Original Article

Etiological Agents of CSOM and its Sensitivity Pattern

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ABSTRACT

Objective: The present study was undertaken with the aim to detect bacteria and fungi as aetiological agent in CSOM and to see susceptibility pattern of bacterial isolates to different antibiotics and to determine the beta lactamase production by the bacterial isolates.

Study Design: Experimental Study.

Place and Duration of Study: This study was conducted in the department of Microbiology, Basic Medical Sciences Institute, JPMC, Karachi, during the period of August 1998 to February 1999.

Materials and Methods: During this period, 110 patients were seen, of them 62 were male and 48 were female with male to female ratio of 1.3:1. Two swabs were taken from each patients ear, one was put immediately in to Brain Heart Infusion (BHI) broth and the other was inoculated on Sabouraud Dextrose Agar (SDA) slant. BHI was incubated for 2-4 hours and subcultured on blood agar, chocolate agar and MacConkey's agar plates. The Chocolate agar was incubated in 5-10% CO_2 atmosphere in a candle jar and they were incubated for 24 hours at 37ºC. SDA slant was incubated for 14 days at 37ºC. Isolates were identified by Gram staining and then confirmed by biochemical test. Fungus growth was stained in Lactophenol Cotton blue (LPCB) and identified microscopically.

Results: Amongst bacterial isolates gram negative rods (41) and Pseudomonas aeruginosa (45) were predominant. 47 gram positive cocci were seen, of those 37 were Staphylococcus aureus. Fourteen fungal isolates were recovered, all of them were found to be Aspergillus species. Bacterial isolates were tested for β-lactamase production the drug sensitivity was noted by disc diffusion method on Mueller Hinton agar. Ciprofloxacin and Enoxacin emerged as the most effective antibiotics. Tobramycin also showed good results against gram negative rods and Pseudomonas aeruginosa. Chloramphenicol, Clarithromycin and Minocycline showed good results against Staphylococcus aureus. And other gram positive cocci. Drug sensitivity of the fungi was not done. Micro-organisms showed least sensitivity to Ampicillin and Cotrimoxazole.

Conclusion: It is concluded that to achieve the maximum benefits of antibiotics, we must use them with discrimination and with the understanding of microbial population and with the knowledge of their indications and limitations. The indiscriminate, haphazard and halfhearted use of antibiotics and poor follow up of patients causes more harm than good.

Key Words: Chronic suppurative otitis media (CSOM), Chronic otitis media with effusion (COME).

INTRODUCTION

Otitis media represents one of the most prevalent forms of disease and is more common in childhood[1,2]. Two distinct forms are recognized. Chronic suppurative otitis media (CSOM) is a recurrent or persistent bacterial infection of the ear[3]. Chronic otitis media is defined as inflammation of the middle ear that lasts longer than three months. It is distinguished by hearing loss, mucopurulent otorrhea and a chronic perforation of tympanic membrane[4]. Chronic otitis media with effusion (COME) is an unresolving inflammation of the middle ear cleft with no otorrhea. It presents with persistent hearing loss and a middle ear filled with thick mucus. The tympanic membrane is intact but markedly retracte[5].

CSOM remains one of the most common childhood chronic infectious diseases worldwide[6,7] . In CSOM the inflammatory process is slow and insidious in its course, tends to be persistent and very often destructive with sometimes irreversible sequelae[2,3,6,7,8]. The aerobic bacteriology of CSOM is widely studied. Pseudomonas aeruginosa and Staphylococcus aureus being found to be the most commonly associated organisms. Staphylococcus epidermidis may be the aetiological agent responsible for purulent otitis media in a small number of cases[10]. Several fungi are found as a cause of inflammatory reaction in the external canal, or blocking the canal and Aspergillus (niger and flavus) leads the list among the isolates[6,10,11,12.]

This study was aimed to isolate the etiological agent of CSOM and COME are its susceptibility pattern in patients attending out door patient of ENT department.

MATERIALS AND METHODS

This study was conducted at the clinical material was obtained from 110 cases, they attended ENT outpatient Department, JPMC, Karachi with the complaints of chronically discharging ears. The study was approved

by Institutional Review Board of Basic Medical Sciences Institute and only consented patients were includes in this study.

