The mode of transmission may be direct contact or through fomites by airborne droplet.

It is an opportunistic pathogen in that it causes infection, most commonly at sites of lowered host resistance, eg. damaged skin or mucous membranes. Those with lower respiratory tract viral infection such as Influenza , Measles and diabetic patients are also suspectible to *Staphylococcus* infections.

EPIDEMIOLOGY

Staphylococcus spp. is a normal component of human indigenous microflora and is carried asymptomatically in a number of sites in the body. Its transmission from these sites causes both endemic and epidemic diseases. The acquisition and carriage of *S.aureus* is a complex problem that is incompletely understood. Colonization of the infant with *Staphylococcus spp.* occurs within a few days after birth, but because of antibodies passively received through the placenta, the carrier rate drops during the first two years of life. By the age of six the child has acquired an adult carrier rate of approximately 30%. Some individuals who harbour *Staphylococcus spp.*are chronic or persistent carriers, but most are intermittent carriers harboring the organism for only a few weeks. *Staphylococcus aureus* is found in the asymptomatic carrier in a number of sites in the body, but the anterior nares is the major reservoir of infection and source of disease.

Pathogenesis : In the typical *Staphylococcal* skin infection, the organisms penetrate a sebaceous gland or hair shaft where they find an environment nutritionally suitable for growth. The defense mechanisms of the host and the size and virulence of the infective dose determine the likelihood of development of *Staphylococcal* infection. Although benign skin infections are common, serious *Staphylococcal* disease is infrequent, which emphasizes the excellent protective barrier provided by the skin and mucous membranes. Any condition that destroys the integrity of these surface areas predisposes the individual to infection. Third-degree burns, traumatic wounds, surgical incisions and certain viral infections are among the many precipitating causes of Staphylococcal disease.

Adhesion : Colonization by *Staphylococcus aureus* requires an initial adherence to host cells. Adherence to nasal mucosal cells is mediated by the teichoic acid component of *S. aureus* [53] and is increased in chronic *Staphylococcal* carriers.

Attachment of *S. aureus* to traumatized or disrupted skin, to foreign surfaces, and to endothelial structures involves interaction with at least five different proteins i.e., fibrinogen, fibronectin, laminin, thrombospondin, and possibly collagen IV.

Invasion : Invasion of the host after colonization requires penetration of the microorganism through the epithelial or mucosal surface.

Chemotaxis : Once *Staphylococcus aureus* has penetrated through the mucosal or epithelial layer, ingestion and killing by PMN, as well as by the monocytomacrophage system, become the major line of defence. Mobilization of phagocytic cells at the site of bacterial growth requires the elaboration of microbial and host-specific signals. Among the former, cell wall-associated and extracellular products of *Staphylococcus aureus* such as peptidoglycan, teichoic acid, and protein A are certainly involved.

Opsonization : The major host signals result from the activation of the complement system; all cell wall components identified, thus far can trigger this reaction, by producing C5a.[65]

Recognition of *S. aureus* by phagocytes is mediated by their receptors for the Fc fragment of IgG immunoglobulins, by their receptors for the activated subunit of the third component of the complement system C3b, and possibly by other complement receptors. This recognition process implies that *S. aureus*, in order to be ingested, has to be coated by C3b and or IgG molecules – a process called opsonization.

In normal serum obtained from healthy non-immune subjects, complement activation via either the classical or alternate pathways provides the major part of the opsonic activity.[66] However, this normal serum also will contain small amounts of antipeptidoglycan antibodies, which result from previous exposure to *Staphylococcus* or other organisms. In striking contrast, opsonization in hyperimmune subjects occurs predominantly by IgG molecules. However, the peptidoglycan present in *Staphylococcus aureus* can also activate to some extent the complement system, as will the IgG molecules bound to the bacterial surface. This complex sequence of events involving complement is usually overshadowed by the IgG opsonization mechanism of the hyperimmune serum.[66] The peptidoglycan matrix is presently considered to be a major determinant of opsonization; in the absence of antibody, peptidoglycan may trigger opsonization by activating the classical and even the alternative complement pathway. Protein A probably plays a triple antiphagocytic role in the bacteria-cell recognition process by virtue of its binding to the Fc portion of IgG. First, extracellular, soluble protein A canreact with the Fc terminal of IgG molecules of human serum, thereby producing immune aggregates that consume complement. Second, extracellular protein A can bind to the Fc portion of specific anti *Staphylococcal* antibodies coating the microorganism by their Fab fragment, thereby preventing further interaction of the complex with the Fc receptor of phagocytes. Third, cell-bound protein A binds to the Fc fragment of any IgG molecule in its neighborhood, thereby eliminating nonspecific and specific antibodies.[67] The capsule of *Staphylococcus aureus*, which may be present in up to 50 percent of human isolates, impairs the phagocytic process – probably by a stearic hindrance mechanism. Specific anticapsular antibodies are required for opsonization of

such strains. During a *Staphylococcal* infection, a number of antibodies are produced against various cell wall antigens, as well as against various toxins of the organisms. At present,none of these has been capable of inducing full protection against *Staphylococcus aureus* infection.

Sensitivity to antibiotics : A cellular immune response to *Staphylococcus aureus* can also be demonstrated experimentally and in patients with recurrent infections. Its specific role and mechanism of action are difficult to assess, because induction of delayed-type hypersensitivity also implies activation of the humoral response.

Staphylococcus aureus and other Staphylococci are inherently sensitive to many antimicrobial agents. Among the most active is Benzylpenicillin, but about 90% of strains found in hospitals are now resistant. Resistance to Penicillin depends on production of the enzyme penicillinase, a β–lactamase that opens the β –lactam ring. Penicillinase also inactivates most of the other Penicillins, but a few, including Cloxacillin and Flucloxacillin, are stable to the enzyme. Cephalosporins and β–lactamase inhibitors are also stable to penicillinase.

Choice of antibiotic for therapy : Pending receipt of susceptibility test results in the treatment of severe infections suspected to be caused by *Staphylococcus aureus* should be started with Flucloxacillin unless MRSA is endemic locally, in which case a glycopeptide such as Vancomycin is indicated. If the patient is hypersensitive to Penicillin, Erythromycin, Clindamycin, Vancomycin (or Teicoplanin) is used. Fusidic acid and Rifampicin are not used alone in serious infections since mutation to resistance arises readily.

Infections caused by bacteria exhibiting reduced susceptibility to glycopeptides may be treated (if susceptible) with other anti-staphylococcal agents.

Streptococcus pyogenes : Strains of *Streptococcus pyogenes* express a large arsenal of virulence factors and, hence, their pathogenicity and the clinical signs that they induce are very diverse. The virulence factors are involved in adherence, evasion of host immunity and tissue damage.

While some factors are expressed by all clinical isolates, others are variably present among *Streptococcus pyogenes* strains. This variation is due to the horizontal transfer of virulence genes among strains, primarily by transduction, and probably explains the temporal variations in the prevalence of severe infections and sequelae. It furthermore explains differences in virulence of individual strains and the different clinical pictures that may associated with infections due to *Streptococcus pyogenes* are also expressed by some of the other species of pyogenic *Streptococci*. In some species pathogenic for animals, the corresponding virulence factors are expressed in a form specifically adapted to interact with their particular host.

Adhesion virulence factors : Interaction with host fibronectin, a matrix protein on eukaryotic cells, is considered the principal mechanism by which *Streptococcus pyogenes* binds to epithelial cells of the pharynx and skin. The structure that recognizes host fibronectin is located on the F protein, which is one of the many proteins expressed on the surface of *Streptococcus pyogenes*. The interaction between the *Streptococcal* F protein and host cell fibronectin also mediates internalization of the bacteria into host cells. In addition to the F protein, surface-exposed lipoteichoic acid and M proteins appear to be involved in adherence to mucosal and skin epithelial cells.

M Proteins : The ability of *Streptococcus pyogenes* to resist phagocytosis by polymorphonuclear leucocytes is to a high degree due to the cell surface-exposed M protein. The M protein is anchored in the cytoplasmic membrane, spans the entire cell

wall, and protrudes from the cell surface as fibrils. Acquired resistance to infection by *Streptococcus pyogenes* is the result of antibodies in secretions and sera to the M protein molecule. However, as a result of genetic polymorphism in the gene encoding the M protein, the most distal part of the protein shows extensive variability among strains. As a consequence, individuals may suffer from recurrent *Streptococcus pyogenes* infections with strains expressing different versions of the M protein. More than 80 different types of M protein have been identified by serological means.

Capsule : Some strains produce two different M proteins with antiphagocytic activity and some, in addition, a structurally related M-like protein. All these proteins can bind various serum proteins of the host, including fibrinogen, plasminogen, albumin, IgG, IgA, the proteinase inhibitor γ2-macroglobulin, and some regulatory factors from the complement system (factor H and C4b-binding protein). Masking the bacterial surface with host proteins some of these affinities are probably responsible for the ability of M proteins to resist phagocytosis. Thus, factor H is capable of destabilizing the important opsonin C3b when deposited on the bacterial surface. Likewise, the C4b binding protein inhibits surface complement deposition by stimulating degradation of both C4b and C3b.

Some strains of *S.Pyogenes* form a capsule composed of hyaluronic acid. Such strains grow as mucoid colonies on blood agar and are highly virulent in animal models. While capsule production is rare among isolates from uncomplicated pharyngitis, a significant proportion of isolates from severe infections have a capsule. Like other bacterial capsules it has an antiphagocytic effect. The relative significance of the M protein and the capsule as antiphagocytic factors differs among strains. The capsule is identical to the hyaluronic acid of the connective tissue of the host and is not

immunogenic. The bacteria may, in this way, disguise themselves with an immunological 'self' substance.

C5a peptidase : The C5a peptidase, which is found also in human pathogenic strains of *Streptococcus pyogenes*, specifically cleaves, and thereby inactivates, human C5a, one of the principal chemoattractants of phagocytic cells.

Streptolysins : *Streptococcus pyogenes* produces two distinct haemolysins, termed streptolysins O (oxygen-labile) and S (serum-soluble), both of which lyse erythrocytes, polymorphonuclear leucocytes and platelets by forming pores in their cell membrane. Streptolysin O belongs to a family of haemolysins found in many pathogenic bacteria. Intravenous injection into experimental animals causes death within seconds, as the result of an acute toxic action on the heart. Streptolysin O may play a role in the pathogenesis of post-*Streptococcal* rheumatic fever. Serum antibodies can be demonstrated after *Streptococca*l infection, particularly after severe infections.

Streptolysin S is responsible for the β-haemolysis around colonies on blood agar plates. It can also induce the release of lysosomal contents with subsequent cell death after engulfment by phagocytes. In contrast to streptolysin O it is not immunogenic.

Pyrogenic exotoxins : Most strains of *S.Pyogenes* produce one or more toxins that are called pyrogenic exotoxins because of their ability to induce fever. Three, SPE A, SPE B and SPE C, have been extensively characterized.

CLINICAL FEATURES :

The most common route of entry of *S.Pyogenes* is the upper respiratory tract, which is usually the primary site of infection and also serves as a focus for other types of infections. Spread from person to person is by respiratory droplets or by direct contact

with infected wounds or sores on the skin. Not all individuals colonized by *Streptococcus pyogenes* in the upper respiratory tract develop clinical signs of infection.

After an acute upper respiratory tract infection, the convalescent patient may carry the infecting *Streptococci* for some weeks. Only a few healthy adults carry Streptococcus pyogenes in the respiratory tract, but the carriage rate in young school children is just over 10%. It may be considerably higher before or during an epidemic.

TREATMENT :

Streptococc spp. are naturally susceptible to Penicillin and to a wide range of other antibiotics. However, acquired resistance to other agents has become an increasing problem. Although *Streptococcus spp*. are intrinsically resistant to Aminoglycosides, these agents interact synergically with Penicillins and the combination is often used in the treatment of *Streptococcal* and *Enterococcal* endocarditis.

Penicillin resistance has never been detected in *S. Pyogenes*. As a result, Benzylpenicillin (Penicillin G) or oral Phenoxymethylpenicillin (Penicillin V) are the drugs of choice for treatment of infections with *Streptococcus pyogenes*. Antibiotic sensitivity tests are currently unnecessary if that species is identified as the infecting organism. In cases of hypersensitivity to Penicillin, Erythromycin is usually the second choice, but resistance occurs and is common in some countries.

Treatment for 3-5 days will limit the effect of severe attacks of *Streptococcal* infection and prevent suppurative complications as otitis media, but the *Streptococci* will be eliminated from the infected area only if treatment is continued for ten days.

Proteus spp. : These bacteria are characteristically highly motile and chemotaxis may play a part in pathogenesis. Strains of *Proteus* species may also express calcium-dependent and calcium-independent haemolysins in addition to a range of proteases such as an IgAase.

Most strains of *Proteus mirabilis* do not produce β-lactamase. They are consequently moderately sensitive to Benzylpenicillin and fully sensitive to Ampicillin, and most other β-lactam antibiotics. *Proteus vulgaris* strains are usually resistant to Penicillins and many Cephalosporins, although they may be sensitive to β-lactamasestable derivatives such as Cefotaxime. All strains are resistant to Polymyxins and Tetracyclines. *Proteus* and *Providencia* strains are inherently sensitive to Aminoglycosides, but resistance, which may be due to enzymic or non-enzymic mechanisms, is now common.

Klebsiella spp. : *Klebsiella spp*. are widely distributed in nature and in the gastrointestinal tracts of humans and animals. They are found in the oropharynx of 1-6% of normal healthy individuals, however, prevalence as high as 20% may be seen in hospitalized patients.

Colonization of the respiratory tract is very common in hospital patients receiving antibiotics, but its clinical significance is often difficult to assess. Some debilitated patients develop bronchopneumonia in which a *Klebsielle species* appears to be the primary infecting agent. In the early days of antibiotic usage, *Klebsiella spp*.were naturally resistant to the available antibiotics and with the passage of time they acquired resistance to the newly developed ones. The emergence of *Klebsiella spp.* as an important cause of infection in hospitals is undoubtedly related to the use of antibiotics.

Long-chain lipopolysacharide (LPS) protects strains from the action of serum complement, and polysaccharide capsules are thought to confer protection against phagocytosis. Adhesion to host tissues has been attributed to the expression of a range of fimbrial and non-fimbrial adhesions. In common with other members of the Enterobacteriaceae, *Klebsiella spp*. expresses type 1 fimbriae that exhibit mannose-sensitive haemagglutination. Type 3 fimbriae also cause haemagglutination of erythrocytes pretreated with tannin. Additional fimbrial structures (type-6 and KPF-28) and non-fimbrial adhesions have been described. Adhesion CF29K is a plasmid-encoded protein that enables strains to adhere to cultured human cell lines.

In common with many enteric bacteria, *Klebsiellae* express an enterobactin – mediated iron-sequestering system, which uses ferric-siderophore receptors antigenically related to those expressed by strains of *E.Coli*. Strains of *Klebsiella spp*. may also express plasmid encoded aerobactin-mediated high-affinity iron uptake system.

Clinical isolates of *Klebsiella spp.* characteristically produce a β-lactamase that renders them resistant to Ampicillin, Amoxicillin and other Penicillins, but combinations of these drugs with β-lactamase inhibitors such as Clavulanic acid are usually effective.

Klebsiella spp. are normally susceptible to Cephalosporins, especially β-lactamase-stable derivatives such as Cefuroxime and Cefotaxime, and to Fluoroquinolones. Resistance to Chloramphenicol and Tetracycline varies from strain to strain; they are often sensitive to Gentamicin and other Aminoglycosides, but transferable enzymic resistance to Aminoglycosides and other antimicrobial agents has become common in strains found in some hospitals.

***Aspergillosis*:**

Aspergillosis is world wide in occurrence, and various species are ubiquitous in nature. *Aspergillosis* is characterized by a noninvasive colonization of the ear canal.

Secondary metabolites produced by species of *Aspergillus spp*.are toxic. Most cases of *Aspergillosis* develop in individuals who have structural abnormalities in the ear canal like rupture of the TM or blocking of eustachian tube. Healthy macrophages are able to contain hyphae, but not conidia. These are not susceptible to neutrophil and monocyte killing mechanisms. *Aspergillus* products have been described that inhibit activation of the alternative pathway of complement. The conidia of Aspergillus species are able to germinate and colonize the surfaces of the ear canals. Both allergy and superficial trauma are necessary for the development of ear infections. Aspergillus niger is the major cause of otomycosis.

Iatrogenic inoculation – by contaminated foreign bodies (such as clips, pins etc.), during surgery, after antibacterial or immunosuppressive chemotherapy, administration of steroids all have dramatically increased the incidence of opportunistic mycoses in recent years. Under these conditions fungi are either introduced directly into the host, by passing normal defense mechanisms, or the host defenses are all sufficiently suppressed leading to patient enhanced fungus invasion.

Candida spp. : *Candida albicans* is an ovoid or spherical budding cell, which produces pseudomycelia both in culture and in tissues. It is a normal inhabitant of the skin and mucosa. As *Candida spp*.can be seen on normal skin or mucosa only the abundant presence is of significance.

Colonization of most surface with *Candida spp.* is higher among patients than the healthy populations. The intact or physiologically normal epithelium is usually resistant to *Candida spp*. invasion. However, *Candida spp*. may invade if there is a marked, increase in the number of *Candida* present. If the skin and mucosa are traumatized or are hormonally altered then *Candida* spp. may attach to endothelial cells.

Strains of *C. albicans* have long been recognized to vary in animal pathogenicity. Hyphae production and resistance to phagocytic killing are associated with virulence. High doses of extracts of *C. albicans* exhibit endotoxin like activity.

Cells of *C. albicans* are able to attach to the epithelium. All these specific ligand-receptor interaction and germ tubes are more adhesive than yeast cells. *C. albicans* adhere to the mucosal cells, endothelial cells and fibrin-platelet matrices. This adherence and penetration of most cells is associated with pathogenicity.

Most strains of *C. albicans* secrete un driable protease(s) capable of digesting most Ig and other substances. Murine virulence of strains correlates to some degree with proteinase production as well as adherence to epithelial cells.

Whole cell or cell walls of *candida* activate the alternate pathway of complement. *C.albicans* has a surface reception similar to that of human cells, that binds C3bi. It also possess a cytoplasmic protein that it binds to cortisone and related steroid hormones and jresembles the mammalian glucorticoid receptor. Another provocative aspect of *C.albicans* is its immunomodulating activity. The most bio-reactive fractions are cell wall glycoproteins. Depending upon the route and fraction of administration either immuno adjuvant or immunosuppressive activity can be observed in experimental animals.

METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS :* (MRSA):[39,101,102]

MRSA are resistant to currently available beta lactam antibiotics including Penicillin and Cephalosporins. The Clinical and Laboratory Standard Institute (CLSI) defines MRSA as an Oxacillin Minimal Inhibitory Concentration (MIC) of 4 microgram/ml or greater.

The first MRSA infection emerged in England in 1961 in hospital environment shortly after Methicillin was introduced. The incidence of MRSA infections has increased steadily since then. Infections that occur in otherwise healthy persons who have not recently been hospitalized or had a medical procedure (such as dialysis, surgery or IV catheter insertion) are known as Community Associated MRSA (CA-MRSA) infections. Infection acquired in hospital is known as Hospital Acquired MRSA (HA- MRSA). 80% of MRSA infections caused by CA-MRSA and soft tissue infections manifest as furuncle, impetigo, cellulitis or small abscesses.

In some circumstances as in cases complicated by invasive infections (Eg. Bactremia, Necrotising fascities) they can become difficult to treat and even cause death. CA-MRSA also causes Necrotising community acquired pneumonia, infective endocarditis, septic arthritis, otitis media, eye infections, sinusitis etc.

Genetics of Methicillin Resistance : Resistance to Methicillin is conferred by the mecA gene. MecA is a chromosomal gene that encodes an altered binding protein that confers resistance to all beta lactums. It acts as a transposable DNA element and translocates to a chromosomal site. This type of system would allow the spread of antimicrobial resistance to sensitive strains.

Detection and identification : Standard culture protocols don't always screen for Methicillin sensitivity. Therefore, a persistent infection warrants consideration of MRSA infection. The physician should also be aware that Nafcillin sodium and Oxacillin are often used as more chemically stable screening alternatives. Thus the physician reviewing culture results should not be complacement if resistance to Nafcillin or Oxacillin is detected. Since such a finding is the equivalent reporting code for MRSA. The Nafcillin and Oxacillin MIC values can be used as they would for Methicillin. Also, it should be noted that in-vitro antibiotic efficacy is not always indicative of in-vivo efficacy, example as is the case with beta lactums to which MRSA is resistance in-vivo but not in vitro.

Restriction fragmented length polymorphism of ribosomal RNA genes (ribotyping) and quantitate antibiograms have been used to screen and type isolates and are an important tool in tracking the evolution of the organisms and in etiologic identifying potential routes and the third sources of infection such as medical personnel, other patients or family members.

Techniques like Real time PCR and Quantitative PCR are employed for rapid identification of MRSA strain. Latex agglutination test which detect PBP2 a protein (pencillin binding protein) which imports the ability of *S.aureus* to be resistant to Oxacillin can also be used.

Treatment : Community acquired MRSA has a greater spectrum of antimicrobial susceptibility including Sulfa drugs, Tetracycline and Clindamycin, but the drug of choice for treating CA-MRSA has not been established .HA-MRSA is resistant even to

these antibiotics and often is susceptible only to Vancomycin. Newer drugs such as Linezolid may be effective against both CA-MRSA and HA MRSA.

Vancomycin and Teicoplanin are Glycopeptide antibiotics used to treat MRSA infections. Oral absorption of these drugs is low, these can be administered intravenously to control systemic infections.Vancomycin intermediate resistant *S. aureus* (VISA) and Vancomycin resistant *S.aureus* (VRSA) can be treated with Linezolid, Dalfoprestin, Daptomycin and Tigecycline.

EXTENDED SPECTRUM BETALACTAMASE PRODUCERS; (ESBL)[103,104]

The presence of extended-spectrum beta Lactamases (ESBL) can be suspected if an isolate of *Klebsiella pneumoniae* or *Escherichia coli* demonstrates resistance to one or more of the indicator Beta lactam antibiotics, but susceptibility to other third generation Cephalosporins. Unfortunately there is no single indicator antibiotic that works in all situations. ESBL occur in other Enterobacteriaceae, such as *Enterobacter* species and *Citrobacter* species but detection is more difficult because of the frequent occurrence of others types of beta-lactamases, especially Amp C enzymes in these species. The CLSI has provided recommendations for *Klebsiella Pneumoniae* and *E.coli*. Once the possibility of an ESBL has been suggested by the initial testing, it is necessary to perform a confirmatory test based on the fact that these enzymes are almost always blocked by inhibitors of Beta-lactamases. The tests that are currently available are :

Double disk test (DDT).

Potentiated disc diffusion test (PDDT).

Comparisons of MIC or inhibitory zone around disk in presence or absence of Beta lactamases inhibitor.

Vitek ESBL test

E-test

Three dimensional tests

The CLSI criteria for screening and confirming ESBL production in *E.coli* and *Klebsiella spp*. have proven reliable, although there is a possibility of missing some strains if the inoculum is too low. These enzymes are carried on plasmids that often mediate resistance to other antimicrobial agents, such Aminoglycosides, Tetracycline and Sulfonamides. So the appearance of unusual resistance pattern can also serve as a clue that the extended spectrum enzymes are present. Several methods have been suggested to augument the detection of this resistance mechanism.

A double disk test was performed with Ampicillin-Clavulanate disks surrounded by Azetreonum and third generation Cephalosporin disks. Distortion of the zone sizes in a synergestic fashion indicated production of enzymes.

Second method was a three dimensional test in which a circular hole in the agar was cut just inside the eventual position of antimicrobial disks. The hole was filled with bacterial inoculum otherwise the procedure followed the standard disk diffusion protocol. Distortion of the zone sizes at the point of the cut in the agar indicated the presence of enzymes.

Antimicrobials used in CSOM :

It appears that the era of antibiotics is giving way to an age of anxiety, as the emergence of antibiotic resistance is becoming more common[43]. Being prolific and so unique, bacteria can replicate a new generation every few minutes, the generation time can sometimes be as little as twenty minutes[67]. Bacteria seem to inherit this property of antibiotic resistance from their predecessor. Now genes mediating resistance are usually transferred from cell to cell, by way of genetic elements such as plasmids, transferon and bacteriophages[40]. Human negligence is also responsible for the development of antibiotic resistance. As soon as symptoms subside, many patients stop taking antibiotics before completion of therapy and allow partially resistant microbes to flourish. This makes the existing bacteria more resistant and result in the production of stronger and more recalcitrant strain. [69] Antibiotics are the main stay in eliminating the organisms.

The medical therapy of CSOM is directed at the eradication of the pathogens from the middle ear. The selected antibiotics should be effective against the isolated pathogens.[70]

Local instillation of appropriate antibiotic drop is sometimes recommended. However the efficacy of the topical antibiotics depends on them reaching the middle ear through the perforated eardrum. This route is often unreliable and inconsistent as it may be blocked by secretions[70]. The strains of yesterday which were sensitive to Streptomycin,Tetracycline and Chloramphenicol, no longer exhibit the old sensitivity pattern today.

