A STUDY OF AEROBIC BACTERIOLOGICAL PROFILE ALONG WITH ANTIBIOTIC SUSCEPTIBILITY PATTERN IN CHRONIC SUPPURATIVE OTITIS MEDIA

Microbiology

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ABSTRACT

Introduction: Chronic suppurative otitis media (CSOM) is a destructive and persistent disease with irreversible sequel and can proceed to serious intra and / or extra cranial complications. Causative agents of infection include bacteria, fungi and viruses with bacteria being the commonest cause. Hence bacteriological studies of CSOM are important for determining effective antibiotic choice and surveillance of bacterial patterns and their relative sensitivities.

Materials and Methods: Total 157 patients having CSOM were included in the study. Ear discharge was processed with standard Microbiological procedures.

Results: On aerobic culture *Staphylococcus aureus* 58 (36.96%) was the most common isolate followed by *Pseudomonas aeruginosa* 35 (21.21%). Fungal culture yielded 19 (11.2%) isolates.

Conclusion: Evaluation of microbiological pattern and antibiotic sensitivity of isolates is necessary to decrease the potential risk of complications of CSOM by early institution of appropriate treatment.

KEYWORDS

Chronic Suppurative Otitis Media, Aerobic bacteriological profile, Antibiotic susceptibility test

INTRODUCTION

Otitis media is common childhood illness second in frequency only to viral upper respiratory tract infection. Chronic suppurative otitis media (CSOM) is a result of an initial episode of acute otitis media (AOM) and is characterised by a persistent discharge from the middle ear through a tympanic perforation. It is an important cause of hearing loss particularly in developing world.³ CSOM is a destructive and persistent disease with irreversible sequel and can proceed to serious intra as well as extra cranial complications.⁴ Causative agents of infection include bacteria, fungi and viruses with bacteria being the commonest cause⁵.

The microbiological flora of middle ear in chronic otitis media includes organisms like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and anaerobic bacteria.⁶

Treatment of CSOM should be guided by culture and suggest choices for instituting narrowest spectrum systemic antibiotics assuring a most effective and cost-effective protocol of treatment.⁷ Indiscriminate, haphazard and half-hearted use of antibiotics and poor follow up of patients has resulted in persistence of low grade infections. *In-vitro* antibiotic susceptibility pattern is very important for the clinicians to plan the general outline of treatment for a patient with CSOM.⁸

Hence bacteriological studies of CSOM are important for determining effective antibiotic choice and surveillance of bacterial patterns along with their relative sensitivities.⁸

The present study was undertaken to provide baseline data on aerobic bacteriological profile of CSOM in our area as this is the first of its kind study from this institute. The study would also provide information regarding antimicrobial susceptibility of the isolates to commonly used antibiotics in this institute.

MATERIALS AND METHODS

The study was conducted in Microbiology Department, at a tertiary care centre during January 2012 to June 2013. A total 157 patients of CSOM (including 12 bilateral cases; hence a total of 169 ear swabs) were examined. Clearance of the institutional ethical committee was obtained before initiation of the study. It was a prospective cross sectional study.

Patients of all age groups and both sexes attending outpatient

department (OPD) and those admitted in our tertiary care centre and clinically diagnosed as suffering from CSOM by ENT Surgeon were included in the study. Patients who have taken topical or systemic antibiotics in last 7 days were excluded from the study.

Sample collection⁹

Written consent of patient / parent of a minor patient was taken before collection of sample.

Patient's detailed history consisting of present complaints, treatment history, and history of investigations was taken.

The external auditory canal of discharging ear was cleaned with sterile cotton. Under all aseptic precautions discharge was collected with two cotton swabs with help of sterile ear speculum, taking care not to touch external ear canal. Swabs were transported in sterile containers to the microbiology laboratory; each specimen was labelled with patient's name, age, sex, registration number, laterality of ear.

Laboratory procedure

Gross appearance of the discharge including colour, odour and consistency were noted.

Microscopy^{10,11}

Primary smear was made from one of the swabs. Gram staining was done and observed under microscope and presence of organisms, pus cells were noted. Potassium hydroxide (KOH) mount was done from the same swab and observed under microscope.

Culture^{9,10,12,13}

Second swab was cultured on Blood Agar (BA), MacConkey agar (MA) and incubated aerobically at 37°C for 24 hours. The same swab was also inoculated on two Sabouraud's dextrose agar (SDA) one was incubated at 37° Celsius and other was kept at room temperature as per standard microbiological procedures.

Blood and MacConkey agar plates were observed for growth of microorganisms. If no growth was present plates were further incubated for next 24 hours, if still there was no growth then it was reported as sterile and plates were discarded. SDA slants were observed thrice weekly for four weeks before reporting sterile.

In case of growth colony / colonies were identified by colony characteristics, morphology, motility and biochemical reactions as per

standard guidelines.

