BACTERIOLOGICAL (AEROBIC) PROFILE OF CHRONIC SUPPURATIVE OTITIS MEDIA WITH ANTIBIOTIC SENSITIVITY TESTING IN A TERTIARY CARE HOSPITAL OF NORTH EAST INDIA.

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ABSTRACT

Background: Chronic suppurative otitis media (CSOM) is a persistent, insidious, notorious infection and a major health problem in developing countries causing serious local damage and threatening complications. The situation is complicated by the increasing antibiotic resistance these days. Early and effective treatment based on the knowledge of causative micro-organisms and their antimicrobial sensitivity ensures prompt clinical recovery and possible complications can thus be avoided. Aims and objectives: To isolate the aerobic bacteria associated with CSOM and to detect the antibiogram of the isolates. Materials and methods: A total of 175 patients clinically diagnosed of CSOM were enrolled in the study and the samples were obtained from each patient using sterile cotton swabs and cultured for microbial flora. Drug susceptibility testing for aerobic isolates was conducted using Kirby-Bauer disc diffusion method. Results: The most common causative organisms isolated were Staphylococcus aureus (42.63%) and Pseudomonas aeruginosa (22.63%) amongst the 190 aerobic isolates. Antimicrobial profile of aerobic isolates revealed maximum sensitivity to gentamicin followed by ciprofloxacin.
Conclusion: Knowing the etiological agents of CSOM and their antimicrobial susceptibility is of essential importance for an efficient treatment, prevention of both complications and development of antibiotic resistance and finally for reduction of the treatment costs.

KEYWORDS: CSOM, Aerobic, Antibiotic Sensitivity

Introduction-
Chronic Suppurative otitis media with its unpleasant symptoms and complications may be a catastrophe for the marvellously structured organ, the ear, on which much of our appreciation of life and human activity depends. It is a privilege for an otorhinolaryngologist to preserve, repair and take utmost care of the structure and function of this wonderful organ, in whatever condition it is presented. It is a challenge especially in children to prevent the progress of acute suppurative otitis media to a chronic disease.1

Chronic suppurative otitis media (CSOM), a chronic inflammation of the middle ear and mastoid cavity, presents with recurrent ear discharges through a tympanic perforation. It usually begins in childhood as a spontaneous tympanic perforation due to an acute infection of the middle ear, known as acute otitis media (AOM) or as a sequel of less severe forms of otitis media (e.g. secretory OM). Generally, patients with tympanic membrane perforations which continue to discharge mucoid material for a duration of 6 weeks to 3 months, despite medical treatment, are recognized as CSOM cases.2

It has multiple etiologies and generally persistent and recurrent in spite of treatment, leading to complications i.e mastoid abscess, facial nerve paralysis, deep-seated suppuration and meningitis.3 Hearing loss associated with CSOM leads on to educational backwardness in children that is well recognized by Otolaryngologists, Paediatricians and Educators. Development of speech, language and learning skills are severely hampered due to poor sensory communication in these children and leads to poor academic potentials, hampered outdoor activities.4 Due to poor hygienic practices and lack of health education, the incidence of CSOM is increasing in the developing countries. The complications of CSOM have been reduced to a greater extent because of the era of intensive care with proper antibiotics. However the morbidity and mortality associated with CSOM should not be underestimated in developing countries due to indiscriminate, hazardous and half hearted use of antibiotics and poor follow up of the patients, leads to persistent low grade infection and development of bacterial resistance.5

Aerobic bacteria, anaerobic bacteria and fungi are the etiological agents responsible for CSOM.3 Aerobes (Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Proteus mirabilis, Klebsiella species etc) are responsible for the disease. Mixed infections of aerobes, anaerobes and fungi are also seen.6 The study of microorganisms commonly associated with CSOM and their in vitro antibiotic sensitivity pattern is very useful to plan a general outline of treatment for the patient with a chronically discharging ear.7

Hence this study is carried out to know the bacterial etiology of CSOM and their antibiotic susceptibility pattern in this belt of Northeast India for appropriate management of the cases and to prevent complications as well as studying the antimicrobial susceptibility pattern will help in preventing the emergence of resistant strains in the community.

