

Original Research Article

Descriptive study of various bacterial flora in chronic otitis media

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ABSTRACT

Background: Chronic otitis media (COM) is the inflammation of the middle ear, which is characterised by ear discharge due to tympanic membrane perforation, at least for over a month. The inflammation is due to the underlying infection, due to which the middle ear mucosa gets inflamed, leading to discharge and eventual perforation of the tympanic membrane. The aim of the study was to study the microbial flora and its culture and sensitivity from ear discharge in chronic otitis media.

Methods: Total of 100 ears with either mucosal COM or squamosal COM were included in this study. Patients coming to the outpatient department (OPD) with discharging ear were subjected to aural suctioning/cleaning with the use of bulls eye lamp. After cleaning the external auditory canal, sterile aural speculum were introduced into the EAC, creating a sterile conduit. Following this, middle ear discharge or the discharge around the tympanic membrane was collected using sterile cotton swabs and sent for laboratory within half an hour for mycological and bacteriological study.

Results: Majority of patients were in an age group of 10-20 years, followed by age group of 21-30 years. The male to female ratio was 4.7:5.3. Out of the 100 swabs sent for culture and sensitivity, 6 swabs had no growth whereas *Pseudomonas aeruginosa* was the highest isolated organism (36 swabs), followed by *Proteus mirabilis* (23 swabs). Aerobic infection was the commonest, isolated in 47.9% of culture positive swabs. Polymyxin B was the most sensitive antibiotic, whereas cefotaxime was the most resistant antibiotic.

Conclusions: *Pseudomonas aeruginosa* and *Proteus mirabilis* are the main bacteria isolated in mucosal COM. There is development of drug resistance in the majority of bacteria in chronic otitis media, due to excessive usage of antibiotics, making the treatment much difficult.

Keywords: Mucosal, Squamosal, Chronic otitis media, Antibiotics

INTRODUCTION

Chronic otitis media (COM) is an inflammation and infection of the middle ear which is characterized by a persistent otorrhoea from a perforated tympanic membrane over a month.¹⁻³

The main subtypes of COM are the following:

Mucosal COM: Tubotympanic COM, also known as mucosal (safe) disease, is characterized by a perforation in the pars tensa, which are usually called safe since there

will be less chances of developing complications.^{4,5} Here, there is chronic inflammation in the mucosa of middle ear and mastoid involving varying degrees of oedema, submucosal fibrosis, and mostly inflammatory infiltrates. Active mucosal COM is sometimes associated with destruction of ossicular chain. It is accepted that infection of upper respiratory tract with virus often precedes bacterial AOM.⁶

Squamosal COM: It is also called as the attic or the unsafe type. The name is given because any involvement of the attic or the antral part can result in formation of cholesteatoma and subsequent bone destruction. Here, a

negative, and a static middle ear pressure may lead to the retraction of tympanic membrane, leading sometimes to the creation of a retraction pocket, where there is invagination of a part of tympanic membrane into the middle ear.

COM has received considerable attention not only because of its high incidence and chronicity, but also because of issues such as bacterial resistance and ototoxicity with both topical and systemic antibiotics.⁷ Hence, a study of microbial flora in COM and its antibiotic sensitivity is of utmost importance.

Discharge from the middle ear: In case of COM, the commonest symptom usually will be discharge from the middle ear, so a study of discharge is of utmost importance in order to study about microbial flora involved in COM. The discharge can be explained as following: scanty/profuse discharge, mucoid/ mucopurulent/purulent, foul smelling/ non-foul smelling and blood stained or non-blood stained.

A profuse, mucoid, non-foul smelling and non-blood stained discharge points towards mucosal/ safe COM.²

Chronic otitis media is an inflammation and infection of the middle ear which is characterized by a persistent otorrhoea from a perforated tympanic membrane over a month.¹⁻³

A scanty, purulent, foul smelling, blood stained discharge associated with pain suggests a squamosal/unsafe COM. In addition, the above discharge characteristics of squamosal COM is because of the osteitic changes in the mastoid in the middle ear.

Having sound knowledge of the frequency of a polymicrobial infection, in particular the extent of involvement of an anaerobe is limited due to the differences in the collection and method of culture.^{8,9} A complicated CSOM was frequent before the antibiotic era. However, due to the introduction of antibiotics, clinicians have been given a tool which is used even without having an accurate causative diagnosis. Adding to this, the irrational use of antibiotics has in fact led to the emergence of the bacteriae which are multi-drug resistant strains, and hence causing disease complication.¹⁰

METHODS

This longitudinal study was carried out in the Department of Otorhinolaryngology, D.Y. Patil Medical College and Hospital, Pune Maharashtra over a period of two years from September 2018 to August 2020 in all patients with history and symptoms suggestive of chronic otitis media. A sample size of 100 ears was planned.

