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Aerobic Bacterial Isolates and Their Antibiotic Susceptibility Pattern in Chronic Suppurative Otitis Media among Patients Attending a Tertiary Care Centre in Bikaner, Rajasthan

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ABSTRACT

INTRODUCTION: Chronic Suppurative Otitis Media (CSOM) has been a source of tremendous health predicament since time immemorial and even today it is immensely intricate both for patients and otologist to deal with.
AIM: The study was conducted with the aim to isolate and identify the bacteria causing Chronic Suppurative Otitis Media in patients attending the ENT department, PBM Hospital, Bikaner to know local pattern aerobic microbes.
MATERIALS AND METHOD: A cross-sectional, descriptive study was done in 100 patients of Chronic Suppurative Otitis Media with ear discharge of more than 6 weeks, attending ENT Department, PBM, Bikaner.
RESULTS: Pseudomonas aeruginosa was commonest organism isolated (57.14%), followed by Staphylococcus aureus which was isolated in 19.04% and Klebsiella species in 7.14% of cases. Fungus as a single isolate was found only in 10.71% of cases.
CONCLUSION: It is concluded from present study that isolation of various aerobic and fungal isolates shows that different conditions of CSOM should be differentiated on microbiological grounds therefore continuous and periodic surveillance of microbiological pattern is desirable in all cases of CSOM.

KEYWORDS: Aerobic Bacterial Isolates, Chronic Suppurative Otitis, Antibiotic Susceptibility, Pseudomonas aeruginosa

INTRODUCTION

Chronic Suppurative Otitis Media (CSOM) has been a source of tremendous health predicament since time immemorial and even today it is immensely intricate both for patients and otologist to deal with. It is a persistent disease of the middle ear, which is capable of causing severe destructive sequelae with the manifestation of deafness, pus discharge and may lead to permanent perforation.[1] Despite advancements in public health and medical care, CSOM is still prevalent.[2]

Incidence of CSOM is greater in developing countries, especially among lower and middle socio-economic society because of malnutrition, poor hygiene, overcrowding and lack of adequate health care systems.[3]

The disease is mainly classified into two types: tubotympanic and attico-antral depending upon which part of the tympanic membrane is affected, whether the pars tensa or the pars flaccida. Tubo - tympanic is also known as the safe type, due to rarity of serious complications and attico-antral as unsafe type because of associated complications.[4]

The infection is usually bacterial in origin, however, viral and fungal agents have also been isolated. Decher and Daum showed that both single and mixed infections of Pseudomonas, Staphylococcus, Proteus and E. coli are important in early stages of CSOM but after a long time Pseudomonas aeruginosa tends to predominate.[5]

Fungal infections are also possibly related to the cases of CSOM as fungi thrive well in moist conditions like pus but the bacterial flora has been the main focus of attention for many authors, and very little is known about their mycological aspects, but the importance of which has been on an increase in the recent years because of the excessive use of broad spectrum antibiotics, immunosuppressant medications like corticosteroids and cytotoxic chemotherapy and an increase in the number of conditions that lead to deterioration of the immune system.[2,4] Most common fungi being Aspergillus spp. and Candida spp.[2]

Keeping the above stated scenario in view, the first line management of CSOM is initiated empirically

on the basis of known bacteriological profile. This study was conducted with the aim of isolation and identification of the bacteria causing Chronic Suppurative Otitis Media in patients visiting the ENT department, PBM Hospital, Bikaner, Rajasthan to know local pattern aerobic microbes.

MATERIALS AND METHOD

The study was a Cross-sectional, Descriptive, Observational type of study.

Study population

Consecutive consenting patients of CSOM with ear discharge of more than 6 weeks, attending ENT Department, PBM, Bikaner.

Sample size determination

The sample was determined by using the formula

$n = 4pq/d^2$

Where,

p= 5.5% , d=5% and q=1-p

Upon calculation,

n = 83.16

For the better precision and power of the study, a sample size of 100 people was taken for the study. Adult and paediatric patients of both the sexes and all age groups with the ear discharge of more than 6 weeks duration were included in the study, while patients with cleft palate, patients on systemic and topical antibiotics, patients with acute suppurative otitis media, patients on follow up, patients /parent /care giver who did not give his or her consent to participate in the study were excluded.

Source of material(s)

The present study was conducted in the Department of Microbiology & Immunology, PBM hospital, Bikaner, during the time frame of January to December, 2016. A total of 100 non- duplicate pus samples from CSOM cases were included in the study. Isolates from both in-patients and out-patients were considered, with no repetition of the same case. Institutional Ethical clearance was obtained. Data regarding age, sex, patient location, history of any clinical illness was obtained from the requisition form submitted to Department of ENT, PBM Hospital, Bikaner.

