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A study to identify and isolate the most common organism causing Chronic suppurative otitis media (CSOM) in patients attending ENT OPD/IPD of tertiary care hospital

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***Abstract*---**Background: Chronic suppurative otitis media (CSOM) is a notorious infection and a major health problem in developing countries causing serious local damage and threatening complications. Early and effective treatment based on the knowledge of causative micro-organisms to ensures prompt clinical recovery and possible complications can thus be avoided. Objective: The of this study was to isolate the organisms associated with CSOM. Materials and Methods: The prospective study was carried out at a tertiary care hospital in a time period of 1 yr (data collection) clinically diagnosed CSOM cases were included in the study.Pus samples from both outpatient and inpatient departments were included.Detailed history was obtained from each patient according to the case proforma. Result: Out of 122 pus samples significant growth was seen in 120(98.36%) samples, were 2(1.63%) sample showed insignificant or no growth. It is mostly seen that aerobic gram negative rods outnumber gram positive cocci organisms in CSOM as reported by some authors [175] which is comparable to the findings in the present study where gram negative rods accounted for 94(78.33%) and gram

positive cocci 26(21.66%). Conclusion: *Klebsiella pneumoniae* (24.16%) was the commonest isolate followed by *Pseudomonas aeruginosa* (18.33%).

***Keywords*---**gram positive, gram negative, CSOM, culture, agar.

Introduction

Chronic suppurative otitis media (CSOM) is chronic inflammation of the middle ear cleft (Eustachian tube, middle ear, and mastoid cavity) which presents with recurrent ear discharge or otorrhoea through a tympanic perforation for two weeks or more.[1] CSOM commonly occurs during the first 6years of a child's life, with a peak around 2 years.[2] According to WHO, about 330 million individuals, globally, have CSOM.As proposedby WHO, CSOM prevalence rates of 1–2% were considered low and 3–6% were high. India has >4% prevalence rate which need urgent attentionto deal with a massive public Health problem.[1]

Chronicsuppurative otitis media (CSOM) is defined as a perforation of the tympanic membrane with persistent drainage of pus from the middle ear lasting at least two weeks.[1] It is a major cause of acquired hearing impairment of varying severity mostly in developing countries.[2] Chronicsuppurative otitis media is common in infants and children of lower socioeconomic status and causes hearing loss, has an impact on speech and language Development and also affects school performance and social interaction.[3]

Environmental factors have also been associated as risk factors; the prevalence is higher in lower socioeconomic groups. In a cohort study, with results of 12,000 children, factors significant for draining ears were general health scores, maternal smoking and day care attendance.[4] Other factors associated with CSOM are ET dysfunction which is more common in patients with CSOM than normal individuals.[5] Craniofacial abnormalities are associated with increased risk of CSOM. The incidence in cleft palate patients is around 20%, 2% of them have cholesteatoma.[6] The tensor veli palatine muscle is hypoplastic in cleft palate children and may predispose to ET dysfunction.[7]

Global burden of illness from CSOM is estimated to involve about 63-330 million individuals with draining ears, 60% of whom (39-200 million) suffer from significant hearing impairment. It accounts for 28,000 deaths and a disease burden of over 2 million Disability Adjusted Life Years (DALYS). Over 90% of the burden is borne by developing countries in South-east Asia, Western Pacific regions and Africa.

Typical pathogens reach the middle ear through insufflations of respiratory pathogens through the ET from the nasopharynx and spread from the external ear canal inwards through a nonintact TM.[8,9] Studies on microbiologic diagnoses of CSOM differ in regard to patient age, geography and the presence of complications such as cholesteatoma and these inconsistencies likely impact some of the variation in reported pathogens. A portion of the variability observed may be related to differences in sampling and processing methods .[8,10]

Aerobes, anaerobes and fungi are all potential pathogens in CSOM. Knowledge of the true frequency of polymicrobial infection, particularly the extent of anaerobic involvement, is limited by differences in collection and culture techniques.In CSOM bacteria can be aerobic (e.g. *Pseudomonas aeruginosa*, *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Proteus mirabilis, Klebsiella species)* or can be anaerobic (e.g. Bacteriodes, *Peptostreptococcus, Proprionibacterium*).[11-13] These bacteria are infrequently found on the skin of the external ear canal, but may proliferate in the presence of trauma, inflammation, lacerations or high humidity. These bacteria may then gain entry to the middle ear through a chronic perforation.[14,15] Pseudomonas is the most commonly isolated organism in CSOM. Various researchers over the past few decades have isolated Pseudomonas from 48 to 98% of patients with CSOM although other studies have shown that *Staphylococcus aureus* is the most common especially when cholesteatoma is present. [16,17,18] Fungi, particularly *Aspergillus* and *Candida species*, although rare are reported as pathogens as well.[19]