Antibiotic susceptibility test¹⁴

It was done for all bacterial isolates by Kirby-Bauer disc diffusion method as per Clinical Laboratory Standards Institute (CLSI) guidelines. Following antibiotic discs procured commercially from Hi-media Laboratories Ltd. were used.

Antibiotic(Symbol-Concentration in mcg): Erythromycin(E-15), Clindamycin(C-2), Cefoxitin(CX-30), Ampicillin(AMP-10), Cotrimoxazole(COT-1.25/23.75), Linezolid(LZ-15), Tetracycline(TE-30), Vancomycin(VA-30), Ciprofloxacin(CIP-5), Gentamycin(GEN10), Ceftazidime(CAZ-30), Piperacillin(PI-100), Amikacin(AK-30), Aztreonam(AT-30), Cefepime(CPM-30), Imipenem(IPM-10), Piperacillin-tazobactam(PIT-100/10), Cefotaxime(CTX-30), Ceftazidim(CAZ-30).

Growth of *Staphylococcus aureus* on Blood agar and Antibiotic susceptibility test on Mueller-Hinton agar showing MRSA

RESULTS

A total of 157 patients having Chronic Suppurative Otitis Media were included. There were 12 patients having bilateral infection. Hence total 169 swabs (145 unilateral cases giving 145 swabs and 12 bilateral cases giving 24 swabs) were examined of which 14 swabs did not yield any growth. On aerobic bacteriological culture they yielded 165 bacterial isolates. While on fungal culture they yielded total 19 isolates.

Table No. 1: Age and Gender wise distribution

Age Group(Years)	Male		Female		Total	
	Number	%	Number	%	Number	%
<10	33	38.37	28	39.43	61	38.85
11-20	18	20.93	14	19.71	32	20.38
21-30	14	16.27	12	16.90	26	16.56
31-40	12	13.95	10	14.08	22	14.01
>40	9	10.46	7	9.85	16	10.19
Total	86	100	71	100	157	100

Chi Square Test = 0.063, $p > 0.05$ hence Not Significant

Out of 157 patients 86 (54.77%) were male and 71 (45.22%) were female showing slight male predominance. Above results also show that maximum number of cases of CSOM were below age of 10 years comprising of 38.85% of total cases.

Out of 157 cases, there were 116 (73.88%) of cases were from rural area while 41 (26.11) cases were from urban area, suggesting higher prevalence of CSOM in rural area which was statistically significant.

Table No. 2: Aerobic bacteriological organisms Isolated

Organism		Number of Isolates	Percentage
Gram Positive	<i>Staphylococcus aureus</i>	58	35.15
	Coagulase negative staphylococci	9	5.45
	<i>Pseudomonas aeruginosa</i>	35	13.94
	<i>Proteus mirabilis</i>	23	5.45
	<i>Proteus vulgaris</i>	9	10.30
Gram Negative	<i>Klebsiella pneumoniae</i>	17	4.24
	<i>Escherichia coli</i>	7	3.03
	<i>Citrobacter freundii</i>	5	1.21
	<i>Acinetobacter baumannii</i>	2	1.00
	Total	165	100

Out of 165 islets, 67 (40.60%) were Gram positive while 98 (59.39%) were Gram negative. Above table shows *Staphylococcus aureus* 58 (36.96%) as most common isolate followed by *Pseudomonas aeruginosa* 35 (21.21%).

Table No. 3: Outcome of fungal culture

Isolates	Number	Percentage
<i>Candida albicans</i>	11	57.89
<i>Aspergillus niger</i>	5	26.32
<i>Aspergillus flavus</i>	3	15.79
Total	19	100

Out of total 19 swabs showing fungal growth, there were 11 isolates of *Candida albicans*, five isolates of *Aspergillus niger* and three isolates of *Aspergillus flavus*.

Table No. 4: Susceptibility of *Staphylococcus aureus*

Organism	Antibiotic- number and percentage of susceptible isolates									
	E	CD	CX	AMP	COT	LZ	TE	VA	CIP	GEN
(no)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
MSSA	36	44	51	16	30	51	24	51	32	39
(51)	(70.59)	(86.27)	(100)	(31.37)	(58.82)	(100)	(47.06)	(100)	(62.74)	(76.47)
MRSA	2	5	0	0	3	7	3	7	4	5
(7)	(28.57)	(71.43)	(0)	(0)	(42.85)	(100)	(42.85)	(100)	(57.14)	(71.43)
MSCO NS	4	7	8	2	4	8	2	8	3	6
(8)	(50)	(87.5)	(100)	(25)	(50)	(100)	(25)	(100)	(37)	(75)
MRCO NS(1)	1(100)	1(100)	0(0)	0(0)	0(0)	1(100)	0(0)	1(100)	1(100)	0(0)

Table No.4 shows that out of 58 isolates of *Staphylococcus* spp. 51 were methicillin sensitive *Staphylococcus aureus* (MSSA) while seven were methicillin resistance *Staphylococcus aureus* (MRSA). Out of nine isolates of Coagulase negative staphylococci (CONS), eight were methicillin sensitive CONS while there was one isolate of methicillin resistant CONS.

