Aetiological Spectrum of Chronic Suppurative Otitis Media in a Tertiary Care Centre in Kerala

Authors

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Abstract

Chronic suppurative otitis media (CSOM) is characterized by persistent purulent discharge from the ear which causes hearing loss in the later stages. This condition occurs as sequelae to inadequately treated acute otitis media causing persistent perforation in the tympanic membrane which could be infected from bacteria in the external auditory canal. Several studies have been done regarding the microbial aetiology of CSOM with widely varying results.

A study was conducted in the Department of Microbiology, Govt. Medical College, Thiruvananthapuram, Kerala with the objective of profiling the aetiological spectrum of CSOM and using this information to formulate an ideal antibiotic protocol to be administered. A total of 322 cases with patients with CSOM including all age groups and both sexes who attended the outpatient, Dept. of ENT, Govt. Medical College Hospital, TVM during the period of March 2013 to February 2014 (1 year) were studied. Majority of CSOM cases were due to bacteria (73%) and fungi accounted for 22% of the total culture positive cases. Among the bacterial isolates 92.8% were monomicrobial and 7.2% were polymicrobial. Combined bacterial and fungal infections was 9%. Among the bacterial isolates, gram negative organisms (65.3%) account for the majority and gram positive bacteria accounted for 34.7%. The most common bacterial isolate was pseudomonas aeruginosa (56%) followed by staphylococcus aureus (34.7%), Klebsiella pneumonia (3.8%), Escherichia coli (3%), proteus mirabilis (1.7%), proteus vulgaris (0.4%), and Acinetobacterbaumani (0.4%). The most common fungi isolated was Aspergillus species (82%) followed by Candida (13.8%) and pencillium species (42%).

Keywords: CSOM, pseudomonas aeruginosa, staphylococcus aureus, MRSA, Aspergillus niger.

Introduction

Suppurative disorders of the middle ear have their earliest descriptions in ancient medical texts. Hippocrates, himself has stated that acute pars in the ear with fever was a dreaded illness. But he then considered it to be sequelae of a brain abscess. It was Morgagni who later showed that the ear was the origin of disease and not the reverse. Boeis is 1959 described chronic suppurative otitis media as a continued suppurative process of the idle ear following acute otitis media. It could also occur as a primary
suppuration of the middle ear with hyperplasia or fibrotic mucosa.

Chronic suppurative otitis media (CSOM) is defined as a chronic infection of the mucosa lining the middle ear cleft. Middle ear cleft includes the eustachean tube, middle ear cavity proper, aditus and mastoid air cells. CSOM usually occurs as a sequelae to inadequately treated acute otitis media. Acute otitis media causing persistent perforation which could be infected from bacteria in the external auditory canal. This condition is known as persistent perforation syndrome clinically CSOM is diagnosed by profuse and mucopurulent ear discharge. Pain in the ear when present is always associated with otitis externa. This commonly occurs when the patients attempts to clean the ear of the purulent secretions with an ear bud.

Materials and Methods

Study Design: Descriptive study

Study period: 1 year (March 2013 to February 2014)

Study population: Clinical cases of CSOM. All age groups and both sexes.

Study setting: Dept. of ENT, Govt. Medical College, Thiruvananthapuram and Dept. of Microbiology, GMC, TVM.

Sample size: 322 (based on std statistical criteria).

Collection of Sample

Ear discharges were collected under aseptic precautions. Excess discharge was mopped and the external auditory canal cleaned using sterile normal saline. The specimen was then collected using fova sterile cotton swabs. One swab was subjected to microscopic examination by KOH wet mount preparation and Gram staining. Second swab was used for bacterial culture. Third swab for fungal culture and fourth swab was inoculated in Robertson’s cooked Meat Medium (RCM) for anaerobic culture. All swabs were processed immediately after collection of the specimen in the 24 hrs clinical Microbiology Laboratory at the Govt. Medical College Hospital, Thiruvananthapuram. Culture media used for inoculation are Blood agar, Mac Conkey agar, Mannitol Salt agar, Sabouraud’s dextrose agar, Anaerobic blood agar and RCM.

All these inoculated media and one tube of SDA were incubated at 37°C and the other SDA tube was incubated at room temperature. The incubated plates were then examined at 24 and 48 hours. The specific identification of bacterial pathogens was done based on microscopic morphology, staining characteristics and biochemical properties wing standard laboratory procedures. Antibiotic sensitivity testing of bacterial isolate was done using Kirby-Bauer disc diffusion method. Antibiotics were selected depending on the type of the organism. Mueller-Hinton agar was used for doing the sensitivity testing. Reports of the bacteriological examination were given only after 48 hours of incubation.

