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Original Research Article

Spectrum of Microorganisms Found in Chronic Suppurative Otitis Media & Their in-Vitro Antimicrobial Sensitivity Pattern

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ABSTRACT

Background: Chronic Suppurative Otitis Media (CSOM) is common health problem in developing countries and has potential to cause severe damage to patients from mild pus discharge to permanent hearing loss. Knowledge of spectrum of microorganisms and their antimicrobial sensitivity pattern is essential so that early and effective therapeutic measure can be taken for better outcome.
Objective: The study was carried out to know the spectrum of microorganisms found in CSOM and their antimicrobial sensitivity pattern.
Materials and Methods: A total of 103 ear discharges collected from clinically suspected cases of CSOM were subjected for isolation and identification of bacterial and fungal pathogen using standard bacteriological and mycological methods respectively. Antimicrobial sensitivity testing of bacterial isolated was performed by Kirby-Bauer's disk diffusion method as per the Clinical Laboratory Standards Institute (CLSI) guidelines.
Result: A total of 86.41% cases were culture positive. Pure bacterial pathogen was isolated from 86.52% followed by pure fungal pathogen 7.87% and mixed pathogen (bacteria and fungus) from 5.62% cases. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the predominant bacterial pathogens and *Aspergillus* species were the predominant fungal pathogen isolated. MRSA and ESBL producers were 60% and 55.56% respectively.
Conclusion: Resistant microorganisms were isolated from cases of CSOM in our hospital. Therefore, such study may help as a baseline data to start empirical therapy while waiting for culture reports.
Key words: Chronic suppurative otitis media, bacteria, fungus, sensitivity tests

INTRODUCTION

Chronic suppurative otitis media (CSOM) is defined as a chronic inflammation of middle ear and mastoid cavity that may present with recurrent ear discharges through a tympanic perforation. [1] The disease is worldwide in distribution. [2] Both Gram positive (*Staphylococcus aureus, Coagulase negative Staphylococcus species (CoNS), Streptococcus pneumoniae*) and Gram negative (*Pseudomonas aeruginosa, Escherichia coli, Proteus* species, *Klebsiella* species) bacteria are involved in the pathogenesis of CSOM. [3] Predominance of *P. aeruginosa* and *S. aureus* has been reported from India [4] and abroad. [5] Indiscriminate usage of antibiotics has been attributed to the emergence of resistant strains which produce both primary CSOM and its post-operative infections. [6] Knowledge of the susceptibility pattern may contribute to an effective management of cases of CSOM and reductions in treatment costs to avoid its

serious complications. Therefore, this study was planned with the objective to know the spectrum of microorganisms found in CSOM in this geographical area and determines their in-vitro antimicrobial sensitivity pattern. Such study may contribute towards cost effective management of cases of CSOM in any Hospital.

MATERIALS &METHODS

This prospective study was carried out for a period of one year from October 2017 to September 2018 in Chhatrapati Shivaji Subharti Hospital (CSSH), a tertiary care Hospital in Meerut City, Uttar Pradesh. The clinical diagnosis of CSOM was made by a consultant Otorhinolaryngologist. Patients of all age groups and either gender with history of unilateral or bilateral ear discharge, moist feeling in ear, otalgia, itching and tinnitus were included in the study. However, patients on local or systemic antibiotics, antifungal or corticosteroid drops, immuno-compromised patient with HIV infection and diabetes mellitus were excluded from the study.

The approval from the Institutional Ethical and Research Committee was obtained before conducting the study. Informed consent was taken from all the patients before collection of clinical samples.

