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Original Article

Microbiology Section

Resistant Microorganisms Isolated from Cases of Chronic Suppurative Otitis Media: A Therapeutic Concern

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ABSTRACT

Introduction: Chronic Suppurative Otitis Media (CSOM) still is a common health care problem in developing countries like India with its potential for serious local damage and complications. Knowledge of causative microorganisms and their antimicrobial sensitivity pattern is essential so that early and effective therapeutic measures can be initiated for better patient outcome.

Aim: The study was carried out to determine the clinico-microbiological spectrum of CSOM from this geographical area and to evaluate the susceptibility pattern of aerobic bacterial isolates.

Materials and Methods: The prospective study was carried out for a period of one year from December 2013 to November 2014 in Chhatrapati Shivaji Subharti Hospital, a Tertiary Care Hospital in Meerut city. A total of 113 ear discharge was collected from clinically suspected cases of CSOM. The discharge was cultured on blood agar and Mac Conkey's agar plates for isolation of bacterial pathogen and Sabourauds Dextrose Agar (SDA) slants for isolation of fungal pathogens. Identification of the bacterial and fungal isolates was done using standard bacteriological and mycological methods respectively. Anti-microbial susceptibility testing of the bacterial isolates was performed by Kirby-Bauer's disc diffusion method as per the Clinical Laboratory Standards Institute (CLSI) guidelines. Further, detection of Metallo Beta Lactamase (MBL) production, Methicillin Resistance in *Staphylococcus aureus* (MRSA) and Extended Spectrum Beta –Lactamase (ESBL) production was carried out by phenotypic methods.

Result: A total of 73.45% cases were culture positive. Pure bacterial pathogen was isolated from 67.46 % followed by pure fungal pathogen 22.89% and mixed pathogen (bacteria and fungus) from 9.63% of cases. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the predominant bacterial pathogen and *Aspergillus* spp. and *Candida* species were the predominant fungal pathogens isolated. Overall the rate of MBL producers, MRSA and ESBL producers were 18.18%, 52.94% and 62.5% respectively.

Conclusion: High level of resistance was observed in cases of CSOM from our hospital. Therefore, knowledge of the causative microorganisms and its susceptibility pattern is required and this data may contribute to an effective management of cases of CSOM.

Keywords: Microbiological spectrum, Otorrhea, Susceptibility pattern

INTRODUCTION

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 World Health Organization (WHO) defines CSOM as "otorrhea that is ear discharge through perforated tympanic membrane present for at least 2 weeks", though few guidelines takes"chronic" as symptoms persisting for more than 6 weeks [2]. The disease is worldwide in distribution [3]. Both Gram positive (*Staphylococcus aureus, Streptococcus pneumoniae*) and Gram negative (*Pseudomonas aeruginosa, Escherichia coli, Proteus* species*, Klebsiella* species) bacteria are involved in the pathogenesis of CSOM [4] Studies from India [5] and abroad [6] have reported predominance of *P. aeruginosa* and *S.aureus*. Recently, indiscriminate antibiotics use has been attributed to the emergence of number of resistant strains which are associated and produce both primary CSOM and its post-operative infections [7]. The study was carried out to determine clinico-microbiological profile of microorganisms causing CSOM and their susceptibility pattern from this geographical area with special reference to prevalence of MRSA, MBL and ESBL producers in CSOM so that efficient empirical treatment can be initiated.

MATERIALS AND METHODS

The prospective study was carried out for a period of one year from December 2013 to November 2014, in Chhatrapati Shivaji Subharti Hospital (CSSH), a Tertiary Care Hospital in Meerut city, Uttar Pradesh, India. The approval from the Institutional Ethical and Research Committee was obtained before conducting the study. Ear discharges was collected from a total of 113 clinically suspected cases of CSOM.

The clinical diagnosis of CSOM was made by a consultant Otorhinolaryngologist. Informed consent was taken from all the patients before collection of clinical samples. The age, sex, population background, seasonal variation, predisposing factors, clinical presentations was recorded for each patient. 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. Patients on local or systemic antibiotics, antifungal or corticosteroid drops, immuno-compromised patient with HIV infection and diabetes mellitus were excluded.

