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Microbiological Profile and Antibiotic Sensitivity Pattern of Active Mucosal Chronic Otitis Mediaand Active Squamous Chronic Otitis Media (with Cholesteatoma) in a Tertiary Care Hospital of Hisar, (Haryana) India

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Abstract

Background: Chronic Otitis media (COM), previously called as Chronic Suppurative Otitis Media (CSOM) is a chronic inflammation of the middle ear cleft, with permanent abnormality of pars tensa or pars flaccida which presents as recurrent otorrhea. Its termed 'active mucosalchronic otitis media' when there is a permanent defect of pars tensa with an inflamed middle ear mucosa, with or without granulations, producing mucopus which keeps discharging for more than 3 months despite medical treatment. Its termed 'active squamous chronic otitis media' or 'chronic otitis media with cholesteatoma' when there is retraction of the pars flaccida or tensa that has retained squamous epithelial debris and is associated with scanty foul smelling blood or pus discharge along with inflammation of the adjacent mucosa. Rationale and adequate use of antibiotics along with surgery remains the mainstay of the treatment of Chronic Otitis media (COM).

Objectives: To study and compare the microbiological profile and antibiogram of chronicmucosal otitis media and chronic squamous otitis media with cholesteatoma.

Materials and methods: This was a descriptive cross sectional study of ear swab with pus sample of 351COM patients studied for the type of microorganism, bacterial isolate, the culture and antibiotic sensitivity pattern.

Results: Out of all the pus samples of active mucosal COM patients (without cholesteatoma) grown on cultures revealed 252 bacterial (77.77%) and 5 fungal isolates (1.54%) and 67 (20.68%) ear swab samples were found to be sterile. The predominant bacterial isolates found were *Pseudomonas aeruginosa* (39.20%) and *Staphylococcus aureus* (32.72%). *Pseudomonas aeruginosa* was found to be highly sensitive (above 80%) with 05 antimicrobials like PB, CL, IPM, PIT. MER. *Staphylococcus aureus* recorded highest sensitivity with as many as 10 antimicrobials giving wide range of options namely PIT, LZ, CFS, CPT, AK, G, DO, VA, TEI, TOB. In patients of active squamous COM (with cholesteatoma), the most common bacterial isolates were gram negative bacteria constituting 93% ($p < 0.05$) whereas only *Staphylococcus aureus* from Gram positive bacteria was isolated.

Conclusion: *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the most common bacterial isolates with pseudomonas having high sensitivity to less antimicrobial then staphylococcus.

Keywords: Chronic otitis media; Cholesteatoma; Bacteriological profile; Pseudomonas aeruginosa; *Staphylococcus aureus*; Antibiotics

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Introduction

Infections of the middle ear space and their sequel have plagued mankind from the beginning of human era. Chronic Otitis media (COM), previously called as Chronic Suppurative Otitis Media (CSOM) is a chronic inflammation of the middle ear cleft, with permanent abnormality of pars tensa or pars flaccida which presents as recurrent otorrhea [1]. Patients presenting with tympanic

perforations and discharging ear for a period of 3 months, despite medical treatment, are recognized as COM cases [2]. The WHO definition requires only 2 weeks of otorrhoea [3] but otolaryngologists tend to adopt a longer duration, e.g. more than 3 months of active disease [4] profuse, intermittent, mucous drainage is commonly noted in chronicmucosal otitis media without cholesteatoma whereas scanty, blood stained discharge is seen in chronic squamous otitis media with cholesteatoma.

Chronic Otitis Media (COM) is a major public health problem and most common illness in Ent. As per WHO, India is one of the countries with highest COM prevalence where urgent attention is needed (WHO, 2004). It is a common cause of hearing impairment and can also lead to fatal intracranial infections [4]. It presents with varied clinical features depending on the duration, severity and progression of the disease. As a result of the complex contiguous relationship of the middle ear and essential intracranial structures, severe complications (intracranial and extracranial) can result from untreated or poorly treated OM [5]. Chronic Otitis Media (COM) can also be differentiated from Acute Otitis Media (AOM) on bacteriological grounds. In AOM the bacteria found in the middle ear include *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae* and *Micrococcus catarrhalis*. In cases of COM with purulent discharge and granulation tissue and COM with cholesteatoma presenting with blood stained discharge have been showing similar microbial growth on culture [6,7]. The bacteria may be aerobic like *Pseudomonas aeruginosa, S.aureus, Escherichia coli, Streptococcuspyogenes, Proteus mirabilis, Klebsiella species* or anaerobic like bacteroides, peptostreptococcus, proprionibacterium.