These drugs have been replaced by Aminoglycosides, Quinolones and Cephalosporins. The systemic use of Aminoglycosides has been known to have a

deleterious effect on the middle ear. The fact that CSOM itself causes a sensori-neural hearing loss has led many to conclude the benefits derived from the usage of topical Aminoglycosides in the treatment of CSOM. There is concern that widespread use of Quinolones such as Ciprofloxacin could lead to the emergence of resistance especially in *Pseudomonas aeruginosa, Staphylococcus aureus* and some *Enterobacteriaceae.*[71]

Commonly used antibacterials are Amoxycillin, Ampicillin, Co-trimoxazole, Erythromycin, and first and second generation Cephalosporins such as Cephalexin. The criteria for antibiotics selection are efficacy, penetration into the middle ear fluid, ease of dosing and side effects. Prophylaxis is recommended for children with recurrent attacks of otitis media. However the use of long term, low dose antibiotics as prophylaxis merely masks symptoms of infection and are not truly prophylactic against the basic condition. The length of treatment varies from ten - fourteen days. If OM persists, the treatment is extend for another two-four weeks with the same or other antibiotics. Antibiotics have radically reduced the incidence of complication but have had much less effect on the mortality of established complications. Of equal importance they have altered the clinical pattern of presentation, introducing an element of masking[72]. Selection of any antibiotic is influenced by its efficacy, resistance to bacteria, safety, risk of toxicity and cost. Knowledge of the local microorganisms pattern and their antibiotic sensitivity is then essential to allow for effective and cost saving treatment[73]. Most patients come after failure of treatment by their respective clinicians. This is significant and this requires proper antibiotics according to the organisms cultured from the discharge. Antibiotics against *Pseudomonas spp.* should only be given when the organism is cultured from middle ear.

In the present scenario it becomes very important that each case of CSOM should be studied bacteriologically so as to prevent administration of unwanted antibiotics.

Dosage and Mechanism of action of Antimicrobials :

Aminoglycosides eg. Amikacin and Gentamicin, Kanamycin. These are bactericidal that penetrate the bacterial cell wall and cytoplasmic membrane and act on the ribososome by binding to proteins in 30S segment. Protein synthesis is there by inhibited and cell death ensues. The drugs are rapidly absorbed after intramuscular injections. Peak concentrations are reached in thirty-ninety min. Half life about two hrs. 85-95% of drug is excreted by glomerular filtration into the urine within 24 hours, hence it is not used in patients with renal failure.

Amikacin is more effective against gram negative bacilli than Kanamycin and also effective against *Pseudomonas aeruginosa*. It is also effective against some strains of Gentamicin resistant organisms. The dose is 15-25 mg /kg / day.

Gentamicin : It is used to treat serious infections caused by *P.aeruginosa*, *Enterobacteriacae* other gram negative bacteria. *S.aureus* including some Methicillin resistant strains. Rapidly absorbed after intramuscular injections. Serum level of 3.5 to 5 mcg / ml occur in 1 hr. The dose is 3-6 mg / kg/ dose.

Erythromycin : It is a Macrolide antibiotic most gram positive cocci, aerobic and anaerobic are susceptible to Erythromycin. However resistent strains are reported. It acts on 50S ribosome to inhibit RNA dependent protein synthesis. It is mainly bacteriostatic. The peak blood level is reached after thirty-ninety minute of oral administration. Dosage is 250-500 mg every six hrs. orally and intravenous 1gm every six hrs. is recommended.

This is particulalry useful in the treatment of infections caused by *S.aureus* when betalactum antibiotics cannot be used because of allergy or bacterial resistance. Adverse reactions are nausea, epigastric discomfort and diarrhoea, cholestasis and hepatitis occurs rarely . IV dose can cause thrombophlebitis.

Beta lactum Antibiotics : This large group involves Pencillins, Cephalosporins and new groups Carbapenems and Monobactum. This class of antibiotics inhibits steps in bacterial cell wall synthesis or maintenance. Recently it is found that Pencillins binds to Pencillin binding proteins located between cytoplasmic membrane and cell wall of bacteria.Pencillin binding proteins play a critical role in maintaining the structure of bacterial cell wall.

Susceptibility of bacteria to a beta-lactum antibiotic depends on three factors :

1) The stability of the antibiotic to enzymatic break down.
2) The ability of the antibiotic to penetrate the cell wall in order to reach the site of action.
3) The ability of the antibiotic to bind to various Pencillin binding proteins.

Pencillins : Pencillinase resistant groups of Pencillin (Methicilln, Nafacillin, Oxacillin, Cloxacillin and Dicloxacillin) are generally less active than Pencillin G but their stability to the action of pencillinases makes them useful for the treatment of infections caused by *S. aureus*. Methicillin resistant *S.aureus* is treated with Vancomycin.

Second generation Pencillins include the Ampicilins (Ampicillin, Amoxicillin, Betacillin etc). These compounds have chemical properties that allow penetration of the cell wall of enteric gram negative rods and *H.influenzae*. Amoxicillin has a longer half life and lower risk of diarrhoea compared to Ampicillin..These drugs are susceptible to

hydrolysis by beta lactamases produced by a number of *H.influenzae* and *Klebsiella pneumoniae*. To combat the beta lactamase produced by these bacteria Clavulanic acid has been added to Amoxicillin. It tightly binds and inhibits many beta lactamases.

Third generation Pencillins include Ticaricillin and Carbenicillin. These have activity against *P.aeruginosa* and are also effective on *B. fragillis*.

Fourth generation Pencillin are called Ureidopencillin – Mezlocillin, Azlocillin and Pipercillin. These are having broad spectrum activity against *H.influenzae, Enterococcus spp., Klebseilla spp*. and *P.aeruginosa*.

Cephalosporins : These drugs inhibit bacterial cell wall synthesis and so these are bactericidal. These antibiotics are used as alternative to Pencillin in the treatment of Pnemococcal and Streptococcal infections when patients have history of skin reactions to Pencillin. These are convenient in initial therapy when bacterial pathogen is unknown, since these possess broad spectrum of antimicrobial activity. These are active against gram positive bacteria including *S.aureus* and *Streptococcus spp.*.

It is also active against gram negative rods including *E.coli, K.pneumoniae* and *Proteus spp*..

First generation Cephalosporins : (Cephalexin, Cefadroxil, Cefazolen, Cephalothin and Cephaloridine). These are active against most gram positive and negative bacilli. They are not effective against *Serratia spp., Enterobacter spp., Enterococcus species*, *P.aeruginosa*, *B.fragilis* and *H.influenzae*.

Second generation Cephalosporins : (Cefamandole, Cefaxitine, Cefuroxime and Cefaclor) These are active against *B.fragilis, H.influenzae*. Cefaclor is the only oral Cephalosporin active against *H.influenzae* including Ampicillin resistant strains.

Third generation Cephalosporins : (Cefotaxime, Ceftizoxime, Ceftriaxone, Ceftazidime, Moxalactum**) :** Mainly active on gram negative bacteria like *H.influenzae* and *N. gonorrhae* which are highly resistant to Pencillins. These are active against *P. aeruginosa* and anaerobes including *B. fragilis*.

Adverse reactions: Immediate reactions include anaphylaxis bronchospasm and urticaria

Doses : Cefotaxime 4-12 gm / day 4 times / day

Ceftazidime 8-12 gm/day 4-6 times / day

Ampicillin 2-12 gm / day 4-6 times / day

Oxacicillin 2-12 gm / day 4-6 times / day

Quinolones : Inhibits DNA synthesis. Effective against gram negative organism Ciprofloxacin including *Pseudomonas spp.*

Dose 500mg 8th hrly orally or 200 mg IV 8-12 hrly

Levofloxacin – Dose 500. OD[3,13,33,44,45,46,47,48,49]

Studies related to CSOM and Observations[56,57,58] : Study done by P.T. Wakode and others showed higher incidence of CSOM in low socioeconomic (53.63%) than in high socioeconomic status (7.01%). common age affected are 13-15 years (28%).

Overall incidence of CSOM is 3%. Peak incidence is in July and October. Tonsillitis, rhinitis and dental caries do not contribute to CSOM occurrence.[50]

Study done by P.K. Maji, and others showed mostly younger age group less than 40 year are affected(86.8%). Most common isolate being *Pseudomonas spp.* (64.4%) followed by *S. aureus* (33.8%). In the anaerobic groups(1.8%) most effective antibiotic is Amikacin followed by Gentamicin and Cefotaxime, where as for anaerobic bacteria

Cefaperozone has better sensitivity. Isolation of aerobic and anaerobic bacteria in July to September and *Pseudomonas spp.* in monsoon. [3]

Study by Itzhak Brook and others showed concordance in isolation of bacteria between sinus aspirates and middle ear effusions. Antimicrobials used help to clear both infections.

Study by B D kulkarni and others- Isolated 86.2% of aerobes and 30% anaerobes 7.5% didnot show any organisms. *Psedomonas spp*. was the predominent aerobe, while *Bacteriodes melaninogenica* was the commonest anaerobe recovered.[7]

Gupta Vineeta and others study - Age group commonly affected 21-30 years (33.12%), male predominates (56.69%).

CSOM without cholesteatoma is seen in 56.94%. Acute on chronic otitis media is seen in 17.02%, 39.49% had chronic persistent and 45.31% had cholesteatoma. Acute on chronic type of otitis media, *S.aureus* is commonest. In chronic persistent otitis media *S. aureus* is followed by *Pseudomonas spp*. and *Klebsiella spp*. were isolated. In unsafe ear *Pseudomonas* was grown followed by diphtheroids. Maximum number of organisms seen in chronic persistent form.[10]

Study by Vijay D and Nagarathnamma T showed - Male predominance, Bacteria (51.2%) Fungi (12%) and mixed growth of bacterial and fungi (19.2%) were isolated. *S. aureus* was the most common bacteria followed by *Klebsiella spp*.

Aspergillus was predominant fungus followed by Candida spp. In mixed cultures *S.aureus* followed by *Klebsiella spp*. were common.

Aspergillus and candida were seen in mixed cultures with bacteria.[53]

Sinha et al studied 92 patients isolated bacteria are 65 monobacterial and 18 are polybacterial mono bacterial isolation seen with *Pseudomonas* spp.and *S.aureus* followed

by *Klebsiella spp*. diphtheroids .Bacteria were more sensitive to Ceftrixone 34%, Ceftazidime 31.6%, Cephalaxin 20.79%, Amoxyclav 18.8% Gentamicin 18.8%, Amikacin 17.8%, Cloxacillin 14.8%, nine swabs yielded no growth.[9]

Gulati et al study showed – Out of 100 patients bacteria was recovered in 67, only fungi in six cases, combination of both in five cases, while twentytwo showed no growth. Predominant bacteria isolated were *Pseudomonas aeruginosa* (30) *Klebsiella spp*.and *S. aureus* (12 each) *E.coli* (8) *Proteus spp*.(4) followed by *Pseudomonas + E. Coli* in 3 cases *S. aureus + Klebsiella.spp.* and *S.aureus +Streptococcus haemolyticus* 1 case each. *Candida spp.(*5*) Aspergillus flavus (*3*) Aspergillus niger* (2*) Aspergillus fumigatus* in (1) cases. Ciprofloxacin, Cefotaxime and Gentamicin were found to be the most effective drugs.[8]

Study by Kshitiz Bhatnagar et al showed that majority were tubotympanic (91.2%) and single bacterial infection, 8.8% had attico antral disease. Acute respiratory illness were the most common predisposing factor (65.6%) followed by trauma (21.8%), vaccine preventable diseases(2.3%), bacteria isolated in 84.4% and fungus in 25.9% cases. Among bacteria *S. aureus* (21.8%) and *Proteus spp.* (17%) *Pseudomonas spp.* and *Streptococcus* spp. (15.6%) each, Micrococci in 11.6% cases, Candida spp. in (17.7%) followed by Aspergillus spp. (8.2%)[51].

Zain AL et al showed-; 44% pure cultures 33.3% were mixed and 18.6% no growth. There were 74% aerobes, 25% fungi 0.9% anerobes. *Pseudomonas spp*. (22.5%) was the most common followed by *S.aureus* and *Aspergillus spp*.[52]

MATERIAL AND METHODS [96,97,98]

The present study **"Bacteriological study of chronic otitis media with reference to aerobes"** was conducted in the Department of Microbiology, KLE University, Jawaharlal Nehru Medical College, Belgaum from Jan 2009 to Dec 2009. Ninety nine patients with CSOM of all age groups and both sexes attending ENT Outpatient Department, DR. Prabhakar Kore Hospital and MRC were selected randomly for the study.

INCLUSION CRITERIA :

- All clinically diagnosed new cases of CSOM (tubo-tympanic type) of all age groups and both sexes attending ENT Outpatient Department at DR. Prabhakar Kore Hospital and MRC, Belgaum.
- Prolonged otorrhoea (more than 2 months duration) were chosen for the study.

EXCLUSION CRITERIA :

- Diagnosed cases of otitis externa
- Foreign body in the external auditory canal
- Diagnosed cases of Attico-antral type of CSOM
- Patient on antibiotic 48hrs prior to collection of sample were excluded from the study

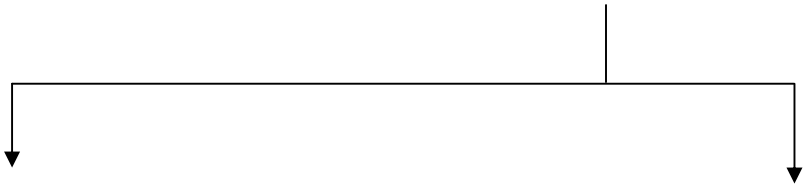
HISTORY TAKING AND EXAMINATION : A proforma was filled for each patient documenting age, sex, address and clinical information including chief complaints, duration of symptoms, predisposing factors and any previous history of treatment, other medical history like diabetes, hypertension, tuberculosis and HIV etc were noted.

COLLECTION OF SAMPLE : Ear discharge was collected under aseptic precaution in clinically diagnosed cases of CSOM attending ENT Outpatient department. Excess discharge was mopped out from external auditory canal and it was cleaned with 70% alcohol first and was allowed to act for 30-40sec to achieve sterile area. Then with the two sterile swabs, specimens were collected. One was for Gram staining and other one was for aerobic culture. Both the swabs were processed immediately in the laboratory.

DIRECT SMEAR EXAMINATION : With one swab thin smear was made on a clear glass side and were fixed by flaming over the Bunsen burner. After fixing, Gram staining was done for the smear and was examined under oil immersion objective to note the various morphological types of bacteria, Gram reaction, presence or absence of inflammatory cells and also to note the numbers of squamous epithelial cells in the sample.

FOR AEROBIC CULTURE:

Days	**Procedure**
Day 1	Gram staining done Inoculated on BA, MA, CA and CA plate Was incubated in a candle jar with 5-10% Carbon dioxide for 18- 24 hrs. at 37^{o}C
Day 2	observed for growth



Growth

1) Colony characters observed
2) Smear for gram stain done
3) Hanging drop for motility
4) Tests for enzymes like Catalase, Oxidase, Coagulase
5) Biochemical tests done
6) Sugar fermentation test- glucose,lactose,sucrose Maltose,mannose,arabinose,xylose
7) Antibiotic susceptibility testing done by using Kirby Bauer disc diffusion method on Mueller-Hinton agar plate.

No Growth → Further incubation → If still no growth -Discarded

Day 3 Observed the biochemical tests, sugar fermentation and sensitivity pattern were Read. The sensitivity pattern reported to The ENT surgeon.

GRAM NEGATIVE BACILLI : For lactose fermenting and non-lactose fermenting colonies, further Gram staining,Hanging drop for motility and catalase and oxidase tests were done. After which the following biochemical tests were done. They include-

- Indole test
- Methyl Red
- Voges Proskauer
- Citrate
- Urease
- Nitrate reduction
- Phenylalanine dehydrogenase
- Mannitol motility
- Triple sugar iron
- O-F glucose fermentation
- Sugar fermentation

GRAM POSITIVE COCCI : For gram-positive bacteria that are gram-positive cocci-catalase test, oxidase tests were done. For cocci in clusters oxidase negative, catalase positive, slide and tube coagulase tests were done. For gram positive cocci in pairs, are arranged at an angle to each other and also in short chains, species-catalase negative-growth at 45^0C, growth in 6.5% Nacl, bile esculin hydrolysis were done. The colonies which were seen on the aerobic culture plates were examined in detail by the methods specified by Mackie and Mc Cartney.

1. Gram's stain : A thin smear from a single colony was made on a glass slide and fixed by flaming over the Bunsen burner. After fixing crystal violet was poured over the smear,

care was taken to completely cover the smear, it was allowed to stand for 1min, washed with tap water. Then Gram's iodine was poured over the slide and kept for one min.,washed with water and was decolourised by 95% alcohol till the blue color of crystal violet disappeared. The slide was washed with water and counter stained by safranine for 1min. Then the slide was washed with water, dried and was observed under oil immersion objective. In all those smears, gram negative bacilli were seen, further motility of these organism were noted by hanging drop method.[96]

Quality control :

Positive control: *Staphylococcus aureus* ATCC 25923

Negative control : *Escherichia coli* ATCC 25922

2. Hanging drop : For this, a small amount of paraffin wax was placed around the lip of the well on the concavity slide. A smooth saline suspension of an individual colony was prepared with the help of a clean sterile loop and loopful of the material was placed on a cover slip. The slide was inverted and pressed over the cover slip, the guiding the drop of bacterial suspension in to the centre of the well. The slide was carefully examined by hanging drop method to know the motility of the different gram negative bacilli. In doubtful cases, motility was confirmed by inoculating onto semisolid media.

One peptone water tube inoculated with the growth. Care was taken to select individual discrete colonies. The tube was kept at 37Ċ for 2 hours and turbidity was compared with 0.5 MacFarland's standard. It was used for sugar fermentation tests, biochemical tests and also for antibiotic sensitivity.

BIOCHEMICAL TESTS:

3. Indole test :

Principle : This test was done to demonstrate the ability of certain bacteria to decompose the amino acid tryptophan in to indole.Here tryptophan rich medium was used**.**

Procedure: Kovac's reagent method was employed.

Kovac's reagent- preparation,

Amyl or isoamyl alcohol	150ml,
β-dimethylaminobenzaldehyde	10gm,
concentrated HCL	50ml.

Individual colonies were inoculated on to tryptophan broth were incubated at 37^{o}C for 18-24hrs. To this 0.5ml of Kovac's reagent was added and gently shaken.[98]

Interpretation : Appearance of red color was taken as indole producer.

Quality control :

Positive control: *Escherichia coli* ATCC 25922

Negative control : *Klebsiella pneumoniae*.

4. Urease test :

Principle : This was done to determine the ability of bacteria to decompose urea in ammonia. Here Christensen,s urea agar medium was used.

Procedure: Inoculate heavily over the entire surface of Christensen's urea agar medium with the peptone water culture and incubate at 37^{o}C. Examine after 4hrs and then overnight incubation.[98]

Interpretation : Positive: when the indicator turned to purple-pink

Negative: no change in colour.

Quality control :

Positive control: *Proteus species,*

Negative control: *Escherichia coli.*

5. Citrate utilization test:

Principle : To determine the ability of bacteria to utilize citrate as sole source of carbon for its growth. Simmon's citrate medium was used to know the utilization of citrate.

Procedure : The citrate slant was inoculated with the suspected single colony and medium was incubated at 37^{o}C for 24 to 48hrs. A positive reaction was indicated by blue colour and streak of growth. A negative reaction if original green color and no growth.

Quality control:

Positive control: *Enterbacter aerogenes,*

Negative control*:Escherichia coli.*

6. Triple sugar iron agar test :

Principle : This was done to determine the ability of bacteria to ferment carbohydrates incorporated in a growth medium and production of hydrogen sulfide**.** Triple sugar iron (TSI) agar medium contains 10 parts Lactose, 10 parts sucrose, 1 part glucose and peptone. Phenol red and ferrous sulphate serve as indicators of acidification and H_2S production respectively. With a sterile straight inoculating wire, touch the top of a well-isolated colony.

Procedure : Inoculate TSI by first stabbing through the centre of the medium to the bottom of the tube and then streaking the surface of the agar slant. Incubate the tube at 37^{o}C for 18 to 24hrs. the results were interpreted as follows.

Interpretation :

Slant/butt	**Color**	**Utilization**
Alkaline slant/Nochange in butt (K/No change)	Red/No Change	Glucose, lactose, sucrose non-utilizers.
Alkaline slant/Acid butt(K/A)	Red/Yellow	Glucose only fermented; peptones utilized
Acid slant/Acid butt(A/A)	Yellow/Yellow	Glucose fermented, lactose and/or sucrose fermented
Alkaline slant/Alkalinebutt bult(K/K)	Red/Red	No fermantatin of glucose, lactose or sucrose. Peptones utilized

A black precipitate in the butt indicates production of Ferrous sulphide and H_2S gas (H_2S^+). Bubbles or cracks in the media indicate the production of CO_2 or H_2.[97]

7. Methyl Red test :

Principle : To determine the ability of bacteria can maintain the low p^H after prolonged incubation.

Procedure : Inoculate the glucose phosphate peptone water medium with a young culture and incubate at 37^{o}C for 48 hrs. To this, add about five drops of the methyl red reagent. Mix and read immediately. Positive tests are bright red and negative tests are yellow.

Quality control :

Positive control: *Escherichia coli* .

Negative control: *Enterobacter aerogenes*

8. Voges Proskauer test :

Principle: This test was done to determine the ability of an organism to produce neutral end products like acetyl methyl carbinol or it reduction product 2,3 butylene glycol from glucose fermentation.

Medium : Glucose phosphate peptone water.

Procedure : Inoculate the glucose phosphate peptone water medium with a young culture, and incubate at 37^{o}C for 48hrs, to this, add 1ml of 40% potassium hydroxide and 3ml of solution of α-naphthol in absolute ethanol.

Interpretation : A positive reaction is indicate by the development of a pink color in 2-5min, becoming crimson in 30 min.

Quality control :

Positive control: *Enterobacter aerogenes,*

Negative control: *Escherichia coli.*

9. Sugar fermentation test:

Principle : This test was done to determine the ability of an organism to ferment a specific carbohydrate that is incorporated in a basal medium, thereby producing acid with or without visible gas.

Procedure : The test was performed on conventional culture media with test sugar.The common sugar fermentation media used for present study were glucose, sucrose, lactose, maltose, mannose, arabinose and xylose. From the peptone water tube (which was incubated for 2hrs. after inoculation) all the sugar fermentation media were inoculated with the help of a clean sterile loop. Care was taken to sterilize the loop everytime, after dipping in different sugars. After the different media were inoculated, these were incubated at 37^{o}C for 18-24hrs. after 24hrs, the sugar media were examined for the production of acid indicated by pink color and gas (presence of an air bubble inside the durham's tube).

Interpretation: Positive test in indicated by change in color to pink with or without gas formation in durham's tube. Negative test is indicated by growth, but no change in colour.[98]

10. Oxidation/ Fermentation test (Modified Hugh and Leifson) :

Principle : This test was done to know the organism uses carbohydrate substrate to produce acid by products either oxidative or fermentative.

Procedure : Hugh-Liefson's basal medium prepared and carbohydrate to be added was sterilized separately and added to give final concentration of 1%. The medium was then tubed to a depth of about 4cm.

Duplicate tubes of medium were inoculated by stabbing. One tube was promptly covered with a liquid paraffin to a depth of 1cm and were incubated at 37^{o}C for 18-24hrs.[98]

Interpretation :

- Fermenting organisms produce an acid reaction throughout the medium is covered (anaerobic) as well as the open (aerobic) tube.
- Oxidizer produce an acid reaction only in the open tube.
- Organisms that cannot breakdown the carbohydrate aerobically or anaerolically produce an alkaline reaction in the open tube and change in covered tube.

11. Nitrate Reduction test:

Principle : The test is used to determine the ability of an organism to reduce nitrate. The reduction of nitrate to nitrite is determined by adding sulfanilic acid and alpha-napthylamine. The sulfanilic acid and nitrate react to form a diazonium salt. The diazonium salt then couples with α-napthylamine to produce a red, water soluble azo dye.

Procedure : This liquid medium was inoculated with the suspected single colony and the medium was incubated for 18-24hrs.

Add 0.1ml of the test reagent to the test culture. The test reagent was prepared by mixing equal volumes of solution A (8.0gm of sulphanilic acid in 1liter of acetic acid 5mol/liter) and solution B (5.0gm of α-napthylamine in 1 liter of acetic acid 5mol/liter). A red color developing within a few minutes indicates the presence of a nitrate. No colour indicated that nitrate have not been reduced or reduced to product other than nitrites, such as ammonia, molecular nitrogen, nitric oxide or nitrous oxide and hydroxylamine. Addition of zinc dust to all negative tests was necessary. Zinc ions reduced nitrates to nitrites and development of red colour after addition of zinc dust indicated the presence of residual nitrates.[98]

Quality control:

Positive control : *Escherichia coli*

Negative control: *Acinetobacter baumannii*

12. Phenylalnine Deaminase test (PPA) :

Principle : To determine the ability of bacteria to deaminate phenylalanine to phenyl pyruvic acid.

Procedure : This test was done to know the ability of the organism to deaminate phenylalanine with the production of phenyl pyruvic acid, which reacts with ferric salts to give green color. Inoculated the agar slope medium containing DL-phenylalanine with a fairly heavy inoculum and incubate $37^{o}C$ for 18-24hrs. After incubation, allow a few drops of 10% solution of ferric chloride to run down over the growth on the slope if the test is positive, a green color will develop in the fluid and in the slope.

Quality control:

Positive control: *Proteus species.*

Negative control: *Escherichia coli.*

13. Amino acid decarboxylase and Arginine dihydrolase test:

Principle : Decarboxyases are agroup of substrate- specific enzymes that are capable of reacting with the carboxyl portion of amino acids, forming alkaline-reacting amines.The conversion of arginine to citrullin is a dehydrolase, in which an NH_2 group is removed from arginine.

Procedure : Here Moellar decarboxylase broth base was used.Test organism was inoculate the medium with straight wire then overlay tubes with sterile mineral oil to cover about 1 cm of the surface. Incubate and read daily for 4 days. The medium first becomes yellow due to acid production during glucose fermentation later if decarboxylation occurs, the medium turned violet colour.

Quality control :

Positive control: *Pseudomonas aeruginosa.* ATCC 27853

Negative control: *Klebsiella pneumoniae*.

14. Catalase test :

Principle : The enzyme catalase mediates the break down of hydrogen peroxide (H_2O_2) into oxygen and water. The presence of the enzyme in a bacterial isolates is evident when a small inoculum is introduced into H_2O_2, and the rapid elaboration of oxygen bubbles occurs. The lack of catalase is evident by a lack of or weak bubble.