All of them showed 100% susceptibility to vancomycin and linezolid while least susceptibility was noted to tetracycline and ampicillin.

Table No. 5: Antibiotic susceptibility of *Pseudomonas aeruginosa*

Antibiotic	Susceptibility	
	Number	Percentage
CIP	24	68.57
GEN	26	74.28
CAZ	29	82.86
PI	27	77.14
AK	28	80
AT	24	68.57
CPM	29	82.86
IPM	31	88.57
PIT	30	85.71

It showed highest susceptibility to imipenem followed by piperacillin-tazobactam, ceftazidime and cefepime. It was least susceptible to ciprofloxacin.

Table No.6: Antibiotic susceptibility of *Enterobacteriaceae* spp.

Organism (no)	Antibiotic- number and percentage of susceptible isolates										
	AMP (%)	COT (%)	CIP (%)	GEN (%)	PI (%)	AK (%)	AT (%)	IPM (%)	PIT (%)	CTX (%)	TE (%)
<i>Proteus Mirabilis</i> (23)	5	11	18	15	17	18	15	20	19	17	10
	(22)	(48)	(78)	(65)	(74)	(78)	(65)	(83)	(74)	(43)	
<i>Proteus Vulgaris</i> (9)	2	4	7	6	8	7	6	7	8	7	3
	(22)	(44)	(78)	(67)	(89)	(78)	(67)	(78)	(89)	(78)	(33)
<i>Klebsiella pneumoniae</i> (17)	4	9	12	12	13	14	12	15	14	11	10
	(24)	(53)	(71)	(71)	(76)	(82)	(71)	(88)	(82)	(65)	(59)
<i>E coli</i> (7)	2	4	5	5	5	6	4	6	6	4	3
	(29)	(57)	(71)	(71)	(71)	(86)	(57)	(86)	(86)	(57)	(43)

<i>Citrobacter freundii</i> (5)	1 (20)	2 (40)	3 (60)	3 (60)	4 (80)	4 (80)	3 (60)	4 (80)	4 (80)	2 (40)	2 (40)
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Table No.6 shows antibiotic susceptibility of *Enterobacteriaceae* species in which maximum sensitivity was seen to imipenem, piperacillin- tazobactam, amikacin while least sensitivity was seen to co-trimoxazole and ampicillin.

DISCUSSION

Present study was undertaken to evaluate aerobic bacteriological and fungal profile in chronic suppurative otitis media (CSOM) and to study their antimicrobial susceptibility.

In our study, higher prevalence of CSOM in males than females can be an incidental finding and there is no gender predilection as mentioned by Ayson *et al.*⁷ and Shyamala and Reddy¹⁵.

Most common age group affected in present study was between 0 – 10 years, comprising 61 (38.85%) of total patients followed by 11-20 years (20.38%). Thus a total of 59.23% of cases were below 20 years. This is consistent with Poorey and Iyer⁴ (0-10yrs), Agrawal *et al.*¹⁶ (0-20yrs), Shyamala and Reddy¹⁵ (0-20yrs), Prakash *et al.* (0-20yrs) and Shazia and Rao¹⁷ (5-20yrs).

In present study, 73.88% of the patients are from rural area, as compared to 26.11% from urban area. The difference is statistically significant as most of the patients coming to our institute are from rural area. Other authors who had predominant rural population in their study were Agrawal *et al.*¹⁶ and Kumar and Seth¹⁸.

In present study, Gram negative organisms (59.4%) outnumbered Gram positive (40.6%) organisms. This is in agreement with Malkappa *et al.*¹⁹ Motayo *et al.*⁶ and Wariso and Ibe *et al.*²⁰.

In present study, the most common organism isolated was *Staphylococcus aureus*, which were 58 in number constituting 35.15% of the total isolates. These findings are consistent with Agrawal *et al.*¹⁶, Kumar and Seth¹⁸, Shyamala and Reddy¹⁵, Nikakhlagh *et al.*⁶, Loy *et al.*²¹

We isolated nine Coagulase negative staphylococci (CONS) which was 5.45% of total isolates. Agrawal *et al.*¹⁶ (6.08%), Shazia and Rao¹⁷ (6.7%) were having similar findings.

In present study *Pseudomonas aeruginosa* was found as the second most common isolates with total number of isolates being 35 (21.21% of total isolates). Our findings are consistent with Singh *et al.*²² (24%) and Prakash *et al.*³ (19.89%).

