International Journal Dental and Medical Sciences Research
Volume 4, Issue 3, May-June 2022 pp 336-348 www.ijdmsrjournal.com ISSN: 2582-6018

Microbiological profile and Antibiogram of Bacterial Isolates from Cases of CSOM at a Tertiary Care Hospital

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Submitted: 20-05-2022 Accepted: 02-06-2022

ABSTRACT:
Chronic suppurative otitis media (CSOM) is a chronic inflammation of the middle ear, characterized by recurrent otorrhea through a permanent tympanic membrane perforation. Chronic suppurative otitis media has received considerable attention, not only because of its high incidence and chronicity, but also because of issues such as innumerable complications and multi-drug resistance. Formulation of Hospital antibiotic policy is the need of hour to control emergence of drug resistant strains.
AIM: To determine the incidence, aetiology of CSOM, and determine their Antibiogram.
MATERIAL AND METHODS: The study was conducted on 200 clinically diagnosed CSOM patients attending ENT OPD and 50 swabs from dry ears as controls. The samples were processed in Microbiology laboratory. Isolation and identification of bacterial agents were done as per standard protocols. Antimicrobial susceptibility testing was performed by Kirby- Bauer's disc diffusion method.
RESULTS: Out of 200 samples, 188 were culture positive. Males were predominant and commonest age group affected was 1-10yrs. 84.40% bacterial agents and 15.60% fungal agents were isolated. Analysis of bacterial flora showed predominance of Gram-negative bacteria (55.96%). The highest incidence was that of Pseudomonasaeruginosa (40.22%), followed by Staphylococcusaureus (20.67%). Incidence of multidrug resistant strains includes, ESBL producers (11.46%), MBL producers (5.04%) and Methicillin resistant strains (12.38%). Antibiogram of all bacterial isolates revealed that Amikacin was the most sensitive drug.
CONCLUSION: Continuous surveillance of etiological agents of CSOM and their antibiogram is necessary to monitor drug resistance and selection of appropriate treatment regimen.
KEYWORDS: Chronic suppurative otitis media, Pseudomonas aeruginosa, Antibiogram.

I. INTRODUCTION:

Chronic suppurative otitis media is a chronic condition, where there is accumulation of purulent fluid in middle ear in addition to tympanic membrane defect [48]. It is one of the most important causes of preventable hearing loss in India and other developing countries [38]. The study of organisms commonly associated with chronic suppurative otitis media is necessary to enable the otologist in management and prevention of complications [50].

Chronic suppurative otitis media has received considerable attention, not only because of its high incidence and chronicity, but also because of issues such as innumerable complications and multi-drugresistance[43]. The indiscriminate use of both topical and systemic antibiotics has led to drug resistance and biofilm formation[2].

More recently, an increase in prevalence of multi-drug resistant (MDR) organisms, mostly Methicillin-resistant Staphylococcus aureus (MRSA) and extended spectrum b-lactamase (ESBL) producing Gram-negative bacteria is menacing the result of anti-infectious treatment in the community and hospitalized patients[6]. Increasing resistance to carbapenem mediated by Metallo beta lactamases (MBL) is a cause for concern because Carbapenemase - producing Pseudomonas aeruginosa strains have been reported to be important causes of nosocomial infections and it adversely affects clinical outcomes and adds to treatment cost[9,27,36,44,54].

II. AIMS AND OBJECTIVES:

To study the incidence, aetiology of chronic suppurative otitis media and to detect drug resistance pattern of the bacterial isolates by phenotypic methods.

III. MATERIALS AND METHODS:

The samples for the present study were collected from patients with chronic suppurative

International Journal Dental and Medical Sciences Research
Volume 4, Issue 3, May-June 2022 pp 336-348 www.ijdmsrjournal.com ISSN: 2582-6018

otitis media (CSOM) of both sexes and of different ages attending Ear, Nose, and Throat, Out- Patient Department and processed in Department of Microbiologyat a tertiary care Hospital.

A prospective study was conducted for a period of 1 year from October 2020 to September 2021 on 200 clinically diagnosed CSOM patients with complaints of ear discharge for more than 3 months. 50 ear swabs were collected from patients with healthy ear as controls. The study was approved by Institutional Ethics Committee ((REG.NO.10/IEC/GMC/2020).

Inclusion Criteria: Patients who were not on any antibiotic treatment for previous 48 hours. Diagnosed CSOM cases of all age groups with ear discharge of more than 3 months.