Procedure : Presence of catalase was demonstrated by test tube method. A small amount of the culture to be tested was picked from a nutrient agar plate with a clean sterile

platinum loop or a clean, thin glass rod and was inserted in to 3% hydrogen peroxide solution held in a small, clean tube.

Interpretation : The production of gas bubbles from the surface of the solid culture material indicates a positive reaction and negative reaction when there were no gas bubbles.[96]

Quality control :

Positive control: *Staphylococcus aureus*

Negative control: *Streptococcus pyogenes*

This test was used to differentiate *Staphylococcus spp.* from *Streptococcus spp*.

15. Oxidase test :

Principle : To determine the presence of an enzyme oxidase, which catalyse the transport of electrons between electron donors in the bacilli and redox dye. The dye is oxidized to indophenol blue producing deep purple colour.

Procedure : Wet filter paper method was used for this test. Strips of Whatman's No. 1 filter paper was soaked with a little freshly made 1% solution of tetramethyl-para-phenylene-diamine dihydrochloride and then with a help of sterile glass rod a single colony from the medium was rubbed over the strip.

Interpretation : A positive reaction was indicated by an intense deep purple blue, appearing within 5-10 seconds and a negative reaction by absence of colouration or by colouration later than 60seconds.

Quality control:

Positive control: *Pseudomonas aeruginosa ATCC 27583*

Negative control: *Escherichia coli ATCC 25922*

16. Coagulase test:

This was done to differentiate between the staphylococcus species.

Both slide coagulase test and tube coagulase test was done.

a) Slide Coagulase test :

Principle : Staphylococcal coagulase is a protein that has prothrombin like activity which can convert fibrinogen to fibrin. A visible clot will result. Slide coagulase test detects bond coagulase which is attached to the bacterial cell wall and is not present in culture filtrate. Fibrin strands are formed between the bacterial cells when suspended in plasma (fibrinogen), causing them to clump into visible aggregates.

Procedure : A smooth milky suspension of the growth was made in normal saline over a clean glass slide. Make similar suspension of control positive and negative strains to conform the proper reactivity of the plasma. To the test suspension a loop full of undiluted human plasma was added and the suspension was observed for the appearance of coarse clumps.

Interpretation : Read as positive when a course clumping of cocci was visible to the naked eye within10 seconds. Read as negative when there was absence of clumping of any reaction within 10 seconds or slow reaction was seen after 10 seconds. Negative and slow reacting stains were re-examined by the tube test.

b) Tube Coagulase test :

Principle: Detects free coagulase, a thrombin like substance, present in culture filtrate. Free coagulase reacts with serum substance (coagulase reacting factor) to form a complex that, in turn, reacts with fibrinogen to produce the fibrin clot .

Procedure : Prepare a 1 in 6 dilution of the plasma in saline (0.85% NaCl) place 1ml volumes of the diluted plasma in a small tube. Emulsify a colony of the *Staphylococcus* under test in a tube of the diluted plasma. With each batch of test include positive and negative control, a tube of unseeded diluted plasma to confirm than it does not clot spontaneously. Incubate the tube at 37^{o}C preferable in a water bath, for upto 4hrs. Examine at 1, 2 and 4hrs for clot formation by tilting the tube through 90^{o}C. Leave the negative tubes at room temperature over night and reexamine.[98]

Interpretation:

Positive: Any degree of visible clot formation or stiff gel formation or if clots was seen floating in the medium.

Negative: When plasma remains wholly liquid or showed only a flocculent or ropy precipitate.

Quality control:

Positive control: *Staphylococcus aureus*

Negative control: Coagulase negative *Staphylococcus*.

17. Salt Tolerence test :

Principle : Ability of grow in the presence of variable amounts of sodium chloride.

Procedure : This test was done to know the *Enterococcus* species. Inoculate 1 to 2 colonies from a 18-24hrs culture into 6.5% NaCl broth which contains glucose and bromocresol purple as the indicator for acid production. Incubate tube at 37^{o}C for 48hrs. Check daily for growth. Positive test was indicated by visible turbidity in broth with or without color change from purple to yellow and negative test was indicated by no turbidity and no colour change.

18. Growth at 45°C:

This test was done to know the ability of the organism to grow at 45°C. inoculate one colony into tube containing Nutrient or Todd Hewitt broth. Place in water bath at 45°C and incubate for 7days. Judge the growth by the turbidity seen after rotating the tube to disperse any sediment.

19. Bile Esculin test :

Principle : This test was done to know the ability of organism to hydrolyse the esculin in the presence of bile(4% bile salts).

Procedure: Inoculate 1 to 2 colonies from an 18-24hrs culture on to the surface of bile esculin agar slant. Incubate at 37°C for 48hrs. positive test was indicated by blackening of the agar slant and negative test was indicated by no blackening of medium.[98]

Quality control : Positive control: *Enterococcus faecalis*

Negative control: *Streptococcus viridians.*

Based on these tests, the following individual organisms were identified with characteristics mentioned below :

Staphylococcus aureus :

They are gram positive, spherical cocci of about 1 micrometer in diameter, arranged in grape like clusters or singly, in pairs and short chains. They are aerobes and facultative anaerobes, with temperature range of 10-42°C, the optimum being 37°C and pH 7.4-7.6. they grow readily on ordinary media forming colonies of 2-4mm diameter, circular, convex, smooth, shiny, opaque and easily emulsifiable. They produce non-diffusible golden yellow pigment. Most of strains are hemolytic.

They are catalase positive and oxidase negative, slide and tube coagulase test positive. They ferment number of sugars, producing acid but no gas. Mannitol is fermented anaerobically.

***Enterococcus fecalis* :**

They are gram positive, ovoid cocci occur in pairs, are arranged at an angle to each other and also in short chains. They are non-motile and non-capsulated. They grow readily on ordinary media and on MacConkey agar, on which they form small (0.5-1mm), magenta coloured colonies. They are usually non-hemolytic but sometimes may produce alpha or beta-hemolysis.

They are catalase negative and are able to grow in the presence of 40% bile, 6.5% sodium chloride, pH 9.6 and at 45^{o}C.They are relatively heat resistant, surviving 60^{o}C for 30minutes. They ferment mannitol, sucrose, sorbitol and aesculin. They form black colonies on tellurite blood agar.

Klebsiella pneumoniae:

They are gram negative, short, plump, straight rods, about 1-2 x 0.5-0.6mm in size. They are non-motile, non-sporing and capsulated. They grow well on ordinary media large, opaque, dome shaped, mucoid colonies of varying degree of stickiness. They produce bright pink colonies on MacConkey agar due to lactose fermentation. They are catalase positive, oxidase negative, reduce nitrates to nitrites. Indole, MR are negative and VP, citrate are positive. They produce acid/acid on TSI with abundant gas. They are urease positive. They ferment a wide range of carbohydrates including glucose, lactose, sucrose, mannitol, xylose, adonitol and inositol with production of acid and abundant gas. Lysine decarboxylase positive but ornithine decarboxylase negative.

Klebsiella oxytoca:

They are similar to *Klebsiella pneumoniae* in their morphology, growth requirement and biochemical reactions except Indole test which is positive.

Citrobacter freundii:

They are gram negative straight rods, 1 micrometer x 2-6micrometer in size, occur singly or in pairs. They are motile, non-capsulated and non-sporing. They produce small, circular, opaque or translucent colonies on nutrient agar. They produce light pink coloured on MacConkey agar due to late lactose fermentation.

They are catalase positive, oxidase negative, reduce nitrates to nitrites. Indole negative, MR positive, VP negative, citrate positive, H_2S is produced. They ferment glucose, lactose(late), sucrose, maltose, xylose, mannitol with production of acid and gas. They are lysine decarboxylase negative.

Proteus mirabilis:

They are gram negative, pleomorphic, straight or curved rods 0.4-0.8 micrometer x 1-3micrometer in size. They are motile, non-sporing and non-capsulated. They grow as discrete pale non-lactose fermenting colonies on MacConkey agar. They produce characteristic swarming growth with a ‘fishy’ or ‘semen’ odour on nutrient and blood agar.

They are catalase positive, oxides negative, reduce nitrates to nitrites, indole negative, MR positive, VP negative and citrate positive. They hydrolyse urea very rapidly. H_2S is produced, phenyl alanine deaminase test(PPA) is positive. They ferment xylose, trehalose with production of acid and gas. They are ornithine decarboxylation positive, lysine and arginine decarboxylation negative.

Pseudomonas aeruginosa:

They are gram negative, slender bacilli 1.5-3micrometer x 0.5 micrometer in size, actively motile, non-sporing, non-capsulated and non-fermentative.

They are obligate aerobes. Growth occurs at a wide range of temperatures 6-42^{o}C, the optimum being 37^{o}C. the optimum pH is 7.4-7.6. They grow well on ordinary media, producing large, opaque, irregular colonies, with a distinctive, musty or earthy smell with bluish green water soluble pigment. They produce diffuse hemolysis on blood agar.

They are catalase positive, oxides negative, reduce nitrates to nitrites, indole, MR positive, VP negative and citrate positive. Produce alkaline/no change reaction in TSI medium. Arginine decarboxylase test is positive but lysine and ornithine decarboxylase tests are negative. They are oxidative but not fermentative on O-F test.

Acinetobacter spp.:

They are gram negative or gram variable bacilli or coccobacilli, aerobic, short, stout, non-motile, non-sporing and often capsulated. They grow well on simple media producing colonies which are white or cream coloured, smooth, circular and opaque. They are non-hemolytic on blood agar. On MacConkey agar they produce a faint pink tint.

They are catalase positive, oxidase negative, do not reduce nitrates to nitrites. Indole negative, produce alkaline/ no change in TSI medium. They utilize citrate. They do not ferment sugars. They are only oxidative on O-F test.

Further these bacterial antibiotic sensitivity pattern was examined.

Antibiogram testing :

Antibiotic sensitivity was tested by Kirby-Bauer's disk diffusion method. Mueller-Hinton agar plate was used. One-two colonies from the culture plate were inoculate into 2ml of peptone water. Incubated at 37^{o}C for 2hrs. turbidity was compared to that of 0.5 McFarland's standard(1.5 x 10^{8} CFU/ml). A cotton swab was immersed

rotated in this inoculum, the swab was then pressed to the sides of the tube so on to remove excess inoculum. The swab was then used to inoculate the plate of Mueller-Hington agar, in three different directions to ensure an even and complete distribution of the inoculum over the entire plate. The antibiotic discs were applied within 15 minutes of inoculation of plate and the plate inverted for incubated for 18-24 hrs. at 37Ċ.

Commercial obtained Himedia discs were used. The strength of discs used and their zone size interpretative standards were according to guidelines by NCCLS guidelines standards.

If these organisms were not sensitive to any of the drugs, then a second line of antibiotics was put up using the same procedure as observed. The drugs used for gram positive organisms were

- Ampicillin (A) (10microgram)
- Gentamicin (G) (10microgram)
- Erythromycin (E) (15microgram)
- Ciprofloxacin (Cf) (5microgram)
- Ceftazidime (Ca) (30microgram)
- Amoxyclav (Ac) (30microgram)
- Oxacillin (Ox) (1microgram)

The drugs used for gram negative organisms were :

- Amikacin (Ak) (30microgram)
- Erythromycin (E) (15microgram)
- Ciprofloxacin (Cf) (5microgram)
- Cefotaxime (Ce) (30microgram)
- Amoxyclav (Ac) (30microgram)
- Levofloxacin (Le) (5microgram)

Methicilline Resistant *Staphylococus aureus* (MRSA):

Disc diffusion test : This test was done to know the methicilline resistant *Staphylococcus* strains. A direct colony suspension of each S. aureus isolated was prepared to a 0.5 McFarland standard and placed on Mueller-Hinton agar containing 2-4% NaCl. An Oxacillin(1microgram) disc was placed on the surface and incubated at 30^{o}C for 24hrs. after incubation, the zone of inhibition was recorded. If zone diameter lesser or equal 10mm was considerd as resistant, greater or equal 13mm as susceptible. Where 11-12mm was considerd an intermediate there, instead of Methicillin. Oxacillin disc was used.

Extended Spectrum Beta-Lactamase by Potentiated Disc Diffusion Test [PDDT]:

This test was done for gram negative bacilli to know the presence of extended spectrum beta-lactmase resistance to one or more of indicator Beta-lactam antibiotics but susceptibility to other third generation cephalosporins. For this test, Mueller-Hinton agar plates were prepared and inocubated with standardized inoculum (corresponding to 0.5 McFarland tube) to form a lawn culture. With a sterile forceps, Ceftazidime (30microgram) disc was placed on the agar near the centre. Giving a center to centre distance of 20-30mm, Ceftazidime/Clavulanic acid (20/10microgram) disc was placed in line with Ceftazidime. The plates were inverted and incubated at 37^{o}C for about 16 to 18hrs. if the strain is ESBL producers, then the zone around Ceftazidime is extended on the side nearest the Caftzidime/Clavulanic acid. If greater 5mm is considered to be ESBL producers.[96]

Quality control:

Positive control: *Klebsiella Pneumoiae* ATCC 700603

Negative control: *Escherichia coli* ATCC 25922 were used.

Follow Up:

On the third day, the antibiogram reports were personally given to the concerned ENT surgeon. During the treatment period follow up examination was performed on the 10^{th} day and 1 month of the start of treatment. Patients outcome were evaluated by otoscopic examination. The cessation of otorrhoea post treatment was taken as indicator for clinical success.

RESULTS

Ninety nine (99) clinically diagnosed cases of Chronic suppurative otitis media attending ENT Outpatient Department, KLE's DR. Prabhakar Kore Hospital and MRC were studied in the Department of Microbiology Jawaharlal Nehru Medical College, Belgaum. Observations made from the study are as follows :

Table 1 : Agewise distribution

Age group	Male		Female		Total	
	No. of cases	%	No. of Cases	%	No. of cases	%
0-10	12	21.4	9	20.9	21	21
11-20	19	33.9	11	25.6	30	30
21-30	14	25	6	13.9	20	20
31-40	4	7.1	9	20.9	13	13
41-50	4	7.1	4	9.3	8	8
51-60	2	3.6	2	4.7	4	4
>60	1	1.8	2	4.7	3	3
Total	**56**	**100**	**43**	**100**	**99**	**100**

Maximum number of cases falls in the age group 11-20 years (31%) and 0-10 years (21%), male to female ratio 1.2 : 1.0. Males (56.6%) predominately affected than female (43.4%) .

Graph- 1 : Graph showing age wise distribution

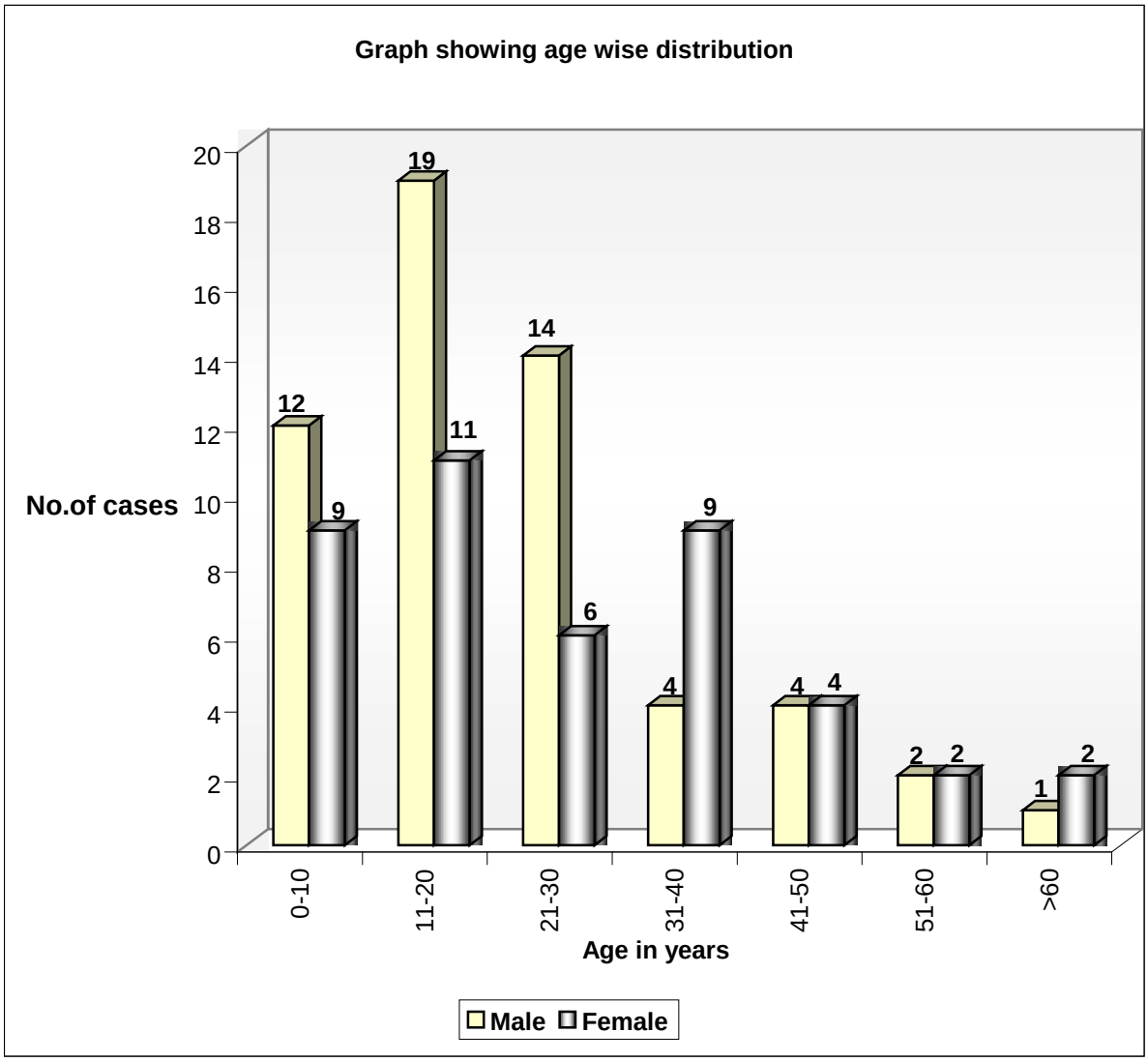


Table – 2 : Sexwise distribution

Sex	Total no. of cases studied	Total no. of positives	Percentage
Male	56(56.6%)	52	60.5
Female	43(43.4%)	34	39.5
Total	**99(100%)**	**86**	**100**

Out of ninety nine cases 56(56.6%) were males and 43(43.4%) were females, out of 56 males studied 52(60.5%) were positive for culture and out of 43 females 34(39.5%) were positive for the culture. Above table shows incidence of CSOM was higher in males compared to females.

Graph- 2 : Graph showing sex wise distribution

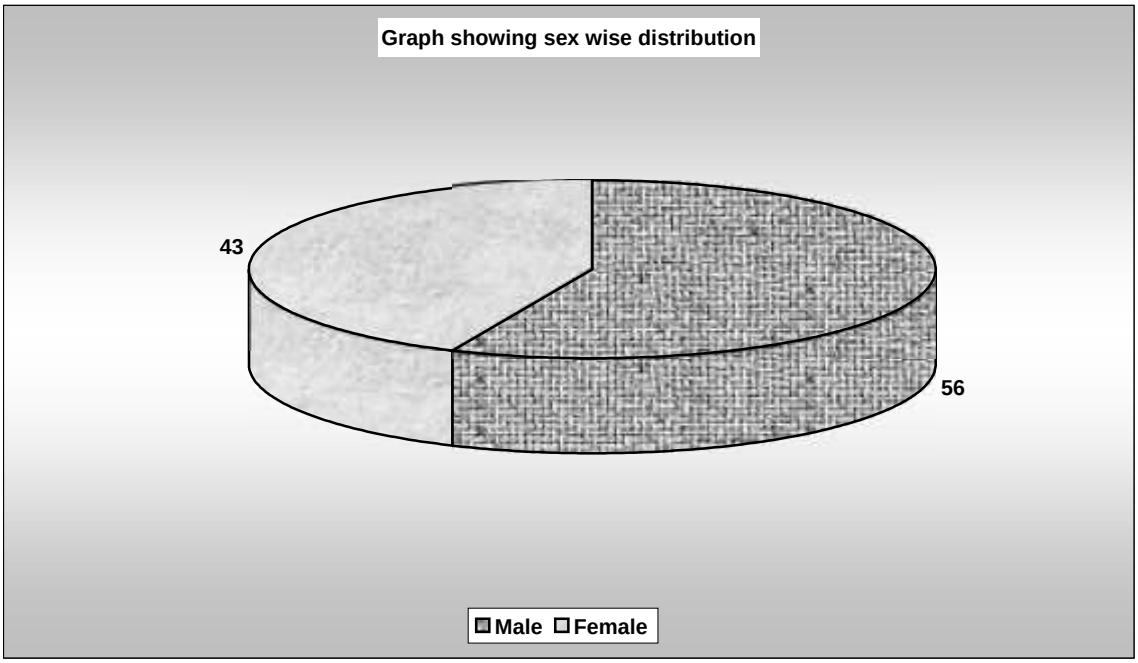


Table – 3 : Distribution of CSOM subjects according to the side of ear affected

Side of the ear	Male	%	Female	%	Total	%
Left	20	35.1	16	38	36	36.4
Right	24	42.1	16	38	40	40.4
Bilateral	13	22.8	10	23.8	23	23.2
Total	**57**	**100**	**42**	**100**	**99**	**100**

The side of involvement showed right ear was predominant (40.4%) compared to left ear (36.4%). Bilateral infection seen in 23(23.2%) of cases.

Graph- 3 : **Graph showing distribution of CSOM cases according to side of ear affected**

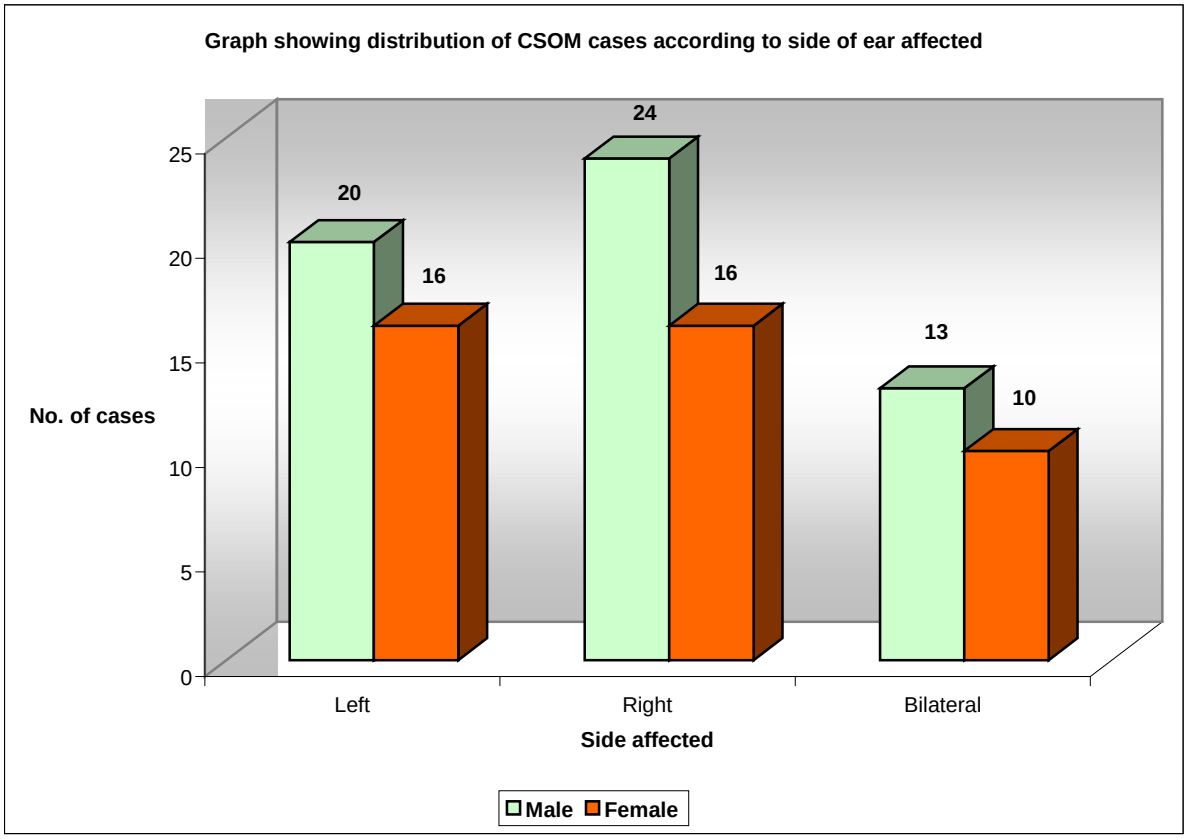


Table – 4 : Area wise distribution

Locality	No. of patients (n=99)	Percentage (%)
Rural	72	72.7%
Urban	27	27.3%
Total	**99**	**100%**

Out of ninety nine cases 72(72.2%) were from rural areas and 27(27.3%) cases were from urban areas. Above table shows that incidence of CSOM was common in rural areas compared to urban areas.