In our study we recovered 32 isolates of *Proteus* spp. out of which 23 were that of *Proteus mirabilis* while nine were *Proteus vulgaris*. *Proteus* spp. accounted for 19.30% of total isolates. Our findings are consistent with Kusuma *et al.*²³ (16.1%), Ashish *et al.*²⁴ (20.6%).

We recovered 17 isolates of *Klebsiella pneumoniae* species, 10.30% of the total isolates. Similar findings were recorded by Singh *et al.*²² (8%), Kumar and Seth¹⁸ (9.1%), and Prakash *et al.*³ (9.42%).

On mycological study of ear discharge we found a total of 19 samples that yielded fungal growth; 11.24% of total number of samples, constituting 11 isolates of *Candida albicans*, five isolates of *Aspergillus niger*, three isolates of *Aspergillus flavus*. Our findings are consistent with Vishwanath *et al.*²⁵ (9.6%) and Prakash *et al.*³ (12.25%).

Antibiotic susceptibility pattern of bacterial isolets

***Staphylococcus aureus*:** In present study, there were 51 isolates of Methicillin sensitive *Staphylococcus aureus* (MSSA) and 7 isolates of Methicillin Resistant *Staphylococcus aureus* (MRSA).

Antibiotic susceptibility pattern of MSSA

In present study, 100% sensitivity was found to linezolid, vancomycin. This is in consistency with various studies.^{8,16} while Kumar and Sheth¹⁸ less sensitivity

In present study, 86.27% of the isolates were sensitive to clindamycin. These findings are consistent with some studies^{22,26}.

Least sensitivity was found to ampicillin this is in consistency with some studies^{7,16}

Antibiotic susceptibility pattern of MRSA

In present study, seven isolates of MRSA were recovered. They showed 100% sensitivity to linezolid and vancomycin and their sensitivity to clindamycin and gentamycin was 71.42% each followed by co-trimoxazole and tetracycline 42.85% each. It showed lower sensitivity to erythromycin (28.57%). All the strains were resistant to ampicillin and cefoxitin. These findings are consistent with findings of Bansal *et al.*²⁷

In present study good sensitivity is shown to non beta-lactam antibiotics this may be due to most of our patients are those attending outpatient department and possibly having community acquired infection.

Antibiotic susceptibility pattern of CONS

In present study, there were 9 isolates of CONS were isolated including 1 isolate of MRCONS. All isolates were sensitivity to vancomycin and linezolid while least sensitivity was shown to tetracycline and ampicillin. These findings are consistent with findings of some studies^{22,19,26,21}

Antibiotic susceptibility pattern of *Pseudomonas aeruginosa*

In present study, *Pseudomonas aeruginosa* 35 (21.21%) was found to be the second most common isolate. It showed highest sensitivity to imipenem (88.57%) followed by piperacillin –tazobactam (85.71%), ceftazidime and cefepime (82.86%). It was least sensitive to ciprofloxacin (68.57%). findings similar to our study were reported by some authors^{3,16,27}. While Shyamala and Sheth¹⁵ reported higher incidence of resistance.

Antibiotic susceptibility pattern of *Proteus* spp.

In present study *Proteus* spp. showed highest sensitivity to imipenem, piperacillin-tazobactam (84.37% each), followed by piperacillin, amikacin and ciprofloxacin (78.12% each). Moderate sensitivity was found in co-trimoxazole (46.87%) and tetracycline (40.63%). While Vishwanath *et al.* reported 100% sensitivity to ciprofloxacin, co-trimoxazole, amikacin and gentamycin. Shyamala and Reddy¹⁵ noticed resistance to most of the drugs

Antibiotic susceptibility pattern of *Klebsiella pneumoniae*

In present study, *Klebsiella pneumoniae* showed highest sensitivity to imipenem (88.2%), followed by amikacin and piperacillin-tazobactam (82.35%). It was moderately sensitive to co-trimoxazole and tetracycline. It showed resistance to ampicillin with sensitive strains being only 23.25%. Kumar and Sheth¹⁸ noticed 100% sensitivity to Piperacillin, Piperacillin-Tazobactam, ciprofloxacin while Shyamala and Reddy¹⁵ noticed resistance to most of the drugs.

Overall good susceptibility was noted to most of the antibiotics as most of the patients in present study were those attending outpatient department and probably had community acquired infection. Majority of patients were from rural areas where exposure to antibiotics is minimal.

CONCLUSION

Our study concludes that continuous and periodic evaluation of microbiological pattern and antibiotic sensitivity of isolates is necessary to decrease the potential risk of complications of CSOM by early institution of appropriate treatment. Our study will help clinicians to decide the empirical antibiotic therapy in management of CSOM patients till reports of culture and sensitivity are available to them. In fact, culture facilities are not available in many of the villages from our area in such conditions also clinicians will be helped by our study findings.

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