The bacteria are infrequently found in the skin of the external canal, but may proliferate in the presence of trauma, inflammation; lacerations or high humidity. These bacteria may then gain entry to the middle ear through a chronic perforation [8]. Cultures with sensitivity are necessary to guide antibiotic therapy because most patients with COM have already been treated at various ENT centres, with multiple antibiotic regimens. For this reason, it is not surprising that two-thirds of all COM patients are infected with β-lactamase-producing microorganisms. Antimicrobial agents that have been included in the treatment of COM are aminoglycosides such as gentamicin, tobramycin, and neomycin in combination with polymyxin B sulfate because of their antipseudomonal properties. More recently, fluoroquinolone antibiotics such as ciprofloxacin and ofloxacin have gained popularity because of their antipseudomonal properties, minimal bacterial resistance, lack of ototoxicity, and potential oral route of administration [9]. Complications of COM can be avoided with early diagnosis and appropriate treatment with antibiotics [10]. Most experts would start with a wide-spectrum antibiotic on an empiric basis and make a request for cultures if drug resistance is suspected [11]. Keeping all this in mind it was thought prudent to find out the microbiological profile and antibiogram of all patients of COM coming having otorrhea or with cholesteatoma.

Aims and objectives

To study the microbiological profile and antibiograms of Chronic Otitis Media (COM) with and without cholesteatoma.

Material and Methodology

A descriptive cross sectional study carried out in department of ent in collaboration with microbiology laboratory of NC Jindal Institute of Medical Sciences (N.C.JIMS), Hisar Haryana. Total 351 Patients of Chronic Otitis Media with and without cholesteatoma coming to Ent OPD from January to May 2016 were included for the study. Study was commenced after the approval from Institutional Ethical Committee of N.C.JIMS. Sample size was calculated using open epi software at the 95% confidence interval.

Inclusion criteria

Patients diagnosed clinically with active mucosal Chronic otitis media (without cholesteatoma) of both genders belonging to adult age group presenting with unilateral or bilateral Purulent ear discharge with granulation tissue of more than 3 months, and patients diagnosed with active squamous chronic otitis media (with cholesteatoma) presenting with blood stained discharge were selected on OPD and IPD basis and willing to give informed written consent were included in the study.

Exclusion criteria

Patients with active ear discharge of less than 3 months (ASOM), ear discharge with intact tympanic membrane (Otitis externa), patients with intra-cranial or extracranial complications (petrositis, facial paralysis, meningitis, abscess), patients with serious medical conditions such as immunodeficiency states, malignancy or blood dyscrasia, were excluded from the study.

Methodology

Study conduct: A diagnosis of COM with or without cholesteatoma was made using otoscope. The diagnosis of COM rests on the verification of a discharging tympanic perforation clinically and confirmed with radiological examination by X-ray mastoid bilateral schullers View [4] and pure tone audiometry.

Specimen / Sample collection: Specimens were collected with all aseptic microsurgical techniques. All pus samples were collected as ear swabs. Each tympanic membrane was adequately visualized. The external auditory canal was swabbed 3 times using sterile cotton pledges soaked in povidone-iodine (betadine). A sterile cotton swab soaked in 70% ethyl alcohol was likewise applied thrice around EAC. A sterile, dry cotton micro cotton swab was applied to the fluid draining from the tympanic membrane with sterile ear speculum avoiding contact with the external auditory canal walls. The samples were transferred in specimen bottle which was labelled with patients name and number and submitted for direct culture aerobes and sensitivity studies at the microbiology laboratory within 15mins of collection [12].

Direct smear examination: With one swab a thin smear is made on a clean glass slide and is fixed with 95% methanol, by pouring one or two drops on the smear and allowed to act for a minimum of 2 minutes or until the methanol dries on the smear. Gram staining is done for the smears so made and is examined under oil immersion objective to note the various morphological types of bacteria, their number, gram reaction, presence or absence of inflammatory cells and also to note the numbers of squamous epithelial cells in the sample [13].