Graph- 4 : Graph showing area wise distribution

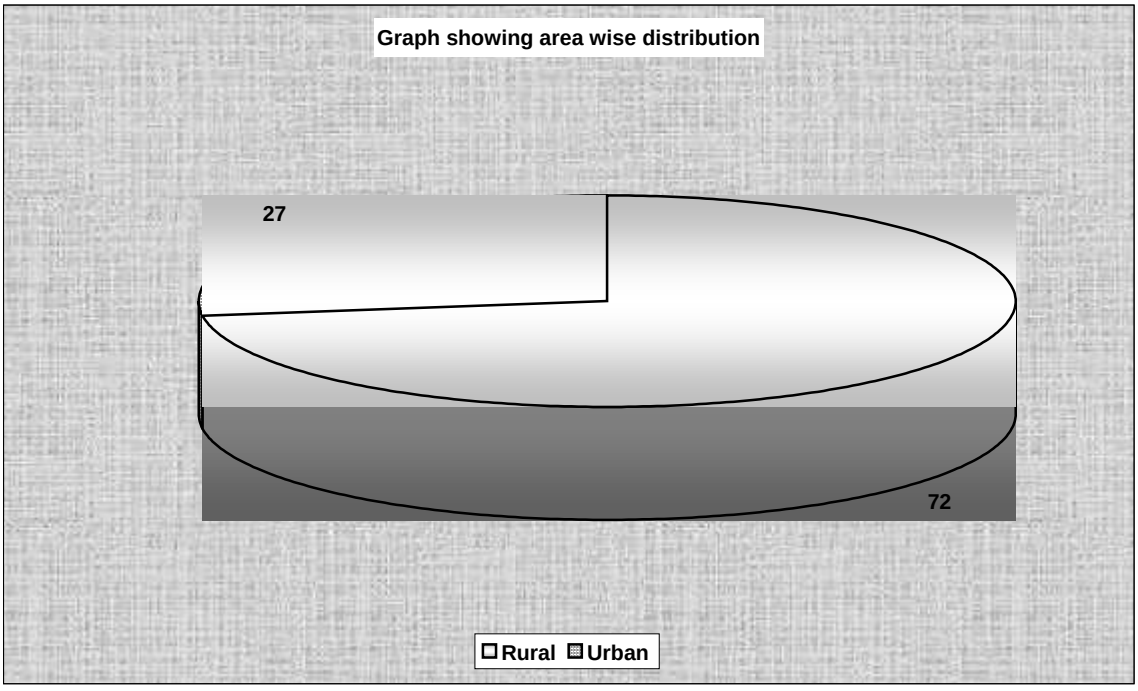
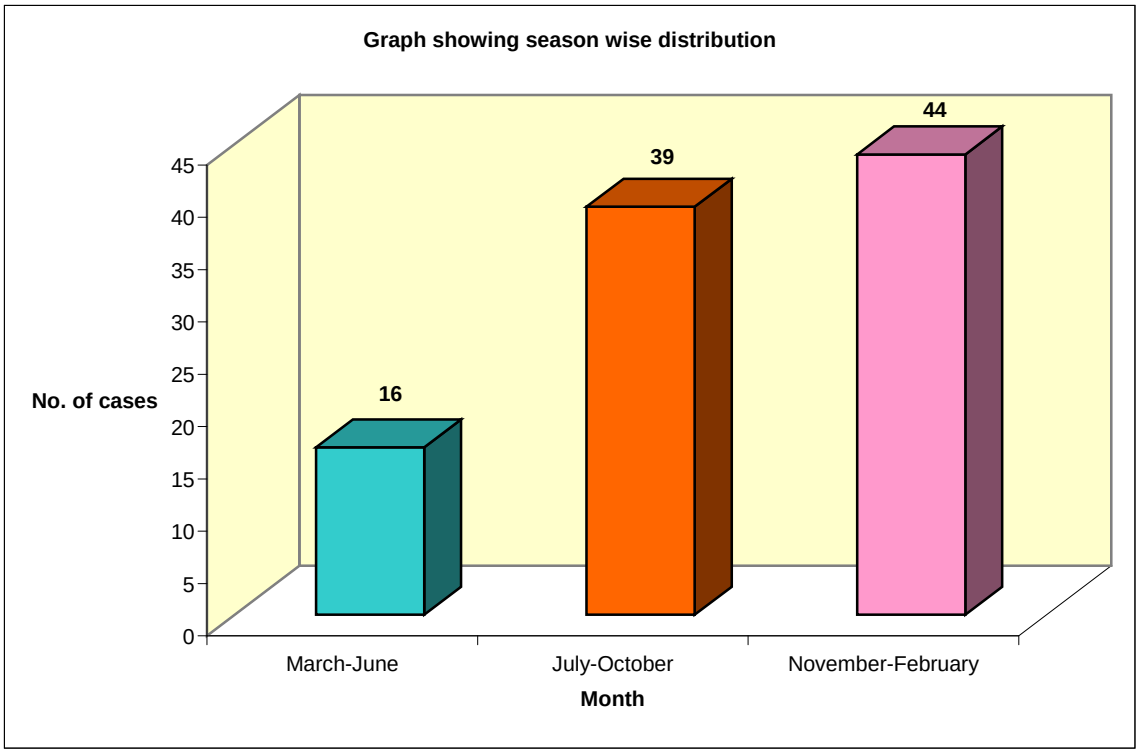


Table - 5 : Season wise distribution

Month	No. of cases (n=99)	Percentage (%)
March-June	16	16.1%
July-October	39	39.4%
November-February	44	44.4%
Total	**99**	**100%**

Out of ninety nine cases 44 (44.4%) were observed during the month of November-February, 39(39.4%) were observed during July-October and 16(16.1%) cases were seen during March-June. Above table shows that incidence of CSOM was more during winter season and early rainy season.

Graph-5 : Graph showing season wise distribution



BACTERIOLOGY

All the ninety nine cases were examined for bacterial infection, both direct smear study and culture methods were done.

Gram Stain : Table 6 shows the analysis of the gram stain smear studied.

Table – 6 : Analysis of the Gram Stain smear studied

Organisms	No. of cases (n=99)	Percentage (%)
Pus cells and organisms	74	74.74%
Only pus cells and no organisms	19	19.19%
Only organisms and no pus cells	6	6.06%
Total	**99**	**100%**

Inflammatory cells and organisms were seen in 78 smears out of ninety nine cases and in 21 smears only inflammatory cells were seen and there were 6 organisms without pus cells in the direct smear studied.

Graph-6 : Graph showing analysis of the Gram Stain smear studied

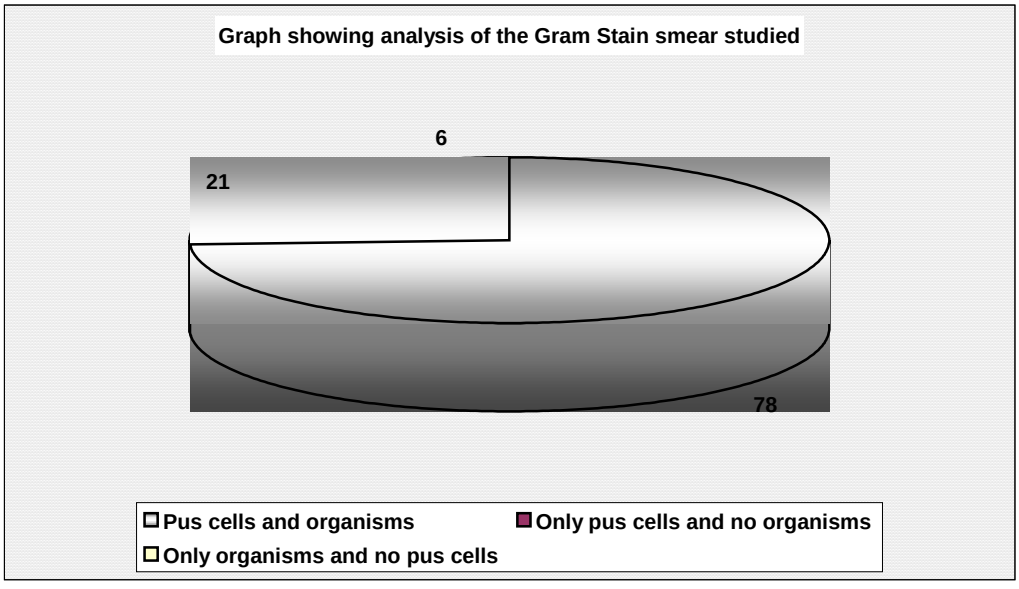


Table – 7 : Results of direct smear and culture

Specimen	Smear positive + culture positive	Smear positive + culture negative	Smear negative + culture negative	Total (n=99)
No. of ear swabs studied	86	8	5	99
Percentage	**86.9%**	**8.1%**	**5.1%**	**100%**

The above table shows that, out of ninety nine ear swabs studied,86(86.9%) were smear positive and culture positive, 8(8.1%) were smear positive and culture negative, 5(5.1%) were smear negative and culture negative.

Graph-7 : Graph showing results of direct smear and culture

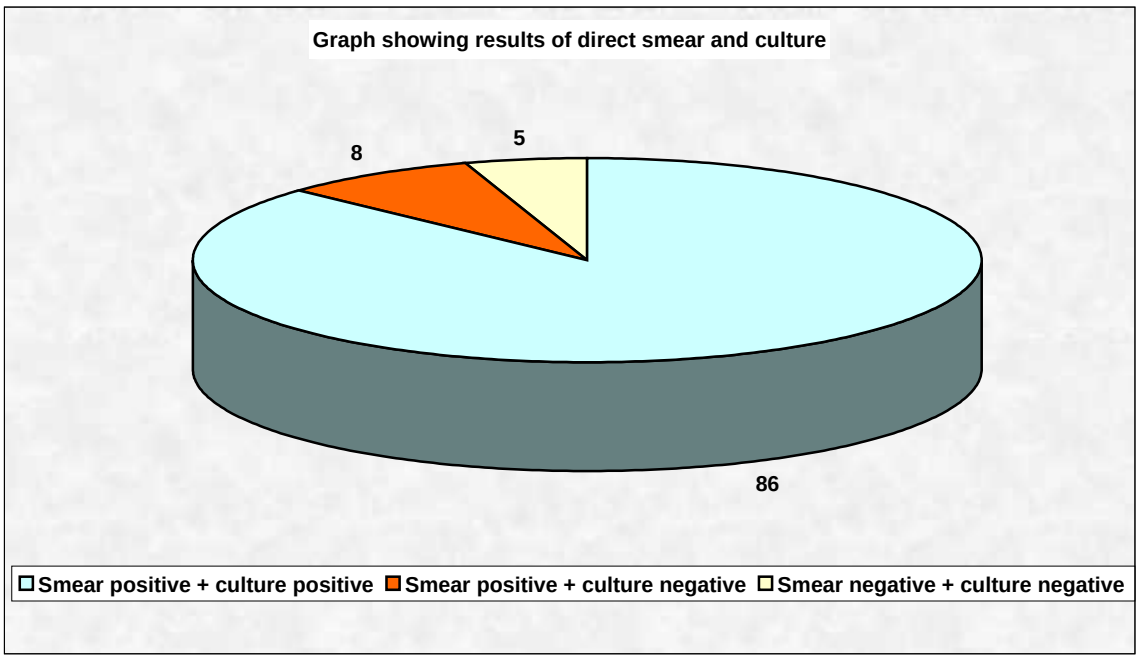


Table – 8 : Gram reaction of culture smears

Gram reaction	No. of culture smears (n=99)	Percentage (n=99)
Gram positive	31	31.3%
Gram negative	41	41.4%
Mixed growth	14	14.1%
No growth	13	13.1%
Total	**99**	**100%**

Out of ninety nine cultures smears 41(41.4%) were gram negative and 31(31.3%) were gram positive. The above table shows that most culture smears were gram negative compared to gram positive. Mixed growth seen in 14(14.1%) and no growth 13(13.1%) cases.

Graph - 8 : Graph showing Gram reaction of culture smears

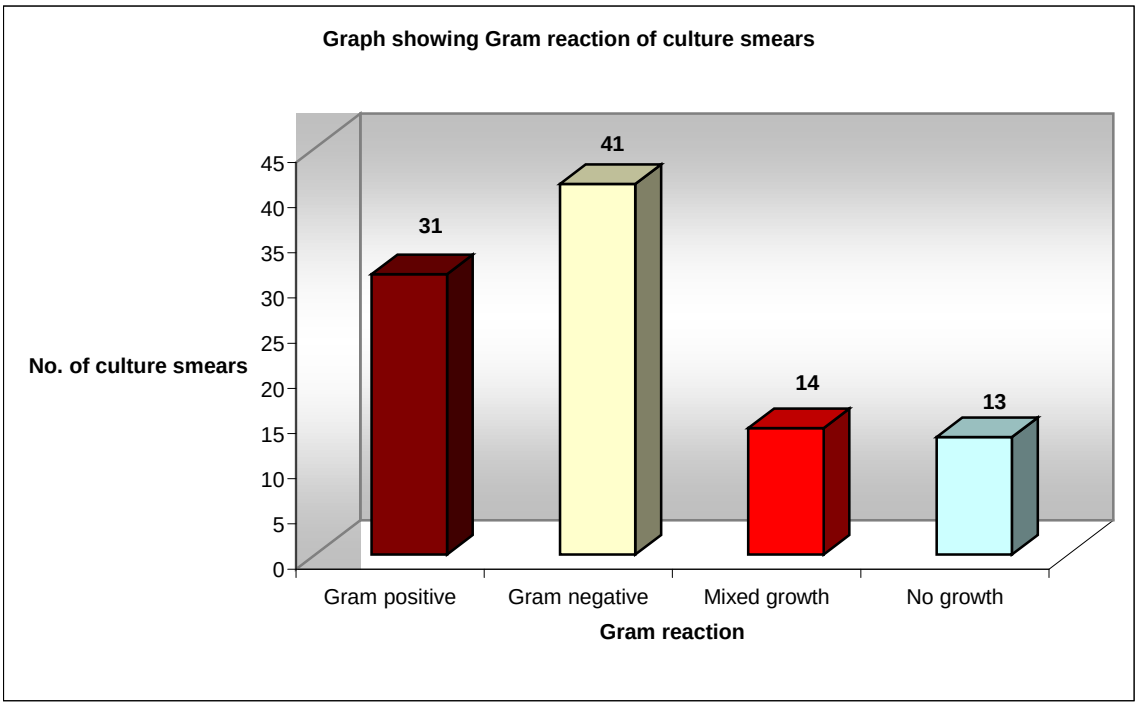


Table – 9 : Incidence of pure and mixed cultures

Organisms	Total no. of strains (n=99)	Percentage (n=99)
Monomicrobial	72	72.7%
Poly microbial	14	14.2%
No growth	13	13.1%
Total	**99**	**100%**

Single organism was isolated in 72 cases (72.7%) of the total cases studied. While 14(14.2%) of the cases yielded mixed growth in 13(13.1%) of the cases the cultures remained sterile.

Graph - 9 : Graph showing incidence of pure and mixed cultures

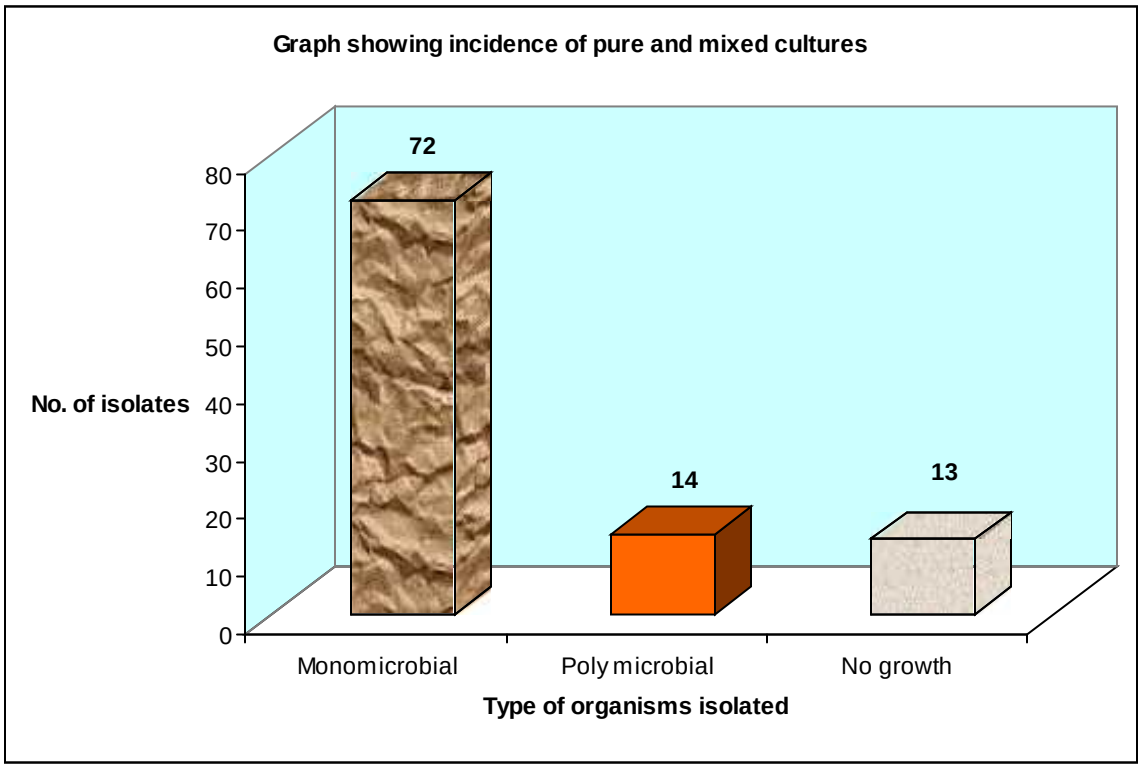


Table – 10 : The individual organisms isolated in the present study and their percentage :

Sl. No.	Organisms	No. of organisms (n=99)	Percentage(%) (n=99)
1	*Staphylococcus aureus*	29	29.3%
2	*Pseudomonas aeruginosa*	25	25.3%
3	*Proteus mirabilis*	5	5.1%
4	*Klebsiella pneumoniae*	4	4.1%
5	*Proteus vulgaris*	2	2.1%
6	*Enterococcus faecalis*	2	2.1%
7	*Acinetobacter spp.*	2	2.1%
8	*Citrobacter freundii*	2	2.1%
9	*Klebsiella oxytoca*	1	1%
10	Mixed infections	14	14.1%
11	No growth	13	13.1%
	Total	**99**	**100%**

In the present study *Staphylococcus aureus* was the commonest organism isolated. Table 10 shows that *S. aureus* was isolated in 29 cases accounting for 29.3% of the total cases studied. The second commonest organism was *Pseudomonas aeruginosa* 25(25.3%) followed by *Proteus mirabilis* 5(5.1%) and *Klebsiella pnuemoniae* 4(4.1%) *Proteus vulgaris,Enterococcus faecalis, Acinetobacter spp*. and *Citrobacter freundii* isolated in 2(2%) of cases each. *Klebsiella oxytoca* was isolated in one case. No growth was observed in 13 cases and mixed infection were seen in 14 cases.

Graph-10 : Graph showing individual organism isolated

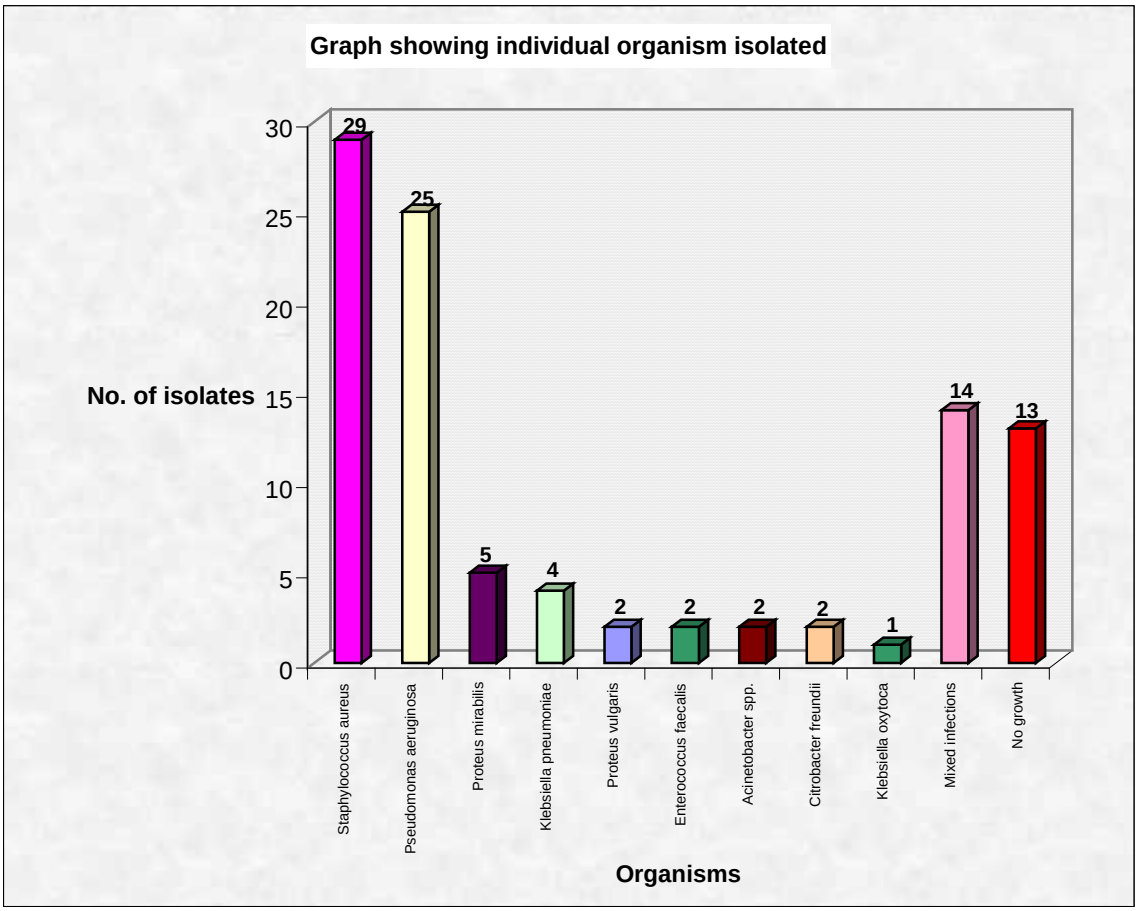


Table – 11 : Incidence of mixed bacterial isolates

Sl. No.	Mixed isolates	No. of cases	Percentage(%)
1	*Staphylococcus aureus + Pseudomonas aeruginosa*	3	21.4%
2	*Staphylococcus aureus + Citrobacter freundii*	2	14.3%
3	*Pseudomonas aeruginosa + Proteus vulgaris*	2	14.3%
4	*Proteus mirabilis + Pseudomonas aeruginosa*	1	7.1%
5	*Staphylococcus aureus + Enterococcus faecalis*	1	7.1%
6	*Staphylococcus aureus + Klebsiella pneumoniae*	1	7.1%
7	*Staphylococcus aureus + Proteus mirabilis*	1	7.1%
8	*Enterococcus faecalis + Proteus mirabills*	1	7.1%
9	*Pseudomonas aeruginosa + Klebsiella pneumoniae*	1	7.1%
10	*Pseudomonas aeruginosa+K.pneumoniae+C.freundii*	1	7.1%
	Total	**14**	**100%**

The above table shows polymicrobial isolates of CSOM case. The common combination of organism isolates are as follows.

S.aureus was associated with *P.aeruginosa* in 3(21.4%) cases. *S.aureus* associated and *C. freundii* in 2(14.3%) and *P.aeruginosa* associated with *P.vulgaris* in 2 and *S.aureus* and *E. faecalis* in 1(7.1%), *P mirabilis* and *P.aeruginosa* in 1, *S.aureus* and *K.pneumoniae* in 1, *S.aureus* and *P.mirabilis* in 1,*E.faecalis* and *P.mirabilis* in 1,*P.aeruginosa* with *K.pnuemoniae* in 1,*P.aeruginosa* with *K.pneumoniae + C.freundii* in 1(7.1%) cases each.

Graph-11 : Graph showing incidence of mixed bacterial isolates

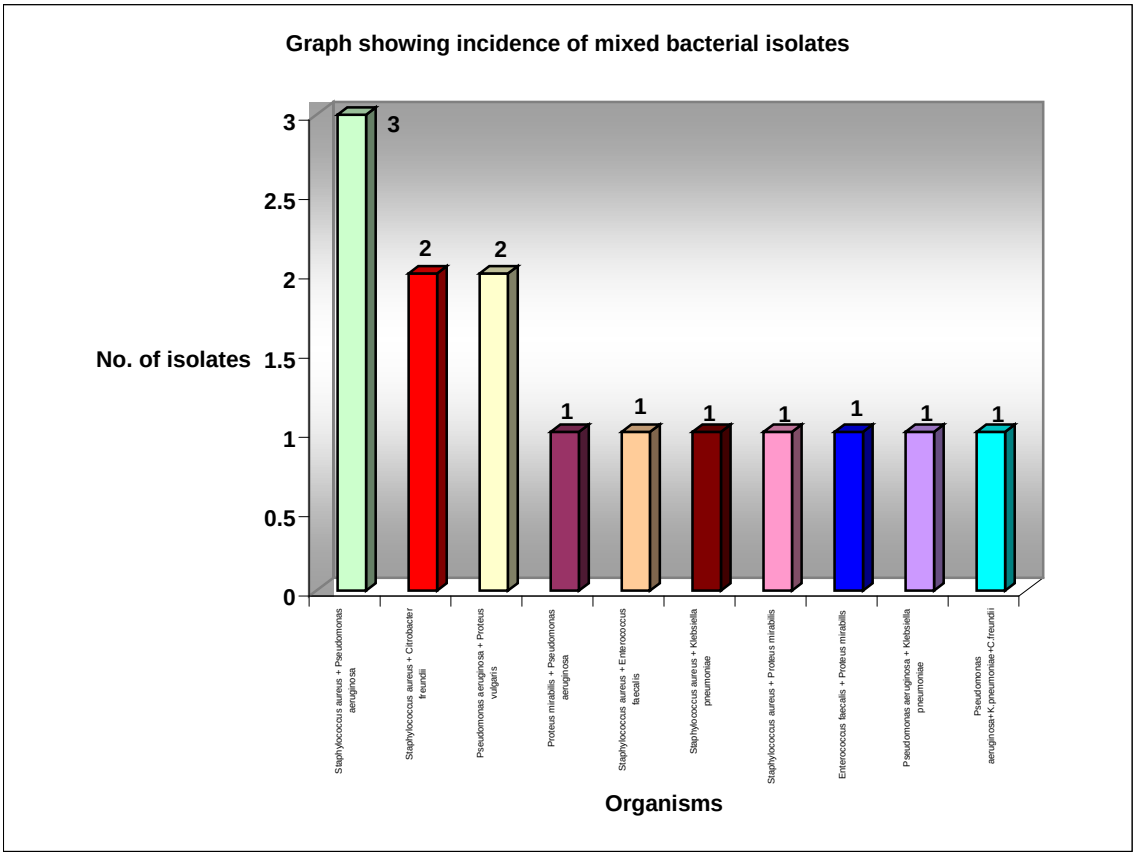


Table – 12 : Showing MRSA Producers

Total no. of S. *aureus* isolated	MRSA isolated	MSSA isolated
29	12(41.4%)	17(58.6%)

Above table shows out of 29 *Staphylococcus aureus* isolated 12(41.1%) are MRSA and 17(58.6%) were MSSA.