MATERIALS AND METHOD—

Study Outline
This community based, Cross sectional study was conducted for a duration of one year from September 2014 to August 2015. The study was carried out in the Department of Microbiology and ENT, Silchar Medical College and Hospital, Silchar, Assam. The study was started after the ethical approval from the institutional review board.

In this study 200 samples of ear discharge are collected from 175 patients. Out of 175 patients 25 patients had bilateral active ear discharge and swabs were collected from both the ears separately. Cases are selected who have fulfilled the inclusion and exclusion criteria from the patients who were clinically diagnosed by ENT surgeons in the department of ENT, Silchar Medical College And Hospital, Silchar, Assam.

Inclusion criteria: 1) The patients diagnosed as suffering from CSOM after thorough clinical evaluation by an E.N.T Surgeon.
The patients giving informed consent for the study.

Exclusion criteria:
1) Patients who have taken antibiotic for CSOM for last 10 days.
2) Ear discharge with intact tympanic membrane.
3) Ear discharge of less than 6 weeks.

Collection of sample:
Under aseptic precautions, the external ear canal was cleaned by suction, then wiped with sterile cotton with 70% alcohol and allowed to dry. Under illumination using a sterile audiology speculum, the sterile swab stick was gently introduced into the external auditory meatus, gently rotated and removed with precautions and immediately put into its container so as not to touch the external ear canal or any other part of the skin. Two specimens from a single ear were collected in this manner, labelled and processed as soon as possible in the Microbiology laboratory for bacterial studies. If processing is delayed, refrigeration was preferable to store at ambient temperature. The fresh samples obtained were subjected for microscopic examination and culture and sensitivity.

Microscopical examination and culture:
Direct Gram staining and Ziehl–Neelsen staining is done with the first swab. The second swab stick was inoculated on 5% sheep blood agar (BA), MacConkey’s agar and nutrient agar for primary isolation of bacteria. The media were prepared as per methods described by Collee JG et al. All plates were incubated at 37°C for overnight under aerobic condition. Evaluation was done at 24 hours, 48 hours and 72 hours. If there was no growth even after 72 hours the samples are considered as sterile and the plates are discarded. In case of isolation of 2 or 3 more types of colonies from one sample are regarded as mixed growth. The bacterial isolates grown were identified based on morphology, cultural characteristics and biochemical reactions according to standard techniques. Antibiotic susceptibility testing is done by Kirby-Bauer disc diffusion method as per the recommendation of Clinical and Laboratory Standard Institute (CLSI).

Results
The information collected was analyzed using statistical package for social science version 20 (SPSS-20). Among the 175 CSOM cases studied, 150 were unilateral cases (85.7%) while bilateral CSOM were recorded in 25 patients (14.3%), thereby making a total of 200 ear cultures available for analysis. Among 150 unilateral cases 57% had right ear involvement and 15% had left ear involvement. In this study distribution of patients were made into 6 groups depending on age. It is evident from the data that the paediatric population (1 to 10 years) is mostly affected. Age distribution of the study group has been tabulated in fig 1.

<table>
<thead>
<tr>
<th>AGE (IN YEARS)</th>
<th>MALE</th>
<th>FEMALE</th>
<th>NO OF MIXED BACTERIAL GROWTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1—10</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>11—20</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>21—30</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>31—40</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>41—50</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>51 and above</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

Out of total 190 bacterial isolates Gram negative bacteria (57.37%) are more responsible for CSOM than Gram positive bacteria (42.63%) in the present study as shown in fig 2.

Fig 2: Distribution of total isolates depending on gram stain result

Among total 190 bacterial isolates Staphylococcus aureus (81.42.63%) is the most predominant isolate out of which 13(7%) isolates were methicillin resistant Staphylococcus aureus (MRSA). It is followed by Pseudomonas aeruginosa 43 (22.63%), Klebsiella pneumoniae 22 (11.57%), Proteus mirabilis 18 (9.47%), Escherichia coli 16 (8.44%), Acinetobacter species 12 (6.74%) and Klebsiella oxytoca 10 (0.5%) as described in table 3.