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 Conkeys agar plates and incubated at 37°C for 24 hours. Identification of bacterial species was done by standard bacteriological technique [8]. The second swab was cultured on slants of Sabouraud Dextrose Agar (SDA) with chloramphenicol (0.05%). The growth was identified by standard mycological technique [9].

Antibiotic susceptibility testing was carried out for bacterial isolates by Kirby-Bauer disk diffusion method on Mueller-Hinton agar as per CLSI recommendations 2014 [10], using commercially available antibiotic discs (Hi Media, Mumbai, India). The antibiotics tested for various microorganisms and their disc potency is as follows:

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

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.

Detection of MBL, MRSA and ESBL production was carried out by phenotypic methods-

Detection of MBL: The meropenam screen positive (zone diameter <21 mm) [10] isolates of *P. aeruginosa* were subjected for confirmation of MBL by Combined Disc Test (CDT) using Imipenem (IPM) and IPM + EDTA disc as per the method used by Yong et al., [11]. An organism demonstrating a zone diameter ≥7 mm around IPM + EDTA disk as compared to IPM disk alone was considered as MBL producer.

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 [10]. A zone diameter of <22 mm 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 [10]. 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.

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

STATISTICAL ANALYSIS

Statistical analysis was carried out using Chi square test.

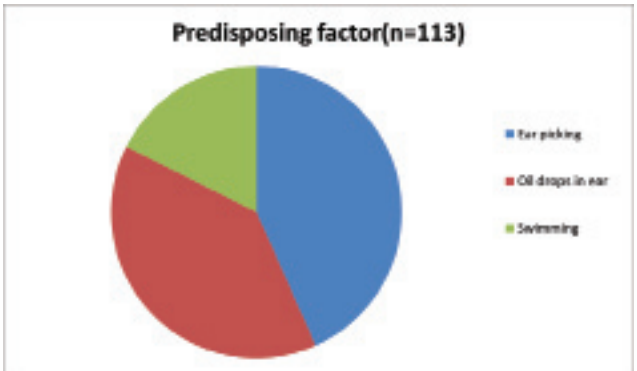
RESULTS

Out of total 113 clinically suspected cases of CSOM 67.25% were males and 32.74% were female patients. Male to female sex ratio was 2:1 with male pre-ponderance. Majority of cases were in the second and third decade of life [Table/Fig-1] (χ^2=3.93; p=0.415). The disease was more common in the patients coming from villages i.e., rural population with an urban: rural ratio of 1:2.5. Maximum number of cases reported to the hospital during the month from April to July, showing that the hot and humid climate in this geographical area may be one of the risk factors (χ^2=1.92; p value =0.998). Ear picking with various objects was the most common predisposing factors identified (80.53%) followed by putting oil drops in ear (72.56%) and swimming (32.745%) [Table/Fig-2]. Otorrhea and otalgia were the most common clinical presentation seen in 100% of cases followed by diminution in hearing (63.71%) and tinnitus (6.19%) [Table/Fig-3].

A total of 83 (73.45%) cases of CSOM were culture positive

Age in years	Clinically suspected cases of CSOM		Total culture positive cases	
	(n=113)	%	(n=83)	%
<10	10	8.84	07	8.43
11-20	40	35.39	35	42.16
21-30	37	32.74	30	36.14
31-40	13	11.50	07	8.43
>40	13	11.50	04	4.81
Total	113	100 %	83	100 %

[Table/Fig-1]: Agewise distribution of clinically suspected and culture positive cases of CSOM.



[Table/Fig-2]: Predisposing factors identified in cases of CSOM.

