



Microbial profile with their antimicrobial susceptibility pattern in ear discharge of CSOM patients at a tertiary care hospital in Northern Rajasthan

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Abstract

Background: Chronic suppurative otitis media (CSOM) is a persistent middle ear disease with high risk of irreversible complications in absence of timely management. It is a massive health problem with India having the highest prevalence rate (>4%) requiring urgent attention.

Objective: To find out risk factors, microbiological profile, with their susceptibility pattern, of ear discharge in CSOM patients to provide guidelines for the empirical treatment.

Material and Methods: Ear discharge samples from 130 clinically diagnosed CSOM patients were collected and processed. Microbial isolates were identified and drug susceptibility testing was conducted using Kirby –Bauer disc diffusion method.

Results: *Pseudomonas species* (59/120,49.16%) was the predominant isolate followed by *Staphylococcus species* (37/120,30.83%) while *Aspergillus species* (10/12,83.33%) was the predominant fungus isolated. No anaerobic bacteria were isolated on culture. Gram negative bacilli were most susceptible to Meropenem (95.45%), Amikacin (91%) and Netilmycin (91%) while *Pseudomonas species* was to Imipenem (98.3%). Gram positive cocci showed 100% susceptibility to Vancomycin, Linezolid and Doxycycline.

Conclusion: A continuous and periodic evaluation of microbiological pattern of CSOM and antimicrobial sensitivity of isolates is necessary for forming the basis of empirical treatment which shall aid in decreasing the potential risk of complications. Further, accurate and timely identification, knowledge of the pathogens and judicious use of antibiotics is the need of the hour.

Keywords: CSOM, middle ear disease, pseudomonas species

Introduction

Chronic Suppurative Otitis Media (CSOM) is chronic inflammation of middle ear cleft. It is a persistent disease of the middle ear, which is capable of causing severe destruction sequelae with the manifestation of deafness, discharge and a permanent perforation [1]. CSOM accounts for 65 to 330 million cases globally.

This disease is mainly classified into two types based on the area of Tympanic membrane involved- Tubotympanic -pars tensa and Atticoantral -pars flaccid.

Tubotympanic is called as a safe type or benign type as there is no serious complication whereas Atticoantral is called as the unsafe or dangerous type because of associated complications which may be life threatening at times [2]. Developing countries like India have higher prevalence (>4%) mostly seen in low socio-economic society. The urban to rural ratio is 1:2 [2, 3].

CSOM is mostly caused by bacteria, but fungi and virus can also be associated with CSOM. The aerobic microorganisms most frequently isolated in CSOM are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus spp.*, *Klebsiella spp.*, *Escherichia spp.*, *Citrobacter spp.*, *Haemophilus influenzae*, and *Moraxella catarrhali* [4, 5, 6].

Untreated/inadequately treated cases cause Intra/ Extracranial complications and emergence of resistant strains.

Since CSOM causes significant morbidity, knowledge of causative microorganisms and their susceptibility patterns guides judicious antibiotics use minimizing complications. This study was conducted to study risk factors and

microbiological profile with susceptibility patterns in CSOM patients to establish empirical treatment guidelines.

Materials and Methods

The present study was a Laboratory based Descriptive type of Observational study conducted in Department of Microbiology, S.M.S. Medical College, Jaipur from May 2014 to April 2015 after approval by the Ethics Committee of SMS Hospital. The samples were collected from 130 clinically diagnosed CSOM patients from ENT Department of SMS Hospital, Jaipur after written informed consent.

Inclusion Criteria: Clinically diagnosed CSOM patients with ear discharge, who did not receive Antimicrobial therapy for the last 7 days, were included in the study.

Exclusion Criteria: Current antibiotic use or use in the preceding 1 week, recent ear surgery or an in-situ grommet or tympanostomy tube, mastoid surgery in the preceding 12 months, congenital ear or hearing problems, obstructed middle ear (eg, polyp), patients with ear discharge due to cholesteatoma.

Procedure of the sample collection

After removing excess discharge and cleaning the External auditory canal to achieve sterile area, swabs(4 in number) or aspirate were collected (using sterile swabs/syringe and properly labelled) for each patient for direct microscopy, aerobic culture, anaerobic and fungal culture respectively.