Table 2: Distribution of organisms isolated from mixed cultures.

<table>
<thead>
<tr>
<th>CULTURES</th>
<th>ISOLATED BACTERIAL PATHOGENS</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed</td>
<td>Staphylococcus aureus+ Pseudomonas aeruginosa</td>
<td>46.15%</td>
</tr>
<tr>
<td></td>
<td>Staphylococcus aureus + Klebsiella pneumoniae</td>
<td>7.69%</td>
</tr>
<tr>
<td></td>
<td>Proteus mirabilis + Klebsiella pneumoniae</td>
<td>7.69%</td>
</tr>
<tr>
<td></td>
<td>Staphylococcus aureus + Escherichia coli</td>
<td>7.69%</td>
</tr>
<tr>
<td></td>
<td>Proteus mirabilis + Pseudomonas aeruginosa</td>
<td>7.69%</td>
</tr>
<tr>
<td></td>
<td>Acinetobacter species + Klebsiella pneumoniae</td>
<td>7.69%</td>
</tr>
<tr>
<td></td>
<td>Acinetobacter species + Proteus mirabilis</td>
<td>7.69%</td>
</tr>
</tbody>
</table>

Table 3: Showing bacteriological profile of CSOM

<table>
<thead>
<tr>
<th>ISOLATED SPECIES</th>
<th>NUMBER</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>81</td>
<td>42.63%</td>
</tr>
<tr>
<td>MSSA</td>
<td>68</td>
<td>35.7%</td>
</tr>
<tr>
<td>MRSA</td>
<td>13</td>
<td>6.8%</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>43</td>
<td>22.63%</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>22</td>
<td>11.57%</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>18</td>
<td>9.47%</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>13</td>
<td>6.84%</td>
</tr>
<tr>
<td>Acinetobacter species</td>
<td>12</td>
<td>6.74%</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>1</td>
<td>0.5%</td>
</tr>
</tbody>
</table>

Antibiotic susceptibility test:
Antibiotic sensitivity testing was carried out for 190 isolates causing CSOM by doing Kirby Bauer disc diffusion method. Results of sensitivity testing are depicted in table 4. The test results of our study depicted that gram positive organisms are fairly susceptible to Vancomycin (100%), linezolid (100%), Gentamicin (88%), Amikacin (83%), Ciprofloxacin (83%), Levofloxacin (75%) but showed 94% resistance to Erythromycin and 39% resistance to Amoxicillin clavulanic acid. Gram negative organisms have highest susceptibility for Imipenem (100%), Meropenem (100%) followed by Gentamicin (87%), Amikacin (82%), Ciprofloxacin (83%), Piperacillin-tazobactam (77%). Among the commonly prescribed topical antibiotics tested, Gentamicin showed the highest susceptibility rate 87%, followed by Ciprofloxacin (83%).

Fig 3: Age and gender wise distribution of cases (in years) male ♀ female

In the study males (96.54%) were more commonly affected than females (79.46%). 125 patients (71%) in the study group belong to lower socioeconomic group and 51 (29%) came from middle socioeconomic class. Major portion of the patients had come from rural area that is 114 patients (65%) and 61 patients (35%) had come from urban background.