Methdology

The prospective study was carried out at a tertiary care hospital in a time period of 1 yr(data collection) clinically diagnosed CSOM cases were included in the study.Pus samples from both outpatient and inpatient departments were included.Detailed history was obtained from each patient according to the case proforma.

Sample collection -The ear discharge is collected from clinically diagnosed CSOM patients using sterile cotton wool swabs under aseptic precautions with the aid of an aural speculum, prior to instillation of any topical medication. Swabs are then transported to the laboratory.
Transport of specimen-Urine sample should be transported immediately to the laboratory and processed. in case of delay, samples should be stored in refrigerator at 4°C.

Identification tests

Processing of the specimen
Following procedure were performed

- Gram staining
- Culture
- Identification of pathogenic organism

Screening test

Gram staning

The swab contening ear discharge was spread on a glass slide covering approximately 1 cm^2 area.the smear was stained with gram stain.presence of one or more becteria per oil immersion field indicates singnificant pathogen.[20]

Culture

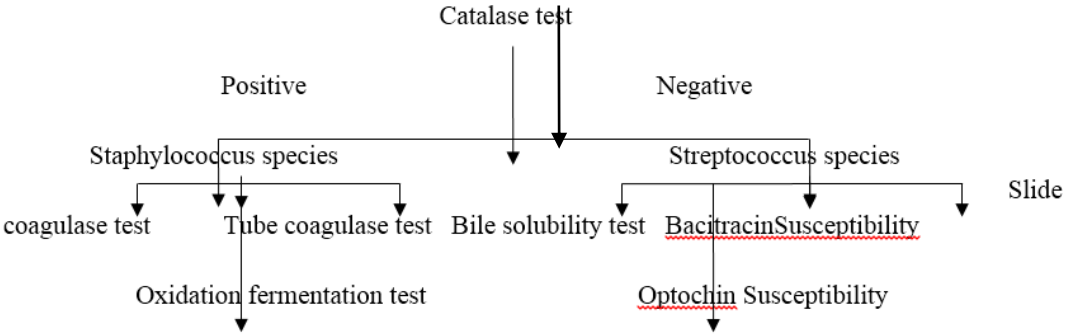
Pus samples were inoculated on blood agar(BA) and MacConkey agar (MA) by direct streak method.The plates were incubated overnight at 37˚C.Pure growth of

an isolated colonies were considered sa singnificant pathogens.Growth of two potential organism considered significant and processed.[21]

Identifications of pathogens

The following tests were performed according to standard methods [22]

1) Grams staining
2) Motility testing by hanging drop preparation method
3) For gram negative bacilli
   - Catalase test
   - Oxidase test
   - Sugar fermentation test (Using Triple Sugar Iron agar)
   - Citrate utilization test
   - Urease production test
   - Indole test
4) For gram positive cocci



The pathogen were identified by standred Micribiological techniques by studying their colony characteristics,morphology, and biochemical reactions

- Colony characteristics: size, shape, elevation, margins, surface, opacity, consistency, change in the medium, pigment production, etc.were studied[23]
- Morphology:the portion of the colony was emulsified in a small drop of seline on a glass slide.After drying of the smear, it was fixed by passing the slide 4-5 times through the flame of Bunsen burner.The fixed smear was subjected to gram stain.[24]Smear was covered with crystal violet and allowed to remain on the surface without drying for 1 minute .After rinsing the slide with water,it was covered with grams iodine for 20 seconds then rince with water.smear was decolorised with acetone till no violet color washed off.The smear is counterstained with safranine for 30 sec the slide washed and gently blotted dry.Gram reaction,size shape ,arrangement,pleomorphism etc. were noted.[24]

Biochemical reactions

Various biochemical reactionslike catalase, oxidase, coagulase, carbohydrate fermentation (lactose, glucose, mannitol, sucrose), indole, methyl red, citrate utilization, urease production, H_2S production test, etc were performed as per

standard procedure. For all biochemical tests suitable positive and negative controls were applied.