Collection of samples: External ear canal was cleaned of cerumen and pus with a blunt curate when indicated. It was then swabbed with 70% alcohol and allowed to dry for two minutes[7]. The pus coming from the middle ear was collected by 'no touch technique' with a sterile swab with all aseptic precautions[8]. Two swabs were taken from each case. One swab was put into brain heart infusion broth, incubated for 2-4 hours at 37ºC, and then inoculated on blood agar, MacConkey agar (Oxoid Ltd, England) and Chocolage agar (Oxoid Ltd, England). The plates were incubated at 37ºC for 24 hours. The chocolate agar plates were incubated in a candle jar at 37ºC for 48 hours. Gram staining of all isolates was done and the organisms were identified by different biochemical tests.

The other swab was inoculated on Sabouraud dextrose agar ((Oxoid Ltd, England) slants and incubated at 37ºC. They were examined on alternate days for presence of growth. The isolates were identified by growth characteristics, and morphological details under microscope for nature of conidiophores and conidia. In cases, where the identification was difficult, slide culture was made to study further details for final identification. Species identification was not attempted. β-lactamase was detected by iodometric method.

Antimicrobial drug susceptibility: Antimicrobial susceptibility was performed on Muellar Hinton agar medium (Oxioid Ltd., England) using modified Kirby Bauer's disk diffusion method according to Clinical Laboratory Standard Institute (CLSI) guidelines. Antibiotic discs of Ampicillin(10ug), Aztreonam (30ug), Amoxil+clavulanic acid (20/10ug), Ceftazidime(30ug), Ciprofloxacin(5ug), Ceftriaxone (30ug), Chloramphenicol(30ug), Cephradine(30ug), Cefixime(30ug), Cotrimoxazole(1.25ug,23.75ug), Clarithromycin(15ug), Enoxacin(10ug), Gentamicin (10ug), Minocycline(30ug), Oxacillin(1ug), Polymaxin B(300ug), Piperacillin(100ug), Tobramycin(10ug), Vancomycin(30ug) Oxacillin (1ug) applied for detection of antibiotic susceptibility[14].

RESULTS

A total of 110 patients were included in the study, out of which 62 were males and 48 were females. The average age of males was 15.8±1.83 standard error of mean (SEM) over a range of 2-72 years and that of female was 18.98±1.88 over a range of 0.75-45 years. The female to male ratio was 1:1.13. The number of patients were predominant in the age group of 0-20 years.

Forty three patients had the duration of pus discharge, 43 patients had duration of 3-11 months, 53 had 1-5 years, 11 had 6-10 years and one each had 11-15 years, 16-20 years and 21-25 years.

The type and nature of otorrhea in 112 chronically discharging ears, was noted that 77 (68.7%) ears were actively discharging, 35 (31.2%) were wet. No dry ears were included in the study. Out of 112 ears the nature of pus discharge was purulent in 82 (73.2%) and 30 (26.7%) were mucopurulent.

Out of the 110 patients, 108 had unilateral ear infection, while only two patients had bilateral ear infection. Out of the total 112 swabs 104 (92.8%) were culture positive. A total of 150 bacterial and fungal isolates were recovered; 104 of 112 swabs (92.8%) were culture positive and 8 (7.1%) had no growth. The most common bacteria isolated were Pseudomonas aeruginosa (30%) followed Staphylococcus aureus (24.6%), Proteus species (13.9%), Klebsiella pneumoniae (4%), Staphylococcus epidermidis (3.3%) and Streptococcus pneumoniae (0.6%). Enterobacter) and Diphtheroids were present in 2% each. Streptococcus pyogenes (0.6%), Serratia marcescen (1.3%)s, Alcaligenes (1.3%) and Providencia (0.6%) .Forty seven percent of bacteria were recovered in mixed growth, while 52.9% in pure culture. Fungi were recovered in 14 (9.3%) cases and all of them were found to be Aspergillus species.