Bacteria were demonstrated in direct smears from 88.5% of specimens and culture could isolate bacteria from those 88.5% of specimens. Analysis of the total 200 cases revealed aerobic bacterial growth was obtained in 177 samples (88.5%) whereas 23 samples (11.5%) showed no growth. 190 total numbers of bacteria were isolated out of which 164 (86%) isolates were found from monomicrobial growth and 26 (13.61%) isolates were found from polymicrobial growth. Table 1 shows the age wise distribution of polymicrobial isolates in CSOM.
Chronic Suppurative otitis media, a middle ear disease are a common disease with approximately 5% global incidence which is characterized by persistant discharge through perforation of tympanic membrane. The chronic inflammation results from the presence of bacteria in the middle ear and mastoid cavity. Bacteria are believed to gain access to the middle ear cleft either from the external auditory canal through perforation or from the nasopharynx via eustachian tube or both. But regardless of the entry mechanism, biofilm formation has been suggested to explain the uncontrollable nature of CSOM. Early bacteriological diagnosis, selection of appropriate antibiotic of all cases assure accurate and appropriate effective therapy

In our study, a total of 175 patients were included, and it was found that majority of the patients belong to age group 1 to 10 years which is similar to the study done by Ghosh et al.16 V K Poorey et al.17 In contrast to this finding Ashish J et al.18 has found that majority of the patients belong to 21 to 30 years age group followed by 1 to 10 years. The high incidence of CSOM in this age group may be due to short and wide Eustachian tube, mother’s breast feeding in supine position, physiological dysfunction of Eustachian tube17, unhygienic practices, swimming habits in pond, lake water, river etc17.

Out of these 175 patients 114 patients came from rural area and 61 patients belonged to urban area. Total 100 patients had swimming history and 125 patients belong to lower socioeconomic group and 50 have middle class socio economic status. Similar finding was also found in the study done by Talukder et al.19 from Bangladesh.

Unilateral disease was observed in 150 patients out of which 57% had right ear involvement and 28.6% had left ear involvement, while bilateral disease was seen in 25 patients. The behavioural nature playing with or inserting contaminated objects into the ears probably makes the prevalence of unilateral disease more common. Predominant unilateral ear involvement was seen in other studies from different researchers like Shyamala et al.20, Shrestha et al.21.

CSOM is observed more commonly in males than females in present study, maybe males normally seek medical attention earlier than females or may be due to their active nature, males are more prone to traumatic conditions. Similar results were seen in studies done by R Shyamala et al.22 Kumar R et al.23. In contrast to our study, studies done by Prakash M et al,24 Rakhee T et al.25 had shown that females are affected more than males. Ashish J et al.26 has shown equal distribution of the disease between both sexes.

In this present study, bacteria are demonstrated in direct gram stain smears in 88.5% of specimens and culture could isolate bacteria from those 88.5% of specimens. Similar kind of observations are seen in study done by Ghosh A et al.15. 177 samples showed aerobic bacterial growth and 23 samples showed no growth. The culture result found to correlate with study done in India by Wahane et al.15, P. N. S. Moorthy et al.16. Sterile cultures may be attributed to 1) Non bacterial organisms, 2) Anaerobic growth, 3) Presence of antimicrobial enzymes, 4) Prior use of antibiotics.

The total numbers of strains isolated are 190, out of which 86.09% are monomicrobial isolates and 13.9% strains are polymicrobial which is similar to the studies done by Ayson PN et al.27, VK Poorey et al.18, Gopichand WR et al.28. Availability and use of topical and systemic broad spectrum antibiotics in the period before consultation was probably responsible for the lower incidence of mixed infection. In contrast to our study SH Geeta et al.15 has from India has found that pure growth was seen in 49% of isolates but mixed growth was seen in 50.4% samples.

It is observed that Gram negative bacteria (57.37%) are more responsible than Gram positive bacteria (42.63%) for infection of the middle ear in present study. This finding of our study correlates with the study done by VK Poorey et al.18. In contrast to our findings Ayson PN et al from Philippines has found gram positive bacteria in 57.5% of isolates and gram negative bacteria in 42.5% of isolates. Mariam et al.29 found predominance of gram positive organisms in CSOM patients.

Most mixed cultures 61% (8/13) were isolated from the adult group suffering from CSOM in our study. Similar kind of observation was seen in the study done by Ayson PN et al, NB Swaroopanir et al.27

In our present study the most predominant organism causing CSOM is Staphylococcus aureus (42.63%) among total 190 aerobic bacterial isolates out of which (35.78%) is Methicillin sensitive Staphylococcus aureus and (6.84%) is Methicillin resistance Staphylococcus aureus. This finding was in accordance with study done by Divya vaishnavi G et al.30. In contrast to our study Shrestha et al.21 isolated 51% MRSA in the year of 2014.

