ORIGINAL ARTICLE

Aerobic bacteriological profile and their antibiotic susceptibility in Chronic Suppurative Otitis Media in patients from Mangalore, Karnataka State

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ABSTRACT

Chronic suppurative otitis media is an infection of the middle ear and mastoid cavity. It is one of the most common causes of deafness. Low social-economic status, overcrowding, malnutrition and poor hygiene are the common risk factors and it commonly affects infants and children. The common pathogens responsible are *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Aspergillus species, Candida species* etc. Several studies similar to this have been done previously from various regions of our country and also from several parts of the world. There are no studies done recently from this region to determine the bacteriological profile of aerobic bacteria and their antibiotic susceptibility in this region. The study was done for six months and the sample size was 80. Ear swab culture was done on blood agar, MacConkey agar, and chocolate agar. The culture plates were processed and identification of the isolates and antibiotic susceptibility were done as per standard methods. Samples with bacterial growth were 66 (82.5%), fungal growth were 10 (12.5%) and 4 (5%) samples had no growth. There were 82 isolates of bacteria and fungi from 80 patients. The predominant bacteria were *Pseudomonas aeruginosa* with 31 (37.80%) isolates followed by *Staphylococcus aureus* 25 (30.49%). All 31(100%) *P. aeruginosa* isolates were sensitive to colistin and polymixin B, while 30 (96.8%) isolates showed sensitivity to piperacillin-tazobactam, cefoperazone-sulbactam and aztreonam. Only 21 (67.7%) isolates were sensitive to gentamicin, 23 (74.2%) isolates were sensitive to ciprofloxacin, whereas 24 (77.4%) isolates were sensitive to levofloxacin. All 25 (100%) *S. aureus* isolates showed sensitivity to vancomycin, teicoplanin and linezolid whereas 23 (92%) isolates showed sensitivity to amikacin, doxycycline and clindamycin. Only one isolate (4%) was sensitive to ampicillin whereas 8 (32%) were sensitive to amoxicillin-clavulanic acid *in vitro*. *P. aeruginosa* and *S. aureus* are the most common aerobic bacterial pathogens in chronic suppurative otitis media. *P. aeruginosa* showed 100% sensitivity to Colistin and Polymixin B followed by Piperacillin-tazobactam (96.8%), Cefoperazone-sulbactam (96.8%) and Aztreonam (96.8%). *S. aureus* showed 100% sensitivity to Vancomycin, Teicoplanin and Linezolid followed by Amikacin, Doxycycline and Clindamycin (92% each). Culture and sensitivity should be done in chronic suppurative otitis media before initiating antibiotic therapy.

Key words: Antibiotic, bacteria, CSOM, mangalore, susceptibility

INTRODUCTION

Chronic suppurative otitis media (CSOM) is an infection of the middle ear and mastoid cavity.[1] It is defined as persistent or intermittent infected discharge of more than three months duration through the perforated or non-intact tympanic membrane caused by bacteria, fungi and viruses resulting in inflammation of the mucosal lining that often results in partial or total loss of the tympanic membrane and the ossicles.[2] CSOM is one of the most common causes of deafness and can cause permanent perforation of the tympanic membrane. Complications of CSOM can be intra and/or extra cranial ranging from facial nerve paralysis, lateral sinus thrombosis, meningitis, brain abscess etc.[1,3-5] World Health Organisation (WHO) has estimated that CSOM prevalence in India, Tanzania, Solomon Islands, Guam, Australian Aborigines and Greenland is highest (>4%) and urgent attention is needed to deal with the health problem.[1] It has also resulted in over 28,000 deaths globally in the year 1990 due to various complications.[6] The common risk factors for CSOM are low socio-economic status, overcrowding, malnutrition and poor hygiene. Infants and children are commonly known to be affected with CSOM and it can affect their intellectual, linguistic and psychosocial developments.[1,7] The common aerobic bacteria causing CSOM are *Pseudomonas aeruginosa, Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* etc. The anaerobic bacteria include

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Bacteroides species, Peptostreptococcus species, Fusobacterium species, Propionibacterium species, Prevotella species, Porphyromonas species etc. Fungi like *Aspergillus species* (*Aspergillus niger*) and *Candida species* are also known to cause CSOM. Aetiological agents of CSOM can vary from one geographical area to the other.[1,2,5,8,9] Several studies similar to this have been done previously from various regions of our country and also from several parts of the world. There are no studies done recently from this region except for one study which was done 23 years ago and another study on anaerobes done 18 years ago.[10,11] The aim of this study was to determine the bacteriological profile of aerobic bacteria and their antibiotic susceptibility in this region.