Identification of Aerobic bacterial pathogens

I Staphylococcus aurens – Gram staining shows Gram positive cocci in groups. Blood agar shows Beta haemolytic golden yellow colonies. Yellow opaque colonies on mannitol salt agar and small lactose fermenting colonies on Mac Conkey agar catalase positive. Slide and tube coagulation tests – positive.

Mannitol fermented. Urea hydrolysed DNAse production and phosphatase production and VP test positive.

II Pseudomonas aeruginosa

Gram negative motile nonsporing noncapsulated bacilli. Diffuse hemolysis on blood agar. Bluish green diffuse water soluble pigment. Catalase and oxidase positive. Oxidative on Hugh-Leifon’s O/F medium. Indole not produced. Gelatin liquefied. Nitrate reduced to nitrite. Arginine dihydrolase test positive.

III) Klebsiella pneumoniae.

Gram negative non-motile bacilli. Mucoid lactose fermenting (pink) colonies on Mac conkey agar.
Catalase positive. Oxidase negative. Indole not produced. Triple sugar iron agar – A/4 no gas. No H2S production. Urea hydrolysed. Citrate utilized. Nitrate reduced to nitrite. Glucose, Lactose, Sacrose and Mannitol fermented with acid and gas. VP positive. MR negative. Decarboxylated Lysine.

IV) Acinetobacter baumanii
Gram negative nonmotile coccobacilli pale lactose fermenting colonies on Mac Conkey agar. Catalase positive. Oxidase – Negative. Oxidative in O/F medium. Lactose, sucrose and mannitol not fermented Glucose fermented with acid and without gas. Triple sugar iron agar – K/No change, no gas, no H2S. Mannitol motility medium – Non fermentative and non motile. Lactose fermentation – Negative on peptone water sugar medium but produce fermenting colonies on 10%. Lactose NA medium. Citrate – utilized. Urea – not hydrolysed. Nitrate not reduced to nitrite.

V) Escherichia coli
Gram negative motile bacilli. Lactose fermenting colonies on Mac Conkey agar. Catalase positive, oxidase – Negative Glucose, Lactose and mannitol fermented with acid and gas. Sucrose – not fermented. Indole produced. Triple sugar iron agar = A/A with gas and no H2S. Urea not hydrolysed. Citrate not utilized. Nitrate reduced to nitrate. MR positive. VP negative. Decarboxylated Lysine.

VI) Proteus species
Gram negative motile pleomorphic bacilli. Non lactose fermenting colonies on Mac Conkey agar. Characteristic swarming growth on blood agar. Fishy or semen odour. Catalase – positive. Oxidase – negative. Hydrolyse urea very rapidly. Indole produced by protens vulgaris and not produced for protens mirabilis. MR positive. VP negative. Citrate utilized. Triple sugar iron agar – K/A with gas and H2S production. Nitrate reduced to nitrite phenyl alaninedeaminase test (PPA) positive. Decarboxylated ornithine and not lysine and arginine.

Antibiotic susceptibility testing
Antibiotic sensitivity testing of the bacterial isolates were done by standard disc diffusion method using Kirby – Bauer method on Mueller – Hinton agar. Minimum Inhibitory concentration (MIC) of the antibiotic testing was done by E-test. The inoculum was standardized by comparing with 0.5 McFarland’s opacity standard. The control strains used were staphylococcus aurens (ATCC 25923), Escherichia coli (ATCC 25922) and pseudomonas paruginosa (ATCC 27853).

Special tests
1) Detection of Metallo Bata Lactamase (MBL)
Screening for MBL production was done in Imepenem resistant isolates of pseudomonas aeruginosa by imepenem – EDTA combined disc test. Test organism was inoculated onto Mueller Hinton agar as recommended by clinical and Laboratory Standard Institute (CLSI) guidelines. Two 10 mg impenem discs were placed on the plate and about 10ml of 0.5 M EDTA solution were added to one of them. The zone of inhibition around Imepenem and Imepenem – EDTA discs were compared after overnight incubation at 37°C. An increase in zone size of at least 7mm around the Imepenem – EDTA disc as compared to Imepenem disc alone was recorded as positive result.