Results

The study was carried out among 122 patients with clinical evidence of COM are suspected to have chronic supurative otitis media (CSOM).The pus samples from the discharging ear was collected and processed

Table 1: Sex wise distribution
A total of 120 clinical isolates grown aerobically having clinic-microbiological significance were tabulated and analysed for there distribution.

Out of total 122 patients it is observed that 56.56% (69) are male patients and female were 43.44% (53).

Gender	No (%)
Male	69 (56.56%)
Female	53(43.44%)
Total	122(100%)

Table 2: Table showing Culture with singnificant &Insignificant growth/No growth

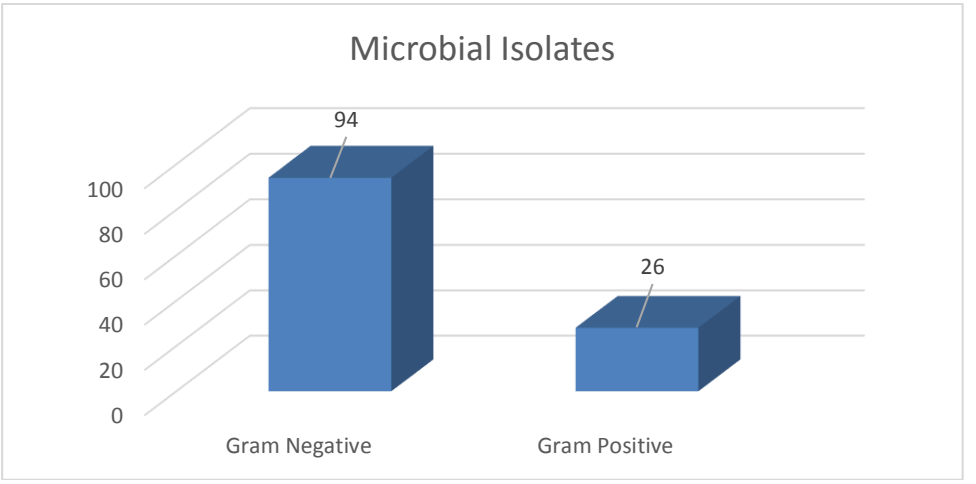
Total	Sample showing singnificant growth No (%)	Sample showing Insingnificant growth/No growth No (%)
122	120(98.36%)	2(1.63%)

Out of 122 pus samples significant growth was seen in 120(98.36%) samples,were 2(1.63%) sample showed insignificant or no growth.

Table 3: Microbial Isolates on the basis of Grams Staining.

Isolates	Total- 120	Percentage
Gram Negative	94	78.33%
Gram Positive	26	21.66%

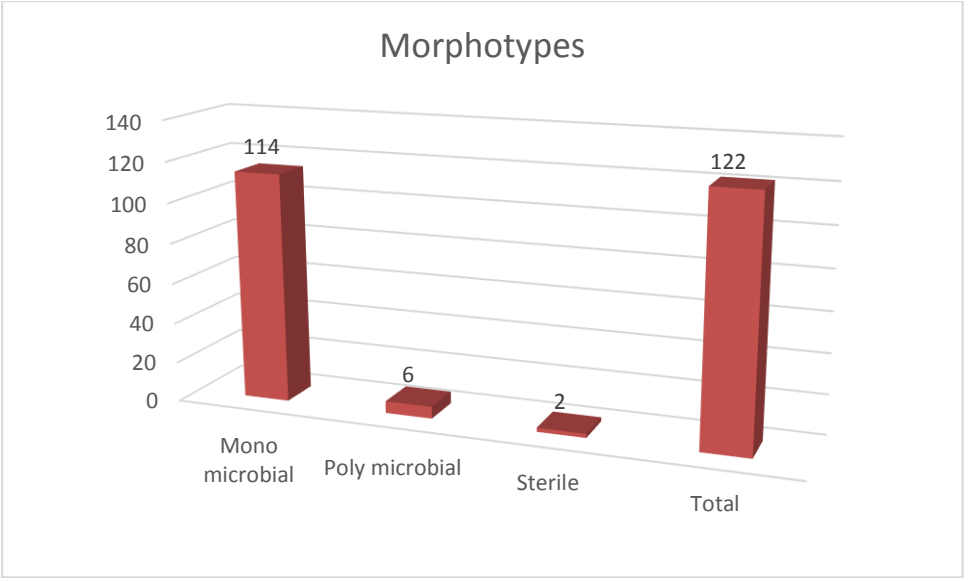
Out of 120 clinical significant isolates majority that is 94(77.04%)were gram negative, and 28(22.95%)were gram positive



Graph 3: Microbial Isolates on the basis of Grams Staining.