Exclusion Criteria: Patients currently on treatment with antibiotics and antifungal drugs.Patients with ear discharge of less than 3 months duration. Patients having Otitis externa with Chronic suppurative otitis media.All known HIV or immunosuppression patients.

Statistical analysis: Data collected was entered into Microsoft Excel- 2010 version. Descriptive variables will be expressed in numbers and percentages. Continuous variables will be expressed as means± standard deviation. Statistical test- Chi square test will used for analyzing qualitative variable and student 't' test for quantitative variable. For all statistical purposes, P value < 0.05 was considered statistically significant.

Collection of Samples:The sterile swab was gently introduced into the ear under direct visualization and the pus specimen was collected. Two pus samples were collected per ear and placed into the sterile containers which were labelled. The samples were immediately transported to Laboratory for microbiological isolates.

Processing of Samples:Processing of samples were carried out in the Department of Microbiology of a tertiary care teaching hospital, Srikakulam. 1st swab was used for Gram stain to see the presence of pus cells, morphology of bacilli. 2nd swab was inoculated on Nutrient agar, Blood agar, MacConkey's agar and incubated at 37°c for 24hrs and observed for growth.

After incubation, the colonial and cultural characteristics of isolates were observed, biochemical tests done for identification and documented as perClinical and Laboratory Standards Institute guidelines[8].

The antimicrobial susceptibility testing was done by the Kirby Bauer disc diffusion method[25].The Mueller-Hinton agar plates with growth suspension equivalent to 0.5 McFarland standards were incubated at 37ºC overnight and the zones were measured as per CLSI guidelines[8]. MRSA was detected using Cefoxitin 30µg disc. ESBL production and MBL production in Gram-negative bacteria was detected by using Potentiated Disc Diffusion test (PDT)[11]. The resistance patterns were further determined by E-test by interpreting Minimum inhibitory concentration (MIC) values (mcg/ml)[5,11].

IV. RESULTS:

Table: 1 The Details of Ear Swabs studied

Study group	Ear swabs	200
Control group	Ear swabs	50
Total		**250**

Table: 2 Culture positivity among Total Samples (n= 200)

Samples	Number of cases	Percentage
Culture positive samples	188	94%
Culture negative samples	12	6%
Total samples	200	100%

Out of 200 ear swabs from chronic suppurative otitis media cases, 188 (94%) samples showed growth, and 12 (6%) samples showed no growth.

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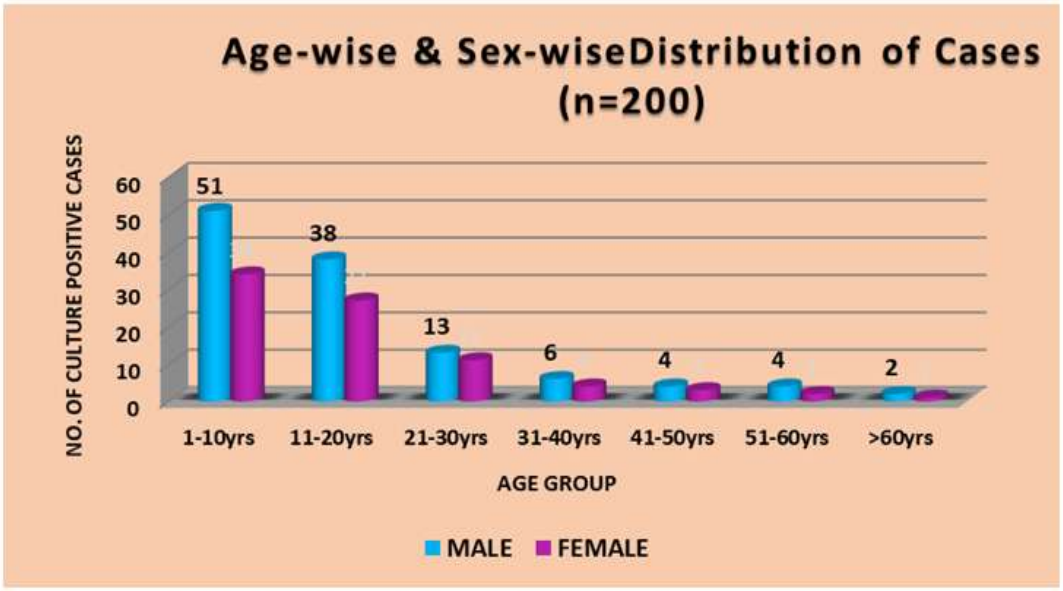
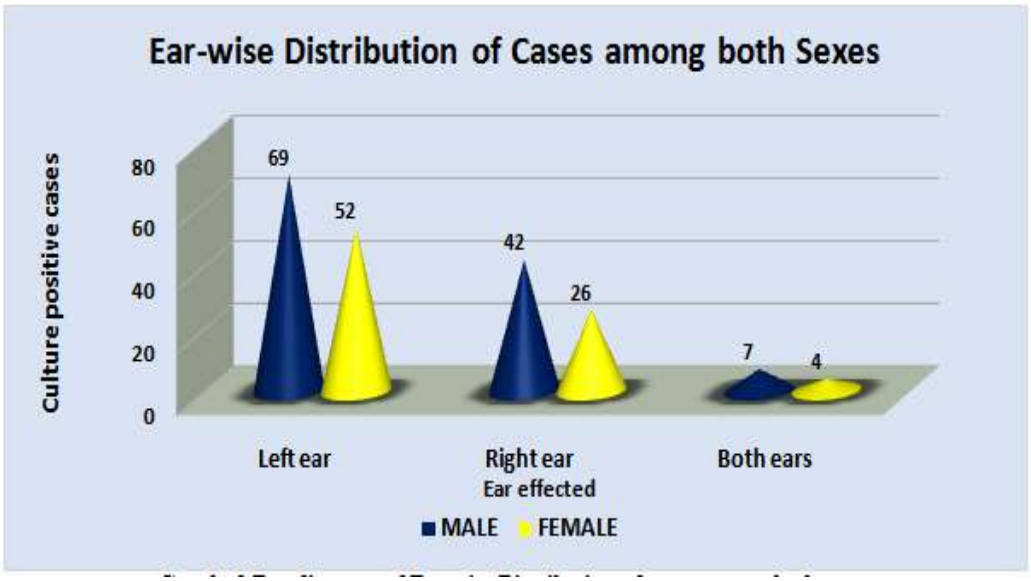