Clinical Presentation	Number of cases	Percentage (%)
Otorrhoea	113	100
Otalgia	113	100
Diminution in Hearing	72	63.71
Tinitus	7	6.19

[Table/Fig-3]: Distribution of various clinical presentation (n=113).

and 30 (26.54%) cases were culture negative. Among the culture positive cases, pure bacterial pathogen was isolated in 56/83 (67.46%) cases followed by pure fungal pathogen in 19/83 (22.89%) and mixed pathogen (both bacteria and fungus in combination) in 8/83 (9.63%). *P. aeruginosa* (44.64%) and *S. aureus* (30.35%) was the predominant bacteria and *Aspergillus* species (52.61%) and *Candida* species (36.84%) was the predominant fungus isolated from cases of CSOM both as pure culture and even as mixed etiology. [Table/Fig-4], *Aspergillus niger* was the predominant species of *Aspergillus* isolated. Distributions of various other bacterial and fungal pathogens isolated are shown in [Table/Fig.-4]. However, association of two bacterial or two fungal pathogens were not seen in the present study.

The clinical isolates of *P. aeruginosa* showed resistance to multiple antimicrobial agents including resistance to meropenem (44%) and imipenem (36%) [Table/Fig-5]. However, they were 100% sensitive to colistin and polymixin B. A total of 18.18% *P. aeruginosa* were MBL producers. Similarly, high level of resistance to penicillin (94.11%), ampicillin (94.11%) and cotrimaxozole (70.78%) was observed in *S.aureus* [Table/Fig-5]. However, all our isolates were sensitive to linezolid and vancomycin. MRSA was isolated from 52.94% cases. The members of Enterobacteriaceae [*K.pneumoniae* (n=4), *E.coli* (n=2) and *Proteus mirabilis* (n=2)] were Multi Drug Resistant (MDR) including resistance to cotrimoxazole (87.5%), ampicillin (75%) and ceftriaxone (62.5%) [Table/Fig- 5]. However, no resistance was seen to colistin and polymixin B. ESBL production was seen in *E. coli* (n=2), *K. pneumoniae* (n=2) and *Proteus mirabilis* (n=1).

Organisms Isolated	Number of Samples	Percentage (%)
Bacterial Pathogen Isolated (n=56):		
S. aureus	17	30.35
CoNS	4	7.14
Enterococcus spp.	2	3.57
K. pneumoniae	4	7.14
E. coli	2	3.57
Proteus mirabilis	2	3.57
Pseudomonas aeruginosa	25	44.64
Total	56	100 %
Fungal Pathogen Isolated (n=19):		
A.niger	6	31.57
A.fumigatus	3	15.78
A.flavus	1	5.26
Candida spp.	7	36.84
Penicillium spp.	2	10.52
Total	19	100%
Mixed Pathogen (Bacteria+Fungus) Isolated (n=8):		
A.Niger + S. Aureus	1	12.5
A.Niger + P. Aeruginosa	1	12.5
A.fumigatus + P. aeruginosa	1	12.5
A.fumigatus + S. aureus	1	12.5
Candida spp. + *P. aeruginosa*	2	25
Candida spp. + CoNS	1	12.5
Candida spp. + *S. aureus*	1	12.5
Total	8	100%

[Table/Fig-4]: Profile of bacterial, fungal and mixed pathogens isolated from cases of CSOM (n=113).

DISCUSSION

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 [1]. In the present study, the disease was more prevalent in second and third decade of life [Table/Fig-1]. Srivastava A et al., [12] and Kumar H et al., [7] reported higher incidence in the first and second decade of life. More number of cases in the first few decades of life may be because of low resistance in young children and infants and a relatively short and straight Eustachian tube. Increased prevalence (30-40 years of age) was seen in Singapore study [13]. Male predominance (male:female ratio-2:1) was seen in this study. Higher incidence in young males may be co-related to the more exposed way of life of males. On the contrary, Prakash M et al., [14] and Shrestha BL et al., [15] have shown female predominance. The difference in results may be due to geographical reasons. Our cases predominantly belonged to the rural population. The reason for this may be because our hospital is a tertiary care center located in out skirts of