MATERIALS AND METHODS

The study was done for a period of six months from June 2013 to November 2013. Patients attending as outpatients of ear, nose & throat department at various clinics and hospitals in Mangalore, Dakshina Kannada district, Karnataka State, India were included in the study. Patients whose ear condition satisfied the definition of CSOM, were included in the study. Only unilateral CSOM cases were included in the study. All types of patients irrespective of age group, prior antibiotic therapy or diabetes mellitus state were included in the study.

Ear swab was collected from the patients using a sterile swab. Two swabs were collected from each patient. The samples were processed at various reputed microbiological laboratories. One swab was subjected to Gram staining. The second swab was used for aerobic culture. Cultures were done on blood agar, MacConkey agar and chocolate agar. The culture plates were processed and identification of the isolates were done as per standard methods.[12,13] The antibiotic susceptibility for bacterial isolates was done by modified Kirby-Bauer method as per the Clinical Laboratory Standards Institute guidelines (CLSI).[14] Antibiotics used for testing were Ampicillin (AMP), Amoxicillin-Clavulanic Acid (AMC), Cefazolin (CZO), Cefuroxime (CXM), Ceftriaxone (CEF), Ceftazidime (CAZ), Cefepime (CPM), Cefoxitin (FOX), Piperacillin-Tazobactam (TZP), Ceforperazone Sulbactam (CSL), Aztreonam (ATM), Gentamicin (GEN), Amikacin (AMK), Ciprofloxacin (CIP), Levofloxacin (LVX), Trimethoprim-Sulphamethoxazole (SXT), Doxycycline (DOX), Azithromycin (AZM), Clindamycin (CLI), Vancomycin (VAN), Teicoplanin (TEC), Linezolid, (LNZ), Colistin (COL), Polymixin B (POL) Imipenem (IMP) and Meropenem (MEM). FOX was used for screening Methicillin-resistant *Staphylococcus aureus* (MRSA). Extended spectrum beta lactamase (ESBL) and inducible CLI resistance detection were also done as per the CLSI guidelines. The antibiotic susceptibility data was analysed using the WHO, WHONET software.[15]

RESULTS

There were 80 patients who fulfilled the inclusion criteria in this period. No formula was used to determine the sample size. There were 82 isolates of bacteria and fungi from 80 patients. Samples with bacterial growth were 66 (82.5%), fungal growth were 10 (12.5%) and 4 (5%) samples had no growth. Out of the 82 isolates, bacterial isolates were 71 (86.59%) and fungal isolates were 11 (13.41%). Mixed bacterial growth with two isolates were seen in 5 (6.25%) samples, mixed fungal growth was seen in 1 (1.25%) and mixed bacterial and fungal growth in 3 (3.75%) samples. Out of the 82 isolates, 42 (51.22%) isolates were Gram negative bacteria, 29 (35.37%) were Gram positive bacteria and 11 (13.41%) were fungi. The details of the bacterial and fungal isolates with their number and percentages are shown in Table 1. The predominant bacteria were *P. aeruginosa* with 31 (37.80%) isolates followed by *S. aureus* with 25 (30.49%) isolates. The Gram positive bacteria isolated in order of their predominance were *S. aureus*, *Streptococcus pneumoniae, Group A Streptococci (Streptococcus pyogenes), Beta Haeamolytic Streptococci (Non-Group A)* and *Enterococcus faecalis*. The Gram negative bacilli isolated in order of their predominance were *P. aeruginosa, E. coli, Klebsiella pneumoniae, Enterobacter species, P. species* (non-*aeruginosa species), Proteus mirabilis, Acinetobacter species* and *Moraxella species*. The antibiotic susceptibility for the bacterial isolates are given in Table 2 and Table 3. *Aspergillus niger* eight isolates (9.76%) was the predominant fungus followed by *Candida albicans* two (2.44%) and *Aspergillus fumigatus* one (1.22%). All 31 (100%) *P. aeruginosa* isolates were sensitive to COL and POL, while 30 (96.8%) isolates showed sensitivity to TZP, CSL and ATM. Only 21 (67.7%) isolates were sensitive to GEN, 23 (74.2%) isolates were sensitive to CIP while 24 (77.4%) isolates were sensitive to LVX. All 25 (100%) *S. aureus* isolates showed sensitivity to VAN, TEC and LNZ whereas 23 (92%) isolates showed sensitivity to AMK, DOX and CLI. Only one isolate (4%) was sensitive to AMP whereas 8 (32%) were sensitive to AMC *in vitro*. Out of the 25 *Staphylococcus aureus* isolates, nine (36%) were MRSA and two (8%) isolates showed inducible CLI resistance. Out of the three *Escherichia coli* and one *Enterobacter species* isolates, two (66.67%) isolates and one (100%) isolate were ESBL producers, respectively. Out of the 82 isolates, 23 (28.05%) were from paediatric (up to 18 years) age group and 59 (71.95%) were from adults. All the isolates from paediatric age group were bacterial and there were no fungi isolated. The sex-wise distributions of the patients were 31 (38.75%) males and 49 (61.25%) females.