Method for isolation & identification of aerobic bacterial isolates:

The scheme for isolation and identification of aerobic bacterial isolates from clinical specimen is given as :-

1. Smear was prepared from the pus swabs collected, and was stained by Gram staining.
2. Stained smear was examined for presence of any epithelial cells, pus cells, and the type of microbial flora.
3. Streak culture method was employed for sample inoculation on Blood agar, Nutrient agar, Chocolate agar, and MacConkey's agar.
4. Culture plates were incubated aerobically at ambient temperature of 37°C for maximum of 48 hours; before declaring it as culture negative.
5. Plates were observed for any bacterial growth within 24 hours along with colony characteristics of growth of isolates on Blood agar (for haemolysis); Chocolate agar; Nutrient agar (pigment) and MacConkey agar (lactose and non lactose fermentation).
6. For mixed culture growths, sub culture was done on blood agar and Mac Conkey agar, and then incubated for 18-24 hours aerobically at 37°C; where ever required.
7. Gram's staining was performed from isolated colonies, and observed for morphology of bacteria along with Gram's reaction, under oil immersion of bright field microscope.

RESULTS

Amongst the 100 cases of CSOM which were studied, 29 cases were in the age group of 11-20 & 21-30 years, followed by 13 CSOM cases in 31-40 years, 10 cases in 41-50 & below 10 years age group, 4 cases in 61-70 years, 3 cases in 51-60 age group and only 2 cases in the age group of 71-80 years. A total of 54 CSOM cases were found in males and 46 in females. Overall, incidence of CSOM was more in males than females. The ratio of female: male was 1:1.17.

In the current study, left side ear was more commonly involved, irrespective of the gender. Among the 54 male patients; left ear was affected 51.85% of the cases, and the right ear in 38.88%. Out of 46 female patients left ear was affected in 50% and right ear in 36.95%. Bilateral involvement

was observed in 9.25% of males and 13.04% of females. Clinically, 65% of our CSOM cases were Tubotympanic type followed by Attico-antral type(35%).

In the present study, growth of microorganisms was seen in 92% of the processed samples and 8% didn't show any growth (Table 1).

Out of 100 processed samples, growth of mono-microbial kind organism was observed in 84% and 08% were poly-microbial. 08% of the samples had no growth (Table 2).

From the total 92 culture positives; 91 isolates were bacterial and 09 were fungal (Table 3).

Out of 92 culture positive samples, Pseudomonas aeruginosa was the commonest organism to be isolated with the rate of 52%, followed next by Staphylococcus aureus which was isolated in 21% of CSOM cases and Klebsiella species in 09% of the CSOM cases. Acinatobacter and CONS species were isolated in 3% of the CSOM cases. Escherichia Coli were isolated in 02% cases and Proteus species were isolated in 01% cases. Both males and females showed similar trends in bacterial isolate. Total Fungal elements were found in 09% of cases (Table 4).

Out of 84 single isolates, Pseudomonas aeruginosa was commonest organism isolated (57.14%), followed by Staphylococcus aureus which was isolated in 19.04% and Klebsiella species in 7.14% of cases. Fungus as a single isolate was found only in 10.71% of cases (Table 5).

Out of total 08 multiple isolates Pseudomonas aeruginosa + Staph aureus species was seen in 37.50% cases followed by Klebsiella spp. + Acinatobacter spp. in 25% of cases. Pseudomonas aeruginosa was the commonest organism isolated in patients of tubotympanic type of CSOM (35.38%) followed by Staphylococcus aureus in 18.46% and Klebsiella species in 6.15% of cases. In 12.30% of cases of tubotympanic type there was no organism isolated (Table 6).

In patients of Attico-antral type of CSOM Pseudomonas aeruginosa outnumbered all organisms, where it was positive in 71.42% of cases followed by Staphylococcus aureus (11.42%) and Klebsiella species (5.71%). All the samples taken from patients of Attico-antral disease were positive for bacterial isolate (Table 7).