Ear discharges was collected from a total of 103 clinically suspected cases of CSOM. The age, sex, presenting symptoms was recorded for each patient. Ear discharge collected from the diseased ear of the patient (minimum of two cotton swabs) was immediately transported to the Clinical Microbiology laboratory under aseptic precaution for isolation and identification of bacterial and fungal pathogens. First swab was cultured on blood agar and Mac-Conkey agar plates and incubated at 37°C for 24 hours. Identification of bacterial species was done by standard bacteriological technique. [7] The second swab was cultured on slants of Sabouraud Dextrose Agar (SDA) with chloramphenicol (0.05%). The growth was identified by standard mycological technique. [8]

Antibiotic susceptibility testing for bacterial isolates was carried out by Kirby-Bauer disk diffusion method on Mueller-Hinton agar plate as per CLSI recommendations 2016, [9] using commercially available antibiotic discs (Hi Media, Mumbai, India).

P. aeruginosa ATCC 27853, *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 (ESBL positive) was used for quality control

The antibiotics tested for various microorganisms and their disc potency is as follows:

Disks tested for Gram positive cocci includes: Penicillin G (10 units), cefoxitin (30µg), erythromycin (15µg), clindamycin (2µg), cotrimoxazole (1.25/23.75µg), ampicillin (10µg)), tetracycline ((30µg), doxycycline (30µg), ciprofloxacin (5µg), moxifloxacin (5µg), gentamicin (10µg), linezolid (30µg), vancomycin (30µg). High content gentamicin (120µg) and high content streptomycin (300µg) discs were used for the detection of high level antibiotic resistance (HLAR) in *Enterococcus* species.

Disks tested for *P. aeruginosa* includes: Piperacillin (100µg), piperacillin-tazobactam (100/10µg), ceftazidime (30µg), aztreonam (30µg), cefepime (30µg), amikacin (30µg), gentamicin (10µg), tobramycin (10µg), ciprofloxacin (5µg), meropenem (10µg), imipenem (10µg), polymyxin-B (300units) and colistin (10µg).

Disks tested for other Gram negative bacilli includes: Ampicillin (10µg), amoxi-clavulanic acid (20/10µg), ampicillin-sulbactam (20/10µg), amikacin (30µg), ciprofloxacin (5µg), meropenem (10µg), imipenem (10µg), ertapenem (10µg) polymyxin-B (300units) and colistin (10µg).

Further, detection of MRSA and ESBL production was carried out by phenotypic methods

Detection of MRSA: Using cefoxitin (30µg) disc on Mueller Hinton agar (Hi-Media Labs, Mumbai) with 16-18 hours

incubation at 35°C as per CLSI recommendations. [9] A zone diameter < 25mm (CoNS) and <22mm*(S. aureus)*was reported as resistant.

ESBL production: All the Enterobacteriaceae were screened for ESBL production by disc diffusion method using indicator drugs and were further confirmed by Phenotypic Confirmatory Test (PCT) as per CLSI guidelines. [9] A 5 mm or more increase in zone of inhibition of either cefotaxime-clavulanic acid or ceftazidime-clavulanic acid disc compared to cefotaxime or ceftazidime disc alone was confirmed as ESBL producer.

RESULT

In the present study out of the clinically suspected cases of CSOM 53 % were males and 47 % were females, showing slight male pre-ponderance [Fig.1]. Majority of patients were in the second and third decade of life [Table-1]

Out of the total 103 ear discharge, 89 (86.41 %) samples were culture positive. Among the culture positive samples, pure bacterial pathogen was isolated in 77/89 (86.52 %) followed by pure fungal pathogen in 7/89(7.87%) and mixed pathogen (both bacteria and fungus in combination) in 5/89 (5.62%) of samples.

Looking at the distribution of bacterial pathogen there was predominance of Gram negative bacteria (70%) as compared to Gram positives (30%). *Pseudomonas aeruginosa* 40(51.96%), *Staphylococcus aureus*15 (19.48%), CoNS 10 (12.98%) and *Klebsiellapneumoniae*6 (7.79%) were the predominant bacterial pathogen isolated [Table2].