100 randomized swabs of ear discharge from patients attending outpatient department of ENT was aged above

10 years and below 60 years were included in the study group.

The following were excluded from the above described study.

All patients having comorbid conditions along with COM such as diabetes, hypertension. Patients who were already on antibiotic ear drops and/or systemic antibiotics for the chronic otitis media. Age group below 10 years and above 60 years. Patients having superadded otomycosis were excluded from the study. Patient refusal. COM occurring post surgery. Myringitis. Otitis externa. Trauma.

Data collection method

Patients coming to the OPD with discharging ear were subjected to aural suctioning/cleaning with the use of bulls eye lamp. After cleaning the external auditory canal, sterile aural speculum were introduced into the EAC, creating a sterile conduit. Following this, middle ear discharge or the discharge around the tympanic membrane was collected using sterile cotton swabs and sent for laboratory within half an hour for mycological and bacteriological study.

Sample collection and processing

All the samples were collected using strict aseptic precautions, were transported in sterile containers and processed immediately as per standard protocols.¹¹

Identifying the organisms with the help of antibiotic susceptibility testing:

Isolates were identified, with sound knowledge of colony morphology and biochemical reactions as per conventional isolation and identification procedure.¹¹

Kirby Bauer disk diffusion was done on all the isolates to check the antibiotic susceptibility as per CLSI 2018 guidelines.¹²

Processing of samples

The clinical samples were processed in various media as per protocol followed in our laboratory example- blood agar and Mac Conkey agar for pus sample. Special media were used whenever required¹¹. These plates were routinely incubated at 37° C aerobically and growth was observed after 24 hours of incubation and colony characteristics were noted.

Isolation and identification of organisms

After performing gram stain, the gram positive and negative bacteria that were isolated were subjected to various biochemical reactions for speciation.¹³

Kirby Bauer sensitivity testing was used to check antibiotic susceptibility as per CSLI 2018 guidelines.¹¹

Method of inoculation of isolated bacteria and placement of discs

Preparation of media

Muller Hinton agar (MHA) was prepared freshly by pouring to a depth of 4 mm (25 ml medium) in flat bottomed 9 cm petri dishes on a level surface. After the preparation of media, one plate was incubated without organism for checking its sterility. Before inoculation, plates were dried, so that there were no droplets of moisture on the agar surface.

Inoculum preparation

The organisms were sub-cultured one day prior to test. A 0.5 McFarland turbidity standard was used to prepare the inoculum in the study. The inoculum was made by suspending a colony in peptone water and matching the turbidity with a 0.5 McFarland standard. With the help of a sterile swab test organisms were inoculated into MHA.

Application of antibiotic

Commercial antibiotic discs (Himedia) stored in refrigerator were brought to room temperature to minimize condensation of moisture, which may lead to hydrolysis of antibiotic when applied on media. Six different antibiotics were applied on one plate 24 mm from center to center of each disc, in 90 mm plate.

Incubation and interpretation

Plates were incubated at 37°C overnight for 18-24 hours. Before results with individual disc were read, plates were examined to confirm that a confluent lawn of growth was obtained. Measurement of diameter of the zone of

inhibition was done in millimeters. Interpretation was done as per CSLI guidelines 2018.¹¹

RESULTS

Age distribution

Total of 100 ear swabs were included in this study and were in the age group of 10-60 years. Majority of patients were in an age group of 10-20 years, followed by age group of 21-30 years.

Table 1: Percentage of isolates.

Organism	No. of isolates	Percentage
<i>Pseudomonas aeruginosa</i>	36	36%
<i>Proteus mirabilis</i>	23	23%
<i>Methicillin sensitive staph. Aureus</i>	6	6%
<i>Methicillin resistant staph. Aureus</i>	6	6%
<i>Escherichia Coli</i>	3	3%
<i>Pseudomonas fluorescens</i>	2	2%
<i>Klebsiella pneumonia</i>	5	5%
<i>Staphylococcus epidermidis</i>	6	6%
<i>Streptococcus pyogenes</i>	5	5%
<i>Citrobacter Freundii</i>	2	2%
No growth	6	6%

Sex distribution

Out of 100 patients included in the study, there was a female preponderance, with 53 cases being females and 47 cases being males.