2) Detection of Extended Spectrum Beta Lactanase (ESBL)
i) Double disc approximation test
A 0.5 McFarland standard inoculum of the test organism was prepared and swabbed on a Mueller-Hinton agar plate. Antibiotic discs of Cefotaxime (30mg), Cefazidime (30mg), Cefepime (30 mg) and Aztreonam (30mg) were placed 20mm apart from Amoxycillin / Clavulante (20/10 mg) disc and incubated overnight. Organisms that showed a clear extension of cephalosporin inhibition zone towards the disc
containing clavulanate were considered as ESBL producers.

ii) CLSI phenotypic confirmation test
Cefotaxime (30mg) and Cefotaxime / Clavulanate (30/10 mg) discs were placed on Mueller Hinton agar plate inoculated with the test organism and incubated overnight. An organism was considered as ESBL producer, if Cefotaxime / Clavulanate disc showed a zone diameter of 5mm or more than the cefotaxime disc alone. Positive control used was Klebsiella pneumonia (ATCC 100603) and the negative control used was Escherichia coli (ATCC 25922)

Identification of fungi
Fungal culture reading was taken daily for four weeks before giving a negative report. Fungal growth obtained on SDA was examined for characteristics like rate of growth, colony morphology, colour of observe and reserve and diffusible pigment production. Fungal growth was examined by a lactophenol cotton blue tease mount preparation slide culture technique was performed for the identification of moulds.

Fungal isolates obtained
1) Aspergillus fumigatus
Macroscopic appearance obverse is velvety or powdery at first, turning to smoky green. Reverse is white to tan.
Microscopic appearance On LPCB tease mount septate hyphae with flask shaped vesicle, uniseriate philadies and conidia covering the upper half of the vesicle.
2) Aspergillus niger
Obverse is first white to yellow, then turning dark brown to black. Reverse is colourless to ivory or pale yellow. On LPCB tease mount, septate hypha, globose vesicle with biseriate phialides, jet black conidia covering entire vesicle.
3. Aspergillus terreus
Obverse is velvety, cinnamon to buff brown. Reverse is white to brown. On LPCB tease mount,

dome shaped vesicle with biseriate phialides with conidia covering only the upper half of vesicle.

4. Penicillium species
Observe is velvety bluish green in colour. Reverse is yellowish cream. On LBCB mount, brush like appearance. Branching of conidiophore into primary metulae and secondary phialides from which chains of conidia arise.

5. Candida species
Candida isolates were identified by standard protocols that included germ tube formation, chlamydospore production on corn meal agar with Tween 80 and sugar assimilation and sugar fermentation tests.

Germ tube test
It is a rapid screening test for the presumptive identification of candida albicans, wherein the production of germ tubes (GT) within 2 – 3 hrs of contact with the serum is indicative of candida albicans. This test must be confirmed with a corn meal agar (CMA) test.

Procedure for germ tube test
Candida colonies are inoculated into 1ml of sterile human serum and incubated at 37°C for 2 – 4 hours. After 2 hours, a drop of serum was taken on a slide and a cover ship was placed on it and examined under 40x objective lens for germ tube formation. Germ tube appears as a cylindrical filament originating from the yeast cell, without any constriction at the point of irigin and without obvious swelling along the length of the filament.

Chlamydomspore formation
Polysorbate (Tween 80) added to corn meal agar to reduce the surface tension to allow for the development of pseudohyphae, hyphae and blastoconidia. Different species of candida develop characteristics morphological features on this medium. Small portion of yeast colony was taken with a straight wire and the medium was inoculated onto corn meal agar such that the agar was stabbed all the way to the bottom of the plate at an angle of 45° (Dalmau inoculation technique). The end of the wire was pushed under the agar and only a small amount of inoculum was used. A
sterile coversly was placed on the inoculated surface of the agar. This provides partial anaerobic environment at the margins of the covership. The plate was incubated at 25°C for upto 3 – 5 days. The petridish itself was placed on the microscope stage and examined under low power (10x) and high power objective (40 x). Observations were made for detection of hyphae, pseudohyphae, blastospores and chlamydospores near the margin of the covership. **Candida albicans:** Elongated pseudohyphal cells with large grape like clusters of blastoconidia along the length of the hyphae. Large single terminal chlamydospore is characteristic, formed most likely near the edge of the covership. Chlamydospores are seen as spherical, thick double walled hyaline structures usually at the terminal end of a hypha.

**Candida tropicalis:** Abundant branching pseudohyphae radiating with clusters of blastoconidia at the centre. Blastospores singly or in very small group all along graceful, long, pseudohyphae are seen.