Graph-12 : Graph showing MRSA Producers

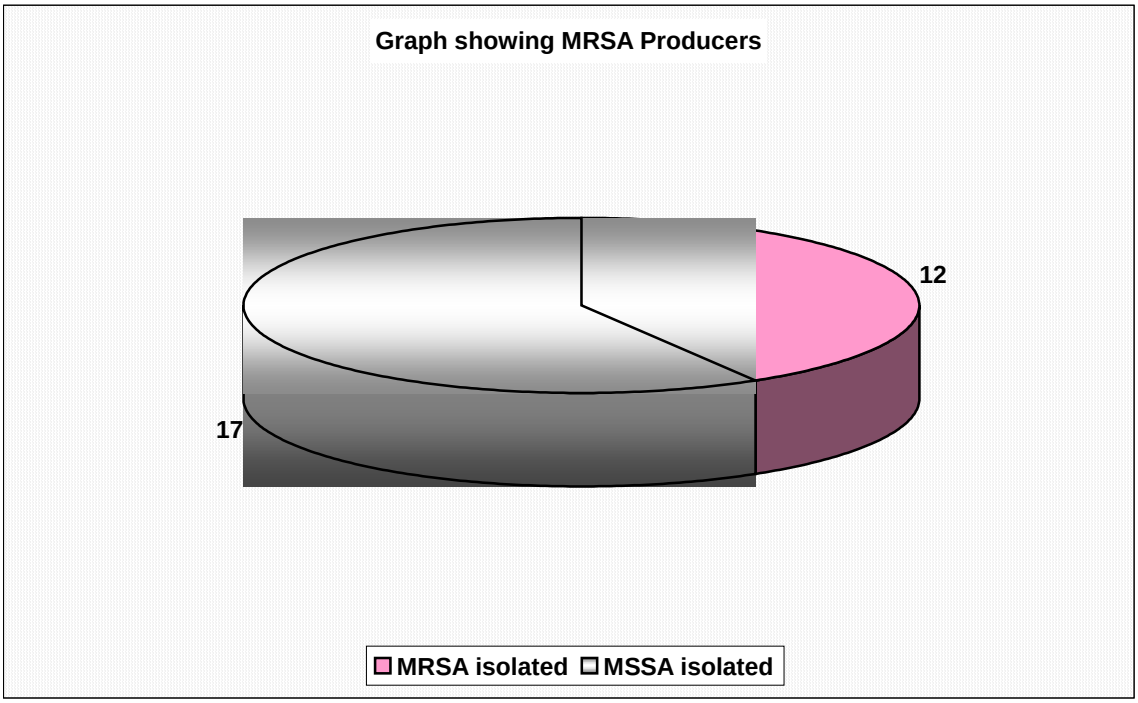


Table – 13 : ESBL Producers and Non-ESBL Producers in Gram negative single isolates

Total no. of gram negative isolates	ESBL producers	Non- ESBL producers
41	14(34.1%)	27(65.9%)

Above table shows out of 41 gram negative single isolates 14(34.1%) are ESBL producers and 27(65.9%) are non- ESBL producers.

Graph-13 : Graph showing ESBL and Non-ESBL producers in gram negative single isolates

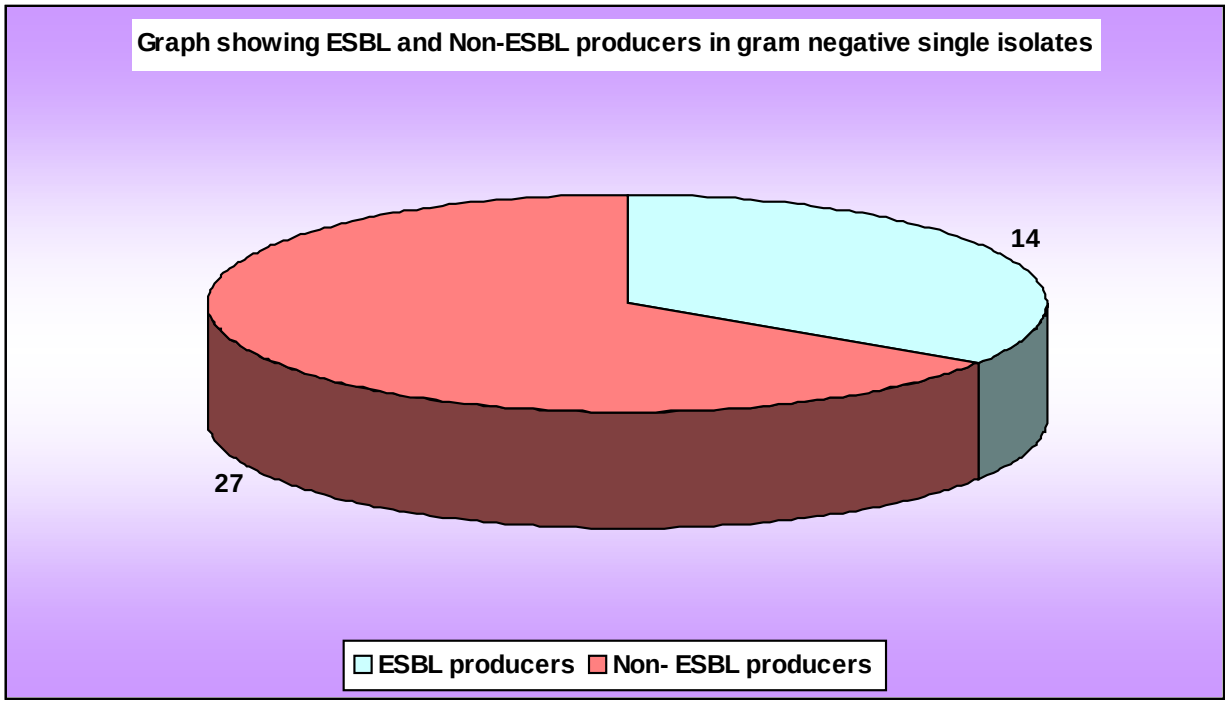


Table – 14 : Different isolates of ESBL are as follows

Sl no.	Isolates	No. of ESBL	Percentage
1	*Pseudomonas aeruginosa*	10	71.4%
2	*Proteus vulgaris*	1	7.1%
3	*Citrobacter freundii*	1	7.1%
4	*Klebsiella oxytoca*	1	7.1%
5	*Proteus mirabilis*	1	7.1%
	TOTAL	**14**	**100%**

Among ESBL producers *P.aeruginosa* was common 10(71.4%) and followed by *P.vulgaris,C. freundii ,K. oxytoca* and *P.mirabilis* 1(7.1%) each.

Graph – 14 : Graph showing different groups of ESBL Producers

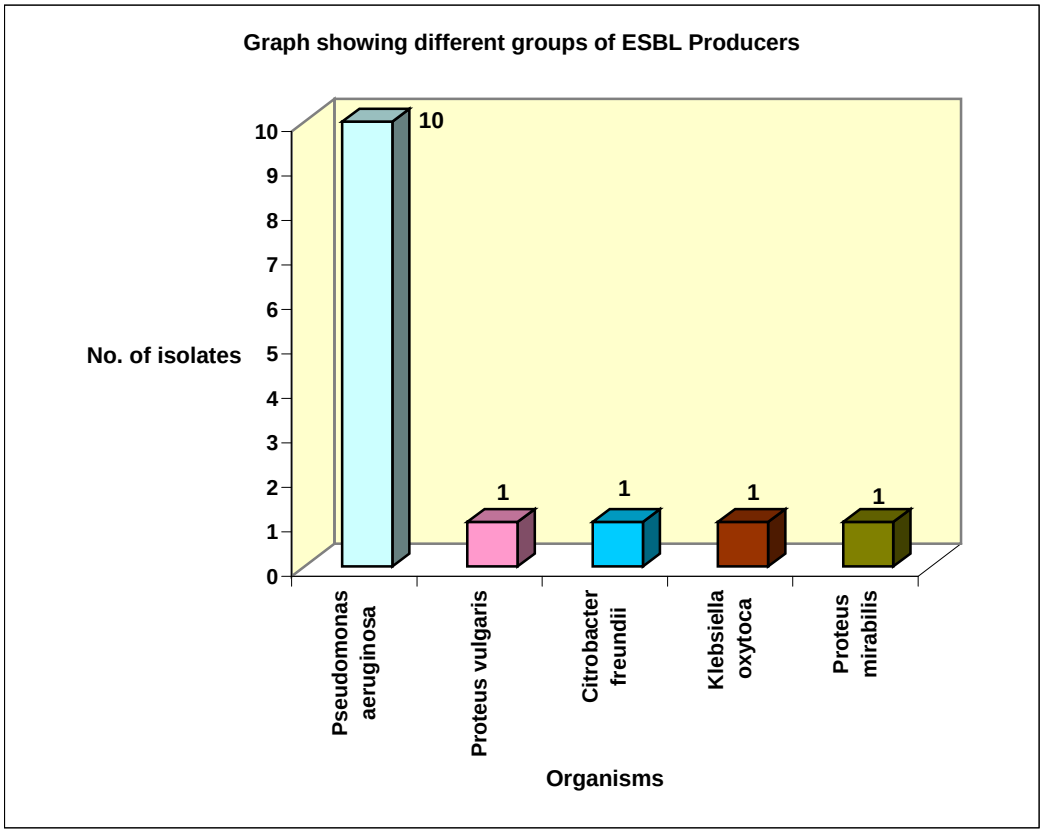


Table – 15 : Mixed isolates showing ESBL and MRSA isolates

SL no.	Mixed isolates	No. of isolates	ESBL and MRSA
1	*Staphylococcus aureus+P. aeruginosa*	2	MRSA+NON-ESBL
2	*Staphylococcus aureus+P. aeruginosa*	1	MRSA+ ESBL
3	*Staphylococcus aureus+ Enterococcus faecalis*	1	MRSA+NON- ESBL
4	*P. aeruginosa+K.pneumoniae+C.freundii*	1	ESBL
5	*Staphylococcus aureus+Proteus mirabilis*	1	MSSA+ESBL
6	*Enterococcus faecalis+Proteus mirabilis*	1	ESBL
	Total cases	**7**	

Above table shows MRSA+ESBL in one case. MRSA+NON ESBL in 3 cases, MSSA+ESBL in one case and only ESBL in 2 cases.

Table 16

The organisms were subjected to sensitivity test to the following antibiotics namely Ampicillin, Erythromycin, Gentamicin, Amoxyclav, Ciprofloxacin, Ceftazidime for gram positive bacteria and Erythromycin, Amoxyclav, Ciprofloxacin, Amikacin, Cefotoxime and Levofloxacin for gram negative organisms.

Most of *Pseudomonas organisms* were sensitive to Amikacin followed by Levofloxacin (68%), Ciprofloxacin(56%) and Cefotaxime(56%). *S.aureus* showed sensitivity of about (72.4%) to Gentamicin and (66.5%) sensitivity to Amoxyclav, 55.2% to Ciprofloxacin.

This is because of *S.aureus* resistant to Ampicillin, Erythromycin than Gentamicin, Amoxyclav, Ciprofloxacin. *Klebsiella spp*. was highly sensitive to Cefotaxime(75%), Amikacin(75%) and Levofloxacin(100%). For Ciprofloxacin(50%) sensitive but resistant to Erythromycin and Amoxyclav.

Proteus mirabilis and *P.vulgaris* were sensitive to Amikacin(100%), Levofloxacin (100%) and Cefotaxime (75%) but resistant to Ciprofloxacin and Erythromycin. *E. faecalis* was sensitive to Gentamicin (100%) and Amoxyclav (100%), resistant to Erythromycin and Ampicillin. *Acinetobacter spp*. was sensitive to Amikacin, Cefotaxime and Levofloxacin, but for Ciprofloxacin and Amoxyclav was 50% sensitive and 50% resistant to Erythromycin. *C. freundii* was equally sensitive (50%) and resistant (50%) to Erythromycin, Amoxyclav, Ciprofloxacin, Cefotaxime but sensitive to Amikacin and Levofloxacin (100%).

It was observed from above study that gram positive organisms were sensitive to Gentamicin and Amoxyclav, but resistant to Ampicillin, and Erythromycin. For Ceftazidime and Ciprofloxacin sensitivity was moderate. Gram negative organisms were sensitive to Amikacin, Cefotaxime and Levofloxacin and resistant to Erthromycin. For Ciprofloxacin and Amoxyclav, all are moderately sensitive except *proteus spp.* which was resistant.

Table 17

In mixed isolates *S.aureus + C.freundii* was sensitive to Gentamicin, Amoxyclav, Ceftazidime, Amikacin, Cefotaxime and Levofloxacin, but resistant to Ciprofloxacin and Ampicillin.*S.aureus+E.faecalis* was sensitive to Gentamicin, Amoxyclav and Ciprofloxacin, but resistant to Ampicillin, Erythromycin and Ceftazidime. *S.aureus + P.aeruginosa* was sensitive to Ciprofloxacin, Ceftazidime, Cefotaxime and Levofloxacin but resistant to Ampicillin, Erythromycin. *P.mirabilis + P.aeruginosa* was sensitive to Ceftazidime, Amikacin, Cefotaxime, Levofloxacin and resistant to Erytromycin, Amoxyclav.

P.aeruginosa + P.vulgaris was sensitive to Amikacin, Cefotaxime and Levofloxcin,but resistant to Erytromycin and showed equal sensitivity and resistance to Amoxyclav and Ciprofloxacin. *S.aureus + K. pneumoniae* sensitive to Ampicllin, Erythromycin, Gentamicin, Amoxyclav, Ciprofloxacin Ceftazidime, Amikacin, Cefotaxime and Levofloxacin.

S.aureus+P.mirabilis was sensitive to Ampicllin, Gentamicin, Amoxycalv, Amikacin and Levofloxacine. Resistant to Erythomycin, Ciprofloxacin, Ceftazidime and Cefotaxime. *E. faecalis + P.mirabilis* was sensitive to Ampicillin, Gentamicin, Amoxyclav, Amikacin and Levofloxacin but resistant to Erythromycin, Ciprofloxacin, Ceftazidime and Cefotaxime.

P.aeruginosa+K.pneumoniae sensitive to Ciprofloxacin, Amikacin,Cefotaxime and Levofloxacin but resistant to Erythromycin, Amoxyclav and Cefotaxime. It was observed from above study that sensitivity pattern was varied with different isolate combinations. It is necessary to choose antibiotics depending on susceptibility when multiple organisms are isolated in culture.

Table – 18 : Sensitivity pattern of MRSA

Total *S.aureus*	MRSA isolated	Ampicillin		Erythromycin		Gentamicin		Amoxyclav		Amikacin		Ceftazidime	
		S	R	S	R	S	R	S	R	S	R	S	R
29	12 (41.1%)	1 (8.3%)	11 (91.6%)	1 (8.3%)	11 (91.6%)	5 (41.7%)	7 (58.3%)	5 (41.7%)	7 (58.3%)	2 (16.6%)	10 (83.3%)	0%	12 (100%)

Table – 19 : Sensitivity pattern of ESBL Producers

Total Gram negative isolation	ESBL producers	Erythromycin		Amoxyclav		Ciprofloxacin		Amikacin		Cefotaxime		Levofloxacin	
		S	R	S	R	S	R	S	R	S	R	S	R
41	14 (31.7%)	0%	14 (100%)	5 (35.7%)	9 (64.3%)	5 (35.7%)	9 (64.3%)	11 (78.6%)	3 (21.4%)	2 (14.3%)	12 (85.7%)	10 (71.4%)	4 (28.6%)

Beta lactamase producing *S.aureus* was sensitive to Gentamicin (41.7%) and Amoxyclav (41.7%) but resistant to Ampicillin, Erythromycin and Ceftazadim. For Ciprofloxacine resistance was (83.5%.)

ESBL producers were sensitive to Amikacine (78.6%) and Levofloxacine (71.4%) but resistant to Erythromycin (100%) and Cefotaxime (85.7%). Sensitivity to Amoxiclav (35.7%) and Ciprofloxacin (35.7%) was moderate.

Table-20

The table shows :

1) *S.aureus+E.faecalis* showed resistance to Ampicllin, Erythromycin, Ceftazidime and sensitive to Gentamicin, Amoxyclav and Ciprofloxacin.
2) Combination of *S. aureus+P. aeruginosa* (MRSA+ESBL) showed resistance to all drugs.
3) Combination of *S.aureus+P.aeruginosa*(MRSA+NON-ESBL) showed resistance to Ampicillin,Erythromycin and sensitive to Gentamicin, Amoxyclav, Ciprofloxacin, Ceftazidime,Amikacin,Cefotaxime and Levofloxacin.
4) Combination of *S.aureus + P.aeruginosa* (MSSA+ESBL) showed resistance to Ampicillin, Erythromycin and sensitive to Gentamicin, Amoxyclav, Ciprofloxacin, Ceftazidime, Amikacin, Cefotaxime and Levofloxacin.
5) Combination of *E.faecalis + P.mirabilis* (ESBL) showed sensitive to Gentamicin, Amoxyclav, Amikacin, and Levofloxacin resistant to Erythromycin, Ciprofloxacin, Ceftazidime and Cefotaxime.
6) Combination of *P.aeruginosa + K.pneumoniae + C.freundii* (ESBL) were sensitive to Ciprofloxacin, Amikacin, Levofloxacin but showed resistance to Erythromycin, Amoxyclav, Cefotaxime.

Above findings observed combination of MRSA+ESBL was resistant to all drugs. Only MRSA combination showed variable resistance and ESBL combination showed resistance to 3rd generation Cephalosporins (Ceftazidime and Cefotaxime), but sensitive to Amikacin, Levofloxacin, and Amoxyclav. For Ciprofloxacin and Erythromycin variable sensitivity were observed.

FOLLOW UP OF CASES

Table shows follow up of cases

Total no. of cases (n=99)	No. of patients follow up done	Cases not come for follow up
99	86	13

Follow up done on 10th day and 1 month after collection of discharge sample. Antibiotics were put depending on the sensitivity pattern of each cases. During follow up of patients Otoscopy, Audiometry were advised. If patients having dry ear and Otoscopy shows perforation they will be posted for Tympanoplasty as advised by ENT surgeons. If discharge was persisting, swab was taken again for culture study and antibiotics were changed depending on sensitivity pattern and follow up done after a course of antibiotics. Patients with complications and surgeries advised for the same were also noted during follow up.

Table 21 : Showing results of follow up cases

SL no.	Cases	Number of cases	Comment
1	No. of patients follow up done	86	Two follow ups done
2	No. of patients became dry ear after treatment	74	Responded to treatment
3	No. of patients with persisting discharge	12	Anaerobic and fungal study advised
4	Tympanoplasty advised / done for dry ear patients	43	Otoscopy showed perforation in follow up
5	No. of patients not advised surgery	25	Advised follow up after 2-3months
6	No. of patients with complications	6	Surgery was advised after ear is dry

Out of ninety nine cases, 86 cases had come for two follow ups after 10 days and 1 month. Out of 86 cases, 74 cases had dry ear as observed by Otoscopy. 12 cases had persisting ear discharge. These 12 cases were advised for Anaerobic and Fungal study. Among these, 6 cases had no growth and 6 cases grown single isolates. Persisting discharge in no growth cases may be due to Anaerobes and Fungal growth and persisting discharge in single isolates may be due to mixture of aerobes with anaerobes or fungus.

Among 74 dry ear cases, Tympanoplasty done/ advised for 43 cases as there was perforation persisting in these cases was seen by Otoscopy. 25 dry ear cases were not advised any surgery but were advised to come for follow up after 2-3 months. 6 patients were with complications like mastoiditis(3), mastoid abscess(2) and brain abscess(1). Mastoidectomy advised / done for all 6 cases after ear was dry with course of antibiotics as shown by sensitivity in culture.

DISCUSSION

In the present study an attempt was made to know the aerobic bacteriology of CSOM, with antimicrobial susceptibility testing of the bacterial isolates. The results were compared with other studies and discussed as follows :

AGE WISE DISTRIBUTION

Sl. no.	Study series	Age group	Cases	Percentage
1	Vijay D and others[53]	11-20years 0-10years	68 52	89.4% 27.7%
2	Sinha and others[9]	21-40years >40years	41 32	44.6% 34.8%
3	Gupta vineeta and others[10]	21-30years 11-20years	52 48	33.1% 30.6%
4	Maji PK and others[3]	10-19years <10years	51 39	31.9% 24.4%
5	Present study	11-20years 0-10years	30 21	30% 21%

Like other studies present study also have high incidence of CSOM in 11-20 years 30 cases (30%) followed by 0-10years 21 cases (21%).

SEX WISE DISTRIBUTION

SL no.	Study series	Male	Female
1	Wakode and others[50]	52(42.3%)	71(57.7%)
2	Gulati and others[8]	61%	39%
3	Rosemary and others[31]	44(60.3%)	29(39.7%)
4	Vijay D and others[57]	226(62.8%)	134(37.2%)
5	Present study	52(60.5%)	34(39.5%)

Males were affected 52(60.5%) than Females 54(39.5%) in the present study. This finding was correlated with the above workers. The male predominace may be because of their more exposed way of life. In the study of Maji PK and others there was no much sex difference in indence.

AREA WISE DISTRIBUTION

In the present study incidence of CSOM cases was more common in rural area[72 cases(72.7%)] compared to urban area[27 cases(27.3%)]. This study was correlated with other workers such as Gulati and others,Wakode PT.[50]

SEASON WISE DISTRIBUTION

In this study CSOM was maximally seen during November-February(44.4%) winter season following rainy season in July-October(39.4%) months. This is correlated with other studies like Wakode PT[50] with maximum cases were seen during the month of July and October. Maji PK[3] and others maximum number of cases were seen during the month of July to September.

SIDE WISE DISTRIBUTION

In the present study CSOM was more common in right ear[40(40.4%)] than left [36(36.4%)] and Bilateral[23(23.2%)]. Gulati[8] and others showed 80% had unilateral involvement and 20% had Bilateral involvement. Rakshak[10] and others showed left side Predominance(51.5%) and Bilateral disease seen in (16.67%) cases.

CULTURE RESULTS OF CASES STUDIED

In the present study 86(86%) specimens were positive and 13(13%) specimens were negative for the culture. Other workers had positive cultures as follows :

Sl no.	Study series	Positive culture	Negative culture
1	Gupta and others[10]	150(95.6%)	7(4.46%)
2	Vijaya D[57]	341(94.7%)	19(5.28%)
3	Oluibekwe and others[52]	83(81.4%)	19(18.6%)
4	Sinha and others[9]	83(90.2%)	9(9.8%)
5	Asiri and others[32]	153(80.5%)	37(19.5%)
6	Present study	86(86%)	13(13%)

Negative cultures can be attributed to non bacterial growth, Anaerobic growth and prior antibiotic therapy.

INCINDENCE OF PURE AND MIXED CULTURES

Sl. no.	Study series	Monomicrobial growth	Polymicrobial growth
1	Sinha and others[9]	65(70.7%)	18(19.6%)
2	Vijay D[57]	208(57.8%)	133(36.9%)
3	Gupta and others[10]	121(76.4%)	29(18.55%)
4	Kshitiz and others[51]	147(92.5%)	26(17.7%)
5	Present study	72 (72.7%)	14(14.2%)

In the present study Monomicrobial etiology was 72(72.7%) and polymicrobial was 14(14.2%). My study is correlated with the above shown workers.Less number of mixed culture due to most of the cases were referred cases and may be due to prior use of antibiotics.

The organisms isolated were - *S. aureus* was the predominant organisms 29(29.3%) followed by *P.aeruginosa* 25(25.3%), Proteus spp. 7(7.1%), *Klebsiella spp*. 5(5.1%), *C.freundii, E.faecalis* and *Acinetobacter spp*.2(2%) each.

Comparative bacteriological study of CSOM cases done by other authors :

Sl no.	Authors	S. aureus	P.aeruginosa	Proteus Spp	Klebsiella Spp	C.freundii	E.faecalis	Acinetobacter spp.
1	Gulathi and others[8]	12 (16.6%)	30 (41.6%)	4 (5.6%)	12 (16.6%)	-	-	-
2	Maji PK and others[3]	33.8%	64.4%	-	-	-	-	-
3	Kashitiz and others[51]	21.8%	15.6%	17%	-	-	-	-
4	Kulkarni BD and others[7]	15 (18.8%)	32 (40%)	7 (8.75%)	9 (11.2%)	2 (2.5%)	-	-
5	Vijay D and others[53]	41 (19.9%)	15 (7.2%)	8 (3.8%)	38 (18.4%)	18 (8.7%)	-	-
6	Asiri S and others[32]	52 (27.4%)	39 (21%)	-	6(3%)	-	-	-
7	Gupta V and others[10]	59 (30.7%)	53 (27.6%)	26 (13.5%)	-	1 (0.5%)	-	3 (1.5%)
8	Sinha A and others[9]	18 (27.7%)	38 (46.1%)	8 (12.3%)	6 (9.2%)	-	-	-
9	Present study	29 (29.3%)	25 (25.3%)	7 (7.1%)	5 (5.1%)	2 (2%)	2 (2%)	2 (2%)

S.aureus was the predominant isolate(29.3%) in the present study. This finding is correlated with other workers, Kshitiz[51] and others,Vijay D and others[53], Asiris and others[32], GuptaV and others[10]. However workers like Gulati and others[8], Maji PK and others[3], Kulkarni and others[7] and Sinha A and others[9] have found *S.aureus* as the second most common organism causing CSOM.