Table No.1: Prevalence of Various Bacteria in Monobacterial and Polybacterial Isolates

Organism	Mono bacterial Isolates	Poly bacterial Isolates	Total
Pseudomonas aeruginosa	25 (55.5%)	20 (44.4%)	45 (33%)
Staph.aureus	19 (51.3%)	18 (48.6%)	37 (27.2%)
Strep.pneumoniae	03 (75%)	01 (25%)	04 (2.9%)
Staph.epidermidis	-	05 (100%)	05 (3.6%)
Strept.pyogenes	-	01 (100%)	01 (0.7%)
Prteus mirabilis	7 (41.1%)	10 (58.8%)	17 (12.5%)
Proteus vulgaris	2 (50%)	2 (50%)	04 (2.9%)
Klebsiella	4 (66.6%)	02 (33.3%)	6 (4.4%)
E.coli	-	06 (100%)	06 (4.4%)
Serratia	-	02 (100%)	02 (1.4%)
Enterobacter	3 (100%)	-	3 (2.2%)
Alcaligenes	1 (50%)	1 (50%)	02 (1.4%)
Providencia	-	01 (100%)	01 (0.7%)
Diphtheroid	-	03 (100%)	03 (2.2%)
Total	64 (47%)	72 (52.9%)	136

Table No. 2: Prevalence of Beta Lactamase Producing Organisms in CSOM

Organism	β-lactamase Positive	β-lactamase Negative	Total
Staph.aureus	29 (78.3%)	08 (21.6%)	37
Pseudomonas aeruginosa	18 (40.0%)	27 (60.0%)	45
Klebsiella pneumoniae	03 (50.0%)	03 (50.0%)	06
Staph.epidermidis	03 (40.0%)	02 (40.0%)	05

Table No.3: Drug sensitivity pattern of commonly occuring bacterilal isolates in CSOM

Drug tests	Gram +ve bacilli (n=86)		Gram +ve cocci (n=47)		Total sensitivity %
	Gram –ve Enteric rods (n=41)	Pseudomonas species (n=45)	Staph. aureus (n=37)	Other Gram + cocci (n=10)	
Vancomycin	-	-	37 (100)	-	100
Ciproxin	31 (75.6)	42 (93.3)	26 (70.7)	9 (90)	81.2
Enoxacin	31 (75.6)	42 (93.3)	24 (64.8)	9 (90)	79.6
Aztreonam	28 (68.2)	36 (80)	-	-	74.4
Ceftazidime	29 (70.7)	39 (86.6)	18 (48.8)	7 (70)	69.9
Piperacillin	26 (63.4)	35 (77.7)	21 (51.6)	7 (70)	61.65
Tobramycin	14 (34.1)	39 (86.6)	18 (48.6)	5 (50}	55.6
Ceftriaxone	20 (48.7)	27 (60)	15 (40.4)	-	51.8
Clarithromycin	-	18 (48.6)	5 (50)		48.9
Gentamicin	19 (46.3)	23 (51.1)	14 (37.8)	-	47.3
Chloramphenicol	3 (7.3)	-	29 (78.1)	7 (70)	44.3
Oxacillin	-	-	15 (40.3)	-	40.5
Minocycline	5 (12.1)	4 (8.8)	21 (51.6)	7 (70)	27.8
Polymaxin B	2 (4.8)	20 (44.4)	-	-	25.5
Amoxil+clavulanic acid	8 (18.5)	-	10 (27)	3 (30)	21.8
Cephradine	-	-	7 (18.9)	1 (30)	21.8
Cefixime	14 (34.1)	4 (8.8)	4 (10)	6 (60)	21.03
Ampicillin	0	-	0	2 (20)	2.2
Cotrimoxazole	0	0	0	1 (10)	0.7

Table No.4: Ddrug Sensitivity Pattern of Pseudomonas Aeruginosa (n=45)

Drug tested	Sensitivity
Ciproxin	42 (93.3%)
Enoxacin	42 (93.3%)
Ceftazidime	39 (86.0%)
Tobramycin	39 (86.0%)
Aztreonam	36 (80.0%)
Piperacillin	35 (77.7%)
Ceftriaxone	27 (60.0%)
Gentamicin	23 (51.1%)
Polymyxin B	20 (44.4%)
Minocycline	4 (8.80%)
Cefixime	4 (8.80%)

The pattern of bacterial isolates were both polybacterial and few were monobacterial. The detail is given in table 1. Out of total 136 bacterial isolates 53 (38.9%) were beta lactamase producing bacteria (BLPB). Majority of Staphylococcus aureus (78.3%) were BLPB, 40% of Pseudomonas aeruginosa, 50% of Klebsiella pneumoniae, and 60% of Staphylococcus epidermidis were also found to be beta lactamase producers. Table,2. Out of 37 Staphylococcus aureus isolates 15 (40.54%) were methicillin resistant.