Graph :1 Bar diagram of Age wise & Sex wise Distribution of Cases

In the present study, the highest incidence of CSOM was observed between 1 – 10 years age group may be due to frequency of Upper respiratory tract infections. Among 200 cases, 118 were males and 82 were females, which shows the incidence of CSOM was higher in males compared to females.



Graph: 2 Bar diagram of Ear wise Distribution of cases among both sexes

The above Bar diagram shows male preponderance in CSOM cases and left ear (60.5%) was more effected when compared to right ear (34%) and both ears (5.5%).

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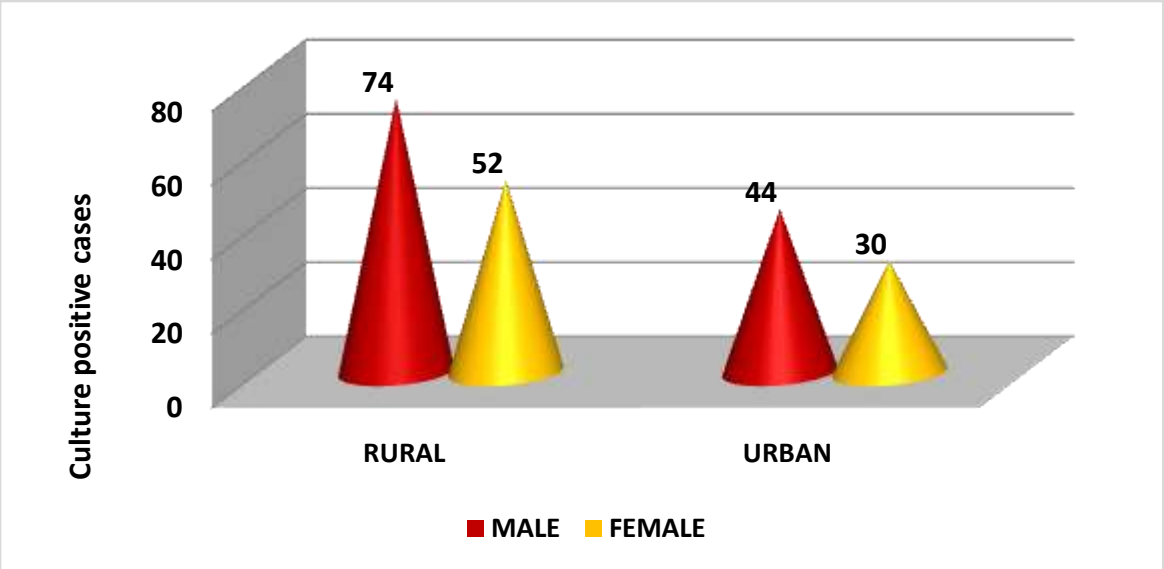
Graph: 3 Distribution of cases based on Demographic area. (n=200)



The study showed predominance of cases in rural population (63%) when compared to urban population (37%).