Table1: Agewise distribution of clinically suspected cases of CSOM (n=103)

Age	Number	Percentage
≤10	4	3.88%
11-20	32	31.06%
21-30	23	22.33%
31-40	13	12.63%
41-50	17	16.50%
51-60	6	05.83%
>60	8	07.77%
Total	103	100%

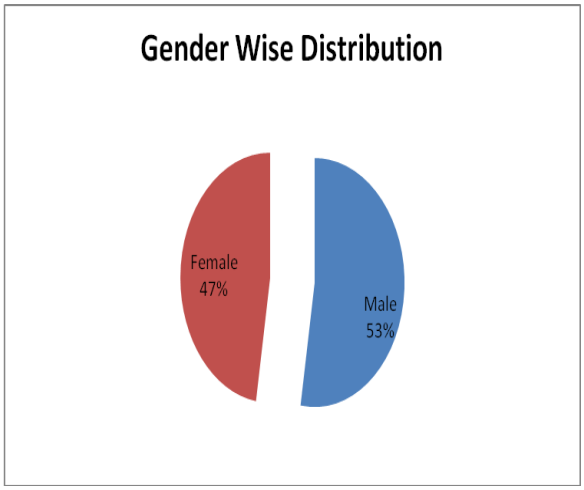

Fig. 1: Gender wise distribution of clinically suspected cases of CSOM (n=103)

Table2: Profile of bacterial pathogens isolated from cases of CSOM (n=77)

Organism isolated	Numbers	Percentage%
Pseudomonas aeruginosa	40	51.96%
Staphylococcus aureus	15	19.49%
Coagulase Negative Staphylococcus Species	10	12.99%
Klebsiella Pneumoniae	6	7.79%
Klebsiella oxytoca	2	2.59%
Morganella morganii	2	2.59%
Proteus mirabilis	1	1.29%
Acinetobacter species	1	1.29%

Table 3: Profile of fungal pathogens isolated from cases of CSOM (n=7).

Organism isolated	Number of samples	Percentage (%)
Aspergillus niger	2	28.57%
Aspergillus fumigatus	1	14.29%
Aspergillus flavus	1	14.29%
Fusarium species	1	14.29%
Candida species	2	28.56%

Table4: Profile of mixed pathogens isolated from cases of CSOM (n=5)

Organism isolated	Number	%
A.niger + K. pneumoniae	1	20%
A.niger+S.aureus	1	20%
A.flavus + K. pnumoniae	1	20%
fumigatus+P. aeruginosa	1	20%
Fusarium spp. + P. aeruginosa	1	20%

Table5: Resistant Profile of pathogens isolated from cases of CSOM

CLASSIFICATION BASED ON DRUG RESISTANCE		
MRSA (n=15)	9	60%
MRCoNS (n=10)	7	70%
ESBL Positive (n=9)	5	55.56%
ESBL Negative (n=9)	4	44.44%

Aspergillus niger was the predominant fungal pathogens isolated from cases of CSOM both as pure culture

(28.57%) and even as mixed etiology (40%). [Table3&4]. *A. fumigatus, A. flavus, Fusarium* species and *Candida* species were the other fungal pathogen isolated. High level of resistance was observed in cases of CSOM. The clinical isolates of *P. aeruginosa* showed resistance to multiple antimicrobial agents including resistance to meropenem (30 %) and imipenem (27.5%)[Fig. 6]. However, all the isolates were sensitive to colistin and polymyxin B. Similarly, high level of resistance to penicillin (100%), ampicillin (100%) and cotrimoxazole (60%) was observed in *S. aureus*. [Fig.7] However, all our isolates were sensitive to linezolid and vancomycin. MRSA was isolated from 9/15 (60%) cases and MRCoNS was seen in 7/10 (70%) cases. The members of Enterobacteriaceae were Multi Drug Resistant (MDR) including ESBL production was seen in 5/9 (55.56 %)[Table 5].