Etiological Agent (No. of Isolates)	Antibiotic Resistant Pattern (%)																	
Gram Negative	**AMP**	**AMC**	**A/S**	**PI**	**PIT**	**TE**	**COT**	**CIP**	**CAZ**	**CTR**	**AT**	**CPM**	**AK**	**GEN**	**TOB**	**IPM**	**MRP**	**ETP**
P. aeruginosa (n=25)	-	-	-	56	32	-	-	68	48	-	32	40	44	56	44	36	44	-
K.pneumoniae (n=4)	100	75	75	50	0	25	75	0	50	50	50	50	0	0	0	0	0	0
E. coli (n=2)	100	50	0	100	0	100	100	100	50	100	50	50	0	0	0	0	0	0
P. mirabilis (n=2)	50	50	50	50	50	0	50	50	50	50	50	50	50	50	50	50	50	50
Gram Positive	**P**	**AMP**	**CX**	**E**	**CD**	**COT**	**TE**	**DO**	**CIP**	**MO**	**GEN**	**LZ**	**VA**	**HLG**	**HLS**			
S. aureus (n=17)	94.11	94.11	52.94	11.76	17.64	70.78	23.52	17.64	29.41	69.23	29.41	0	0	-	-			
Enterococcus spp.(n=2)	100	100	-	50	50	100	0	0	50	50	-	0	0	50	50			

[Table/Fig-5]: Antibiotic resistance pattern of bacterial isolates in cases of CSOM.
AMP: Ampicillin; AMC: Amoxycillin -Clavulanic acid; A/S: Ampicillin-sulbactum; PI: Piperacillin; PIT: Piperacillin-Tazobactum; COT: Cotrimoxazole; CIP: Ciprofloxacin; CAZ: Ceftazidime; CTR: Ceftrioxone; AT: Azteronam; CPM: Cefipime; AK: Amikacin; GEN: Gentamicin; TOB: Tobramycin; IPM: Imipenem; MRP: Meropenem; ETP: Ertrapenem; CX: Cefoxitin; E: Erythromycin; CD: Clindamycin; DO: Doxycycline; MO: Moxifloxacin; LZ: Linezolid; VA: Vancomycin; HLG: High Level Gentamicin; HLS: High Level Streptomycin.

the city where we receive majority of cases from the villages nearby. Isolation rate was more during the month from May to July & August showing that hot and humid climate was one of the risk factors for infection along with ear picking with different objects, putting oil drops in ear and swimming [Table/Fig.-2]. Similarly, Nwokoye NN et al., [16] have reported poor hygiene and unorthodox approach to treatment like use of unconventional ear drops and concoctions such as oil and honey into the middle-ear may initiate the proliferation of opportunistic pathogens leading to blockage of eustachian tube. Another study by Kumar S et al., [17] has also reported similar results. Otalgia and otorrhoea was the commonest clinical presentation in our cases followed by diminution in hearing. Similar findings have been observed in the study done by Mugliston T et al., [18]. Tinnitus was an uncommon clinical presentation seen in few (6.19%) of our cases.

The rate of culture positivity in the present study was 73.45%. Culture positivity rate varying from 84% to 91.18% have been reported in different Indian studies [7,12]. Out of the culture positive cases, bacterial and fungal etiology could be established in 67.46% and 22.89% cases respectively. However, in 9.63 % cases mixed etiology (bacteria + fungus) was seen [Table/Fig-2]. No association between two bacteria was found in any of our cases as observed by Kumar H et al., [7]. Comparatively high rate (26.54%) of culture negativity in the present study may be due to following reasons; firstly our center being a tertiary care hospital patients usually come to us after having sought medical advice from local doctors and having taken multiple or incomplete course of antibiotics, which they could not tell us in initial history taking, which might have led to sterile cultures in clinically suspected cases of CSOM. Secondly, these infections may have been caused by certain other microorganisms which were not looked for such as anaerobic bacteria, mycoplasma and chlamydia. Culture negativity in 12.6% and 16.9% of cases has been reported in other studies from India [19, 20].

Our study showed predominance of Gram negative bacilli (GNBs) (58.92%). Similar findings have been reported by various authors [7,21,22]. *P. aeruginosa* (44.64%) was the predominant bacteria isolated followed by *S.aureus* (30.35%) [Table/Fig-3]. Similar findings have been reported from India [5] and abroad [6]. Higher rate of isolation of *P. aeruginosa* has its own implications, as *Pseudomonas* is an important cause of nosocomial infections and it possess the potential to transmit plasmid carrying resistance to other species as well [22]. Coliforms such as *K. pneumonia* (7.14%) and *E. coli* (3.57%) could be isolated from few cases. Similar findings were reported by Mansoor T et al., [22]. A study done on 2012 by Shyamala and Reddy showed a higher rate of isolation of Enterobacteriaceae (*E. coli* in12% ,Klebsiella in 5%); which might be due to other contributing factor like exposure to hospital environment or heavy contamination of local water with coliforms [21].