Table: 3 Prevalence of Pure and Mixed Infections (n= 188)

Culture positivity	**Number**	**Percentage**
Monomicrobial (Pure)	158	84.05%
Polymicrobial (Mixed)	30	15.95%
Total	**188**	**100%**

Out of 188 culture positive cases, 158 (84.05%) cases showed monomicrobial growth. 30 (15.95%) cases showed polymicrobial growth.

Total number of isolates obtained from monomicrobial and polymicrobial samples were 218. Out of 218 isolates, bacterial species isolated were 184 (84.40%) and fungal species isolated were 34 (15.60%).

Table: 4 Distribution of Bacterial Isolates in Pure and Mixed cultures (n=184)

Organism	**Total**		**Pure**		**Mixed**	
	No.	%	No.	%	No.	%
Pseudomonas aeruginosa	74	40.22%	56	30.43%	18	9.78%
Staphylococcus aureus	38	20.67%	26	14.13%	12	6.52%

Proteus species Proteus mirabilis Proteus vulgaris	24 (16) (8)	13.05%	10 (6) (4)	5.43%	14 (10) (4)	7.60%
Coagulase negative Staphylococci	20	10.86%	16	8.69%	4	2.17%
Klebsiella pneumoniae	14	7.61%	8	4.34%	6	3.26%
Escherichia coli	6	3.26%	6	3.26%	-	-
Acinetobacter baumanii	4	2.17%	2	1.08%	2	1.08%
Micrococci	2	1.08%	2	1.08%	-	-
Corynebacterium species	2	1.08%	2	1.08%	-	-
TOTAL	**184**	**100%**	**128**	**69.56%**	**56**	**30.43%**

Out of total 184 bacterial isolates,128 were from in pure growth and 56 were from mixed growth. The predominant bacterial isolate was Pseudomonasaeruginosa 74 (40.22%) followed by Staphylococcusaureus 38 (20.67%), Proteusspecies 24 (13.05%), CONS 20 (10.86%), Klebsiellapneumoniae 14 (7.61%), E. coli 6 (3.26%), Acinetobacter baumanii 4 (2.17%), Micrococci 2 (1.08%), and Corynebacteriumspecies 2 (1.08%).

Out of total 34 fungal isolates, the predominant isolate was Candidaalbicans 12 (35.29%), Aspergillusniger 12 (35.29%) followed by Aspergillusflavus 6 (17.64%).

Table: 5 Combination of Organisms in Mixed cultures (n=30)

ORGANISM/S	No. of samples	No. of isolates
1. Pseudomonas aeruginosa + Proteus mirabilis	6	12
2. Pseudomonas aeruginosa + Klebsiella pneumoniae	6	12
3. Pseudomonas aeruginosa + Staphylococcus aureus	4	8
4. Proteus vulgaris + Staphylococcus aureus	4	8
5. Proteus mirabilis+Coagulase negative Staphylococci	4	8
6. Acinetobacter baumanii + Staphylococcus aureus	2	4
7. Pseudomonas aeruginosa + Aspergillus niger	2	4
8. Staphylococcus aureus + Candida albicans	2	4
TOTAL	30	60

Out of 30 mixed infections, 60 isolates were reported. The most common combination of organisms was Pseudomonasaeruginosa and Proteusmirabilis was seen in 6 (20%) samples, Pseudomonasaeruginosa and Klebsiellapneumoniae seen in 6 (20%) samples.

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Table: 6 Antibiotic sensitivity patterns of Gram-positive Isolates (n=122)

Organisms	AMP	CIP	AK	GEN	CAZ	IMP	PIT	CAC	CTX	AT	CO
P. aeruginosa	24%	35%	91.5%	60%	77%	82%	92%	80%	35%	92%	88%
E. coli	35%	70%	85%	68%	65%	90%	100%	90%	65%	NT	NT
K. pneumoniae	25%	85%	75%	65%	75%	85%	90%	90%	50%	NT	NT
Proteus spp	20%	20%	80%	60%	50%	95%	98%	92%	57%	NT	NT
A. baumannii	-	-	25%	25%	50%	100%	75%	75%	25%	NT	NT