Direct microscopy of all the clinical samples was done by Gram staining and KOH mount. Aerobic bacterial isolation-Sample was inoculated on Blood Agar, Mac Conkey Agar and Thioglycollate (TG) broth (Himedia, Vadhani Ind. Est., LBS Marg, Mumbai, India). Plates/tubes were incubated and identification of bacterial pathogens was done according to standard laboratory procedures [6]. Antimicrobial susceptibility testing was done by Kirby Bauer Disc Diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [7]. Anaerobic bacterial isolation-Sample on collection was immediately inoculated into Robertson's Cooked Meat broth, transported to the lab and incubated at 37°C for 48 hrs. After 48 hours, gram staining and subculture on blood agar was done. Plates were then put in Anaerobic Gas Jar with Gas pack (BD BBL™ GasPak™ Anaerobic System), incubated at 37°C and examined at 24 hours, 48 hours, and 72 hours for any growth. If no growth was observed at 72 hours, results were recorded as sterile. It was proposed that if growth was seen then Gram Staining would be done and further identification shall be done by Vitek 2 using identification strips [8].

Fungal isolation-Sample was inoculated on Sabouraud's Dextrose Agar with (Gentamicin and Chloramphenicol) and without antibiotics and incubated at 25° C for 21 days and examined daily for first week then weekly upto 21 days. The growth was observed for rate of growth, colony morphology, texture and pigmentation. Microscopic identification by Lactophenol Cotton Blue mount was done. Microslide culture was put in case sporulating structures were not observed on LCB mount. Gram staining was done for identification of yeast and yeasts like cells. Antifungal Susceptibility Testing for yeasts was done according to CLSI guidelines [9].

Results

A total of 130 samples, including 80 ear swabs and 50 aspirates, were collected from clinically diagnosed CSOM patients. Age wise distribution of these patients is shown in table 1.

Among the patients, 59.2% (77/130) were males and 40.8 % (53/130) were females, with male to female ratio being 1.5:1. The growth positivity was higher in male patients (75/77, 97.4%) as compared to females (47/53, 88.67%) and the difference was statistically significant (p value = 0.04).

In this study, 70/130 (53.84 %) patients were from rural area and 60/130 (46.15%) from urban area. The growth positivity was higher in patients living in rural areas (98.57%, 69/70) as compared to patients from urban area (88.33%, 53/60) and the difference was statistically significant (p value = 0.02).

Of the 130 samples processed, 122 (93.84%) samples showed growth positivity in which 120 (92.3%) showed bacterial growth while 12 (9.20%) had fungal growth. Pure growth was seen in 112 (86.15%) samples and mixed growth in 10 (7.69%) samples. All the 120 bacterial isolates were aerobic. No anaerobic bacteria were isolated on culture.

Various bacteria and fungi isolated from the clinical samples in our study are shown in table 2 Antimicrobial susceptibility pattern of the various microorganisms isolated in our study are shown in tables 3, 4 & 5

Discussion

The present study was undertaken on 130 clinically diagnosed

Chronic Suppurative Otitis Media (CSOM) patients. Out of 130 cases, majority of the CSOM patients 43(35.24%) were in 11-20 years of age group which is in concordance with the findings of various other studies [10-13] while Gulati *et al.*, Baroah *et al.*, A Nandy and Shivrajan *et al.*, reported higher incidence of CSOM in the first decade of life [14-16].

In the present study, males were affected more than female which is in concordance with the other studies [10, 17-19] but in contrast with the studies done by Rajat Prakash *et al.*, Tahira Mansoor *et al.*, Prakask M *et al.* and Loy *et al.* who reported a higher percentage of females being affected in their study [2, 20-22]. The cause of predominance of male patients over female is unknown.

Among the patients, 53.84% (70/130) were from rural area and 86.92% (113/130) were of low socioeconomic status. Our study correlates well with Lasisi AO *et al.*, Agrawal *et al.*, Gulati *et al.* and Waqar-uddin *et al.* [23-25]. Increased incidence in lower socio-economic status and rural population might be due to unhygienic surroundings, overcrowding and lack of nourishment.

In the present study, 93.84% (122/130) samples were culture positive which is in accordance with other studies [2, 16, 26-28] However, Tahira Mansoor *et al.* [20] have reported lower culture positivity i.e. 78% and 77% respectively. High percentage of negative cultures could be probably due to presence of lysozyme in samples, initiation of antimicrobials before sample collection or poor patient selection criteria or sample collection technique [29].

Percentage of pure bacterial isolates of 90.16%, pure fungal isolates 1.64%, mixed growth i.e fungus plus bacteria 8.19% is in agreement with many previous researchers [30, 31]. This study revealed that gram negative organisms 67.5% (81/120) outnumber the gram positive organisms 32.5% (39/120) in CSOM which is reported similarly in other studies [2, 32-34]. *Pseudomonas species* (59/122, 49.17%) was the commonest isolate followed by *Coagulase Positive Staphylococcus* (COPS) (21/120, 17.5%) in this study which is in accordance with the other studies [17-19, 22, 29]. This could be attributed to the fact that *Pseudomonas* is known to be resistant to antibiotics and can survive in nutrient deficient environment. Its ability to produce bacteriocins, presence of pili and enzymes like proteases gives it added advantage of survival over the other bacteria in middle ear infections [35]. However certain authors have reported *Staphylococcus species* as the most common isolate [2, 19, 21, 24, 36]. This signifies the need for studying the microbiological profile for each geographical area.