**Candida parapsilosis**

Blastoconidia, single or in small clusters are along the pseudo-hyphae which are crooked or curved in appearance and relatively short. Occasional giant cells and which are large hyphal elements are seen.

**Candida glabrata:** No pseudohyphae are seen. Small oval yeast cells with terminating budding / short chins of ovoid cells are seen.

**Colony characteristics on CHROM agar**

Readymade CHROM agar media in petri plates were obtained from Hi Media. Colonies of the candida isolates from a fresh 24 to 48 hrs subculture were directly streaked on the plates and incubated at room temperature from 24 – 48 hours. Colour of the growth of each isolate was noted and compared with the manufacturer’s standard.

**Distribution of Candida isolates in CHROM Agar**

| Colour | Isolate          |
|--------|------------------|
| Light  | Candida albicans |
| Blue   | Candida tropicalis |
| Cream  | Candida parapsilosis |
| Pink   | Candida glabrata |

**Carbohydrate Assimilation test**

A suspension of colonies in saline (0.85%) or distilled water was prepared and turbidity was adjusted to a density equivalent to McFarland No.4. The surface of yeast nitrogen base was covered with the suspension. The various carbohydrate impregnated discs (Hi Media) were placed onto the surface of the agar plate in a well spaced manner (30mm apart) and the plates were incubated at 37°C for 24 – 48 hrs. Growth around individual discs indicated assimilation of that carbohydrate.

| Species   | Glucose | Maltose | Sucrose | Lactose | Galactose | Trehalose |
|-----------|---------|---------|---------|---------|-----------|-----------|
| C.albicans | +       | +       | +       | -       | +         | +         |
| C.tropicalis | +      | +       | +       | -       | +         | +         |
| C.glabrate | +       | -       | -       | -       | -         | +         |
| C.parapsilosis | +   | +       | +       | -       | +         | +         |

**Carbohydrate Fermentation Test**

This is done to test the ability of Candida species to ferment a number of sugars producing acid and gas. Hence pink colour in the presence of Andrade’s indicator.

**Method**

Candida species grown on SDA at 37°C for 24 hrs inoculated in test tubes containing 2% carbohydrate solution of dextrose, maltose, sucrose, lactose, galactose, and trehalose. These tubes were incubated at 25°C for 7 days and examined every 48 – 72 hrs interval for the production of acid (pink colour) and gas (in Durham’s tube). Fermentation was indicated by production of gas in the tube while only acid production might simply indicate that carbohydrate was assimilated.
Interpretation

| Species       | Glucose | Meltose | Sucrose | Lactose | Galactose | Trehalose |
|---------------|---------|---------|---------|---------|-----------|-----------|
| C. albicans   | F       | F       | F       | -       | F         | F         |
| C. tropicalis | F       | F       | F       | -       | F         | F         |
| C. glabrata   | F       | -       | -       | -       | -         | F         |
| C. parapsilosis| F      | -       | -       | -       | -         | -         |

Results

Total number of 322 patients clinically diagnosed with chronic suppurative otitis media who attended the ENT outpatient Department were examined and the samples obtained from these cases were processed in the 24 hrs clinical microbiology Laboratory. Among the 322 samples processed, 306 (95%) were culture positive and 16 cases (5%) were culture negative.

Table 1 Gender distribution of CSOM cases

| Sex         | Culture positive | Culture negative | Total cases |
|-------------|------------------|------------------|-------------|
| Male        | 143 (95.9%)      | 6 (4.1%)         | 149 (46%)   |
| Female      | 163 (94.2%)      | 10 (5.8%)        | 173 (54%)   |
| Total       | 306 (95%)        | 16 (5%)          | 322         |

Table 2 Geographical distribution of CSOM

| Locality | No. of patients |
|----------|-----------------|
| Rural    | 193 (60%)       |
| Urban    | 129 (40%)       |
| Total    | 322 (100%)      |

Table 3 Seasonal distribution of CSOM

| Season                  | No. of patients |
|-------------------------|-----------------|
| Summer (March to June)  | 42 (13%)        |
| Monsoon (July to October) | 77 (24%)    |
| Winter (November to February) | 203 (63%)  |
| Total                   | 322             |

Table 4 Clinical Symptoms distribution of CSOM

| Serial No. | Symptom  | Number of Patients |
|------------|----------|--------------------|
| 1          | Otorrhoea| 306 (95%)          |
| 2          | Itching  | 290 (90%)          |
| 3          | Earache  | 294 (91.3%)        |
| 4          | Fever    | 176 (54.6%)        |
| 5          | Hearing loss| 102 (31.6%)      |
| 6          | Headache | 80 (24.8%)         |
| 7          | Tinnitus | 2 (0.6%)           |