Cultures used for the specimens: Direct culture material was seeded on, blood agar, mac conkey's agar, chocolate agar plates. All plates were incubated aerobically at 37^0C and evaluated at 24 hours, 48 hours and 72 hours and the plates were discarded if there was no growth. The specific identification of bacterial pathogens was done based on microscopic morphology, staining characteristics, cultural and biochemical properties using standard laboratory [13,14].

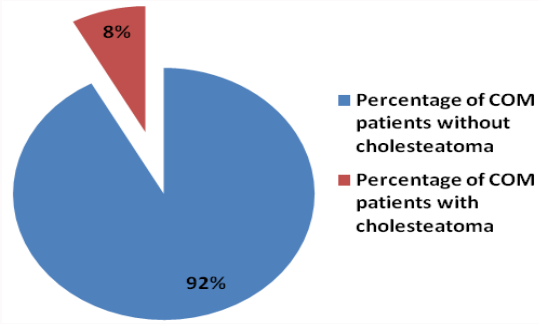

Figure 1: Shows percentage distribution of patients of com without cholesteatoma was 92% and with cholesteatoma were 8%.

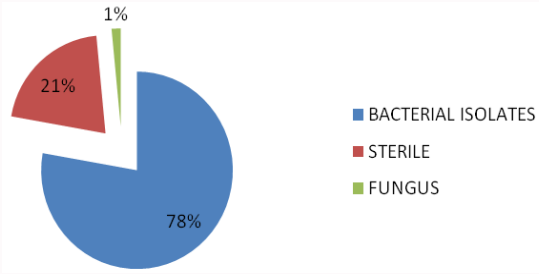

Figure 2: Microbiological profile of patients of com: percentage of bacterial, fungal and sterile swabs. The microbiological profile of ear discharge found in the pus samples of COM revealed bacteria was 78%, 21% samples were sterile and 1% showed fungal growth. The proportion of bacterial growth was statistically significant (p <0.05).

Antibiotic Sensitivity pattern: Isolates yielding pure cultures were further studied for antimicrobial sensitivity and resistance, using drugs chosen from commonly prescribed medications for patients with COM in our institution. This study was limited to identification of aerobic bacterial isolates from the samples submitted for culture. No studies were done for anaerobes, viruses or fungi. Antibiotic sensitivity testing was done by Kirby Bauer disk diffusion method.

The following parameters were recorded

Type of microorganism: Bacterial isolates, Fungi, Sterile, if any percentage distribution of patients of COM with and without cholesteatoma

Bacterial profile: Bacterial strains.

Antibiotic Sensitivity pattern: Recorded as sensitive (S), Intermediate (I), Resistant (R).

The data was analysed using unpaired "t" test of significance.

Discussion

In our study, total 351 patients of either sex coming to the ent OPD of NC. Jindal Institute of Medical Sciences (N.C.JIMS), Hisar Haryana were enrolled for the study. Clinically out of 351 patients, 324 patients (92%) where diagnosed with active mucosal COM (without cholesteatoma) whereas 27 patients (8%) had active squamous COM with cholesteatoma (8%) (Figure 1). All the patients were in the adult age group. India is a developing country and majority are still living under poverty level. After developing perforation in tympanic membrane in childhood due to recurrent upper respiratory tract infections, activities such as swimming, bathing and washing clothes. In contaminated water supply, pouring oil in the ear due to traditional beliefs also attribute to chronic ear infections [12,15,16].

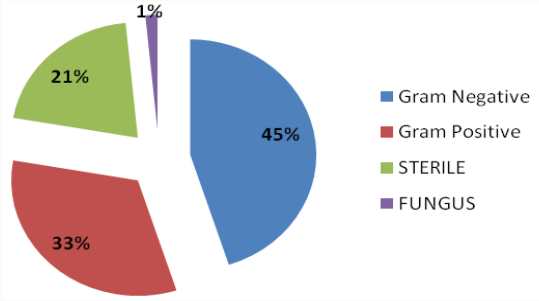

Figure 3: Gram positive and gram negative bacterial distribution.The most common bacterial isolates were gram negative bacteria constituting 45% of all the ear swabs of COM patients without cholesteatoma (p <0.05).