Table 1: organisms isolated in chronic suppurative otitis media

Name of the organisms	Number (%)
Pseudomonas aeruginosa	31 (37.80)
Staphylococcus aureus	25 (30.49)
Aspergillus niger	8 (9.76)
Escherichia coli	3 (3.66)
Klebsiella pneumoniae	3 (3.66)
Candida albicans	2 (2.44)
Streptococcus pneumoniae	1 (1.22)
Group A Streptococci (Streptococcus pyogenes)	1 (1.22)
Beta Haeamolytic Streptococci (Non-Group A)	1 (1.22)
Enterococcus faecalis	1 (1.22)
Enterobacter species	1 (1.22)
Pseudomonas species (Non-aeruginosa species)	1 (1.22)
Proteus mirabilis	1 (1.22)
Acinetobacter species	1 (1.22)
Moraxella species	1 (1.22)
Aspergillus fumigatus	1 (1.22)
Total number of isolates	82

DISCUSSION

CSOM is commonly seen in developing countries. It is estimated by WHO that CSOM prevalence in India, Tanzania, Solomon Islands, Guam, Australian Aborigines and Greenland as the highest (>4%); whereas it is high (2-4%) in Nigeria, Angola, Mozambique, Republic of Korea, Thailand, Philippines, Malaysia, Vietnam, Micronesia, China, Eskimos; low (1-2%) in Brazil, Kenya and; lowest in (<1%) in Gambia, Saudi Arabia, Israel, Australia, United Kingdom, Denmark, Finland and among American Indians.[1] Low social-economic status, overcrowding, malnutrition and poor hygiene are the common risk factors. CSOM is commonly known to affect infants and children and it can affect their intellectual, linguistic and psychosocial developments.[1,7] The common aerobic bacterial aetiological agents of CSOM include *P. aeruginosa,*

Table 2: Antibiotic profile for bacterial isolates and their percentages of sensitivity

Bacteria	AMP	AMC	CZO	CXM	CEF	CAZ	CPM	FOX	TZP	CSL	ATM	GEN	AMK
Staphylococcus aureus	4	32	48	64	64	64	64	64	64	64	—	68	92
Streptococcus pneumoniae	100	100	100	100	100	100	100	100	100	100	—	100	100
Group A Streptococci (*Streptococcus pyogenes*)	100	100	100	100	100	100	100	100	100	100	—	0	0
Beta Haeamolytic Streptococci (Non-Group A)	100	100	100	100	100	100	100	100	100	100	—	0	0
Enterococcus faecalis	100	100	—	—	—	—	—	—	100	—	—	0	0
Pseudomonas aeruginosa	—	—	—	—	—	90.4	87.1	—	96.8	96.8	96.8	67.8	83.9
Escherichia coli	33.3	33.3	33.3	33.3	33.3	33.3	33.3	—	100	100	33.3	33.3	100
Klebsiella pneumoniae	0	66.6	100	100	100	100	100	—	100	100	100	100	100
Enterobacter species	0	0	0	0	0	0	0	—	100	100	0	100	100
Pseudomonas species (Non-*aeruginosa* species)	0	0	0	0	0	100	100	—	100	100	100	0	0
Proteus mirabilis	100	100	100	100	100	100	100	—	100	100	100	100	100
Acinetobacter species	0	100	0	0	0	0	0	—	100	100	0	100	100
Moraxella species	100	100	100	100	100	100	100	—	100	100	100	0	0