Table 2: Culture and sensitivity if isolates.

	<i>Proteus mirabilis</i>	<i>Pseudomonas aeruginosa</i>	<i>MS SA</i>	<i>MR SA</i>	<i>E. Coli</i>	<i>Pseudomonas fluorescens</i>	<i>Klebsiella pneumoniae</i>	<i>Staphylococcus Epidermidis</i>	<i>Streptococcus pyogenes</i>	<i>Citrobacter freundii</i>
Ciprofloxacin	S	R	S	S	S	S	R	S	S	S
Polymyxin B	S	S	S	S	S	S	S	S	S	S
Ofloxacin	S	S	S	R	S	S	R	S	S	S
Gentamycin	S	R	S	S	S	S	R	-	S	S
Amikacin	S	R	-	-	S	S	S	-	S	-
Chloramphenicol	S	-	-	-	S	R	-	-	-	-
Piptaz	S	S	-	-	S	S	R	-	S	S
Cotrimoxazole	S	-	S	S	-	S	-	-	R	-
Cefotaxime	R	R	R	R	R	R	R	R	R	R
Cefoxitin	R	R	S	R	R	R	R	R	R	R

Organisms isolated

All of the 100 patients with COM, included in the study, were having ear discharge as a presenting complaint. After aural cleaning, the middle ear discharge or discharge around the tympanic membrane (as in squamosal COM), from either ear, was sent for microbial examination. All of them had growth in the culture media. Out of 100 isolates, majority of the organism was *Pseudomonas aeruginosa*, and *Pseudomonas fluorescens* and *Citrobacter freundii* were the least isolated organism. 6 of the swabs, which were sent for culture and sensitivity, showed no growth.

Based on aerobic or anaerobic

Out of 94 isolates, 45 were aerobic organisms, 44 were facultative anaerobic, and 5 were aerobic and facultative anaerobic both.

Culture and sensitivity

The swabs taken from the middle ear were sent to test culture and sensitivity, and also antibiotic sensitivity and resistance were determined. The sensitivity and resistance pattern of each of the isolates are shown in the table below. *Klebsiella pneumonia* was the most resistant of all the isolates, and *proteus mirabilis* being the most sensitive.

DISCUSSION

This study was done aiming at isolating the various microorganisms in patients presenting with COM. With proper implementation of the inclusion and exclusion criteria, the study population was subjected to aural cleaning and culture swab from the middle ear or the retracted part of tympanic membrane, and the result obtained is explained below.

In our study, majority of the study population were in age group of 10-20 years of age. 30% of population was in 10-20 years group, and the least (11) were in 51-60 years age group. In a similar study by Kumar et al, maximum patients, i.e. 37% were in 0-10 years age group.¹⁴ Chronic otitis media is common in younger population, including the pediatric and adolescent age groups, but owing to the exclusion criteria of <10 years, our study holds true the fact that COM is common in younger age group, mostly due to the Eustachian tube being wider and more horizontal compared to adults.

In our study, there was a female preponderance, with 53% of the study population being females, and 47% being males. This was in contrary to the study done by Kumar et al, where there was a male preponderance with 61.3% of study group being males and 38.7% of population being females.¹⁵

Out of the 100 swabs taken from 100 ears of patients who presented with COM, 94 swabs were positive for isolates, whereas in the remaining 6 isolates, there was no growth.

Out of the 100 culture study, *Pseudomonas aeruginosa* was the highest isolated organism, with 36 culture being positive, which was followed by *Proteus mirabilis*, with 23 cultures being positive. *Staphylococcus aureus* was the third most common in this study, with 12 cultures being positive for it. Out of the 12, 6 isolates were MSSA and 6 isolates were MRSA. Out of 94, 6 isolates were positive for *Staphylococcus epidermidis*, 5 isolates positive for *Klebsiella pneumonia*, 5 positive for *Streptococcus pyogenes*, 2 isolates positive for *Pseudomonas fluorescens* and 2 isolates positive for *Citrobacter freundii*. Similar study was done by Kumar et al, in patients of CSOM.^{15,16} Their study matches with the present study with 46% *Pseudomonas aeruginosa* isolates, followed by *Staphylococcus aureus* (33.19%), followed by *proteus species* (6.95%), *E. coli* (3.47%), *Klebsiella species* (6.95%), and *Citrobacter* (1.73%).

Also, in the present study, there was a predominance of aerobic bacterial growth (47.9%), followed by anaerobic growth (46.8%), followed by mixed (5.3%). But compared to this, a study done by Sweeney et al, isolated aerobic bacteria in 61% cases and anaerobic bacteria in 44 % of cases.¹⁷ There was no much difference of aerobic and anaerobic infection in COM infections in our study.