Of the 12 fungal isolates 10(83.33%) were *Aspergillus species* and 2 (16.66%) were *Candida species* which is in accordance with the studies of Saraswati Jayanti *et al.* and Loy *et al.* [22, 37]

Even though anaerobes play a role in the pathogenesis of CSOM, this study did not find any isolate, which is in concordance with the results of Suman Yeli *et al.* and Swayamsidha Andhale *et al.* [38, 39]

All the pathogenic strains isolated in the present study were tested against various antibiotics as per standard protocol.

Various studies from different parts of India in the past couple of years report 100% sensitivity of *Pseudomonas* to Imipenem as compared to 98.3% in present study [21, 24, 40, 41]. This though small but presence of resistance to Imipenem in *Pseudomonas*

is an alarming scenario of emerging resistant strains which could be due to indiscriminate use of Imipenem without using first line empirical antibiotics for therapy or horizontal spread of resistant strains in the hospital setup. This calls for strict monitoring to prevent the spread of resistance to other susceptible strains. At the same time high rate of resistance in *Pseudomonas* to Gentamicin in present study (61%) as compared to that reported by similar other studies (12% to 24%) indicates that there is a need to review empirical treatment of CSOM from time to time [21, 40, 41].

On the other hand, if we see the susceptibility pattern of Gram positive cocci and gram negative bacilli (other than *Pseudomonas*) resistance to Gentamicin is very less (5.13% and 27% respectively). This indicates that susceptibility pattern of different microorganisms to same drug is different, so there is a need for preliminary identification of pathogens in ear discharge of CSOM patients before starting the empirical treatment which is possible in less than 24 hours, so as to prevent the emergence of resistant strains.

A study from Agra (neighbouring area) showed high sensitivity of *Pseudomonas* to Levofloxacin (68.3%) and Tobramycin (80.5%) as compared to 37.28% and 52.54% in our study [26]. Difference in sensitivity patterns signifies the geographical variations prevalent in our country and the need to study these patterns so as to have knowledge about the most prevalent pathogen in a particular area and their sensitivity pattern.

Coagulase positive Staphylococcus (COPS) showed 100% sensitivity to Vancomycin, Linezolid and Doxycycline in our study, which is a heartening situation but calls for their judicious use so that resistance to them can be prevented and regular monitoring for emergent resistance.

In case of *Escherichia coli*, *Proteus species* and *Citrobacter species*, Amikacin was found to be the most effective drug to which the sensitivity was 100% while Indudharan *et al.* [42] reported 100% sensitivity to Ceftriaxone and Yang *et al.*, [43] Sharma *et al.* [44] and Rao and Reddy *et al.* [45] to Ciprofloxacin. When the results of various studies were compared, it became obvious that the bacteriology and antibiotic sensitivity pattern of CSOM has been changing from region to region and over time period too.

Coagulase negative Staphylococcus (CONS) is a normal skin flora but can sometimes become an opportunistic pathogen. Majority of them were sensitive to almost all the antibiotics tested. In our study highest sensitivity was towards Vancomycin and Gentamicin 100%, followed by Linezolid 93.7% and Doxycycline 87.5%.

Antifungal Susceptibility pattern of *Candida species* showed 100% sensitivity to Voriconazole while sensitivity to Amphotericin B, Flucytosine and Fluconazole was 50%. We cannot draw any inference from this pattern due to very small number of *Candida* isolates (2/12) in our study.

Conclusion

The increasing rate of micro-organisms' resistance to traditional antimicrobials has had an important effect on empirical selection of antimicrobials. Thus, we recommend that constant evaluation of the antimicrobial susceptibility

pattern of locally prevalent etiological agents of CSOM against commonly used antimicrobials is necessary to formulate a protocol for empirical antibiotic therapy and second line treatment and also to prevent the emergence and spread of resistant pathogens. Further, we also recommend a basic preliminary identification of all CSOM ear discharge for *Pseudomonas* (the most common isolate in our region), as its resistance to Gentamicin, a first line topical antibiotic is much higher than other gram negative bacilli and gram positive cocci.