Table 4: The Distribution of Sample isolates (Morphotypes) on the basis of singular or multiple growths.

Monomicrobial No (%)	Polymicrobial No (%)	Sterile No (%)	Total No (%)
114(93.44%)	6(4.91%)	2(1.63%)	122(100%)



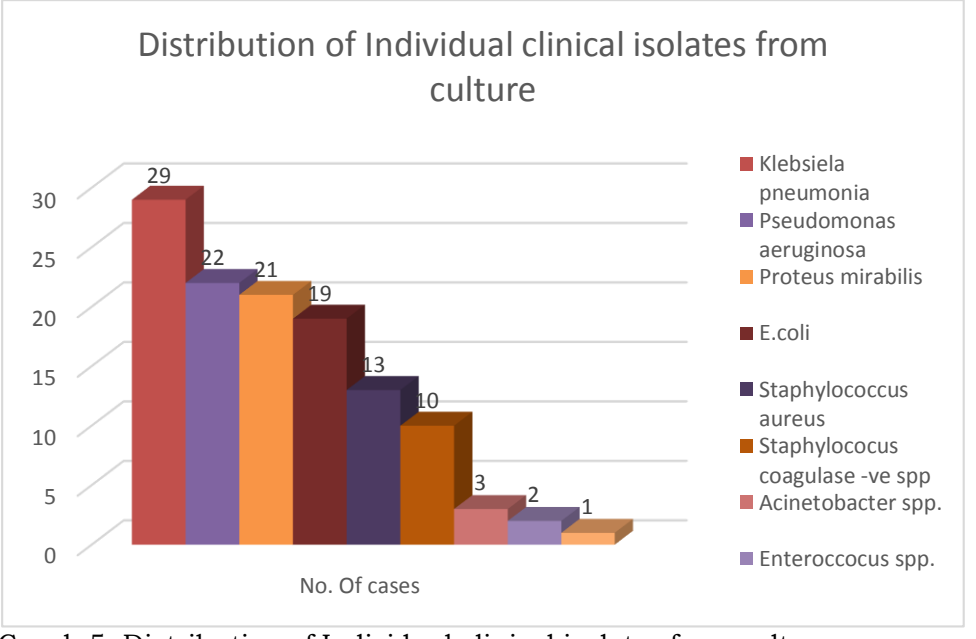
Graph 4: The Distribution of Sample isolates (Morphotypes) on the basis of singular or multiple growths

Table 5: Distribution of Individual clinical isolates from culture.

Organism	No. Of cases	Percentage
Klebsiela pneumonia	29	24.16%
Pseudomonas aeruginosa	22	18.33%
Proteus mirabilis	21	17.5%
E.coli	19	15.83%
Staphylococcus aureus	13	10.83%
Staphylococus coagulase -ve spp	10	8.33%
Acinetobacter spp.	3	2.50%
Enteroccocus spp.	2	1.66%
Proteus vulgaris	1	0.83%

Out of 120 clinical significant bacterial isolates the pre dominant becrerial isolate is *Klebsiela pneumoniae* 29(24.16%) followed by *Proteus mirabillis* 21(17.5%) and *Pseudomonas aeruginosa 21 (17.5%),E.coli* 19(15.83%), *Staphylococcus aureus* 13(10.83%) *Staphylococus coagulase -ve spp* , *Enteroccocus spp,and Proteus vulgaris*

All the samples were analysed for presence/absence of growth along with its significance in relation to clinico-microbiological correlation



Graph 5: Distribution of Individual clinical isolates from culture.