Next Predominate organism in the present study was *P.aeruginosa* correlated with studies with Asiris and others [32] Gupta V and others[10] also *P.aeruginosa* was second

common isolate but studies like Gulati and others[8], Kulkarni BD and others[7] and Sinha A and others[9] showed *P. aeruginosa* as the predominate isolate.

The third most common isolate was *Proteus spp*. 7(7.1%), which is correlated with other studies as shown in table. But, according to one study - Kashitiz and others[51] showed *Proteus spp.* was the second common isolate.

Klebsiella spp. Isolated in 5 (5.1%) cases, which is correlated with other studies.But, studies like Gulati and others and Vijay D and others observed *Klebsiella spp*. as second common isolate.

C.freundii isolated in 2 (2%) cases which is also isolated with Kulkarni BD and others [7](2.5%), Vijay D and others [53](8.7%) and Gupta V and others[10](0.5%).

E.faecalis isolated in 2(2%) cases and even *Acinetobacter spp*. isolated in 2(2%) cases. Gupta V and others isolated *Acinetobacter spp*. in 3(1.5%) cases.

The increased frequency of *S.aureus* in the middle ear infections can be attribute to their ubiquitous nature and high carriage of resistant strains in the external auditory canal and upper respiratory tract.

The organism like *Pseudomonas spp.* and *Proteus spp*. are considered mostly as secondary invaders from external auditory canal gaining access to middle ear via a defect in tympanic membrane resulting from an acute episode of otitis media. *Klebsiella spp*. become opportunistic pathogen in the middle ear when resistant is low. *C.freundii*, *E.faecalis* and *Acinetobacter spp.* in 2(2%) cases only indicating rear pathogens in CSOM.

Antibiotic sensitivity pattern of isolates

SL no.	Bacteria isolates	Ampicillin	Erythromycin	Gentamicin	Amoxyclav	Ciprofloxacin	Ceftazidime	Amikacin	Cefotaxime	Levofloxacin
1	*P.aeruginosa*	-	16%	-	48%	56%	-	72%	56%	68%
2	*S. aureus*	27.61%	35.5%	72.4%	66.5%	55.2%	44.8%	-	-	-
3	*Klebsiella spp.*	-	0%	-	25%	50%	-	75%	75%	100%
4	*P.mirabilis*	-	0%	-	40%	20%	-	100%	75%	100%
5	*P. vulgaris*	-	0%	-	0%	0%	-	100%	50%	100%
6	*E .faecalis*	0%	0%	100%	100%	50%	50%	-	-	-
7	*Acinetobacter spp.*	-	0%	-	50%	50%	-	100%	100%	100%
8	*C .freundii*	-	50%	-	50%	50%	-	100%	50%	100%

Above table showed sensitivity pattern of single isolates in the present study, *S.aureus* was more sensitivity to Gentamicin(72%) followed by Amoxyclav(66.5%). *P.aeruginosa* showed more sensitive to Amikacin(72%) and Cefotaxime(56%) followed by Levofloxacin,and Ciprofloxacin(68% and 56%).Other gram negative bactria were more sensitive to Amikacin, Cefotaxim and Levofloxacin.

Both *Pseudomonas* and *S. aureus* were least sensitive to Erythromycin.

Other studies like

1) Maji PK and others[3] showed – different isolates had sensitive to following antibiotics,
   a) For *S.aureus* - Ampicillin(7.1%), Gentamicin(87.5%),Ciprofloxacin(64.3%), Amikacin(100%), Cefotaxime(80.3%) ,Levofloxacin(41.1%).

b) *P.aeurginosa*-Ampicillin(0%),Gentamicin(86%),Ciprofloxacin(46.6%), Amikacin(100%), Cefotaxim(85.4%)and Levofloxacin(29.4%).

2) Study by Vijay D and others [57] showed sensitive to following antibiotics,

a) *S.aureus*-Amoxicillin(41.2%), Erythomycin(15.8%), Gentamicin(33.3%), Norflox(44.8%), Amikacin(66.6%) and Ciprofloxacine(55.9%)

b) *P.aeruginosa* - Erythromycin(45%), Amoxicillin(41.2%), Amikacin(68%), Ciprofloxacin(45.5%) and Amikacin(68%).

c) *Klebsiella spp*. Amoxicillin(9%), Ciprofloxacin(31.8%), Amikacin(59%),and Norflox(72.7%)

d) *Proteus spp*.- Amikacin(80%), Ciprofloxacin(100%) and Norflox(60%).

e) *Citrobacter spp.*- Erythromycin(9%), Amoxicillin(9%), Ciprofloxacin(81.8%), Amikacin(63.6%), and Norflox(63.6%).

f) *Enterobacter spp*.- Erythromicin(33.3%), Gentamicin(50%), Ciprofloxacin(66.6%) and Amikacin(33.3%).

3) Gulati and others [8] showed- following antibiotic sensitivity,

a) *P. aeruginosa*- Ciprofloxacin 20(60.6%), Cefotaxime 1(3%), and Amikacin 24(72.7%).

b) *S. aureus* - Ampicillin 4(28.5%), Erythromycin 7(50%), Ciprofloxacin 14(100%) and Gentamicin 11(78.6%).

c) *Klebsiella spp*.- Ciprofloxacin 8(61.5%), Cefotaxime 9(69.2%), Amikacin 3(23%) Gentamicin 9(69.2%).

d) *Proteus spp.*-Ciprofloxacin 2(50%) and Cefotaxime 3(75%).

4) Study by Sinha A and others [9] showed sensitivity as follows;

a) Ceftriaxone 41.5%, Ciprofloxacin 36.6%, Cefotaxime 34.6%, Ceftazidime 31.6%, Cephalexin 20.8%, Amoxyclav18.8%, Gentamycin 18.8% and Amikacin 17.8%.

MIXED CULTURE OF ISOLATES

The present study showed common polymicrobial culture with combination of *S. aureus + P. aeruginosa* 3(21.4%) followed by *S.aureus + C.freundii* and *P. aeruginosa+P. vulagris* of 2 cases (14.3%) each. Other mixed culture of *S.aureus + E.faecalis, P.mirabilis + P. aeruginosa, S.aureus + K.pneumoniae, S.aureus + P.mirabilis, E.faecalis + P.mirabilis,* one case (7.1%) each. *P. aeruginosa + K. pneumoniae + C. freundii* 1(7.1%) case was identified.

Others studies showed mixed culture as follows; Vijay D and others[57] showed mixed culture of *S.aureus+P.aeruginosa* was predominant which is compared to present study Asiri SA and others[32] also showed *P. aeruginosa* was common with mixed cultures.

Sinha A and others[9] also isolated *P.aeruginosa* in mixed cultures B D Kulkarni and others[7] showed mixed cultures in 19(7.5%) patients. Sensitivity pattern was varied in different mixed growths but are mostly sensitive to Amikacin, Cefotaxime and Levofloxacin. But for Ampicillin, Erythromycin, Gentamicin and Ciproflxacin. Sensitivity varied for different mixed growth.

MRSA and ESBL producers :

In this study MRSA group comprises 12(41.1%) in *S.aureus* and ESBL produces 14(31.7%). This was compared with Choi and others[108] which showed MRSA of 28% in CSOM. Park DC and others showed MRSA 45.9% in CSOM[109]. Park MK and others showed MRSA in 4.9% of CSOM.[107]

Susceptibility pattern of MRSA showed sensitivity was more with Gentamicin and Amoxyclav (41.7%) but resistant to Ampicllin, Erythomycin and Ceftazidime. It was compared to the study by Baba A and others[110] showed resistant to Levofloxacin and Erythromycin but sensitive to Gentamycin, Rifampicin, Trimethoprim, Sulfamethaxizole and Vancomycin.

Sensitive pattern of ESBL producers showed sensitivity to Amikacin(78.6%) and Levofloxacin (71.4%) Amoxyclav and Ciprofloxacin (35.7%). Resistant to Erythromycin (100%), Cefotaxime(85.7%).This was compared with other studies. Varsha G and other[105] showed ESBL producers in urine, pus and sputum of 24%. Mathur et al found 68% and Tankhiwal et al found in 48%. Most of the studies showed E.coli was commonest ESBL producers followed by *K.pneunomiae*, *Citrobacter spp*., *P.aeruginosa* and *Proteus spp*. in pus,urine and sputum. Sensitivity of ESBL producer in Varsha G and other showed as resistance to Amikacin(24%), Gentamicin(75%), Ciprofloxacin (65%), Cefotaxime (90%), Amoxyclav (69%) which is comparable to present study.[106]

CONCLUSION

Chronic otitis media is major health problem in many populations around the world and a significant cause of morbidity and mortality. The disease and its sequale produce, substantial economic and social costs. Its is particularly common in developing countries. Despite of advances in public health and medical care, CSOM is still prevalent.

In this study, ninety nine clinically diagnosed cases of CSOM attending ENT Outpatient Department at DR. Prabhakar Kore Hospital and MRC were included.

The commonest isolates of CSOM are *S. aureus* followed by *P. aeruginosa*. Among *S. aureus*, 50% of them are MRSA. Mixed isolates were also found significantly.

Antibiotic sensitivity showed susceptibility of single isolates to newer antibiotics like Amikacin,Cefotaxime and Levofloxacin. Moderate susceptibility to Ciprofloxacin and Amoxyclav, Gentamicin and most of them were resistant to Ampicillin and Erythromycin. Among mixed isolates *S.aureus+P.aeruginosa* was more common. Mixed isolates showed varying sensitivity pattern.

ESBL producers constitute 34.1% and showed sensitivity to Amikacin, Levofloxacin and resistant to Cefotaxime, Ciprofloxacin, Amoxyclav and Erytromycin. Most of MRSA were resistant to most of drugs.

Hence, it is necessary to know the causative agent and drug sensitivity pattern for better treatment where antibiotics are commonly abused. This will enhance better treatment and reduce the burden of the infection on the patients and in long term, it may reduce the cost of treatment. Proper selection of antibiotics also helps in preventing drug resistance and also clearing of infection. Hence isolation of bacteria and sensitivity study is important for all CSOM cases.

Follow up of patients after a course of antibiotics will help in cure of some patients with only medical treatment and in preparing patients for surgery either tympanoplasty or mastoidectomy. It also helps in preventing development of complications of CSOM.

SUMMARY

This study was conducted in the Department of Microbiology, J.N Medical College, Belgaum for period of one year from January 2009 to December 2009.

Ninety nine patients of clinically diagnosed CSOM cases attending ENT Out Patient Department at KLE's DR. Prabhakar Kore Hospital and MRC, Belgaum, and patients were selected for study to know the aerobic microbial flora and antibioitic susceptibility pattern of bacterial isolates, MRSA and ESBL producers.

1) 31% of patient were in the age group of 11-20 years followed by 21% in the age group of 1-10 years. Maximum number of patients were males 56(56.4%) than the females 43 (43%).
2) Majority of patients were from rural areas 72(72.7%) compared to urban areas 27(27.3%).
3) Unilateral infection 76(76.8%) was more common than bilateral infection 23(23.2%). Among unilateral cases right side commonly affected 40(40.4%) than left side 36(36.4%).6)
4) Maximum no of cases were seen during November-February 44(44.4%) i.e winter season and July-October i.e. early rainy season 39(39.4%)
5) In Gram stain showed presence of inflammatory cells and organisms were seen in 74(74.74%) and only inflammatory cells without organisms seen in 19(19.19%) and 6(6.06%) organisms without pus cells.
6) Total number of ear swab studied were ninety nine of which 86(86.9%) were culture positive and 13(13.1%) were culture negative.

7) Gram negative organisms were more common 41(41.4%) than gram positive 31(31.3%) organisms.
8) Among positive cultures monomicrobial isolates were seen in 72(72.7%) and only poly microbial isolates were seen in 14(14.2%).
9) The most common organisms isolated was *S.aureus* 29(29.3%) followed by *P.aeruginosa* 25(25.3%) *Proteus spp*. 7(7.1%), *Klebsiella spp*. 5(5.1%), *E.faecalis* 2(2%), *Acinetobacter spp*. 2(2%), *C. freundii* 2(2%).
10) Among mixed isolates *S. aureus* + *P. aeruginosa* 3(21.4%) was common followed by *S. aureus* + *C. freundii* and *P. aeruginosa* + *P. vulgaris* 2(14.3%) cases each. *S.aureus* + *E. faecalis*, *P. mirabilis* + *P. aeruginosa*, *S. aureus* + *P. mirabilis*, *Efaecalis* + *P.mirabilis*, *P. aeruginosa* + *K. pneumoniae* were seen in 1(7.1%) case each. *P.aeruginosa*+*K. pneumionae* + *C. freundii* was seen in 1(7.1%) case.
11) Out of 29 *S. aureus*, MRSA were isolated in 12(41.4%).
12) Out of 41 gram negative organism, 14(34.1%) were ESBL producers.
13) Among mixed isolates MRSA+ESBL were seen in 3 patients and MRSA+NON-ESBL was seen in one patient. Only ESBL isolates seen in 2 cases and MSSA+ESBL seen in one case.
14) *S.aureus* and *E.faecalis* were sensitive to Gentamicin (72.4%), Amoxyclav(66.5%) and Ciprofloxacin(44.8%).Showed Less sensitivity to Ampicillin (27.6%) and Erythromycin (35.5%) .
15) *P. aeruginosa* and other gram negative organisms were sensitive to Amikacin(70-100%), Cefotaxime(50-100%), Amoxyclav(50-100%), Levofloxacin(68-100%) and

observed less sensitive to Erythromycin(16-50%). Sensitivity to Ciprofloxacin was moderate(50-60%).

16) Sensitivity pattern for mixed cultures were different for different isolates, but sensitivity to Amikacin, Cefotaxime and Levofloxacin was more compared to Ampicllin, Erythromycin. Also observed moderate sensitivity to Ciprofloxacin and Amoxyclav.

17) Sensitivity pattern of MRSA was observed as moderate sensitive to Gentamicin, Ciprofloxacin and Amoxyclav and resistant to Ampicillin, Erythromycin, and Ceftazidime. ESBL producers were showed sensitivity to Amikacin and Levofloxacin, but resistant to Erythromycin, Cefotaxime. Sensitivity to Amoxycalv and Ciprofloxacin was moderate.

18) Combination of MRSA+ESBL producers were resistant to most of the drugs. Combination with only MRSA showed variable sensitivity pattern. However, Combinations with only ESBL producers resistance to third generation Cephalaosporins (Ceftazidime and Cefotaxime) but sensitive to Amikacin, Levofloxacin and Amoxyclav. For Ciprofloxacin and Erythromycin variable sensitivity pattern was also observed.

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PROFORMA

TOPIC : BACTERIOLOGICAL STUDY OF CHRONIC SUPPURATIVE OTITIS MEDIA WITH REFERENCE TO AEROBES.

Case No. : IP /OP No. :

Name : Unit / Ward :

Age : Date of sample collection :

Sex : Specimen :

Address :

Chief complaints :

Any other complaints :

Treatment history :

General physical examination :

Local examination :

Systemic examination :

CVS CNS RS P/A

Clinical diagnosis

Microbiological investigations :

Macroscopic examination :

Direct Smear (Gram stain) :

Culture :

Nutrient agar :

Blood agar :

MacConkey agar :

Gram staining of smear from colony :

Motility :

Catalase :

Oxidase :

A. Gram positive cocci :

Coagulase :

Mannitol fermentation :

Growth at 45^0 :

Growth in 6.5% Nacl :

Aesculin hydrolysis :

Antibiogram :

Ampicillin (10μg), Erythromycin (15μg) :

Gentamicin (10μg), Amoxyclav (30μg) :

Ciprofloxacin (5μg), Ceftazidime (30μg) :

MRSA producers – Oxacillin / Methicillin
Incubated at 30^0C.
ESBL producers – Potentiated Disc Diffusion Test (PDDT).
Ceftazidime (30μg) / Cefotaxime (30μg)
Ceftazidime + Clavulanic acid (30μg/10μg)

B. Gram Negative bacilli :

1. Lactose fermentors :

Nitrate reduction test :

IMVIC test :

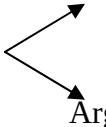
Urease test :

TSI test :

Sugar fermentation :

Glu	Lac	Suc	Malt	Man	Arab	Xyl

Lysine :

Decarboxylation 

Arginine :

2. Non-lactose fermentors :

Nitrate reduction test :

IMVIC test :

Urease test :

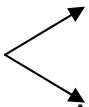
TSI test :

Sugar fermentation :

Glu	Lac	Suc	Malt	Man	Arab	Xyl

OF test :

Lysine :

Decarboxylation 

Arginine :

Identification of isolates :

Report :

Antibiogram :

Amikacin (30μg):
Cefotaxime (30μg):
Ciprofloxacin (5μg):
Erythromycin (15μg):
Amoxyclav (30μg):
Levofloxacin (5μg) :

CONSENT FOR PARTICIPATION IN RESEARCH WORK

We are requesting you to enrollee yourself in study titled **BACTERIOLOGICAL STUDY OF CHRONIC SUPPURATIVE OTITIS MEDIA WITH REFERENCE TO AEROBES,** conducted by PG student in Microbiology, Belgaum KLE's DR. Prabhakar Kore Hospital and Medical Research Centre, KLE University, Belgaum.

You have been requested to participate in research because you are into the study group. During the study you will be asked some questions and you are supposed to answer to the best of you knowledge.

Your participation in research is voluntary. Your decision whether or not to participate in the study will not affect your relationship with Jawaharlal Nehru Medical College. If you decide to participate you are free to withdraw at any time.

The purpose of research is to isolate and identify the aerobic bacterial from clinically diagnosed cases of CSOM and to carry out antibiotic susceptibility testing for aerobic isolation.

PROCEDURE INVOLVED :

Microbiological study of the material obtained from earswab will be done to detect the aerobic bacteria causing chronic suppurative otitis media.

RISKS AND BENEFITS

There are no extra risks involved and benefits are to be evaluated.

PRIVACY AND CONFIDENTIALITY

The only people to know that you are a research subject are members of the research team. No information about you or provided by you during research will be disclosed to others without your written permission, except :

1. In emergency to protect your rights and welfare.
2. If required by law.

AUTHORIZATION TO PUBLISH RESULTS

When the results of the research are published or discussed, in a conference no information will be displayed that would disclose your identity. Any information that is obtained in connection with this study and that can be identified with you will remain confidential.

FINANCIAL INCENTIVES FOR PARTICIPATION

You will not paid / offered any free gifts for participating in the research. You will not be reimbursed for expenses.

I undersigned _____ have been explained in my vernacular language about the study and my participation in the study is voluntary. If I want, I can withdraw at any time. Also I have been given enough time to clear my doubts and rights as study participant.

In case you have any questions about your rights as a study participant, you can contact Dr. V. D. Patil.

CONSENT STATEMENT

Signature or left hand thumb print of participant or legally authorized representative.

Participants Name _____ Signature _____

Witness Name Signature _____ Signature _____

Experimenter's Name Signature _____ Signature _____

Date :

Place :

Fig. 3 : Direct smear showing Gram negative bacilli and inflammatory cells

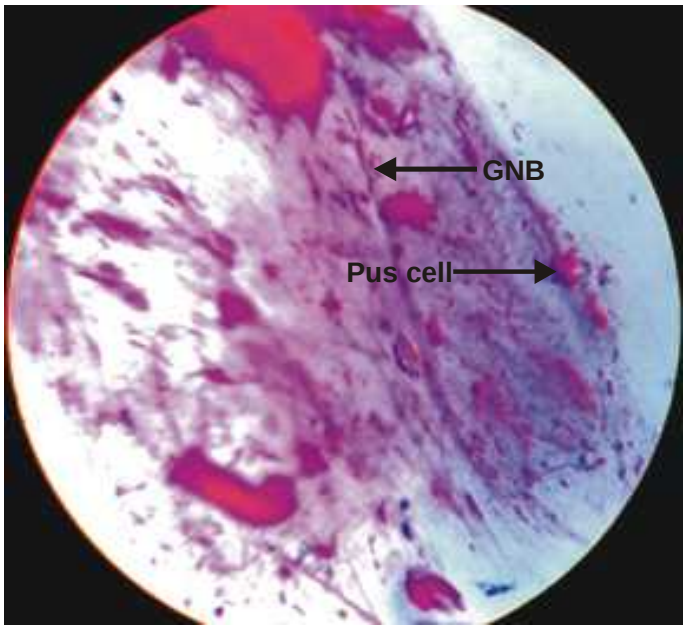
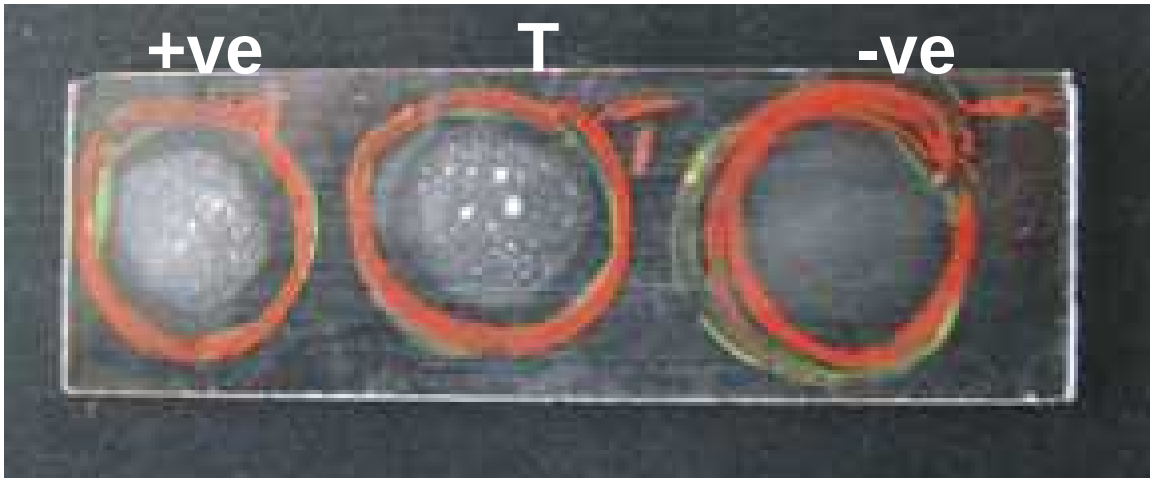


Fig. 4 : Gram stain showing the Gram positive cocci arranged in clusters

Fig. 5 : Blood agar showing the growth of *S.aureus*

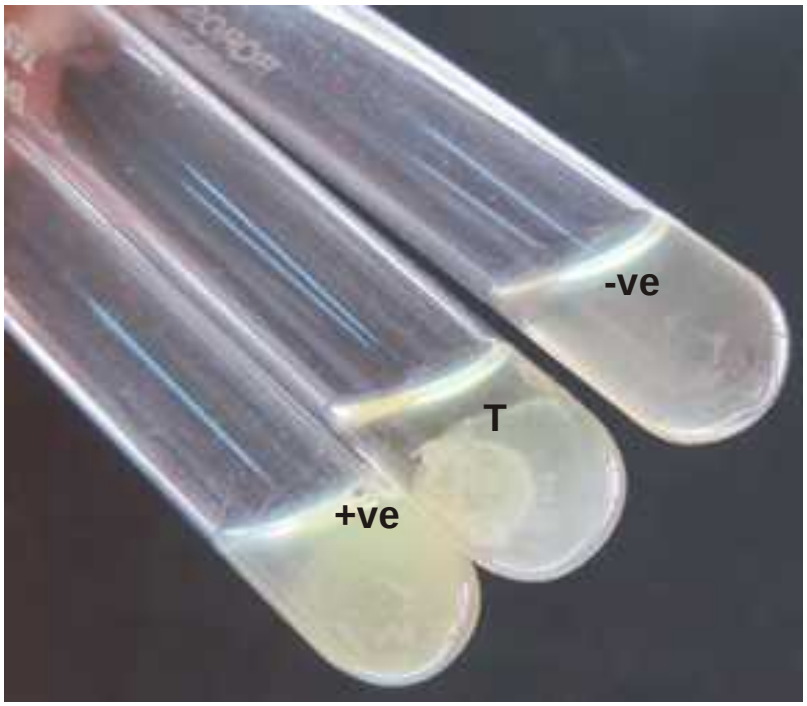
Fig. 6 : Growth of *S.aureus* on Nutrient agar

Fig. 7 : Slide Coagulase



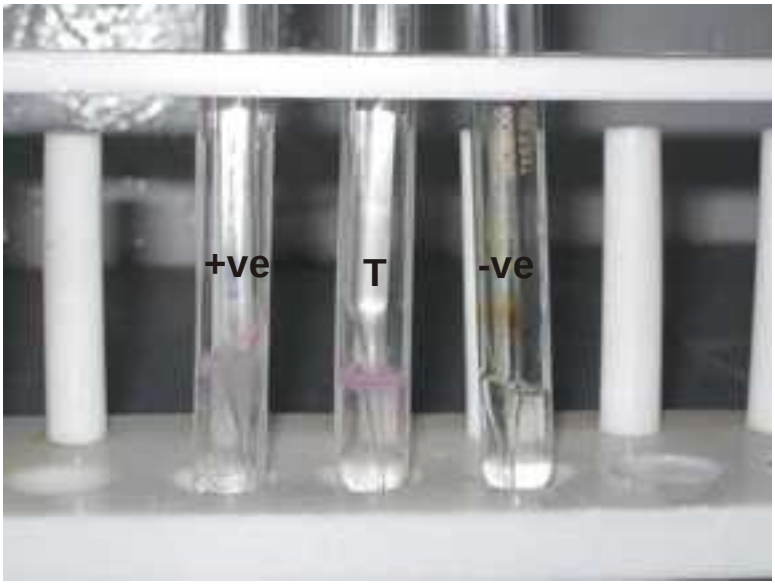
1. Positive Control : S.*aureus* 2. Test : S.*aureus* 3. Negative Control : S.*epidermidis*