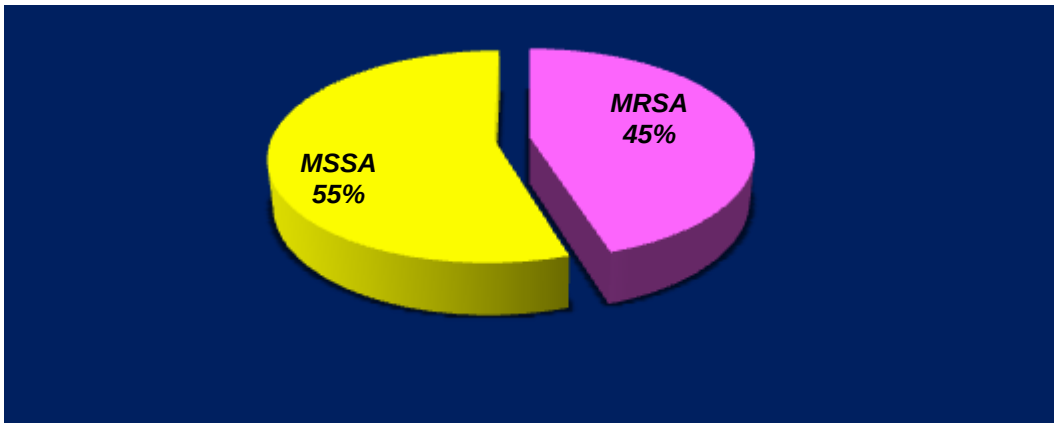
Most of Gram-negative isolates are sensitive to Imipenem, Amikacin, Piperacillin+ tazobactam, Ceftazidime + clavulanic acid, Ciprofloxacin and Gentamycin.

Table: 7 Antibiotic sensitivity pattern of Gram -positive Isolates (n=62)

Organism	AMP	AK	TEI	CD	CTX	CX	LZ	CIP	VA	AZ	LE
S. aureus	74%	89%	100%	85%	65%	55%	97%	42%	95%	93%	79%
CONS	42%	78%	100%	78%	55%	42%	100%	58%	100%	85%	78%

Most of Gram-positive isolates were sensitive to Teicoplanin, Linezolid, Vancomycin, Azithromycin, Amikacin Levofloxacin and Clindamycin.

Graph: 4 Pie Diagram showing Distribution of MRSA strains in Staphylococcus aureus (n= 38)



In the present study, out of 38 Staphylococcus aureus isolates, 18 (45%) were Methicillin Resistant Staphylococcus aureus (MRSA) and 20 (55%) were Methicillin Sensitive Staphylococcus aureus (MSSA).

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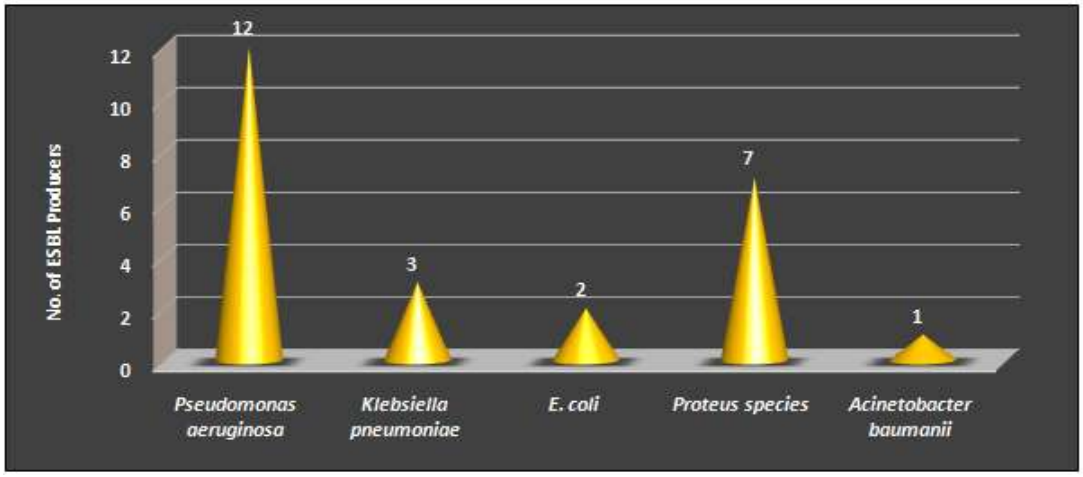
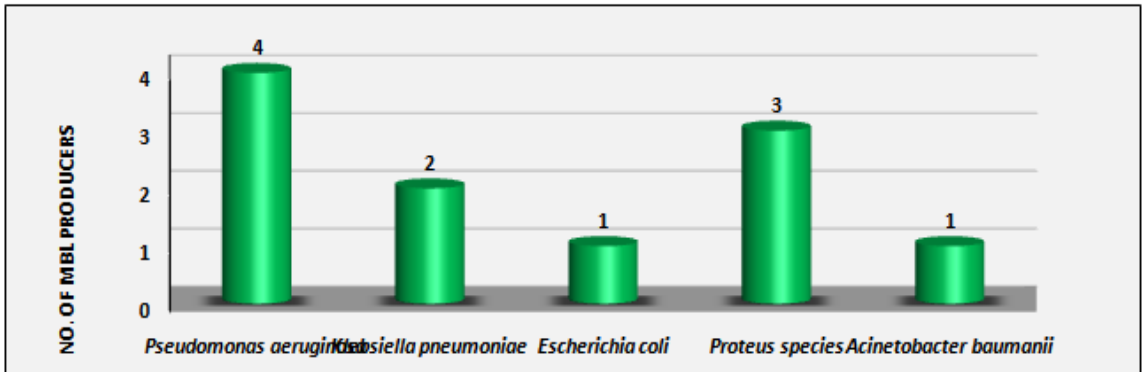