In our present study the most predominant organism causing CSOM is Pseudomonas aeruginosa (22.63%). The frequency of Staphylococcus aureus in the middle ear infections can be attributed to their ubiquitous nature and high carriage of resistant strains in the external auditory canal and upper respiratory tract.31 Various studies conducted by different researchers from different
parts of world Patricia N. Ayson et al., Shrestha et al. have found Staphylococcus aureus as the most predominant organism followed by Pseudomonas aeruginosa in CSOM. In contrast to our study several researchers have isolated Pseudomonas aeruginosa as the most predominant organism followed by Staphylococcus aureus like Raakhee T et al., Mansoor S et al. This variations may be due to effect of climate and due to variation of organisms in different community and locality.

Klebsiella pneumoniae is the third common organism in present study which comprises 11.57% of total aerobic bacterial isolates which correlates with the findings of different studies, Gopichand WR et al., Divya vaishnavi G et al(1.4%)But in contrast to our finding one research work done in Italy by Ricciardiello F et al has shown Klebsiella pneumoniae as the second most common organism(25.4%)in CSOM. The next common organism found was Proteus mirabilis which comprises 9.09% of our total isolates. Similar findings are found in studies done by V. K. Poorey et al., Ricciardiello F et al., Divya vaishnavi G et al. But in contrast to our findings one study done by Olajide TG et al has found Proteus mirabilis as the second most common organism whose isolation rate was 24.2% respectively.

Less common aerobic bacteria isolated in our study is Escherichia coli 6.84%, Acinetobacter species 6.3%, Klebsiella oxytoca 0.52% which is in accordance with another study to some extent which is done by Saranya SK et al. The organisms like Pseudomonas and Proteus species are considered to be secondary invaders from external auditory canal which gets access to middle ear cavity through defective tympanic membrane.

Antimicrobial sensitivity testing was done as per CLSI guidelines. In our study MSSA has revealed 100% sensitivity to vancomycin, Linezolid and cephalosporin followed by Gentamicin, Amikacin, Ciprofloxacin, Cotrimoxazole. In our study 65% of MSSA isolates showed resistance to Amoxicillin. Sensitivity pattern to Cefoxitin and Cefuroxime of our study is in accordance with study done by Divya vaishnavi G et al., K.S., Wahane et al. In contrast to our findings study done by Agrawal et al.(2014) has found Sensitivity with ciprofloxacin to be 55.3% which was 74% in our study. One study done in Ethiopia by Abera B et al in 2011 has concluded that only 4.6% isolates of Staphylococcus aureus was resistant to Ciprofloxacin. Raakhee T et al has found sensitivity to Ciprofloxacin to be 90.47%. In present study MSSA has shown 52% to 66% sensitivity to Erythromycin, Cotrimoxazole and Levofloxacin. One study done in Ethiopia by Abera B et al has shown 60% and 66% sensitivity to Cotrimoxazole and Erythromycin which is more or less similar to our finding.

MRSA isolated in our study has shown 100% sensitivity to Vancomycin, good sensitivity against Ciprofloxacin(76%), Amikacin (76%) thus making these agents as the drug of choice for the same. But MRSA has shown 100% resistance to amoxicillin clavulanic acid ampicillin and cephalosporines which is in accordance with the study done by Divya vaishnavi G et al(10) to some extent. But in contrast to our study another study done by Swaroopanri NB et al has shown sensitivity of MRSA to gentamicin (41.7%) and amoxycyclav (41.7%).