Table 1: Age wise Distribution of patients

Age	Number of samples	Growth Positivity
0-10	25 (19.23 %)	24 (19.67 %)
11--20	46 (35.38 %)	43 (35.24 %)
21-30	31 (23.84 %)	29 (23.77 %)
31-40	13 (10 %)	12 (9.83 %)
41-50	8 (6.15 %)	7 (5.73 %)
51-60	5 (3.84 %)	5 (4.09 %)
61-70	2 (1.53 %)	2 (1.63 %)
Total	130	122

Table 2: Various Microorganisms Isolated from clinical samples

Bacterial Isolates	Number	Percentage (%)
<i>Pseudomonas species</i>	59	49.17
Coagulase Positive <i>Staphylococcus</i>	21	17.5
Coagulase Negative <i>Staphylococcus</i>	16	13.33
<i>Escherichia coli</i>	7	5.83
<i>Enterobacter cloacae</i>	7	5.83
<i>Proteus mirabilis</i>	3	2.5
<i>Enterobacter aerogenes</i>	2	1.67
<i>Enterococcus species</i>	2	1.67
<i>Proteus vulgaris</i>	1	0.83
<i>Acinetobacter species</i>	1	0.83
<i>Citrobacter species</i>	1	0.83
Fungal Isolates		
<i>Aspergillus niger</i>	4	33.33
<i>Aspergillus flavus</i>	3	25
<i>Aspergillus fumigatus</i>	3	25
<i>Candida species</i>	2	16.66

Table 3: Antimicrobial Susceptibility Pattern of Gram Positive cocci

Antibiotics	COPS (21)	CONS (16)	Enterococcus (2)
Ampicillin	13 (61.9 %)	10 (62.5 %)	1 (50 %)
Amoxyclav	17 (80.95 %)	11 (68.75 %)	1 (50 %)
Ceftriaxone	19 (90.47 %)	10 (62.5 %)	0 (0%)
Cephalexin	20 (95.23 %)	12 (75 %)	0 (0%)
Clindamycin	17 (80.95 %)	13 (81.25 %)	0 (0%)
Erythromycin	14 (66.66%)	12 (75%)	0 (0%)
Gentamicin	20 (95.23%)	16 (100%)	1 (50%)
Piperacillin	16 (76.19%)	7 (43.75 %)	1 (50%)
Vancomycin	21 (100%)	16 (100%)	2 (100%)
Cephoxitin	19 (90.47%)	10 (62.5%)	1 (50%)
Doxycycline	21 (100%)	14 (87.5%)	2 (100%)
Linezolid	21 (100%)	15 (93.75%)	2 (100%)

Table 4: Antimicrobial Susceptibility Pattern of Gram Negative bacilli

Antibiotics	E.coli (7)	E.cloacae (7)	P.mirabilis (3)	E.aerogenes (2)	P. vulgaris (1)	Acinetobacter spp (1)	Citrobacter spp(1)
Amikacin	7 (100%)	7(100%)	3 (100%)	1(50%)	1(100%)	0	1(100%)
Ampicillin	2(28.57)	0	2(66.66%)	0	1(100%)	0	1(100%)
Cefepime	7(100%)	6(85.71%)	3(100%)	0	0	0	1(100%)
Cefixime	4(57.14%)	3(42.85%)	3(100%)	0	0	0	1(100%)
Ceftriaxone	7(100%)	5(71.42%)	(100%)	1(50%)	0	0	1(100%)
Cefoperazone + sulbactam	7(100%)	6(85.71%)	3 (100%)	1(50%)	0	0	1(100%)
Cefuroxime	4(57.14%)	3(42.85%)	3(100%)	0	0	0	0
Cephalexin	3(42.85%)	2(28.57%)	2(66.66%)	0	0	0	0
Ciprofloxacin	2(28.57%)	3(42.85%)	3(100%)	0	1(100%)	0	1(100%)
Gentamycin	7(100%)	4(57.14%)	3(100%)	0	1(100%)	0	1(100%)
Netilmycin	7(100%)	7(100%)	3(100%)	0	1(100%)	1(100%)	1(100%)
Doxycycline	6(85.71%)	3(42.85%)	1(33.33%)	0	0	1(100%)	0
Meropenem	7(100%)	7(100%)	3(100%)	2(100%)	1(100%)	0	1(100%)

Table 5: Antimicrobial Susceptibility Pattern of *Pseudomonas species*

Antibiotics	Percentage Susceptibility
Aztreonam	76.27%
Carbencillin	35.59%
Cefoperazone	76.27%
Ceftazidime	71.18%
Cefoperazone Sulbactam	83.05%
Gentamycin	38.98%
Imipenam	98.30%
Piperacilin Tazobactum	86.44%
Tobramycin	52.54%
Meropenam	93.22%
Polymyxin –B	94.91%
Colistin	93.22%
Levofloxacin	37.28%

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