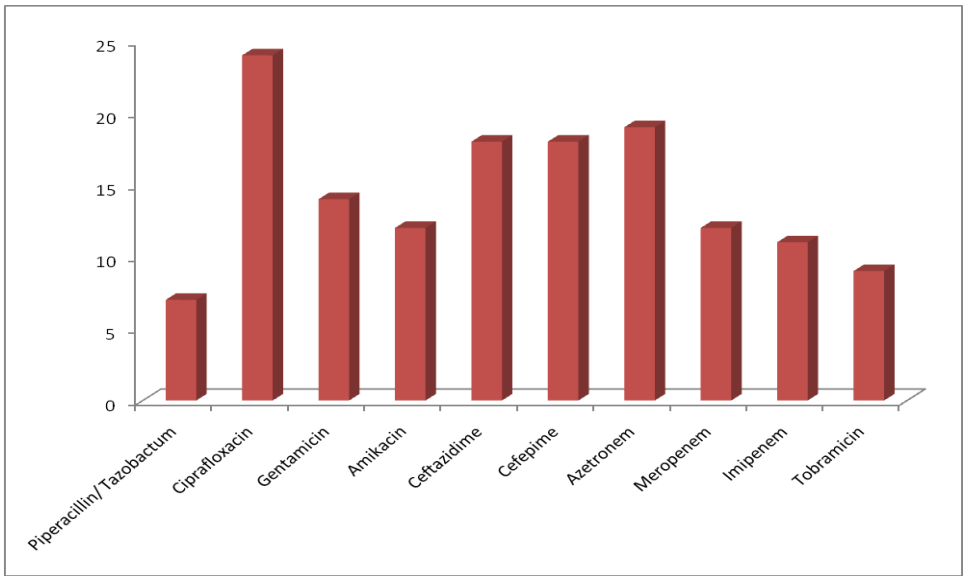

Figure 6: Resistance pattern of *Pseudomonas aeruginosa*(n=40)

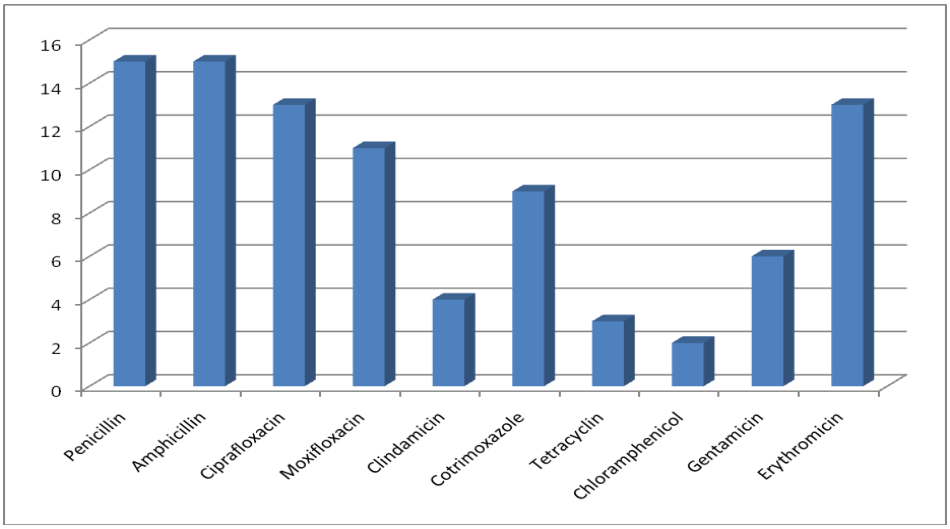

Figure 7: Resistance pattern of *Staphylococcus aureus*(n=15)

DISCUSSION

Knowledge of the causative microorganisms and its susceptibility pattern may contribute to an effective management of cases of CSOM. In the present study; the disease was more prevalent in second and third decade of life. Previous study by Kumar *et al*. [10] have also reported higher incidence in the first and second decade of life. More number of cases

in these decades may be because of low resistance in young children. However studies from abroad have reported increased prevalence in 30-40 years of age. [11] Our study showed pre-ponderance in male patients which may be co-related to the more exposed way of life of males. On the contrary, few studies have shown female predominance. [12,13] This difference may be due to geographical variation.