Fig. 8 : Tube Coagulase



1. Positive Control : S.*aureus* 2. Test : S.*aureus* 3. Negative Control : S.*epidermidis*

Fig. 9 : Catalase test



1. Positive Control : S.*aureus* 2. Test : S.*aureus* 3. Negative Control : Streptococcus *spp.*

Fig. 10 : Biochemical reactions of *S.aureus*

Fig. 11 : Gram stain showing the Gram negative bacilli

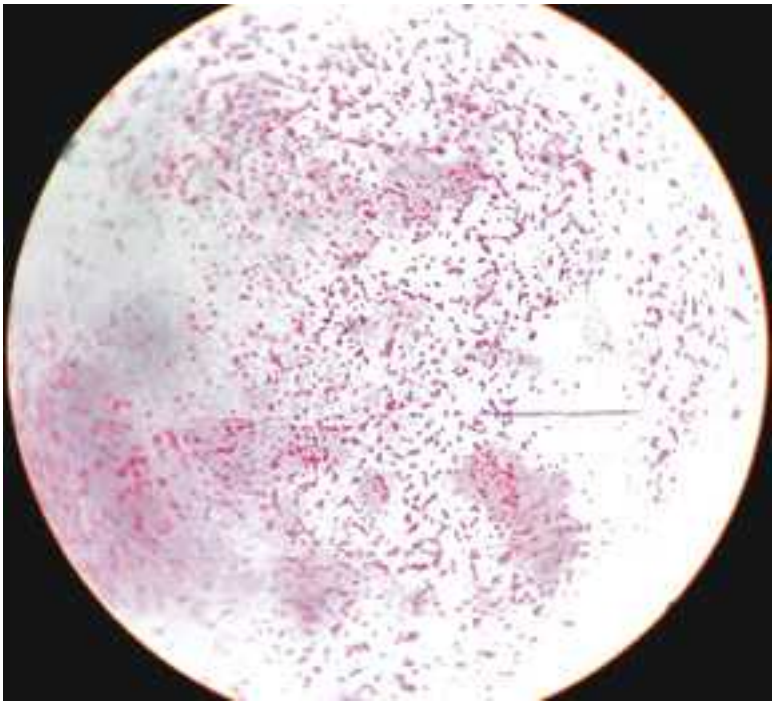


Fig. 12 : Growth of P.*aeruginosa* on Nutrient agar

Fig. 13 : Growth of Non-Lactose fermenter on MacConkey agar

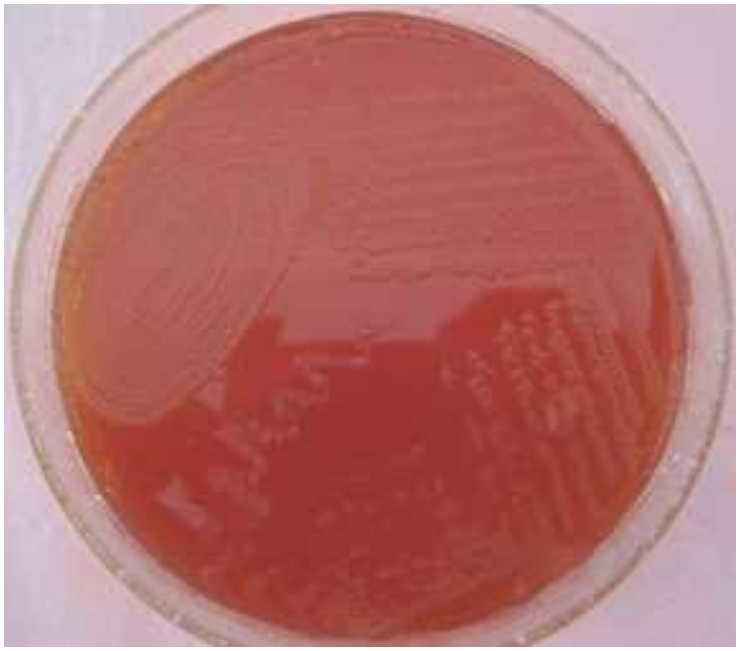
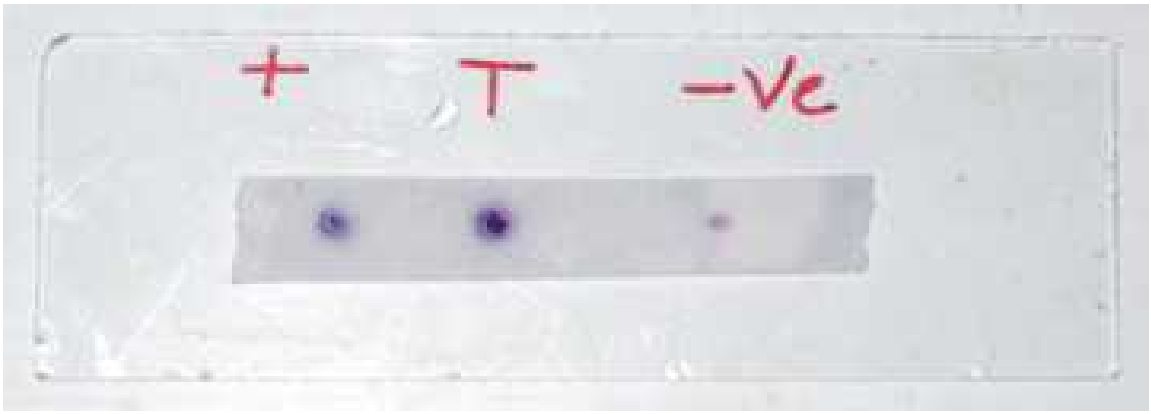
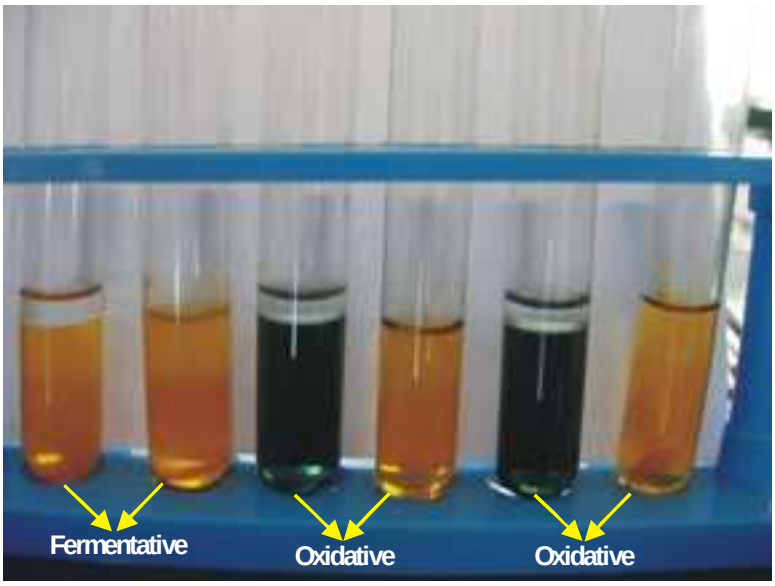


Fig. 14 : Oxidase test



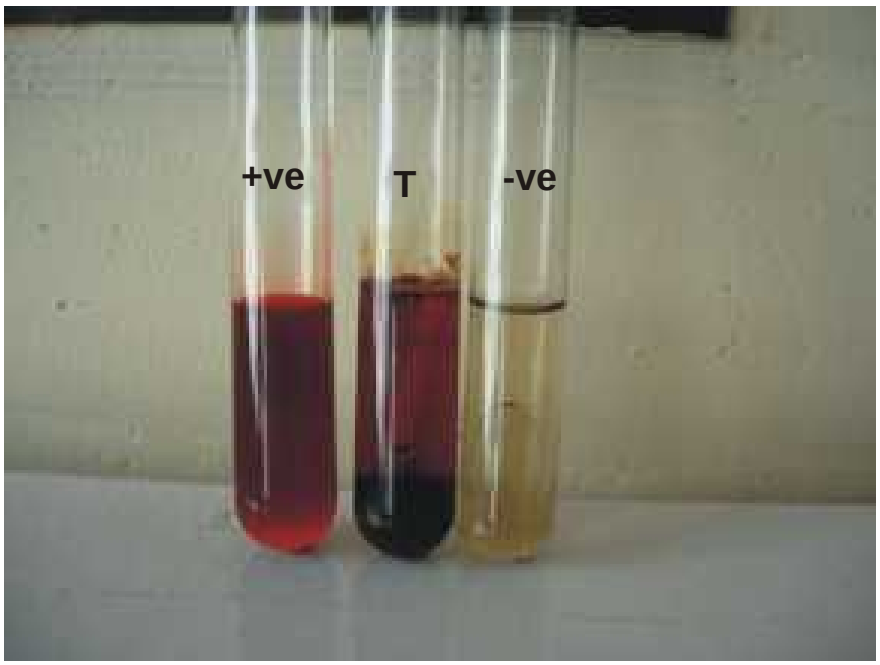
1. Positive Control : P.*aeruginosa* 2. Test : P.*aeruginosa* 3. Negative Control : E.*coli*

Fig. 15 : Oxidative-Fermentation (Hugh Leifson) test



1. Fermentative Control : E.*coli* 2. Oxidative control : P.*aeruginosa* 3. Test : P.*aeruginosa*

Fig. 16 : Nitrate reduction test



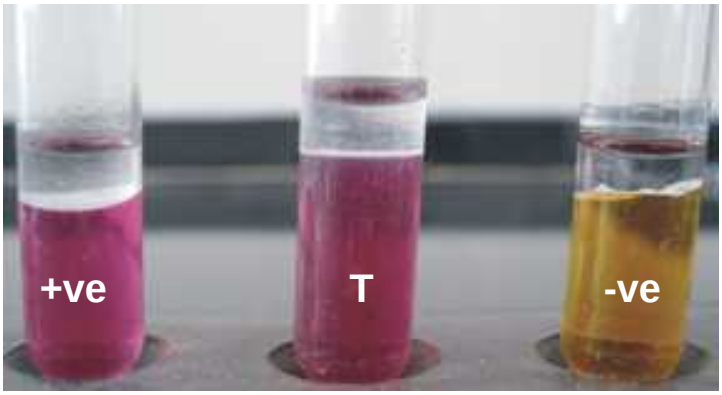
1. Positive Control : E.*coli* 2. Test : P.*aeruginosa* 3. Negative Control : Ancinetobacter *spp.*

Fig. 17 : Biochemical reaction of P.*areuginosa*

Fig. 18 : Triple sugar iron agar

i) Uninoculated ii) K/A with abundant H_2S : P.*mirabilis* iii) K/A : Serralia *spp.* iv) K/NC : P.*aeruginosa* v) A/A with gas : K.*pneumoniae*

Fig. 19 : Arginine Dihydrolase test

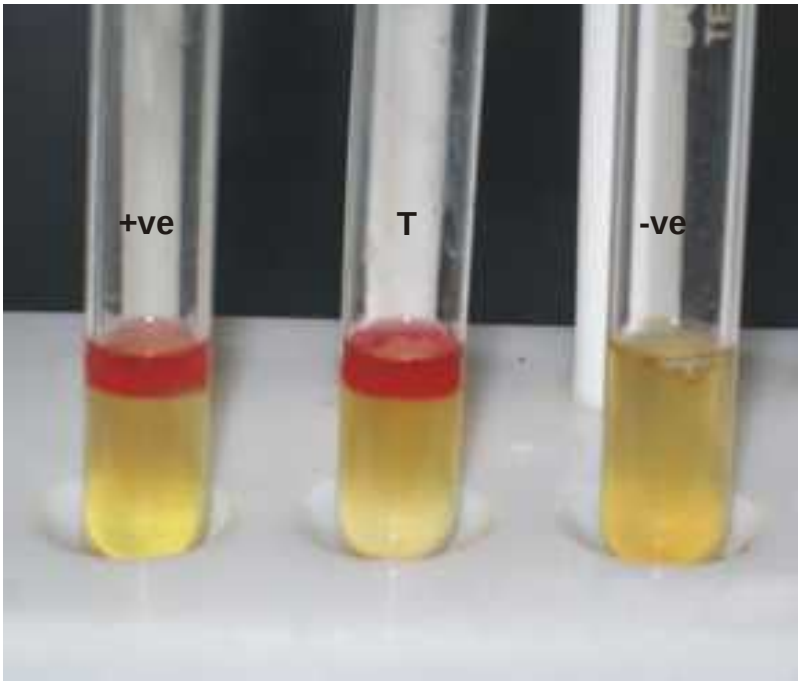


1. Positive Control : P.*aeruginosa* 2. Test : P.*aeruginosa* 3. Negative Control : K.pneumoniae

Fig. 20 : Blood agar showing swarming of Proteus *spp.*

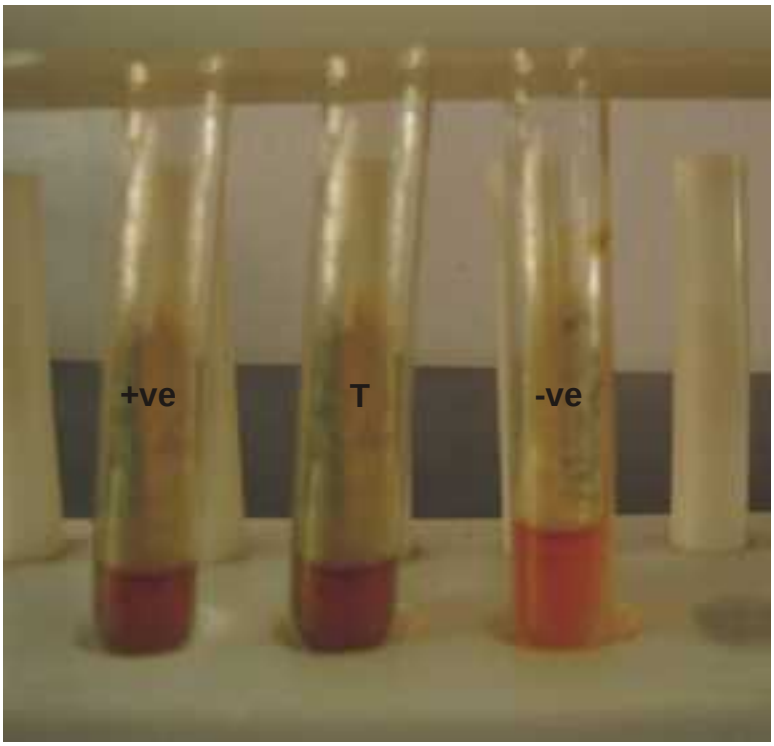
Fig. 21 : Biochemical reaction of Proteus *spp.*

Fig. 22 : Methyl Red test



1. Positive Control : E.*coli* 2. Test : P.*mirabilis* 3. Negative Control : K.*pneumoniae*

Fig. 23 : Phenylalnine Deaminase test (PPA)

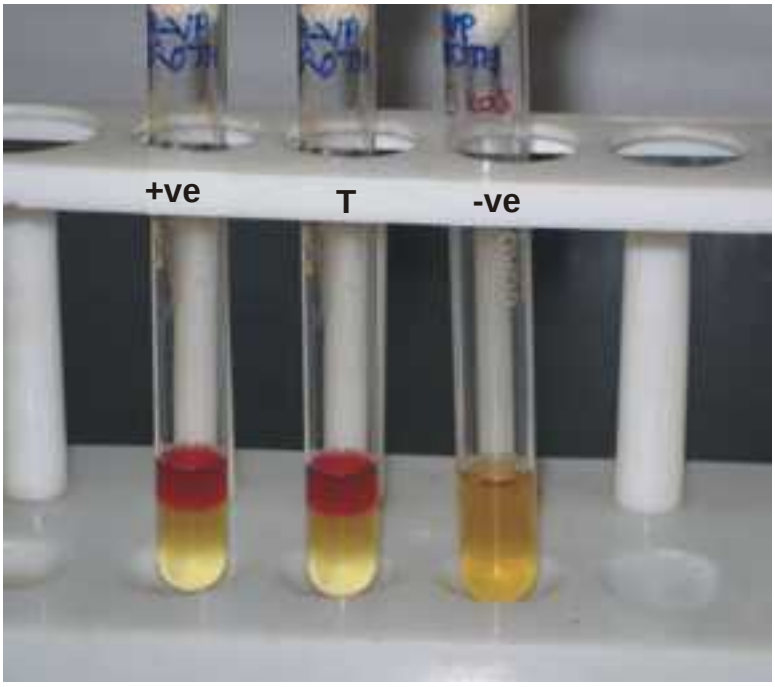


1. Positive Control : Proteus *spp.* 2. Test : P.*mirabilis* 3. Negative Control : E.*coli*

Fig. 24 : Klebsiella *spp.* on MacConkey agar

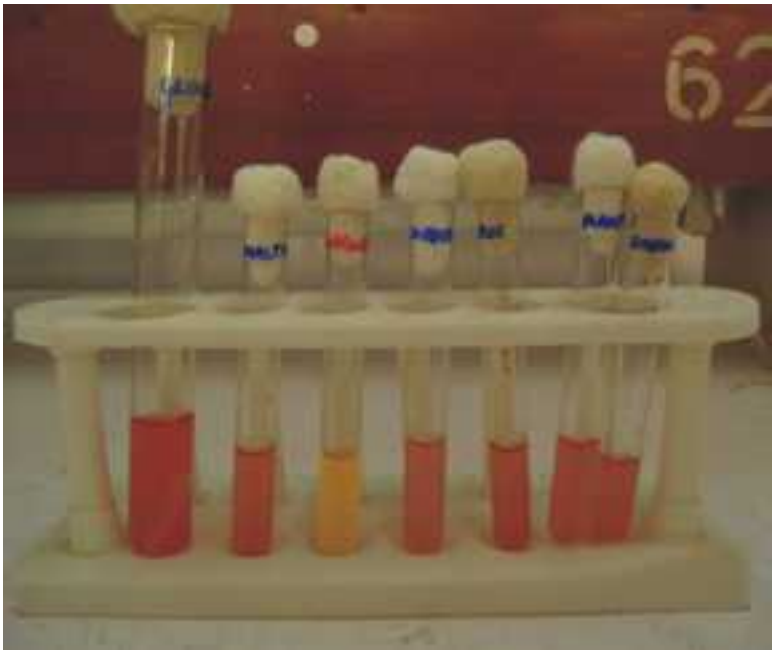
Fig. 25 : Biochemical reaction of K.*pneumoniae*

ig. 26 : Voges-Proskauer test



1. Positive Control : K.*pneumoniae* 2. Test : K.*pneumoniae* 3. Negative Control : E.*coli*

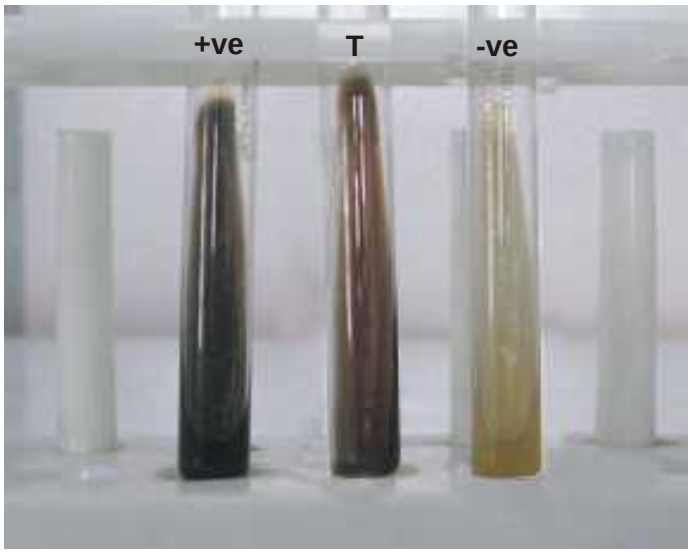
Fig. 27 : Sugar fermentation test



Glucose : Fermented with gas **Sorbitol : Fermented** **Sucrose : Not fermented**
Maltose : Fermented **Xylose : Fermented**
Arabinose : Fermented **Mannose : Fermented**

Fig. 28 : Growth of E.*faecalis* on Blood agar

Fig. 29 : Bile esculin test



1. Positive Control : Enterococcus *spp.* 2. Test : E.*faecalis* 3. Negative Control : Streptococcus *viridians*

Fig. 30a : Antibiotic sensitivity of P.*aeruginosa*

Fig. 30b : Antibiotic sensitivity of S.*aureus*

Fig. 31 : Disc diffusion susceptibility test (DDT) for Methicillin Resistant Strains of S.*aureus*

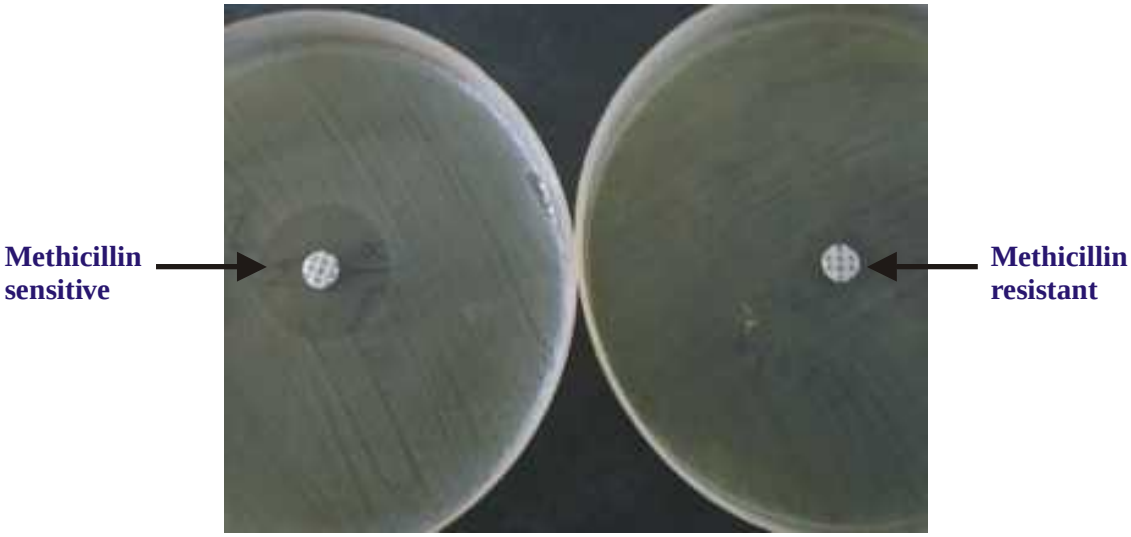
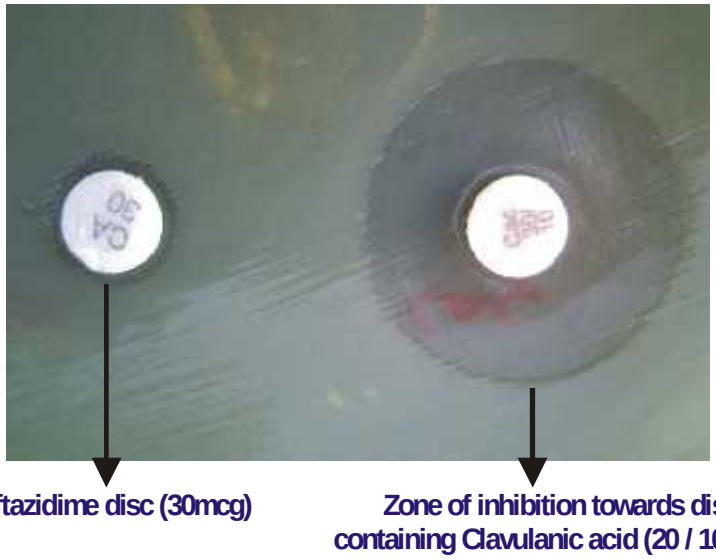


Fig. 32 : Potentiated disc diffusion susceptibility test (PDDT) for ESBL producers