Table 5 Age and Sex wise distribution of CSOM

| Age group | Number of cases | Total cases |
|-----------|-----------------|-------------|
| Male      | Female          | Total       |
| 0 – 10    | 2               | 3           | 5 (1.5%)    |
| 11-20     | 24              | 13          | 37 (11.5%)  |
| 21 – 30   | 49              | 77          | 126 (39.13%)|
| 31 – 40   | 55              | 61          | 116 (36%)   |
| > 40      | 19              | 19          | 38 (11.8%)  |
| Total     | 149             | 173         | 322 (100%)  |

Table 6 : Types of CSOM

| Clinical Type          | Number of cases |
|------------------------|-----------------|
| Tubotympanic disease   | 294 (91.3%)     |
| Atticoantral disease   | 28 (8.7%)       |
| Total                  | 322             |

Table 7 : Profile of isolates in CSOM

| Pathogen            | Number of isolates |
|---------------------|--------------------|
| Bacterial           | 234 (73%)          |
| Fungi               | 72 (22%)           |
| Total               | 306 (100%)         |

Table 8 : Incidence of pure and mixed culture

| Organism            | Number of cases |
|---------------------|-----------------|
| Monomicrobial       | 287 (93.8%)     |
| Polymicrobial       | 10 (3.3%)       |
| Bacteria + fungi    | 9 (2.9%)        |
| Total               | 306             |

Table 9 : Profile of bacterial isolates

| Sl. No. | Bacteria isolated  | No. of isolates |
|---------|--------------------|-----------------|
| 1       | Pseudomonas aeruginosa | 131 (56%)      |
| 2       | Staphylococcus aureus    | 81 (34.7%)     |
| 3       | Klebsiella pneumonia     | 9 (3.8%)       |
| 4       | Escherichia coli         | 7 (3%)         |
| 5       | Proteus mirabilis        | 4 (1.7%)       |
| 6       | Proteus vulgaris         | 1 (0.4%)       |
| 7       | Acinetobacter baumanii    | 1 (0.4%)       |
| Total   |                      | 234             |

Table 10: Polymicrobial isolates

| Organism isolated                   | Number of cases |
|-------------------------------------|-----------------|
| Pseudomonas aeruginosa + Staphylococcus aureus | 8 (80%)        |
| Klebsiella pneumonia + Staphylococcus aureus    | 1 (10%)        |
| Escherichia coli + Staphylococcus aureus       | 1 (10%)        |
| Total                                           | 10 (100%)      |
### Table 11: Antibiotic Sensitivity pattern of pseudomonas aeruginosa (n = 131)

| Antibiotic         | Sensitive | Resistant |
|--------------------|-----------|-----------|
| Gentamicin         | 67 (51.1%) | 64 (48.9%) |
| Amikacin           | 85 (64.8%) | 46 (35.2%) |
| Ceftazidime        | 120 (91.6%) | 11 (8.4%)  |
| Ciprofloxacin      | 49 (37.4%) | 82 (62.6%) |
| Piperacillin + Tazobactam | 120 (91.6%) | 11 (8.4%)  |
| Polymyxin B        | 131 (100%) | Nil       |
| Imipenem           | 120 (91.6%) | 11 (8.4%)  |
| Meropenem          | 120 (91.6%) | 11 (8.4%)  |
| Netilmicin         | 76 (58%)   | 55 (42%)   |
| Tobramycin         | 74 (56.4%) | 57 (43.6%) |

### Table 12: ABST pattern of Staphylococcus aureus isolates (n = 81)

| Antibiotic | Sensitive | Resistant |
|------------|-----------|-----------|
| Pencillin  | 14 (17.2%) | 67 (82.8%) |
| Gentamicin | 42 (51.8%) | 39 (48.2%) |
| Amikacin   | 81 (100%)  | Nil       |
| Erythromycin | 10 (12.3%) | 71 (87.7%) |
| Cefoxymycin | 79 (97.5%) | 2 (2.5%)   |
| Vancomycin | 81 (100%)  | Nil       |
| Linezolid  | 81 (100%)  | Nil       |