Antibiotic susceptibility testing of Pseudomonas aeruginosa has shown 100% sensitivity to Imipenem, Meropenem, Meropenem ,80 to 90% sensitivity to Piperacillin- tazobactam, Aztreonam, Cefazidime, Gentamicin, and Ciprofloxacin which is consistent with another study done by Agrawal, et al., Mansoor T et al, Rakhee T et al. 90% of Pseudomonas aeruginosa was found to be sensitive to ciprofloxacin in our study which is in accordance with different studies done by Wahane et al., Saranya SK et al. 88% of Pseudomonal isolates were found to be sensitive to Cefazidime and it is also comparable to other studies done by Mansoor et al., Wahane et al., Saranya SK et al. 88% of Pseudomonal isolates were found to be sensitive to Cefazidime and it is also comparable to other studies done by Mansoor et al. and co.

So from observations made in our study we have come to a conclusion that gram positive organisms are fairly susceptible to Vancomycin, Linezolid ,gentamicin Amikacin Levofloxacin and Ciprofloxacin but 39% of the gram positive isolates are resistant to amoxicillin-clavulanic acid. Gram negative organisms have highest susceptibility to Imipenem, Meropenem followed by Gentamicin, Amikacin, Ciprofloxacin and Piperacillin Tazobactam. Most of the gram negative organisms have shown fair susceptibility towards Aztreonam and Cephalosporins.

In our study; based on the antibiogram pattern, 87% and of the isolates which includes Pseudomonas, Staphylococci and members of Enterobacteriaceae family were sensitive to Gentamicin. 83% of these isolates are sensitive to Amikacin and Ciprofloxacin. Pseudomonal organisms are commonly resistant to macrolides, Vancomycin, Aztreonam, Vancomycin, Aztreonam, Vancomycin and Erythromycin. But in contrast to our findings one study done by Wahane et al. and Moorhy PNS et al., has found very high sensitivity of Ciprofloxacin against all isolates which correlates to our present study to some extent.

In this study special interest was taken into account of the common topical antibiotics prescribed by the ENT physicians in treating ear discharge namely Gentamicin and ciprofloxacin. Among them sensitivity to gentamicin (87%) was highest followed by ciprofloxacin (83%). Review of literature in the course of our study has found that topical therapy is more likely to be effective than systemic therapy because of the high concentrations of antimicrobial agents. Studies comparing systemic administration to topical administration show that topical cure rates nearly double systemic rates. Topical therapy does not fail because even if the organism is resistant it supposedly succumb to these very high concentrations. Another character of topical delivery systems is the absence of systemic effects. Because no appreciable systemic delivery of topically administered agents occurs, the normal flora in the respiratory and gastrointestinal tracts is not exposed to antibiotics.

One study done by Loy AH et al in Singapore in 2002 published that gentamicin was the most effective among topical ear drop antibiotic. However, there are controversies over the question of ototoxicity with the topical usage of aminoglycosides, such as gentamicin. Among the various antibiotics available as topical ear drops, gentamicin was highly effective against our study isolates. However concerns regarding its ototoxic potential may limit its use. Newer antibiotics available like ciprofloxacin were also found to be fairly susceptible to antibiotic bacterial agents isolated in present study with added advantage of not being ototoxic. Systemic therapy should be reserved for cases of CSOM that fail to respond to topical therapy. Topical therapy presumably fails because the antibiotics cannot reach the organism in the ear. Systemic therapy should be reserved for cases of CSOM that fail to respond to topical therapy.

Conclusion:
Staphylococcus aureus was the most commonly isolated bacteria in CSOM in this part of north east India and gentamicin appeared to be the first line antibiotic followed by ciprofloxacin. Organisms are found to be less susceptible to erythromycin and co.
trimoxazole. With the development and widespread use of antibiotics, the types of pathogenic micro-organisms and their resistance to antibiotics have changed. Continuous and periodic evaluation of microbiological pattern and antibiotic sensitivity of isolates is thus necessary in order to determine the magnitude of risk of complications by early institution of appropriate treatment. As higher incidence of disease was seen among children so educating parents and guardians on possible risk-factors of the disease may be a preventive strategy that might reduce disease occurrences. We believe that our data may contribute to an effective management of CSOM.

Declaration of interest: None

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