MASTER CHART

SL.No	Age in Years	Sex M/F	OP NO	EAR B/R/L	ASSOCIATED COMPLAINTS	GRAM STAIN	ISOLATES	ANTIBIOTIC SUSCIPTIBILITY											COMPLICATIONS	FOLLOW UP
								A	Ery	Gent	AC	cf	Ca	Ak	Ce	Le	MRSA	ESBL		
1	32	M	1081050	Rt	-	GNB	*P.mirabilis*	-	R	-	R	S	-	S	S	S	-	NON	-	Dry ear/tympanoplasty
2	22	M	1051506	Rt	-	GPC	*S. aureus*	R	R	S	R	R	R	-	-	-	`+ve	-	-	Persisting discharge
3	30	M	1083125	Lt	Repeated attack	GNB	*P.aeruginosa*	-	R	-	R	S	-	S	R	S	-	`+ve	-	Dry ear/tympanoplasty
4	28	F	1007132	Rt	-	-	*NOGC*	-	-	-	-	-	-	-	-	-	-	-	-	persisting discharge
5	9	F	1099738	Lt	Repeated attack	GPC	*S .aureus*	R	R	R	S	S	R	-	-	-	`+ve	-	-	Dry ear/tympanoplasty
6	35	F	1008275	Lt	H/o tinnitus	GPC	*S. aureus*	R	R	S	S	R	R	-	-	-	`+ve	-	-	Dry ear
7	16	F	1105955	Lt	Repeated attack	GPC	*S.aureus*	R	S	S	S	S	S	-	-	-	MSSA	-	-	Dry ear/tympanoplasty
8	8	F	982300	Lt	Repeated attack	GPC	*S.aureus*	R	R	S	S	S	R	-	-	-	MSSA	-	-	Dry ear
9	45	M	1109749	Rt	Repeated Rhinitis	GPC	*S.aureus*	R	R	S	S	S	S	-	-	-	MSSA	-	-	Not done
10	25	M	1113492	B/L	H/O Fever&blood stained	GNB	*P.vulgaris*		R		R	R	-	S	R	S	-	`+ve	-	Dry ear/tympanoplasty
11	30	M	1103119	Lt	Post aural fistula	GPC	*S.aureus*	R	R	S	R	S	R	-	-	-	MSSA	-	mastd.abcs	Dry ear/Mastoidectomy
12	13	F	1113760	Rt	H/o tinnitus/ear pain	GNB	*P. vulgaris*	-	R		R	R	-	S	S	S	-	NON	Brn. abscs	Dry ear/Mastoidectomy
13	28	M	1116164	Lt	H/oRhinitis	GPC	*S.aureus*	R	R	S	R	S	S	-	-	-	MSSA	-	-	Dry ear
14	30	F	1118099	Rt	H/o pain	GPC	*S .aureus*	R	R	S	S	R	R	-	-	-	`+ve	-	-	Dry ear/tympanoplasty
15	15	F	1114312	Rt	-	-	*NOGC*	-	-	-	-	-	-	-	-	-	-	-	-	persisting discharge
16	32	F	1095995	Lt	decrease hearing	GNB	*P.aeruginosa*	-	S	-	S	S	-	S	S	S	-	NON	-	Dry ear/tympanoplasty
17	11	M	1095608	Lt	H/o URTI	GPC	*S.aureus*	R	R	S	R	S	R	-	-	-	MSSA	-	-	Dry ear
18	3	M	1115086	Rt	Reptd. Attack	GPC	*S. aureus*	R	S	S	S	S	S	-	-	-	MSSA	-	-	Dry ear/tympanoplasty
19	11	M	878656	Rt	-	GNB	*P. aeruginosa*	-	R	-	S	R	-	S	R	R	-	`+ve	-	persisting discharge
20	58	F	1120834	Rt	Rept. attack	GPC	*S. aureus*	R	R	S	S	R	S	S	S	S	MSSA	-	-	Dry ear/tympanoplasty
21	40	F	1102352	Lt	H/o tinnitus	GPC	*S. aureus*	R	R	S	S	R	S	-	-	-	MSSA	-	-	Dry ear/tympanoplasty
22	18	M	1121549	B/L	H/o tinnitus	GNB	*P. mirabilis*	-	R	-	S	R	-	S	S	S	-	NON	-	Dry ear/tympanoplasty
23	6	M	953543	Lt	H/o epilepsy	GNB	*P. mirabilis*	-	R	-	R	R	-	S	S	S	-	NON	-	Dry ear
24	20	M	747894	Lt	Repeated attack	GPC	*S. aureus*	R	S	S	S	S	R	-	-	-	-	-	-	Dry ear/tympanoplasty
25	16	M	106597	Lt	H/o decrease hearing	GNB	*P. mirabilis*	-	R	-	R	R	-	S	S	S	-	NON	-	Dry ear/tympanoplasty
26	35	F	1008271	Lt	-	GPC	*S. aureus*	S	S	S	S	S	S	-	-	-	MSSA	-	-	Dryear/tympanoplasty
27	67	M	1105317	Lt	H/o decrease hearing	GPC	*S. aureus*	R	R	R	R	R	R	-	-	-	`+ve	-	-	persisting discharge
28	13	M	1127009	B/L	H/o tinnitus	GNB	*K.pneumoniae*	-	R	-	R	S	-	R	S	S	-	NON	-	Dry ear
29	60	F	949584	Rt	H/o tinnitus	-	*NOGC*	-	-	-	-	-	-	-	-	-	-	-	-	Dry ear/tympanoplasty

MASTER CHART

SL.No	Age in Years	Sex M/F	OP NO	EAR B/R/L	ASSOCIATED COMPLAINTS	GRAM STAIN	ISOLATES	ANTIBIOTIC SUSCIPTIBILITY											COMPLICATIONS	FOLLOW UP
								A	Ery	Gent	AC	cf	Ca	Ak	Ce	Le	MRSA	ESBL		
30	26	M	1108588	B/L	H/o tinnitus	GNB	*P. aeruginosa*	-	S	-	S	R	-	S	S	R	-	NON	-	Dry ear/tympanoplasty
31	26	M	1060312	Lt	-	GPC	*S. aureus*	S	S	S	S	S	S	-	-	-	MSSA	-	-	Dry ear/tympanoplasty
32	17	M	1132116	Rt	H/o pain	GPC	*S. aureus*	R	R	R	R	R	R	-	-	-	`+ve	-	-	Dry ear/tympanoplasty
33	11	F	1132957	Lt	-	GPC+GNB	*S.aureus + C. freundii*	R	S	S	S	R	S	S	S	S	MSSA	NON	-	Dry ear
34	43	F	1132964	Rt	Post operative	-	*NOGC*		-		-	-	-	-	-	-	-	-	-	Dry ear
35	8	M	1133135	Lt	Repeated attack	GNB	*P. aeruginosa*		R	-	R	R	-	R	R	R	-	`+ve	-	persisting discharge
36	3	M	1137856	Rt	H/o tinnitus	GNB	*C. freundii*	-	R	-	R	S	-	S	R	S	-	`+ve	-	Dry ear
37	11	F	1132957	Lt	-	GPC+GNB	*S. aureus + C. freundii*	R	R	S	S	R	S	S	S	S	MSSA	NON	-	Dry ear/tympanoplasty
38	2	M	1695914	B/L	-	GPC	*S. aureus*	R	S	R	R	R	R	-	-	-	`+ve	-	-	Dry ear/tympanoplasty
39	23	M	1090447	Lt	DNS	GPC	*S. aureus + E. faecalis*	R	R	S	S	S	R	-	-	-	`+ve	-	-	Dry ear/tympanoplasty
40	28	M	1138401	B/L	-	GPC	*S. aureus*	S	S	S	S	S	S	-	-	-	MSSA	-	-	Dry ear
41	19	M	326704	Rt	-	-	*NOGC*	-	-	-	-	-	-	-	-	-	-	-	-	persisting discharge
42	1	F	1143456	B/L	URTI	GNB	*p.mirabilis + P.aeruginosa*	-	R	-	R	-	S	S	S	S	-	NON	-	Dry ear/tympanoplasty
43	15	M	1139118	Lt	-	GNB	*P. aeruginosa*	-	S	-	S	S	-	S	S	S	-	NON	-	Dry ear/tympanoplasty
44	8	F	1144271	B/L	Hearing loss	GNB	*K.pneumoniae*	-	R	-	R	S	-	S	S	S	-	NON	-	Dry ear/tympanoplasty
45	44	M	1130154	Lt	-	-	*NOGC*	-	-	-	-	-	-	-	-	-		-	-	Not done
46	26	F	1138057	Lt	-	GPC	*NOGC*	-	-	-	-	-	-	-	-	-		-	-	Persisting discharge
47	18	M	1149300	B/L	Repeated attack	GNB	*P. aeruginosa*	-	R	-	R	S	-	S	S	S	-	NON	-	Persisting discharge
48	13	F	1091209	Rt	Repeatd attack-	GNB	*P. aeruginosa*	-	R	-	S	S	-	S	S	S	-	NON	-	Dry ear/tympanoplasty
49	14	F	1153416	Lt	-	GPC	*S .aureus*	S	S	S	S	S	S	-	-	-	MSSA	-	-	Dry ear/tympanoplasty
50	17	M	919872	Rt	Repetead attack	GNB	*P. aeruginosa*	-	R	-	R	R	-	R	S	S	-	`+ve	-	Dry ear/tympanoplasty
51	9	F	1160601	Lt	URTI	GNB	*K.pneumoniae*	-	R	-	S	R	-	S	S	S		NON	-	Dry ear
52	16	M	1120363	Rt	-	GNB	*P. aeruginosa*	-	R	-	R	R	-	R	S	R	-	NON	-	persisting discharge
53	9	M	950501	Rt	Tonsillitis	GNB	*P. aeruginosa*	-	R	-	R	R	-	R	S	R	-	NON	-	Dry ear/tympanoplasty
54	34	F	1115846	Lt	-	GNB	*P. aeruginosa*	-	R	-	R	S	-	S	S	S	-	`+ve	-	Dry ear/tympanoplasty
55	21	F	1168736	Lt	Repeatd attack-	GNB	*K.pneumoniae*	-	R	-	R	R	-	S	R	S	-	NON	-	Dry ear/tympanoplasty
56	20	M	1130055	Rt	-	GNB	*P .aeruginosa*	-	S	-	S	R	-	S	S	R	-	NON	-	Dry ear
57	1	F	1141785	B/L	-	GNB	*P. aeruginosa*	-	R	-	R	S	-	S	S	S	-	NON	-	Dry ear/tympanoplasty
58	16	M	1176549	B/L	decrease hearing	GPC	*E. faecalis*	R	R	S	S	R	S	-	-	-	-	-	-	Dry ear

MASTER CHART

SL.No	Age in Years	Sex M/F	OP NO	EAR B/R/L	ASSOCIATED COMPLAINTS	GRAM STAIN	ISOLATES	ANTIBIOTIC SUSCIPTIBILITY											COMPLICATIONS	FOLLOW UP
								A	Ery	Gent	AC	cf	Ca	Ak	Ce	Le	MRSA	ESBL		
59	48	M	1169092	Lt	H/o pain	GNB	*P. aeruginosa*	-	R	-	S	S	-	S	S	S	-	NON	-	Dry ear/Mastoidectomy
60	44	F	1173218	Rt	Repeated attack	GNB	*P.aeruginosa*	-	R	-	S	R	-	S	S	S	-	NON	Mastoiditis	Dry ear/Mastoidectomy
61	13	F	1091209	B/L	Tonsillitis	GNB	*P.aeruginosa*	-	R	-	S	S	-	S	R	S	-	`+ve	-	Dry ear/tympanoplasty
62	12	F	332247	Rt	Repeated attack	GNB	*P. aeruginosa*	-	R	-	R	S	-	R	R	S	-	NON	-	Dry ear/tympanoplasty
63	17	M	335256	Lt	Repeated attack	GPC	*NOGC*	-	-	-	-		-	-	-	-	-		-	Not done
64	27	M	337388	B/L	Repeated attack	GPC+GNB	*S. aureus + P.aeruginosa*	R	R	R	R	R	R	R	R	+	`+ve		Mastoid abscess	Dry ear/Mastoidectomy
65	48	F	1136306	B/L	Hearing loss	GPC+GNB	*S.aureus+ P.aeruginosa*	R	R	S	S	S	S	S	S	S	`+ve	NON	-	Dry ear
66	17	M	929872	B/L	Repeated attack	GNB	*K .oxytoca*	-	R	-	S	R	-	S	R	R	-	`+ve	-	Dry ear/tympanoplasty
67	53	M	1190952	Rt	Repeated attack	GPC	*S. aureus*	R	R	S	S	S	R	-	-	-	`+ve	NON	-	Dry ear/tympanoplasty
68	13	F	1091209	B/L	-	GNB	*P. aeruginosa*		R	-	S	S	-	S	R	S	-	`+ve	-	Dry ear
69	10	F	923684	Rt	decrease hearing	GPC	*S .aureus*	S	S	R	S	R	S	-	-	-	MSSA	-	-	Dry ear
70	30	M	335309	Lt	Acute pharyngitiS	GPC	*S aureus*	S	R	S	S	R	R	-	-	+	-	-	-	Dry ear/tympanoplasty
71	15	M	1201789	Rt	-	GPC	*NOGC*	-	-	-	-		-	-	-	-	-	-	-	Not done
72	18	M	1210841	B/L	Drecrease hearing loss	GNB	*P .aeruginosa*	-	R	-	S	S	-	R	R	R	-	NON	-	Dry ear
73	1	F	342455	Rt	Repeated attack	GPC	*S. aureus*	R	R	R	R	R	R	-	-	-	`+ve	-	Mastoiditis	Dry ear/Mastoidectomy
74	7	M	1213804	Rt	H/o pain	GPC	*E. faecalis*	R	R	S	S	S	R	-	-	-	-	-	-	Dry ear
75	35	F	1113951	Rt	Repeated attack	GNB	*P.aeruginosa*	-	R	-	R	R	-	R	R	R	-	`+ve	-	persisting discharge
76	40	F	666947	B/L	Repeated attack	GNB	*P. aeruginosa*	-	R	-	R	S	-	S	S	S	-	NON	-	Dry ear
77	40	M	342867	Rt	-	GPC+GNB	*S aureus + P aeruginosa*	R	R	S	S	S	S	S	S	S	`+ve	NON	-	Dry ear
78	10	M	1219787	Rt	Repeated attack	GPC	*S. aureus*	R	R	R	R	R	R	-	-	-	`+ve	-	-	persisting discharge
79	22	M	1219166	Lt	-do-	GPC	*S. aureus*	S	R	S	S	R	S	-	-	-	MSSA	-	Mastoiditis	Dry ear/Mastoidectomy
80	30	F	1224095	B/L	H/o tinnitus	GNB	*P.aeruginosa+ P.vulgaris*	-	R	-	S	R	-	S	S	S	-	-	-	Dry ear
81	14	F	12224636	B/L	Repeatd attack-	GNB	*C. freundii*	-	S	-	S	S	-	S	S	S	-	-	-	Dry ear/tympanoplasty
82	82	F	1206146	B/L	H/o DM&HTN	GPC	*NOGC*	-	-	-	-	-	-	-	-	-	-	-	-	Note done
83	1	M	1221722	Rt	-	GPC	*S. aureus*	R	R	R	R	R	R	-	-	-	`+ve	-	-	Not done
84	4	F	1228815	Lt	-	GPC	*NOGC*	-	-	-	-	-	-	-	-	-	-	-	-	Not Done
85	11	M	1229013	B/L	H/o Repeated sore throat	GNB	*P.aeruginosa + P. vulgaris*	-	R	-	R	S	-	S	S	S		`-VE	-	Dry ear/tympanoplasty

MASTER CHART

SL.No	Age in Years	Sex M/F	OP NO	EAR B/R/L	ASSOCIATED COMPLAINTS	GRAM STAIN	ISOLATES	ANTIBIOTIC SUSCIPTIBILITY											COMPLICATIONS	FOLLOW UP
								A	Ery	Gent	AC	cf	Ca	Ak	Ce	Le	MRSA	ESBL		
86	21	M	1231756	Rt	Repeatd attack-	GNB	*P.aeruginosa +K.pneumoniae + C.freundii*	S	R	-	R	S	-	S	R	S	-	`+VE	-	Dry ear
87	10	M	236947	Lt	-do-	GNB	*P. aeruginosa*	-	R		S	S	-	S	S	S	-	NON	-	Dry ear/tympanoplasty
88	45	F	941831	Rt	-do-	-	*NOGC*	-	-	-	-	-	-	-	-	-	-	-	-	Not Done
89	35	F	124977	Rt	Trauma	GPC+GNB	*S.aureus+ K.pneumoniae*	S	S	S	S	S	S	S	S	S	MSSA	NON	-	Dry Ear
90	42	M	125035	Rt	--do-	GNB+GPC	*S. aureus+ P.aeruginosa*	S	R	S	S	R	R	S	R	S	MSSA	`+ve	-	Dry ear
91	36	F	347988	Lt	HIV I & II	-	*NOGC*	-	-	-	-	-	-	-	-	-	-	-	-	Not Done
92	13	M	1221310	B/L	Tonsilitis+Nose block	GPC	*S. aureus*	S	S	S	S	S	S	-	-	-	MSSA	-	-	Dry ear/tympanoplasty
93	53	M	1252608	Lt	Repeated attack	GNB	*P. aeruginosa*	-	R	-	R	R	-	S	R	S	-	`+ve	-	Not Done
94	75	F	1252712	Rt	Repeated attack	GPC+GNB	*E .faecalis + P. mirabilis*	S	R	S	S	R	R	S	R	S	-	`+ve	-	Dry ear
95	32	M	1113492	Rt	-do-	GNB	*P. aeruginosa*	-	R	-	R	R	-	S	R	S	-	`+ve	-	Dry ear/tympanoplasty
96	35	M	1237219	Lt	Repeatd URTI	GNB	*K.pneumoniae+ P.aeruginosa*	-	R	-	S	S	-	S	S	S	-	-	-	Not done
97	26	M	353259	Rt	H/o Repeatd headache	GNB	*P. mirabilis*	-	R		S	R	-	S	R	S	-	`+ve	-	Not done
98	6	M	355230	Rt	Tonsillitis	GNB	*Acinetobacter spp.*	-	R		S	R	-	S	S	S	-	-	-	Dry ear/tympanoplasty
99	26	F	355380	Lt	Repeated attack	GNB	*Acinetobacter spp.*	-	R		R	S	-	S	S	S	-	NON	-	Not done

KEY TO MASTER CHART

A	–	Ampicillin
Ac	–	Amoxyclav
AK	–	Amikacin
B/L	–	Bilateral
Ca	–	Ceftazidime
Ce	–	Cefotaxime
Cf	–	Ciprofloxacin
DM	–	Diabetes Mellitus
DNS	–	Deviated Nasal Septum
E	–	Erythromycin
ESBL	–	Extended Spectrum Beta Lactamase
F	–	Female
G	–	Gentamicin
GPC	–	Gram Positive Cocci
GNB	–	Gram Negative Bacteria
HIV	–	Human Immuno Deficiency Virus
HTN	–	Hypertension
Le	–	Levofloxacin
Lt	–	Left
M	–	Male
MRSA	–	Methicillin Resistant Staphycoccus aureus
MSSA	–	Methicillin Sensitive Staphycoccus aureus
NOGC	–	No Organisms Grown in Culture
R	–	Resistant
Rt	–	Right
S	–	Sensitive
URTI	–	Upper Respiratory Tract Infection

TABLE 16 : ANTIBIOTIC SUSCEPTIBLITY PATTERN OF SINGLE ISOLATES

Sl no	Isolates	No. of isolates	Ampicillin		Erythromycin		Gentamcin		Amoxyclav		Ciprofloxacin		Ceftazidime		Amikacin		Cefataxime		Levoflo
			S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
1	*S.aureus*	29	8 -27.60%	21 -72.40%	10 -35.50%	19 -65.50%	21 -72.40%	8 -27.10%	19 -66.50%	10 -34.50%	16 -55.20%	13 -44.80%	13 -44.80%	16 -55.20%	_	_	_	_	_
2	*P. aeruginosa*	25	_	_	4 -16%	21 -84%	_	_	12 -48%	13 -52%	14 -56%	11 -44%	_	_	18 -72%	7 -28%	14 -56%	11(44%)	17(68%)
3	*K . pnemoniae*	4	-	-	0(0%)	4(100%)	-	-	1 (25%)	3(75%)	2(50%)	2(50%)	-	-	3(75%)	1 (25%)	3(75%)	1(25%)	4 (100%)
4	*K . oxytoca*	1	-	-	0(0%)	1(100%)	-	-	1 (100%)	0(0%)	0 (0%)	1(100%)	-	-	1 (100%)	0(0%)	0(0%)	1(100%)	0 (0%)
5	*P . mirabilis*	5	-	-	0(0%)	5(100%)	-	-	2(40%)	3(60%)	1 (20%)	4(80%)	-	-	5 (100%)	0(0%)	4(75%)	1(25%)	5 (100%)
6	*P . vulgaris*	2	-	-	0(0%)	2(100%)	-	-	0(0%)	2 (100%)	0 (0%)	2(100%)	-	-	2 (100%)	0(0%)	1(50%)	1(50%)	2 (100%)
7	*E. faecalis*	2	0(0%)	2 (100%)	0(0%)	2(100%)	2(100%)	0(0%)	2(100%)	0(0%)	1 (50%)	1(50%)	1(50%)	1(50%)	-	-	-	-	-
8	*Acinatobacter spp.*	2	-	-	0(0%)	2(100%)	-	-	1(50%)	1(50%)	1(50%)	1(50%)	-	-	2 (100%)	2(100%)	2(100%)	2(100%)	2 -100%
9	*C.fruendii*	2	-	-	1(50%)	1(50%)	-	-	1(50%)	1(50%)	1(50%)	1(50%)	-	-	2 (100%)	0(0%)	1(100%)	1(0%)	2(100%)
Total		72																	

oxacin
R
–
8(32%)
0 (0%)
1 (100%)
0(0%)
0(0%)
-
2 (100%)
0 (0%)

TABLE 17 : ANTIBIOTIC SUSCEPTIBLITY PATTERN OF MIXED ISOLATES

Sl no.	Isolates	No. of isolates	Ampicillin		Erythromycin		Gentamicin		Amoxyclav		Ciprofloxacin		Ceftazidime		Amikacin		Cefotaxime		Levofloxacin	
			S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
1	*S. aureus* + *C .freundii*	2	0 0%	2 -100%	1 -50%	1 -50%	2 -100%	0 0%	2 -100%	0 0%	0 0%	2 -100%	2 -100%	0 0%	2 -100%	0 0%	2 -100%	0 0%	2 -100%	0 0%
2	*S. aureus* + *E. facecalis*	1	0 0%	1 -100%	0 0%	1 -100%	1 -100%	0 0%	1 -100%	0 0%	1 -100%	0 0%	0 0%	1 -100%	-	-	-	-	-	-
3	*S. aureus* + *P. aeruginosa*	3	0 0%	3 -100%	0 0%	3 -100%	2 -66.70%	1 -33.30%	2 -66.70%	1 -33.30%	2 -66.70%	1 -33.30%	2 -66.70%	1 -33.30%	2 -66.70%	1 -33.30%	2 -66.70%	1 -33.30%	2 -66.70%	1 -33.30%
4	*P .mirabailis* + *P.aeruginosa*	1	-	-	0 0%	1 -100%	-	-	0 0%	1 -100%	-	-	1 -100%	0 0%	1 -100%	0 0%	1 -100%	0 0%	1 -100%	0 0%
5	*P. aeruginosa* + *P. vulgaris*	2	-	-	0 0%	2 -100%	-	-	1 -50%	1 -50%	1 -50%	1 -50%	-	-	2 -100%	0 0%	2 -100%	0 0%	2 -100%	0 0%
6	*S. aureus* + *K. pneumoniae*	1	1 -100%	0 0%	1 -100%	0 0%	1 -100%	0 0%	1 -100%	0 0%	1 -100%	0 0%	1 -100%	0 0%	1 -100%	0 0%	1 -100%	0 0%	1 -100%	0 0%
7	*S .aureus* + *P .mirabilis*	1	1 -100%	0 0%	0 0%	1 -100%	1 -100%	0 0%	1 -100%	0 0%	0 0%	1 -100%	0 0%	1 -100%	1 -100%	0 0%	0 0%	1 -100%	1 -100%	0 0%
8	*E .faecalis* + *P. mirabilis*	1	1 -100%	0 0%	0 0%	1 -100%	1 -100%	0 0%	1 -100%	0 0%	0 0%	1 -100%	0 0%	1 -100%	1 -100%	0 0%	0 0%	1 -100%	1 -100%	0 0%
9	*P.aeruginosa* + *K. pneumoniae*	1	-	-	0 0%	1 -100%	-	-	0 0%	1 -100%	1 -100%	0 0%	-	-	1 -100%	0 0%	1 -100%	0 0%	1 -100%	0 0%
10	*P. aeruginosa* + *K .pneumoniae* + *C. freundii*	1	-	-	0 0%	1 -100%	-	-	0 0%	1 -100%	1 -100%	0 0%	-	-	1 -100%	0 0%	0 0%	1 -100%	1 -100%	0 0%
	TOTAL	**14**																		

TABLE 20 : SENSITIVITY PATTERN OF A MIXED ISOLATES OF MRSA AND ESBL

Sl. no	Isolates	-	No. of Isolates	Ampicillin		Erythromycin		Gentamicin		Amoxyclav		Ciprofloxacin		Ceftazidime		Amikacin		Cefotaxime		Levofloxacin	
				S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
1	*S. aureus* + *E. faecalis*	MRSA + NONESBL	1	0 0%	1 -100%	0 0%	1 -100%	1 -100%	0 0%	1 -100%	0 0%	1 -100%	0 0%	0 0%	1 -100%	-	-	-	-	-	-
2	*S. aureus* + *P. aeruginosa*	MRSA + ESBL	1	0 0%	1 -100%	0 0%	1 -100%	0 0%	1 -100%	0 0%	1 -100%	0 0%	1 -100%	0 0%	1 -100%						
3	*S. aureus + P. aeruginosa*	MRSA + NONESBL	2	0 0%	2 -100%	0 0%	2 -100%	2 -100%	0 0%	2 -100%	0 0%	2 -100%	0 0%	2 -100%	0 0%	2 -100%	0 0%	2 -100%	0 0%	2 -100%	0 0%
4	*S. aureus* + *P. mirabilis*	MRSA + ESBL	1	1 -100%	0 0%	0 0%	1 -100%	1 -100%	0 0%	1 -100%	0 0%	0 0%	1 -100%	0 0%	1 -100%	1 -100%	0 0%	0 0%	1 -100%		
5	*E. faecalis* + *P. mirabilis*	ESBL	1	1 -100%	0 0%	0 0%	1 -100%	1 -100%	0 0%	1 -100%	0 0%	0 0%	1 -100%	0 0%	1 -100%	1 -100%	0 0%	0 0%	1 -100%	1 -100%	0 0%
6	*P. aeruginosa* + *K. pneumoniae* + *C. freundii*	ESBL	1	-	-	0 0%	1 -100%	-	-	0 0%	1 -100%	1 -100%	0 0%	-	-	1 -100%	0 0%	0 0%	1 -100%	1 -100%	0 0%
	TOTAL CASES		**7**																		