DISCUSSION

In the present study the highest percentage of CSOM cases were observed in the second and third decade of life i.e. 29% each followed by fourth decade of life where numbers of cases were 13%. These findings were in accordance with studies conducted by Ahmed S et al.[6], Tan AL et al. [7], Kumar H et al.[1], and Srivastava et al.[2], where maximum number of CSOM patients were in the second and third decade of life. The effect of age on the prevalence of Otitis media varies from one study to another.[9] Several studies on CSOM were done among children and most developing countries have predominantly young population,[10] in whom CSOM is most prevalent; CSOM can affect both paediatric and adult groups.[11]

In present study, CSOM was more commonly found in males (54%) in comparison to (46%) females with the male to female ratio 1.17:1. The findings of the present study was consistent with studies conducted by Parveen S et al.[12], Nikakhlagh S et al. [13], Gulati et al.[14], Shyamala R et al.[15], and Ayson et al. [16] who also reported male predominance with 56%, 54%, 61%, 57% and 59.37% respectively. In contrast, female preponderance was reported by Prakash M et al.[17], Hirapure PV et al.[18] with 53.92%, 55%, 54.76% cases.

In the present study a total of 100 processed samples, 92(92%) were culture positive and 8 (8%) samples were culture negative. Similar studies by various authors have reported frequency of culture positivity ranging in between 85% to 95%. Our culture positivity findings are in accordance with the study of Prakash M et al.[17], Parveen SS et al.[12], Shyamala R et al.[15] and Ahmad S et al.[6] where they have reported 93.75%, 88%, 93%, and 90.2% respectively. A low culture positivity was reported by Srivastava et al.[2] and Gulati et al.[14] (80.3% of cases). The difference rate of culture positivity may be affected by factors such as instillation of prior antibiotic therapy and presence of antimicrobial enzymes such as lysozymes alone or in combination with immunoglobulin's that suppress bacterial growth and infection with organisms such as Mycoplasma, Chlamydia, and

anaerobic bacteria which are difficult to grow using routine culture methods.[19]

It has been reported that polymicrobial infections are more severe then mono microbial infections.[10] In the present series of CSOM, the monomicrobial etiology was found in 84 (91.30%) and polymicrobial was noted in 08(08.69%). Our findings are similar with the studies of Agrawal et al.[5], Vaishnavi D et al.[19] and Srivastava et al.[2], where mono-microbial isolate were 90%, 86%, 80% and polymicrobial 10%, 14% and 19.3% respectively. In another studies done by Ayson et al.[16] and Parveen S et al.[20] mono-microbial growth was observed in 74%, 77.8% and 16.7% and 14% were positive for polymicrobial flora. The variation in number of isolates of various authors, may be due to the differences in the patient population studied, the changing pattern of disease process, increasing trend of using combined antimicrobials for the treatment of CSOM and geographical variation.[2,3]

Both Gram positive and Gram negative organisms are responsible for infection of middle ear. In the current study gram negative rods outnumbered the gram positive organisms in CSOM.[4]

Out of 91 bacterial isolates, gram negative bacilli were predominant isolates and accounted for 67 (73.62%) and gram positive cocci were 24 (26.37%). Our result is in agreement with study done by Kumar H et al.[1], and Sharma K et al.[21] who also had reported predominance of gram negative bacilli 59.74% and 60% respectively. While, Prakash M et al[17] and Vaishnavi D et al.[19], have reported predominance of gram positive cocci which was 41.25% and 41.66% respectively.

In our study, the predominant bacterial isolate was Pseudomonas aeruginosa in 52(57.14%) cases, followed by Staphylococcus aureus, second most common isolate in 21(22.10%) cases, followed by Klebsiella species, Acinetobacter species, Escherichia coli and Proteus species in 09(9.89%), 03(3.29%), 02(2.19%) and 01(1.09%) of cases and rest 03(3.29%) cases were CONS. These finding were similar to the reports by Kumar H et al.[1], and Hirapure PV et al.[18] who reported the same pattern of predominance of gram negative bacilli (59.74%) and revealed Pseudomonas aeruginosa to be most common organism (45.5%), and (28.35%) followed by Staphylococcus aureus (37.7%) and (24.37%) and Klebsiella species (8.9%) and (11.44%) respectively.

A Study by Sharma K et al.[21] also reported Pseudomonas aeruginosa as predominant organism (60%). No significant difference was found between the distribution pattern of various microorganisms in males and females. The occurrence of Pseudomonas aeruginosa as major offending organism can be attributed to several factors, as stated by Pollock et al.[22] that Pseudomonas survive in competition with other organism due to minimal nutritional requirement, relative resistance to antibiotics and antimicrobial products such as pyocyanin and bacteriocin. Contrary to our study, Prakash et al.[3], Parween N et al.[23] and Singh AH et al.[24] reported that Staphylococcus aureus as predominant isolate and this observation was in line with diversity of microbial flora of CSOM in colder regions as compared to tropical region as in our study where Pseudomonas was the more common isolate.[10] The frequency of Staphylococcus aureus in the infections of middle ear can be attributed to their pervasive ubiquitous nature, and high carriage of resistant strains in the external auditory canal and upper respiratory tract.[19]