The clinical isolates of *P. aeruginosa* showed resistance to multiple commonly prescribed anti-pseudomonal agents such as ciprofloxacin (68%), amikacin (44%), tobramycin (44%), meropenem (44%) and imipenem (32%) [Table/Fig-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. MBL producer in 18.18% isolates of *P.aeruginosa* in our study differ from a recent study by Khatoon A et al., where MBL production was not detected in GNB [4]. MBL production was not looked for in other Gram negative isolates in our study. Overall, the high level of resistance seen in our isolates of *P. aeruginosa* may be because incomplete or partially

treated cases reach our tertiary care referral center. However, all our isolates were sensitive to polymyxin-B and colistin, the last resort drug that's all we are left with in this era of desperation. The isolates of *S.aureus* showed resistance to multiple antimicrobial agents [Table/Fig-5]. Similar findings have been reported [5,23]. In our set up 52.94% isolates were MRSA. Such high level of MRSA from cases of CSOM is a matter of concern as MRSAs are resistant to beta lactams, cephalosporins, beta lactamase inhibitors leaving very few treatment options. Similarly, recent studies by Khatoon A et al., [4] and Rejitha IM et al., [24] have reported MRSA ranging from 29% to as high as 83.3% respectively. Overall, ESBL producers were seen in 8.9% cases of Enterobacteriaceae in our study. However, studies published earlier showed the rate of isolation of MRSA and ESBL to be 33.33% and 31.57% respectively by Rejitha IM et al.,[24] and ESBL 6.6% by Sattar A et al., [25] which proves that rate of ESBL strains has gone up over the years.

In the present study, fungal etiology was found in 22.89% cases as pure culture and 9.63 % cases as mixed culture, out of which *Aspergillus* was the predominant species isolated [Table/Fig-4]. Similar findings have been reported by other workers [13,26]. High isolation rate of fungus may be due to traditional unhygienic habit persisting in our rural community such as ear picking with different objects and orthodox habit of instillation of oil in ear canal. Fungal infection of the middle ear is common as fungi thrive well in moist pus [25]. In this study *A.niger* (29.62%) was the predominant species followed by *A.fumigatus* (18.51%) and *A.flavus* (3.70%), consistent with the study carried out by Prakash M et al., [14]. On the contrary Kumar and Seth reported predominance of *Candida* spp. [7]. This difference in rate of isolation may be attributed to the environmental effect. In short the present study show that *P. aeruginosa* and *S. aureus* are the predominant bacterial pathogen and *Aspergillus* spp. and *Candida* species are the predominant fungal pathogens isolated from cases of CSOM which is in complete agreement with the published data [5,6]. There is emergence of antimicrobial resistance thus knowing the spectrum of microorganisms causing ear discharge is important for the treatment of patients that is weather to start antibacterial agents, antifungal agents or in mixed etiology (9.6%) where the treatment may require both groups of drugs.

LIMITATIONS

Our study had few limitations due to lack of resources- i) Microorganisms like anaerobes (mycoplasma and chlamydia) were not looked for in this study; ii) Quality control for hyphal fungus, antifungal susceptibility testing and genotypic methods of detection of resistance could not be carried out. Still this study highlights the common microorganisms associated with CSOM from the semi urban and rural population of Western Uttar Pradesh which was previously undocumented and it has seen a rise in the resistance pattern of the organism isolated with increased number of MRSA and ESBL production in these organisms.

CONCLUSION

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. Therefore, knowledge of the spectrum of microorganisms causing ear discharge is important for the treatment of patients that is weather to start antibacterial agents, antifungal agents for effective therapy and reduction in treatment costs. Finding of mixed etiology of infection in 9.63% of cases also requires attention as these cases need to be treated with both the antibiotic and antifungal agents.

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