Table 6: OPD / IPD Distribution of Sample Isolates
Sample received /collected from OPD/IPD of clinically suspected patients are cultured to find out presence of bacteria and are analysed

	Singnificant Growth (n= 120)		Insingnificant / No growth (n= 2)
	Single growth (monomicrobial) n(%)	Mixed growth (polymicrobial) n(%)	
OPD	97(85.08)	5(83.33)	2(100)
IPD	17(14.91)	1(16.67)	0(00)
Total	114	6	2

Above table shows that out of 120 culture positive samples,114(93.44%) showed monomicrobial growth and 6(5.0%) samples showed 2 predominant organism. No growth was observed in 2(1.63%) samples.
Out of 114 (93.44%) samples showing significant growth of single organism OPD contributed 97(85.08%)samples against IPD contributing 17(14.91%) only. Among IPD patients growth having 2 organism is observed in 1(16.67%)sample.

Table 7: Types & Pattern of pus discharge.

Type of discharge	Pattern of discharge	
	Continuous No (%)	Recurrent No (%)
Mucopurulent	25(37.87)	11(20.37)
Mucoid	30(45.45)	20(37.03)
Purulent	9(13.63)	23(42.59)
Blood stained	2(3.03)	0(0.0)
Total	66	54

Pus drainage was mucoid in 30(45.45%), followed by mucopurulentin 25(37.87%), purulent in 9(13.63%) and blood stained in 2(3.03%) ears. The pattern of drainage was continuous in 66(55.1%) and recurrent in 54(44.9%) ears.

Table10: Seasonal variation found in isolates

Month	*Proteus mirabillis*	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Klebsiela pneumoniae*	*Acinetobacter spp.*	*E.coli*	*Staphylococus coagulase -ve spp*	*Staphylococus aureus*	*Enterococcus spp.*
March	0	0	2	0	1	1	0	0	0
April	0	0	1	01	0	2	1	1	0
May	1	0	2	01	0	0	1	1	0
June	1	0	1	02	1	1	1	1	0
July	04	0	4	06	0	3	1	2	0
August	5	1	6	10	0	3	0	2	0
September	7	0	4	08	1	4	3	2	2
October	2	0	1	01	0	2	2	2	0
November	1	0	0	0	0	2	1	1	0
December	0	0	0	0	0	1	0	1	0

Discussion

One hundred and twenty two patients with clinical evidence of CSOM attending ENT Clinic who met the inclusion criteria for the study were recruited. CSOM was most prevalent in children and young adults than in the older age group where 26(21.31%) were aged below 10 years and 34 (27.86%) were aged 11-20 years and In the age group of 21-30 years the CSOM was found in21(17.21%) and in the age group of 31-40 years is 18(14.75%) age 40 and above the cases found 21(17.21%)[table 1/graph 1] respectively similar to studies conducted by other authors[25,26]. There are several reasons to explain this observation like the eustachian tube in children is shorter, narrower and more horizontal than in adults and also frequent upper respiratory tract infections which are more common in children.[4] However, these findings differ from the findings of another study which showed that the disease was more prevalent among the age group of 31-40 years[25].

.Out of 122 pus samples significant growth was seen in 120(98.36%) samples,were 2(1.63%) sample showed insignificant or no growth [table 3/graph 3].It is mostly seen that aerobic gram negative rods outnumber gram positive cocci organisms in CSOM as reported by some authors [27] which is comparable to the findings in the present study where gram negative rods accounted for 94(78.33%) and gram positive cocci 26(21.66%)[table 4/graph 4].

Analysis of the total 122 specimens collected revealed that pure and mixed culture growth were obtained 114(93.44%) and 6(4.92%) each respectively while in 2(1.64%) specimens there was no growth[table 5/graph 5]. Figures reported by other authors vary significantly where pure cultures were isolated in more patients than mixed cultures.[28]The proportion of different organisms isolated vary from study to study like in this study where the most common bacteria isolate causing CSOM were aerobic bacteria- *klebsiella pneumonia*29 (24.16%), *Pseudomonas aeruginosa* 21(17.50%) and *proteus merabilis* 21(17.50%) [table