### Table 13: ABST pattern of gram negative isolates

| Antibiotic | Klebsiella | E coli | Pseudomonas aeruginosa | Proteus spp | Acinetobacter |
|------------|------------|--------|------------------------|-------------|---------------|
| Ampicillin | NT         | 2 (28.5%) | 3 (60%)                | 1 (100%)    |               |
| Gentamicin | 3 (33.3%)  | 2 (28.5%) | 2 (40%)                | 1 (100%)    |               |
| Cephalosporin I | 2 (22.2%) | 2 (28.5%) | NT                     | 0           |               |
| Cephalosporin III | 7 (77.75%) | 6 (85.7%) | 5 (100%)               | 1 (100%)    |               |
| Ceftoperazone + Sulbactum | 9 (100%) | 7 (100%) | 5 (100%)               | 1 (100%)    |               |
| Amikacin   | 9 (100%)   | 7 (100%) | 5 (100%)               | 1 (100%)    |               |
| Ciprofloxacin | 6 (66.6%) | 7 (100%) | 5 (100%)               | 1 (100%)    |               |
| Imipenem   | 9 (100%)   | 7 (100%) | 5 (100%)               | 1 (100%)    |               |
| Meropenem  | 9 (100%)   | 7 (100%) | 5 (100%)               | 1 (100%)    |               |
| Piperacillin + Tazobactam | 9 (100%) | 7 (100%) | 5 (100%)               | 1 (100%)    |               |

### Table 14: Mechanism of drug resistance among gram negative isolates

| Resistance mechanism | Escherichia Coli | Pseudomonas aeruginosa | Klebsiella pneumonia |
|----------------------|------------------|------------------------|----------------------|
| ESBL                 | 1                | NT                     | 2                    |
| MBL                  | NT               | 11                     | NT                   |

ESBL – Extended spectrum Beta Lactamase
MBL – Metallo Beta Lactamase

### Table 15: Fungal isolates in CSOM

| Sl. No | Fungus isolated | No. of cases |
|--------|-----------------|--------------|
| 1      | Aspergillus niger | 29 (40.3%) |
| 2      | Aspergillus flavus | 17 (23.6%) |
| 3      | Aspergillus fumigatus | 12 (16.7%) |
| 4      | Aspergillus terreus | 1 (1.4%)   |
| 5      | Pencilium species | 3 (4.2%)    |
| 6      | Candida species  | 10 (13.8%)   |
|        | Total           | 72           |

### Table 16: Mixed fungal and bacterial growth

| Sl. N | Organisms | No. of cases |
|-------|-----------|--------------|
| 1     | Staphlococcus aureus + A. Nigor | 3 (33.33%) |
| 2     | Pseudomonas aeruginosa + A.nigor | 2 (22.2%) |
| 3     | Pseudomonas + A.flavus | 2 (22.2%) |
| 4     | Pseudomonas + A fumigatus | 1 (11.11%) |
| 5     | Staph. Aureus + Pencilium spp. | 1 (11.11%) |
|       | Total     | 9            |

### Table 17: Distribution of candida species

| Sl. No | Species          | Number of cases |
|--------|------------------|-----------------|
| 1      | Candida albicans | 2 (20%)         |
| 2      | Candida tropicalis | 4 (40%)       |
| 3      | Candida parapsilosis | 2 (20%) |
| 4      | Candida glabrata | 2 (20%)         |
|        | Total            | 10              |

### Table 18: Complications of CSOM (n = 19)

| Sl. No | Complication        | Number of cases |
|--------|---------------------|-----------------|
| 1      | Mastoiditis         | 12 (63.2%)      |
| 2      | Facial Nerve palsy | 5 (26.4%)       |
| 3      | Temporal lobe abscess | 1 (5.26%) |
| 4      | Post aural fistula | 1 (5.26%)       |
|        | Total No. of patients | 19 (100%)   |

**Antibiotic Sensitivity pattern**

Pseudomonas aeruginosa isolates in this study were 100% sensitive to polymyxin. Sensitivity to Ceftazidime, Piperacillin – Tazobactam, Imipenem and Meropenem were 91.6% Sensitivity to Amikacin was 64.8%. Ciprofloxacin and Gentamicin showed lower sensitivity values such as 37.4% and 51.1% respectively.
Antibiotic Sensitivity testing
ABST was carried out for 306 isolates obtained from cases of CSOM in our study. Staphylococcus aureus was the only gram positive organism isolated. Only 17.2% of the isolates were sensitive to penicillin and 97.5% were sensitive to Cefoxitin. Sensitivity to gentamicin was 51.8%. All the isolates were sensitive to vancomycin and Linezolid. Resistance of Staphylococcus aureus to Penicillin seems to be high in the reported studies.