Graph:5Bar diagram of ESBL producers among various gram –negative isolates (n = 25)

In the present study, Extended spectrum beta-lactamase (ESBL) production was seen in 25 (20.49%) isolates.



Graph: 6 Bar Diagram showing MBL producers among various gram- negative isolates (n = 11)

Out of total 122Gram- negative isolates, 11 (9.01%) were Carbapenemase producers.

Total isolates		MDR strains		MRSA		ESBL producers		MBL producers	
No.	%	No	%	No	%	No.	%	No.	%
218	**100%**	**63**	**28.89%**	**27**	**12.38%**	**25**	**11.46%**	**11**	**5.04%**

Table: 8 Distribution of MDR strains among total isolates (n= 218)

Out of 218 microbial isolates, Multi drug resistant (MDR) strains were 63 (28.89%). Among total Multi drug resistant strains, MRSA isolates were 27 (12.38%), ESBL producers were 25 (11.46%) and Carbapenemase (MBL) producers were 11 (5.04%).

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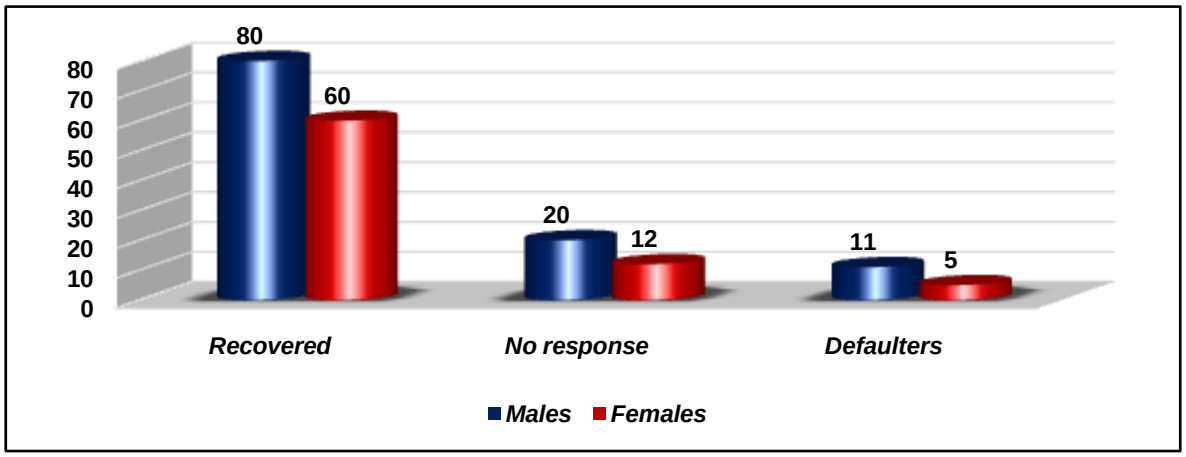
Graph: 7 Bar diagram showing Therapeutic Response among cases (n= 188)



Out of total 188 cases, 140 recovered with antibiotics, 32 showed no response to treatment and 16 were defaulters.