Staph aureus, Pseudomonas aeuroginosa and Aspergillus spp were most common pathogens causing CSOM. Ciprofloxacin Enoxacin have shown mostly susceptible antibiotic against Gram negative bacilli and Minocycline and Clarithromycin were effective against Gram positive cocci. Table 4,5.

Table No.5: Drug Sensitivity Pattern of Staphylococcus Aureus (n=37)

Drug tested	Sensitivity
Vancomycin	37 (100.0%)
Chloramphenicol	29 (78.3%)
Ciproxin	26 (70.7%)
Enoxacin	24 (64.8%)
Piperacillin	21 (56.7%)
Minocycline	21 (56.7%)
Clarithromycin	18 (48.6%)
Tobramycin	18 (48.6%)
Ceftriaxone	15 (40.5%)
Oxacillin	15 (40.5%)
Gentamicin	14 (37.8%)
Amoxicil clavulanic acid	10 (27.0%)
Cephradine	7 (18.9%)
Cefixime	4 (10.0%)

The antibiotic sensitivity in gram positive and gram negative bacteria is shown in table 3. There were a total of 136 bacterial isolates, 47 gram positive cocci, 96 gram negative rods and 3 diphtheroids species.

There were a total of 86 Gram negative and 47 Gram positive isolates. Ciproxin showed sensitivity in 73 Gram negative and 35 Gram positive isolates. The total sensitivity in 108 (81.2%). Enoxacin showed the similar pattern in Gram negative rods and in Gram positive. 33 isolates were sensitive. The overall sensitivity was 79.6%. Ceftazidime was sensitive in 68 Gram negative and 25 Gram positive isolates. The total sensitivity was in 93 (69.9%) isolates.

The commonly used drugs, Cephradine 21.2 %, Ampicillin 10% and Cotrimaxazole has shown 0.7% susceptibility against gram positive rods.

DISCUSSION

Chronic suppurative otitis media (CSOM) is a persistent and insidious disease that often leads to destructive changes and irreversible sequelae. Since the disease is very common and often overlooked or treated haphazardly, many complications may occur. For this reason the present study was planned to see the prevalence of bacteria and fungi causing CSOM, their susceptibility pattern, and beta lactamase production. It was hoped that such a study would give a guideline to the clinicians to select appropriate antibiotics with clinical correlation.

We studied the prevalence of different micro-organisms in CSOM irrespective of type of tympanic membrane perforation and our study is in line of agreement with other studies. The most common pathogens found in our study were *Pseudomonas aeruginosa* (30%) and enteric Gram negative rods (27.3%). Amongst the Gram positive isolates *Staph. aureus* was the most predominant isolate (24.6%) with *Staph. epidermidis* (3.3%), *Strep. pneumoniae* (2.6%) and *Strep. pyogenes* in few cases. The organisms recovered in the present study were 64 (47%) in pure culture, 72 (52.9%) in mixed growth. Amongst the fungi three (21.4%) were pure culture and 11 (78.5%) were in mixed growth with bacteria. The present study showed, 92.8% positive cultures

In a multicentre study carried out in Karachi by Anwarus Salam in 596 consecutive ear swabs specimens, Staph. aureus was present in 40.4%, *Pseudomonas* in 29.6%, Aspergillus in 6.2% and *Candida* in 3.1%,14.4% of swabs were culture negative[11].

In a study carried out at Gomal University D.I. Khan, the causative organisms of CSOM were found to be Pseudomonas aeruginosa (37%), Staph. aureus (27%), Strep. pyogenes (18%), Proteus (15%) and E.coli (3%) [13]. Munir lodhi et al, found Staphylococcus aureus and pseudomonas aeruginosa more common in CSOM cases in Multan in patients under 15 years age. The CSOM cases were more common in children and prevalence reducing with increase of age. This pattern of decrease in infection rate with increase of age is same as in our study. In our study Ciprofloxacin and Enoxacin are most susceptible drugs as compare to penicillin group of drugs in pseudomonas and other gram negative rods. Same results were also found by other researchers in children[14,15,16,17]

CONCLUSION

It is concluded that to achieve the maximum benefits of antibiotics, we must use them with discrimination and with the understanding of microbial population and with the knowledge of their indications and limitations. The indiscriminate, haphazard and halfhearted use of antibiotics and poor follow up of patients causes more harm than good.

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