AMP: Ampicillin; FOX: Cefoxitin; AMC: Amoxicillin-Clavulanic acid; TZP: Piperacillin-Tazobacatam; CZO: Cefazolin; CSL: Cefoperazone-Sulbactam; CXM: Cefuroxime; ATM: Azteronam; CEF: Ceftriaxone; GEN: Gentamicin; CAZ: Ceftazidime; AMK: Amikacin; CPM: Cefepime; —: Not reported/Not tested

Table 3: Antibiotic profile for bacterial isolates and their percentages of sensitivity

Bacteria	CIP	LVX	SXT	DOX	AZM	CLI	VAN	TEC	LNZ	COL	POL	IMP	MEM
Staphylococcus aureus	36	40	48	92	52	92	100	100	100	—	—	64	64
Streptococcus pneumoniae	100	100	0	100	0	100	100	100	100	—	—	—	—
Group A Streptococci (Streptococcus pyogenes)	100	100	0	100	0	100	100	100	100	—	—	—	—
Beta Haeamolytic Streptococci (Non-Group A)	100	100	100	100	0	100	100	100	100	—	—	—	—
Enterococcus faecalis	0	0	—	0	100	—	100	100	100	—	—	100	0
Pseudomonas aeruginosa	74.2	77.5	—	—	—	—	—	—	—	100	100	93.6	83.9
Escherichia coli	33.3	33.3	33.3	33.3	—	—	—	—	—	100	100	100	100
Klebsiella pneumoniae	100	100	100	66.6	—	—	—	—	—	100	100	100	100
Enterobacter species	100	100	100		—	—	—	—		100	100	100	100
*Pseudomonas species (*Non-*aeruginosa species)*	100	100	—	—	—	—	—	—	—	100	100	100	0
Proteus mirabilis	100	100	100	100	—	—	—	—	—	0	0	100	100
Acinetobacter species	100	100	100	100	—	—	—	—	—	100	100	100	100
Moraxella species	0	0	0	0	—	—	—	—	—	—	—	—	—

AMK: Amikacin; TEC: Teicoplanin; CIP: Ciprofloxacin; LNZ: Linezolid; LVX: Levofloxacin; COL: Colistin; SXT: Co-Trimoxazole; POL: Polymixin B; DOX: Doxycycline; IMP: Imipenem; AZM: Azithromycin; MEM: Meropenem; CLI: Clindamycin; —: Not reported/Not tested; VAN: Vancomycin