Control group:

Out of 50 ear swabs, 28 (56%) were culture positive and 22 (44%) culture negative.

Table: 9 Organisms Isolated in Control Group (n= 28)

S. No	Isolate	No	%
1.	Coagulase negative Staphylococci	11	39.28%
2.	Micrococci	10	35.71%
4.	Corynebacterium species (diphtheroid)	6	21.42%
6.	Candida tropicalis	1	3.57%
	TOTAL	28	100%

Out of 28 culture positive samples, CONS 11(39.28%) was the predominant isolate followed by Micrococci 10 (35.71%), Diphtheroid 6 (21.42%) and C. tropicalis 1 (3.57%).

V. DISCUSSION

The present study was conducted over a period of 12 months to evaluate the aetiological agents (bacterial & fungal) of CSOM and their antimicrobial susceptibility. Males (59%) were more affected than females (41%) in the present study which correlated with Narayana R.S. et. al[40] (59%), N. Lakshmi (58.9%)[29], Saranya SK. et. al.[45] (61.42%) Raghu Kumar KG et. al.[37] (62.26%) Whereas, Ramakrishna PJ. et. al.[38] (54.7%), Nikakhlagh S.et. al.[53] (54%) and Sharma M.et. al.[52] (52%) reported slightly lower incidence.

Maximum cases of CSOM were seen between the age group of 1-10 yrs (42.5%) which correlates with the study of N. Lakshmi (46.1%)[29], Sarathbabu (43.75%)[46] and Bhumbla U.et. al. (38.5%)[6], Vaidya K et. al.[23](28.57%)and Sharma M.et. al.[52](24.8%). Whereas the studies of Kumar H.et. al.[15](35.71%), Prakash et. al.[35](26.47%)and Sagar Kashyap et al.[43](42.16%) reported higher incidencein the age group of 11- 20 years and the study of Raghu Kumar KG et. al.[37](25.42%) reported higher incidence in 21- 30 years.Studies of Ramya SN et. al.[40](37.1%) and Loy AH.et. al.[24](23.3%)reported higher incidence in age group of 31- 40 years.

In the present study, left ear (60.5%) was more affected when compared to right ear which correlates with study of Sarath babu R. (61.25%)[46], Saranya SK. et. al[45] (60%) Kamal N

et. al.[20](46.7%)and N. Lakshmi (56.6%)[29] Whereas, Ramakrishna PJ et. al.[38](32%) andHirapure PV et. al.[18] (33.8%)reported less incidencein left ear.

Majority of CSOM cases were from rural areas (63%) which correlates with the study of Sarath babu R. (66.25%)[46], Harshika YK. et. al.[17] (60%) and Kaur P.et. al.[21] (59%) Whereas, Sharma M. et. al[52](72%), Kumar H. et. al.[15](75%), Harrison Phiri. et. al.[16](81%) and Bhumbla U. et. al.[6](81.91%) reported higher incidencedue to low socio-economic status, lack of personal hygiene and poor education.

Analysis of the 200 samples of CSOM revealed that culture positivity was seen in 94% of the cases. This correlates with study of Loy AH et.al.[24]who reported (96.5%), PajorA.et.al.[30](96.5%), Sangeeta Baskaran[44] (93%), Shashidhar V. et.al.[49](88.5%), Bhumbla. U.et.al.[6] (88.5%)and Sharma M. et.al.[52] (91.2%). Monomicrobial growth seen in 84.05%, mixed growth was seen in 15.95% in the present study which coincides with Sarath babu (83.2% & 16.8%)[46], Harrison phiri et al[16] (85.8% & 14.2%) and Pajor et. al.[30] (82.5% & 17.5%).

In the study, 78.40% of bacterial isolateswere reported which coincides with the studies of Fatima G et. al.[11]who reported 80.07%, Bhumbla. U. et. al.[6](79%). V.C. Suresh Chander et al.[54] (74.7%) Shashidhar V. et. al.[49](74.8%), Saranya SK et. al.[45](69.79%), and Kumar H et.al[15].(69%). Whereas, Narayana R. S. et. al.[40](62.4%) and Attalah MS. et. al.[2](48%)reported relatively lower incidence.A higher incidence was reported by Sharma M. et.al[52] (89.6%),PajorA. et. al.[30](88.6%), Loy AH et. al.[24](87.7%) and Sangeeta Baskaran[44](87%).Pseudomonas aeruginosa (40.22%) was the predominant isolate followed by Staphylococcusaureus (20.67%), which coincides with the study of V.C. Suresh Chander et al.[54] (52.56%) Attallah MS et al.[2] (51.7%), Wariso BA et. al.[57] (41%), Malkappa SK et. al.[26], (45.2%), and Raghu Kumar KG et. al.[37] (42.2%) Fatima G et. al.[13], Raghu Kumar KG et. al[37]. and Sagar Kashyap et al[43]. whereas, Vaidya K et. al.[23] (54.5%) and GH. Ettehad et al.[14] (31.15%) reported Staphylococcus aureus as the predominant isolate followed by Pseudomonas aeruginosa in their studies.