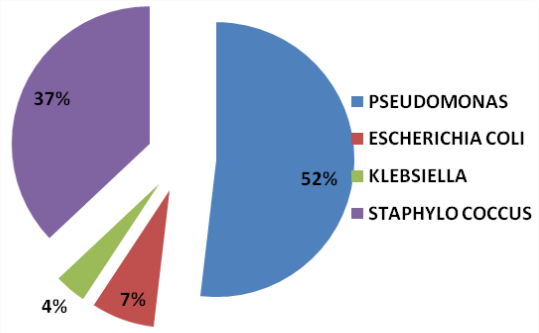

Figure 4: Percentage of gram positive and gram negative bacterial distribution. The most common bacterial isolates were gram negative bacteria constituting 93% of all the ear swabs of COM patients with cholesteatoma (p <0.05) whereas only staphylococcus Aureus from Gram positive bacteria was found in the samples of COM with cholesteatoma.
PB: Polymixin B; CL: Colistin; IPM: Imipenem/Cilastin; MRP: Meropenem; PIT: Piperacillin-Tazobactam; CPM: Cefepime; G: Gentamycin; AK: Amikacin; TOB: Tobramycin; AT: Aztreonam; LE: Lefloxacin; CFS: Cefaperzone/ Sulbactam: PI: Pipercillin; LOM: Lomifloxacin; CPT: Cefepime/Tazobactam; TCC: Ticcarcillin/Clauvlanin acid; CTR: Ceftriaxone

In this study, in patients with active squamous COM (with cholesteatoma), the proportion of bacterial swabs were found significantly higher than other type of swabs (p <0.05). The bacterial growth was found in 78%, fungal in 1% while 21% swabs were found to be sterile (Figure 2). These results are similar to the study done by Harvinder Kumar and Sonia Seth [17] Suman Yeliand Heba Abdel Fattah [18], Shreshta BL et al. [19] in which the proportion of bacterial swab were highly significant for pseudomonas in patients of COM with and without cholestetoma [20].

Whereas in cases of active mucosal COM (without cholesteatoma), the most common bacterial isolates were gram negative bacteria constituting 45% of all the ear swabs of COM without cholesteatoma followed by gram positive bacteria contributing 33%. Our finding coincides with the findings of many studies [18,21,22] (Figure 3) where, gram negative bacteria constituted 93% of all the ear swabs of COM patients with cholesteatoma (p <0.05) (Figure 4). Among all the total twenty seven bacterial isolates the predominant bacteria isolated in our study was *Pseudomonas sp*. (52%) from gram negative bacteria followed by *Staphylococcus aureus* (37.04%) while *Ecoli,* Klebsiella and Proteus were in very few in cases up to 15% which is insignificant. Our results were in accordance with many of the previous studies which showed pseudomonas to be the most common bacteria isolated from COM with cholesteatoma cases [23-25] (Figure 4). Our result shows that pseudomonas seems to be the most common bacterial isolates in

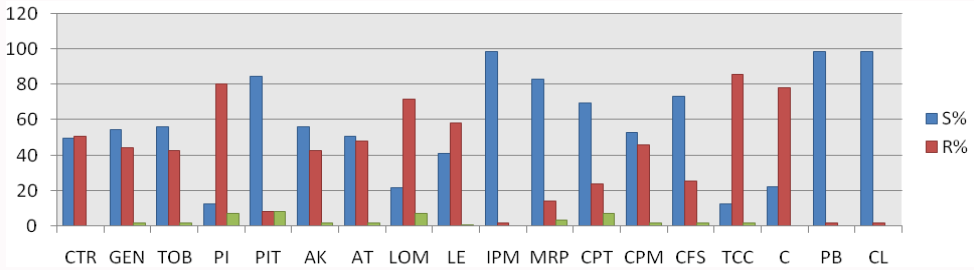

Figure 5: Antibiogram for the most common bacterial isolate pseudomonas a.
Pseudomonas A.washighly sensitive (above 80%) with 4 antimicrobials like PB, CL, IPM-, PIT, MRP.
PIT: Piperacillin-Tazobactam; CPM: Cefepime; CPT: Cefepime/Tazobactam; CXM: Cefuroxime; CFS: Cefoperzone/Tazobactam; G: Gentamycin; CX: Cefoxitin; AK: Amikacin; TOB: Tobramycin; AS: Ampicillin/Sulbactam; LZ: Linexolid; Le: Levofloxacin; A: Ampicillin; DO: Doxycycline; MO: Moxafloxacin, E: Erythromycin; VA: Vancomycin; TEI: Teicoplanin; LOM: LomifloxaciN