S. aureus, E. coli, Klebsiella pneumoniae, Proteus mirabilis etc.[1,2,5,8,9] In our study, *P. aeruginosa* was the most common with 31 isolates (37.8%) followed by 25 isolates of *S. aureus* (30.49%). The percentage of other isolates [Shown in Table 1], were less when compared to them. Similar findings have been reported in majority of the studies done earlier in India and other parts of the world.[3,5,7,8,10] Few studies have reported *S. aureus* as the most common isolate followed by *P. aeruginosa*.[2,4,6] All 31 (100%) *P. aeruginosa* isolates were sensitive to COL and POL, while 30 (96.8%) isolates showed sensitivity to TZP, CSL and ATM. Only 21 (67.7%) isolates were sensitive to GEN, 23 (74.2%) isolates were sensitive to CIP while 24 (77.4%) isolates were sensitive to LVX. All 25 (100%) *P. aeruginosa* showed better sensitivity to CAZ, CPM and CSL than to IMP and MEM. All 25 (100%) *S. aureus* isolates showed sensitivity to VAN, TEC and LNZ while 23 (92%) isolates showed sensitivity to AMK, DOX and CLI. Only one isolate (4%) was sensitive to AMP while 8 (32%) were sensitive to AMC. Studies have shown increase in MRSA isolates. In this study, nine (36%) isolates were MRSA. All (100%) the MRSA isolates were sensitive to VAN, TEC and LNZ. The antibiotic susceptibility pattern of *P. aeruginosa* and *S. aureus* were different when compared to the studies done earlier from various geographical areas and there was no common antibiotic for either *P. aeruginosa* or *S. aureus,* which showed highest susceptibility. As per Cochrane review the most effective treatment for CSOM is aural toilet combined with antibiotic therapy. Topical antibiotics are better than systemic antibiotics and combined is no better than topical alone. Topical antibiotics like Neomycin-Polymixin, Gentamicin and fluoroquinolones are usually recommended.[1] In this study, both Gram positive and Gram negative bacteria showed high resistance to topical antibiotics like GEN, CIP, LVX [Tables 2 and 3]. Gram positive bacteria showed highest sensitivity to DOX and Gram negative bacteria to POL and COL [Table 3]. Though DOX topical solutions are not easily available, tetracycline can be used as an alternative since DOX represents tetracycline susceptibility.[14] Fluoroquinolones like CIP and LVX and beta lactam drug AMC have been one of the favourite choices for therapy. In contrast to the studies done earlier,[5,8] this study showed high resistance to CIP, LVX and AMC for both Gram positive and Gram negative bacteria [Tables 2 and 3]. The widespread use and misuse of antibiotics have been implicated as the reasons for emergence of antibiotic-resistant bacteria.[3,5]

Aspergillus species and *Candida species* are the most common fungi encountered in CSOM.[1,2] The findings in this study were similar to where *Aspergillus niger* was the most common fungus isolated followed by *Candida albicans* and *Aspergillus fumigatus* [Table 1]. Though anaerobic bacteria like *Bacteroides species, Peptostreptococcus species, Fusobacterium species, Propionibacterium species, Prevotella species, Porphyromonas species* etc. can cause CSOM, they are rarely isolated in culture.[1,9,11] Anaerobic culture was not done in this study. There are few studies done on anaerobes and this area can be further explored.

Gram staining can be used for early screening of the type of bacteria present in the specimen. Due to the chronic nature of CSOM and the possibility of the patient having already undergone prior therapy it would be wiser to initiate appropriate antibiotic therapy only after performing a culture and antibiotic susceptibility. The exact number of patients on prior antibiotic therapy before or at the time of culture and the risk factors were not determined in this study. But all the patients had received one or several courses of antibiotics at one point of time earlier.

Infants and children are commonly known to be affected with CSOM and it can affect their intellectual, linguistic and psychosocial developments.[1,7] In this study, CSOM was uniformly seen in all age groups and there was uniform distribution of the isolates among all age groups. No predominance of any isolate was seen to a specific age group, except for the *Streptococcus* species, which were isolated only from paediatric age group. There was no predominance of organisms with respect to sex and there was fairly equal distribution of isolates among males and females.

It was not possible to follow up all patients included in this study after the antibiotic therapy was initiated. The patients who could be followed up responded well after initiating specific therapy. This study cannot recommend one single drug for therapy as the antibiotic susceptibility was variable depending on the organism. The antibiotic susceptibility also varies depending on the region. Empiric therapy is also not recommended as blind therapy without a culture and the antibiotic sensitivity report may lead to prolonged duration of therapy and huge burden to the patient, if the bacteria isolated is resistant to the antibiotic used. Hence, culture and antibiotic sensitivity must be done before initiating the therapy. Studies have to be done on regular intervals in different regions to determine the most common isolates and their susceptibility.

CONCLUSION

P. aeruginosa and *S.aureus* are the most common aerobic bacterial pathogens in CSOM. *P. aeruginosa* showed 100% sensitivity to Colistin and Polymixin B followed by TZP, CSL and ATM. *S. aureus* showed 100% sensitivity to Vancomycin, Teicoplanin and Linezolid followed by Amikacin, Doxycycline and Clindamycin. There was no

predominance of organism specific to age group or sex. Culture and sensitivity should be done in CSOM before initiating antibiotic therapy.

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