Discussion
In the present study it was found that females were slightly more affected than males. Out of the 322 cases studied, 149 (46%) were males and 173 (54%) were females. Prior studies conducted by different groups such as Srivaalsava V.K et al and Asini Saad et al showed male predominance. However, this finding may not have any statistical significance.

The incidence of CSOM was more common in rural areas (60%) compared to urban areas (40%). This finding correlated with studies conducted by Gulathi et al, Urmil Mohan et al, and M. Koppad et al. The reason may be due to non availability of trained specialists and lack of education and awareness. The other reasons may be contaminated water sources used for personal hygiene and the use of crude objects for cleansing the external ear.

CSOM may be more prevalent during winter season. In our study, 63% of cases occurred during winter. This correlates with studies by Maji P.K. et al and Charles D Bluestones. The increased incidence during winter season can be attributed to higher incidence of viral and bacterial upper respiratory tract infections.

In the present study culture positivity was 95% and negative cultures accounted only for 5% of cases. 93.3% of cases of CSOM were found to be culture positive as per the results of a study conducted in Turkey by Dincer et al. Indian studies conducted by Gupta v. et al and Nandy A. et al reported 95.8% and 95.54%. Culture positivity respectively which correlate with our study. Negative cultures may be attributed to multiple factors like prior antibiotic therapy or presence of antimicrobial enzymes and also because of nonbacterial on non-fungal aetiological agents.

In the present study, CSOM was more prevalent in the age group between 21 – 30 years (39.13%). This correlates with studies conducted by Erkan Mustaya et al and Gulathi et al. Bacteria are the most common causative agents of CSOM. In the present study bacteria accounted for 73% of cases. Among the bacterial isolates, 65.3% were gram negative and 34.7% were gram positive organisms, pseudomonas aeruginosa was the predominant gram negative isolate accounting for 56% of cases. Staphylococcus aureus was the only gram positive organism accounted for 34.7% of cases. The other gram negative isolates are Klebsiella pneumonia (3.8%), Escherichia coli (3%), Proteus species (2.1%) and Acinetobacter baumanii (0.4%) only. Many studies correlated with the findings of the present study. Pseudomonas aeruginosa is the most common organism is CSOM reported by Ballal M. et al and Gulathi et al in 1997, Urmil Mohan et al in 1998, Hiremath S.L. et al from Karnataka in 2001, Loy A.H.C et al from Singapore, Nwabuisi in 2002 from Nigeria and Tahira Mansoor et al at Karachi in 2006. Staphylococcus aureus is the second most common organism isolated in this study. Most of the studies mentioned above also reported the same. In the present study, Klebsiella pneumonia comes as the third causative agent. This finding correlates with studies by Ballal M. etal, Vijayaetal and Loy et al. E.Coli, Proteus mirabilis, protens vulgaris and Acinetobacter baumanii were the other isolates in the current study as observed in other earlier studies by Singh et al and Poorey et al.

Profile of Fungal isolates
As per the results of the present study, 22% of cases of CSOM had fungal aetiology. This is significantly igher than the results of most of the other reported studies. Fungal pathogens
accounted for 7% of cases in Nigeria in 1983. Another study by Rio de Juneiro reported 8.9% of fungal isolates in a study. However, a study from Saudi Arabia reported 18.5% of fungal aetiology in CSOM. Aspergillus species were the major fungal pathogen accounting for 82% followed by Candida (13.8%) and penicillium species (4.2%). Urmil Mohan et al observed the same findings in a study conducted in Punjab and many other studies in North India and South India have made the same observations. Among candidate isolates, the major difference seen in the present study was that Non albicans and candidealbicans (20%) constituted 80% of the total isolates. Candida tropicalis was the most common isolate (40%). Two cases of candida parapsilosis (20%) and candida glabrata (20%) were also detected. Candida parapsilosis is an important emerging fungal pathogen. It is now the second most common isolated organism after Candida albicans. MRSA isolates were resistant to gemtamicin, Erythromycin and cefoxin but sensitive to Amikacin, Vancomycin, Linezolid, Clindamycin and Rifampicin. Meca gene was identified in both the isolates using PCR. These two patients were treated with topical Amikacin and oral Linezolid and both were cured. All the gram negative isolates, showed 100% sensitivity to Amikacin, Imepenem, Cefoperazone + Sulbactum and Piperacillin – Tazobactum. Sensitivity to first generation cephalosporine and gentamicin were only 22.2% and 33.3% for Klebsiella and 28.5% each for E.coli.