In the present study Klebsiella species and E. coli were isolated from 9.80% and 2.19% cases and this observation was in line with studies of many previous investigators, namely Vaishnavi D et al.[19] and Loy at al.[7], where it was isolated in 11.4% and 7.5% of samples. In the present study Acinetobacter species and Proteus species were isolated from 3.29% and 1.09% cases and this is in agreement previous investigators Raghu Kumar KG et al.[25] where it was isolated in 4.44% and 4.44% of samples, however in a study conducted by Malkappa SK et al.[26] Acinetobacter and Proteus species were reported to be found in 2.38% and 6.35% of the cases, respectively.

Amongst the 08 polybacterial cultures; majority of association between bacterial combinations included Staphylococcus aureus along with Pseudomonas aeruginosa which was 37.50%. Second highest association was found between bacterial isolates i.e. with Klebsiella species and Acinatobacter species in 25%.Our study was consistent with Kumar H et al,[1] where Pseudomonas aeruginosa and Staphylococcus aureus was 50%.

In our study, Pseudomonas aeruginosa was the predominant isolate and showed sensitivity rate of 59.61% for Amikacin followed by 61.53% for Gentamicin. Similar findings were reported by Agrawal et al.[27] and Hirapure PV et al.[18] for Amikacin (87%) and Gentamicin (60%).

In present series, Staphylococcus aureus was the second most common isolate that showed 100% sensitivity to Linezolid, Vancomycin and Tetracycline. Singh AH et al.[24] reported similar results in their study where Staphylococcus aureus was highly sensitive to Ciproflaxacin (89%), Clindamycin (89%), Gentamicin (85%), Tetracycline (79%) and Cotrimoxazole (75%) with low sensitivity for Penicillin (21%). Agrawal et al.[27] also reported 100% sensitivity for Vancomycin and Linezolid, 68.1% for Gentamicin and 61.7% for Erythromycin which was similar to ours. In another study by Maji et al., a high sensitivity was reported for Gentamicin (87%), Ciprofloxacin (64.3%).[28] Srivastava et al. also reported high sensitivity for Aminoglycosides (55%), Cephalosporins (76%), Fluoroquinolones (83%).[2]

For Gram positive isolates in our study there was 100% sensitivity for Linezolid, Tetracycline, Vancomycin and followed by Gentamicin 91.66%, Amoxyclav 83.33%, Ciprofloxacin 75%, Erythromycin 66.66%, Cotrimoxazole 62.50% and Clindamycin 50%, These findings were consistent with the findings of Prakash M et al. where gram positive cocci were highly susceptible to Cephalosporins and Gentamicin.[17] Agrawal et al. showed 100% sensitivity for Vancomycin.[27] Srivastava et al.[2] also reported maximum sensitivity for Cephalosporin's and Flouroquinolones.[2] Agrawal et al.[27] also reported 100% sensitivity for Linezolid and Vancomycin which was similar to ours.

CONCLUSION

It is concluded from the present study that different conditions of CSOM should be differentiated on microbiological grounds by isolation of various micro-organisms and periodic surveillance of microbiological pattern is desirable in all cases of CSOM. In the current era, the emergence of resistance to antibiotics has become a common medically significant condition and human negligence is one of the factors responsible for the development of the same. Therefore, continuous and thorough evaluation of antibiotic pattern of microbiological isolates along with the sensible use of antibiotic medication is of supreme importance in prescribing corrective antibiotics for successful treatment of Otitis Media, thus, leading to minimum complications and emergence of resistant strains. Large sample size along with culture for anaerobes should be included in further studies. Hence for better management of CSOM, classification of infection on microbial basis as well as conduction of antibiotic sensitivity test of isolated organism are essential to come up with appropriate decisions regarding anti-microbial therapy that will eventually help in the eradication of the concerned pathogen.