Antibiotic sensitivity results

In our study, the isolates obtained in the culture were subjected to antimicrobial sensitivity testing to infer the sensitivity and resistance of the isolated flora towards the various antibiotics. The results obtained are as follows:

Pseudomonas aeruginosa, which was the most common isolate obtained, was sensitive to polymyxin B, Ofloxacin, piperacillin-tazobactam, ticarcillin, and colistin, Whereas it was resistant to most of the antibiotics such as gentamycin, amikacin, ciprofloxacin, cefotaxime, ceftazidime, meropenem, amoxicillin-clavulanic acid, and levofloxacin. In a similar study done by Kumar et al, the *Pseudomonas aeruginosa* strains were sensitive to ticarcillin, and colistin, whereas there was reduced sensitivity to cefepime, amikacin, ciprofloxacin, and gentamycin.¹⁶ Where as in a similar study done by Deb et al, *pseudomonas* was the most common gram negative isolate but was sensitive to ciprofloxacin.¹⁸ Compared to these studies, our study shows a variation where there is resistance noted for gentamycin, amikacin, ciprofloxacin, cefotaxime, ceftazidime. This could be possibly due to the emergence of antibiotic resistance strains of *Pseudomonas aeruginosa*, which emphasizes on the limited use of antibiotics to be done.

Proteus mirabilis, the second most isolated organism, showed sensitivity to most of the antibiotics, including cotrimoxazole, polymyxin B, ofloxacin, gentamycin, amikacin, chloramphenicol, piperacillin-tazobactam, ciprofloxacin, norfloxacin, meropenem, amoxicillin-clavulanic acid, ceftriaxone, ticarcillin, nitrofurantoin, and levofloxacin, whereas it was resistant to cefotaxime and

cefoxitin. In the study done by Kumar et al, *Proteus mirabilis* was sensitive to all above mentioned antibiotics except cefazolin.¹⁶ We can note the difference in our study compared to this study, where *Proteus mirabilis* is resistant to 2nd and 3rd generation cephalosporins, compared to resistance for the first generation cephalosporin in the comparative study mentioned above. This again could be due to drug resistance developed by *Proteus* species for cephalosporins, which again emphasizes on the importance of limited usage of antibiotics.

Methicillin sensitive Staphylococcus aureus was sensitive to cotrimoxazole, polymyxin B, ofloxacin, gentamycin, cefoxitin and colistin, and was resistant to ciprofloxacin. This was similar to the study by Rakesh et al, where MSSA had reduced sensitivity towards ciprofloxacin.¹⁶

In our study methicillin resistant *Staphylococcus aureus* was sensitive to cotrimoxazole, polymyxin B, gentamycin, ciprofloxacin, and colistin, but was resistant to cefoxitin. This roughly corresponds to the study done by Ahmed, where MRSA showed resistance against cephalothin, which is a first generation antibiotic.¹⁹

Klebsiella pneumoniae was resistant to gentamycin, piperacillin tazobactam, ciprofloxacin, cefotaxime, cefoxitin, meropenem, amoxicillin-clavulanic acid, ofloxacin, cefuroxime, ceftazidime, and nitrofurantoin whereas it was sensitive to amikacin, polymyxin B, tigecycline and colistin. Similar study by Kumar et al implies *Klebsiella pneumoniae* being sensitive to meropenem.¹⁶ This change in sensitivity pattern could be due to emergence of resistant strains of *Klebsiella pneumoniae*.

6 of the total of 100 swabs showed no growth, which is similar in comparison to study done by Kumar et al, where there was no growth in 5.72% of culture study.¹⁵

CONCLUSION

Our study has demonstrated that chronic otitis media can affect wide range of age groups, with adolescents being mostly involved. *Pseudomonas aeruginosa*, and *Proteus mirabilis* are the main isolated bacteria in chronic otitis media, whether it be mucosal, or squamosal COM. *Klebsiella pneumoniae* plays an additional role in predominantly squamosal COM. Antibiotics such as second and third generation cephalosporins and amoxicillin-clavulanic acid are no longer effective in treating chronic otitis media, due to development of bacterial resistance by majority of bacteria towards it. Sending a swab from the middle ear for culture and sensitivity is of utmost importance, since it gives accurate sensitivity of the isolated bacteria towards a specific antibiotic group, thereby preventing further development of bacterial resistance.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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