6/graph 6]. In a study conducted in the rural area of Malawi in 1998 also showed that *Proteus mirabilis* was the commonest aerobic bacteria[27]. The findings from the rural setting were similar to findings in this study from an urban setting of Malawi in terms of CSOM causing microorganisms. Comparative findings were in a study done in a rural area of Kenya which showed that *Proteus mirabilis* was the commonest isolate and also in urban areas of Congo and Ethiopia. [29,30] Contrary to this study, other studies have reported *Pseudomonas aeruginosa* as the major organism causing CSOM.[31,32,33] This could be attributed to effect of climate and variation of organisms in different communities and localities and different study sites which are either hospital or community based. In this study *E.Coli* was isolated in 19(15.83%) specimens and *Klebsiella* species 29(24.16%) comparable to findings as reported by some authors in literature[26,27]. More frequent isolation of faecal bacteria like *E.Coli, Klebsiella* species indicates that individuals are at high risk of infection due to poor hygiene conditions. Some studies have reported isolation rates of anaerobes of 20% to 50%.[34,35]There was significant association between CSOM causing micro-organisms and quantity of pus drainage, mode of onset, otalgia, hearing loss, location of TM perforation and mucosalappearance contrary to a study where bacteriological findings had no significant effect on symptoms and signs. [36]The dominant microbiological isolate was *klebsiella pneumoniae 29*(24.16%), a gram-negative aerobe in age group 11-20 years found in this study[table 11/graph 10]. Thiswas followed by *Proteus mirabilis21*(17.50%) and *pseudomonas aeruginosa* 22 (18.33%) are gramnegative aerobe. The finding of *Proteus mirabilis* as the mostcommon isolate is similar to findings by Chirwa in 2014 inMalawi where *Proteus mirabilis accounted for* 28.6%,[37]Adubaet al in 2010inGarissa (Kenya) where *Proteus mirabilis* accounted for 32.7%,[29] and Muluye et alin 2013 in Ethiopiawere *Proteus mirabilis* accounted for 27.5%.[30] These findingsare different from those of other studies where they found *thatpseudomonas aeruginosa* was the most common isolate.[38]The difference in the pattern of microbiologicalisolates may be explained by differences in the geographicalconditions and population dynamics.[39]The dominant microbiological isolate was *klebsiella pneumoniae 29*(24.16%), a gram-negative aerobe . This was followed by *Proteus mirabilis21*(17.50%) and pseudomonas aeruginosa 22 (18.33%) are gram negative aerobe. In this study male(56.66%) were effected more than females(43.33%)[table 12/graph11]. The finding of *Proteus mirabilis* as the mostcommon isolate is similar to findings by Chirwa in 2014 in Malawi where *Proteus mirabilis accounted for* 28.6%,[37] Adubaet al in 2010inGarissa (Kenya) where *Proteus mirabilis* accounted for 32.7%,[29]and Muluye et alin 2013 in Ethiopiawere *Proteus mirabilis* accounted for 27.5%.[30] These findingsare different from those of other studies where they found *thatpseudomonas aeruginosa* was the most common isolate.[38]The difference in the pattern of microbiological isolates may be explained by differences in the geographical conditions and population dynamics.[39]The bacteriological profile is to some extent dependent on the seasonal variations[table 13/graph 12]. A month wise data have been arranged according to the number of isolations. This projects that there is considerable aggregation of case in the months of July to September. 82.75% cases of *klebsiella pneumoniae* cases and 76.19% of cases belonging to *Proteus mirabilis* have occurred during monsoon and in post monsoon periods. This needs further study to delineate the relationship of humidity, temperature and effect of bacteriological load in the cases of chronic suppurative otitis media.The similer study was done by P.K Maji et al.[40]/P.K Maji T.K. Chatterjee S. Chatterjee J.

Chakrabarty B.B. Mukhopadhyay Ab Indian J. Otolaryngol. Head Neck Surg. 128 (April-June 2007) [42-46]]

The difference in the pattern of microbiologicalisolates may be explained by differences in the geographicalconditions and population dynamics. *Proteus* species arewidely distributed in places with poor sanitary conditions, beingfound in feces, decomposing meat and sewage.[41] This couldaccount for its high frequency in this study where the majority of the patients (60.66%) stayed in peri-urban areas which are associated with poor sanitary conditions. In this study most of the patients comes from hindu religion (66.39%),followed by muslims (26.22)[table 14/graph 13].

Conclusion

CSOM have a major impact on the quality of life of patients with the condition. They are a major cause of hospital admission and health care utilization.

- CSOM was more common in males (57.5%) then female (43.33%).
- Bacterial infection in CSOM was seen more in the age group of 11-20 years.
- *Klebsiella pneumoniae* (24.16%) was the commonest isolate followed by *Pseudomonas aeruginosa* (18.33%).

Pus culture is a good and simple diagnostic tool to study the aetiology due to bacteria in CSOM.

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