The rate of culture positivity in the present study was 86.41 %. Culture positivity rate varying from 84% to 91.18% have been reported by various workers in the past. [14- 17] Out of the culture positive cases, pure bacterial etiology could be established in 86.52 % and pure fungal etiology in 7.87 % cases. However, in 5.62% cases mixed etiology (bacteria + fungus) was seen [Table2,3&4]. We could not isolate two bacteria or two fungus in any of our cases as observed in previous study. [6]

However, out of the clinically suspected cases of CSOM, 13.39% were culture negative. Culture negativity in our study may have been due to following reasons; first ours being a tertiary care hospital patients usually come to us after having sought some antibiotic treatment from local doctors, second these infections may have been caused by anaerobic bacteria, mycoplasma and chlamydia which we have not looked for. Similarly, culture negativity in 12.6% and 16.9% of cases has been reported by other Indian studies. [18,19]
There was predominance of Gram negative bacilli (GNB) (70%) as compared to Gram positive cocci (GPC) (30%) in our study. *P. aeruginosa* (51.96 %)was the predominant GNB and *S.aureus*(19.48%) was the predominant GPC isolated. Similar findings have been reported from India [4] and abroad. [5] Coliforms such as *K. pneumonia* (7.59%) and *Proteus* spp. (1.29%) were isolated from few cases. Study by Mansoor *et al*., [20] have reported similar findings. The clinical isolates of *P. aeruginosa* showed resistance to multiple commonly prescribed anti-pseudomonal agents including meropenem (30%) and imipenem(27.5%) which is a matter of great concern. Such high level of resistance to newer drugs like meropenem and imipenem is an alarm for the judicious use of carbapenems. One of the limitations of our study was that metallo-betalactamases (MBL) production was not looked for in clinical isolates obtained from cases of CSOM.

Predominance of Methicillin resistance in *Staphylococcus* species was observed in our study, 70% cases were MRCoNS and 60% cases were of MRSA. Such high level of resistance in *Staphylococcus* species is a matter of concern as we are left only with linezolid and vancomycin as the treatment for these cases. ESBL production was seen in 55.56 % cases. However, studies published earlier showed the rate of isolation of MRSA was 33.33% and ESBL to be and 31.57% & 6.6%. [21–24] Thus the finding clearly highlights that the rate of MRSA and ESBL producers have definitively gone up over the years which indeed is a matter of therapeutic concern.

Looking at the profile of fungal pathogens isolated from cases of CSOM we observed that *Aspergillus* species was the predominant fungus isolated. Similar findings have been reported by other workers in the past. It is known that fungal infection of the middle ear is common as fungi thrive well in moist pus. Among the Aspergillus, *A.niger* was the predominant species isolated in our study followed by *A.fumigatus* and *A.flavus.* Similar findings have been reported in the past by other workers. The fungal pathogen isolated in our study occurred both as pure growth and also as mixed pathogen with bacteria [Table 4 &5]. Looking at the total profile of microorganisms isolated this study shows that *P. aeruginosa* and *S. aureus* are the predominant bacterial pathogen and *Aspergillus* species was the predominant fungal pathogen isolated from cases of CSOM which is in complete agreement with the earlier published data.

CONCLUSION

To conclude, high level of resistance to various antimicrobial agents was observed in cases of CSOM and the emergence of antibiotic resistant strains has led to treatment failure. Early, microbiological diagnosis in cases of CSOM is needed for prompt and effective treatment to avoid its serious complications as well as it will help us to know the common microbes associated with the diseases in that locality. Another important point our study highlights is that it is important to have knowledge regarding the spectrum of microorganisms causing ear discharge from the point of view of treatment of patients; that is weather to start antibacterial agents or antifungal agents. Finding of mixed etiology of infection in 5.62% of cases may require attention as these cases need to be treated with both antibiotic and antifungal agents.

Limitations

Our study had few limitations: i) MBL detection in Gram negative bacteria was not looked for ii) Microorganisms like anaerobes (mycoplasma and chlamydia) were not looked for in this study iii) antifungal susceptibility testing for fungus and genotypic methods of detection of resistance could not be carried out.

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