Imipenem (90%) followed by Amikacin (85%) and Piperacillin+ tazobactam (89%) were the most sensitive drugs for Gram-negative isolates which coincides with study of Saranya SK et al.[45], Kiran Yadav et. al [22]Harrison Phiri et. al.[16], Fatima G et. al.[13]and Raghu Kumar KG et. al[37]. Pseudomonas aeruginosa isolates were sensitive to Colistin (88%), Imipenem (82%), Aztreonam (92.4%), Amikacin (91%), Piperacillin+ Tazobactam (92%), Ceftazidime (77%) which coincides with study of Saranya SK et al[45]., Fatima G. et. al.[13] and Soumya S. et. al.[54] Vancomycin (100%) followed by Amikacin (89%) were found to be the most sensitive antibiotics for gram positive isolates which correlated with study of Saranya SK et. al.[45], Sagar Kashyap et. al.[43] and Kumar H. et. al.[15]

Among the total CSOM isolates 218, Multi drug resistant strains were 63 (28.89%). Out of them, MRSA isolates were (12.38%) which correlated with study of S. Nanarayan R et. al.[40] (11.79%), Ramakrishna PJ et. al.[38] (13.63%) and Fathy Mohamed ES. Et. al[12] (10.83%). Bhumbla U. et. al[6] reported 33.33%. Among 122 Gram-negative isolates, (20.49%) were ESBL producers which correlated with the studies of Prasanth D.P. et. al.[36](22.22%), Ramesh A. et. al.[39] (24.5%). Whereas Sagar Kashyap et.al.[43](62.5%),Rejitha I.M. et. al[41].(31.57%)andBhumbla U. et. al.[6] (33.33%) reported higher incidence of ESBL producers.Fathy Mohamed E.S. et. al.[12] (3.7%) and Sattar A et. al.[47](6.6%) reported lower incidence of ESBL producers respectively.Metallo beta Lactamases were (9.02%) which correlated with the studies of Chakraborthy B. et. al.[7](10.71%), Ramesh A. et. al.[39](6%) and Sagar Kashyap et. al.[43](18.18%). Whereas the studies of Sowmya S. et. al.[54](30%), Neelaveni D.et. al.[28](29.1%) reported higher incidence and Harshika YK. et. al.[17](5.13%) reported relatively lower incidence

VI. SUMMARY AND CONCLUSION

The present study showed that Pseudomonasaeruginosa was the most common aerobic bacterial isolate causing CSOM followed by Staphylococcusaureus. Antibiotic susceptibility test showed that most of Gram-negative isolates are sensitive to Imipenem, Amikacin, Piperacillin+ tazobactam, Ceftazidime + clavulanic acid, Ciprofloxacin, and Gentamycin. Most of Gram-positive isolates were sensitive to Teicoplanin, Linezolid, Vancomycin, Azithromycin, Amikacin and Clindamycin. Recently, the multidrug resistant strains are increasing gradually due to injudicious use of drugs, availability of over-the-counter drugs and increase use of steroid drops. Majority of cases responded to antibiotic treatment. No response in few cases could be mainly, due to development of cholesteatoma or mastoiditis which needs surgical treatment.

Therefore, periodic evaluation of microbiological pattern and their antibiotic sensitivity pattern in local area becomes important & helpful in prescribing empirical antibiotics for successful treatment of CSOM and thus minimizing its complications and emergence of resistance strains.

VII. ACKNOWLEDGEMENTS:

Iam thankful to staff of Department of Microbiology and Department ofOtorhinolaryngology, for providing support for the project from which this paper grew. We acknowledge the infrastructure and support of Government Medical College and Hospital, Srikakulam.

Disclosure:

All the authors listed have made a substantial direct and intellectual contribution to the work and approved it for the publication.

1. Dr. B.S.V.V. Subhashini*[1]
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Funding: No funding sources

Conflicts of Interest: Nil

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