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LEGENDS

CULTURE	NO OF CASES (%)
Positive	92 (92%)
Negative	08 (08%)
Total	**100**

Table 1. Distribution of CSOM cases based on culture positivity

ORGANISM	NO. OF CASES
Mono-microbial growth	84
Poly-microbial growth	08
No growth for any organism	08
Total	**100**

Table 2. Distribution of CSOM cases according to Morpho types

BACTERIAL ISOLATES	MALE (%)	FEMALE (%)	TOTAL (%)
Pseudomonas aeruginosa	28	24	52 (52%)
Staphylococcus aureus	12	09	21 (21%)
CONS	02	01	03 (03%)
Klebsiella spp.	07	02	09 (09%)
Escherichia coli	02	00	02 (02%)
Acinetobacter spp.	02	01	03(03%)
Proteus spp.	00	01	01 (01%)
Fungal elements	04	05	09 (09%)
Total	**57**	**43**	**100**

Table 3. Distribution of various isolation in CSOM (n = 100)

ORGANISM	NO. OF CASES (%)
Pseudomonas aeruginosa	48 (57.14%)
Staphylococcus aureus	16 (19.04%)
CoNS	03 (3.57%)
Klebsiella spp.	06 (7.14%)
Escherichia coli	01 (1.23%)
Proteus spp.	01 (1.23%)
Fungal elements	09 (10.71%)
Total	**84 (100%)**

Table 4. Distribution of single isolates in Chronic Suppurative Otitis Media

S No.	ORGANISM	CASES
1	Pseudomonas aeruginosa+ Staph aureus	03
2	Staph aureus+ Klebsiella spp.	01
3	Staph aureus + E coli	01
4	Pseudomonas aeruginosa + Acinatobacter spp.	01
5	Klebsiella spp. + Acinatobacter spp.	02
6	**Total**	**08**

Table 5. Distribution of multiple isolates in Chronic Suppurative Otitis Media

ANTIBIOTIC	PSEUDOMONAS AERUGINOSA (N=52)	KLEBSIELLA (N=09)	E. COLI (N=2)	ACINATOBACTER SPP. (N=3)	PROTEUS SPP. (N=1)	OVERALL SENSITIVITY OF GNB (N=67)
	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	
Amikacin	31 (59.61%)	8(88.8%)	2 (100%)	3 (100%)	1(100%)	45 (67.16%)
Gentamicin	32 (61.53%)	8 (88.8%)	2 (100%)	3 (100%)	1(100%)	46 (68.65%)
Polymyxin B	49(94.23%)	Not Applied	Not Applied	Not Applied	Not Applied	49 (94.23%)
Colistin	50(96.15%)	Not Applied	Not Applied	Not Applied	Not Applied	50 (96.15%)
Ampicillin + Sulbactam	Not Applied	8 (88.8%)	1 (50%)	3(100%)	1(100%)	13 (19.40%)
Piperacillin + Tazobactum	46 (88.46%)	8 (88.8%)	2 (100%)	3(100%)	1(100%)	60 (89.55%)
Ceftriaxone+ Sulbactum	38(73.07%)	5 (55.5%)	2 (100%)	2 (66.66%)	1(100%)	48 (71.64%)
Cefoperzone	37 (71.15%)	6 (66.6%)	1 (50%)	3(100%)	1(100%)	48 (71.64%)
Cefepime	33 (63.46%)	4 (44.4%)	2(100%)	3(100%)	1(100%)	43 (64.17%)
Ciprofloxacin	29(55.76%)	6 (66.6%)	2(100%)	2(66.6%)	1(100%)	40 (59.70%)
Co-Trimoxazole	Not Applied	5 (55.5%)	01(50%)	2(66.6%)	00	8(11.94%)
Aztreonam	36(69.23%)	2 (22.2%)	00	2(66.6%)	1(100%)	41 (61.19%)
Imipenem	52(100%)	9 (100%)	2 (100%)	3(100%)	1(100%)	67 (100%)

Table 6. Antibiotic sensitivity pattern of gram negative isolates in cases of CSOM

ANTIBIOTIC	STAPHYLOCOCCUS AUREUS (N=21)	COAGULASE NEGATIVE STAPHYLOCOCCAL SP. (N=3)	OVERALL SENSITIVITY OF GPC (N=24)
	Sensitive	**Sensitive**	**Sensitive**
Penicillin G	02 (9.52%)	01 (33.33%)	03(12.50%)
Amoxyclav	17 (80.95%)	03 (100%)	20 (83.33%)
Gentamicin	19 (90.47%)	03 (100%)	22 (91.66%)
Erythromycin	15 (71.42%)	01 (33.33%)	16 (66.66%)
Clindamycin	10 (47.61%)	02 (66.66%)	12 (50%)
Ciprofloxacin	17 (80.95%)	01(33.33%)	18 (75%)
Tetracycline	21 (100%)	03 (100%)	24 (100%)
Cotrimoxazole	13(61.90%)	02 (66.66%)	15(62.50%)
Vancomycin	21 (100%)	03 (100%)	24 (100%)
Linezolid	21 (100%)	03 (100%)	24 (100%)

Table 7. Antibiotic sensitivity pattern of Gram positive isolates in cases of CSOM