Management of CSOM cases
All patients were treated with aural toileting with acetic acid and topical amikacin or ciprofloxacin with 88.5% cure rate. In long standing cases, oral antibiotics like ciprofloxacin, amoxicillin + clavulanic acid and linezolid (MRSA) were used – based on the antibiotic sensitivity patterns. A total of 37 patients (11.5%) underwent surgical treatment Tympanoplasty / myringoplasty was done in 15 cases (4.6%). Cortical mastoidectomy with tympanoplasty was performed in 10 cases (3.1%). Modified Radical Mastectomy for atticoantral disease was done in 12 cases (3.8%). Among the 322 cases of CSOM studied, 19 cases (5.9%) developed complications such as mastoiditis, temporal lobe abscess and facial nerve palsy which were managed accordingly. No case fatalities reported.

Conclusion
The study was conducted in the Department of Microbiology, Govt. Medical College, Thiruvananthapuram to know the aetiological spectrum of CSOM and to formulate an ideal antibiotic protocol. A total of 322 cases studied in detail. Culture positivity was (95%). Bacterial pathogens (73%) and fungal pathogens (22%) were isolated in this study. Being a disease that is associated with significant morbidity if untreated, CSOM requires diligent laboratory evaluation with comprehensive antibiotic sensitivity testing. The incidence of pure fungal and combined bacterial and fungal growth in our study, only highlights the need for a fungal culture in all cases referred for organisms isolation in chronic suppurative otitis media.

Conflicts of Interest
There are no conflicts of interest.

Acknowledgement
The authors greatly thank Dr.Mohan J, Professor and Head of the Dept. of ENT, Govt. Medical College, Thiruvananthapuram for providing necessary help during the study and for granting permission to collect samples to carry out the study.

References
1. Ballal M, Joythirlatha, Kishor J et. al. Chronic suppurative otitis media – A bacteriological and mycological study. Indian journal of otolaryngology and Head and Neck 1992 : 1 (1) 10 – 13
2. Brook Itzhak. Microbiology of otitis media. Indian journal of Otolaryngology 1996 : 2 (1) : 26 – 29
3. Gulathi, Sudesh Kumar. Investigative profile in patients of chronic suppurative otitis media. Indian journal of Otolaryngology 1997; 3 (2) 59 – 62
4. Kenna MA Treatment of chronic suppurative otitis media. Otolaryngol Clin. North Am, 1994, 27 (3). 457 – 472.
5. Maqbool M. Text book of Ear, Nose and Throat Disease. 8th edition, New Delhi, Jaypee Brothers, 2008
6. Nikakhlaghs, Khosravi AD, Fazlipour A. et al. Microbiological findings in patients with chronic suppurative otitis media J. Medical Science 2008, 8 : 503 – 506.
7. JagadishChander. Text Book of Medical Mycology, 3rd Edition, 2012, 266-290.
8. Nandy A, Mully PS, Sivarajan K, Chronic suppuratie otitis media – A bacteriological study. Indian Journal of Otolaryngology 1991, 43 (3), 136-138.
9. Mohan Urmil, Jindal Neeraja, Fungal and bacterial flora of chronic suppurative otitis media in Amritsar. IJO and HNS 198, 50 (2) 175-177.
10. Mansoor, Musani, G Khalid et al. Pseudomonas aeruginosa in CSOM. Sensitivity spectrum against various antibiotics in Karachi. J. Ayub Med. Coll. Abottabad 2009; 21 (2) : 120 – 123
11. Kumar S, Sharma R, SaxenaAk et al study of bacterial flora and sensitivity to antibiotics in cases and CSOM. Indian J otol 2008 : 14 : 20 – 24
12. Kwon – Chung KJ and Bennet. Medical Mycology 1992.
13. Vijaya D, Nagaratnamma, T. Microbiological study of Chronic suppurative otitis media. Indi. J. of Otol 1998; 4 (4) 172-174
14. Gupta V, Gupta A and Sivarajan K. Chronic suppurative otitis media, an aerobic microbiological study. Indian Journal of otology. 1998 June, 4 (2) : 70 – 82
15. MosafariNia K, Sepheri G, Khatmihetal Isolation and antimicrobial susceptibility of bacteria from CSOM patients in Kerman, Iran. IRCMJ 2011, 13 (12) : 891 – 894
16. Purnajaf A, Ardebili A, Goudarzi L etal, PCR based identification of MSRA strains and their antibiotic resistant profiles. Asian Pac J Trop Biomed 2014, 4 (SI) : S 293 – 297.