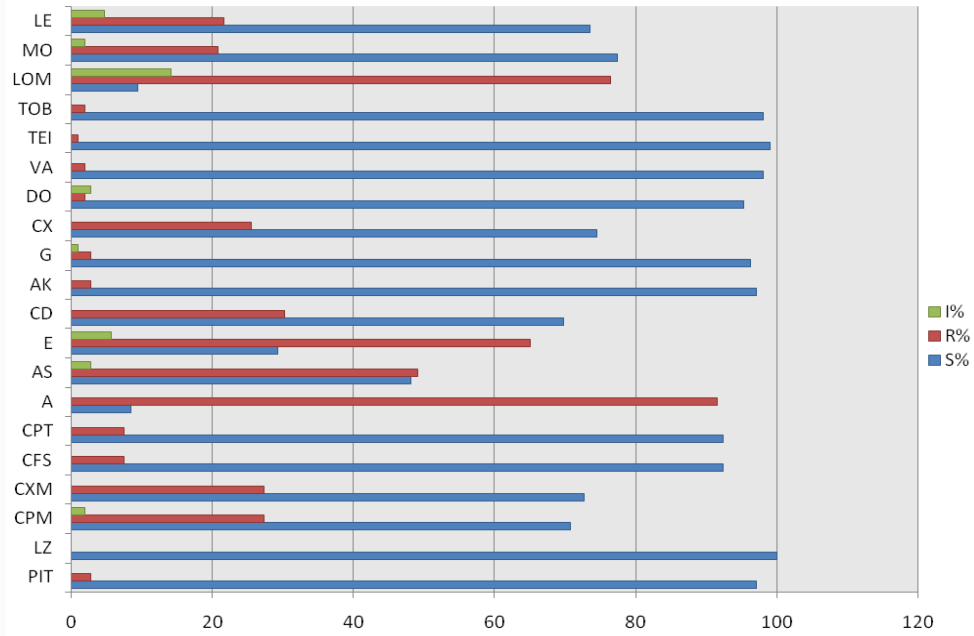

Figure 6: Antibiogram for staphylococcus a.
For staphylococcus Aureus the figure shows as many as 10 antibiotics with sensitivity more than 80% giving wide range of options namely PIT, LZ, CFS, CPT, AK, G, DO, VA, TEI, TOB

COM with or without cholesteatoma.

Several studies elsewhere in the world have reported that the most common isolated organisms were *Pseudomonas spp.* followed by *S. Aureus* [26]. Pseudomonas aeruginosa is a gram-negative rod that is extremely common in moist environments and is generally found colonizing the EAC. Possible explanation to this difference in isolation rate might be related to the effect of climate. Bacterial colonization of otitis media increases as temperatures rises which in-turn increases the isolation rate of bacteria [27]. The ability of *P. aeruginosa* to survive in competition with other organisms may be due to minimum nutrition requirement. Ability of pseudomonas to use pile to attach to the necrotic or diseased epithelium of the middle ear. After attachment pseudomonas produces enzymes like proteases, lipopolysaccharides to elude from normal defence mechanism of body required for fighting infections [27]. *Staphylococcus aureus* and pseudomonas aeruginosa are the common aerobic isolates in COM. Both are indigenous microorganisms. *Staphylococcus aureus* is gram-positive coccus that colonizes the nares. Thus, it is not surprising that these are the important pathogens in chronic middle ear disease [9].

The antibiogram pattern revealed that the most common gram negative isolate *Pseudomonas aeruginosa* was found highly sensitive to following antibiotics PB-Polymixin B (98.4%) , CL- Colistin, (98%), MRP-Meropenam (82.68%) IPM- Imipenem/Cilastin (82.6%), PIT- Piperacillin-Tazobactam (84.25%), for all these antibiotics sensitivity was more than 80% *Pseudomonas areguinosa* displayed high resistance against TCC- Ticcarcilin/ Clavulunic acid (85.83%), PI- Pipercillin (80.31%), LOM-Lomifloxacin (71.65%) (Figure 5). The sensitivity and resistance pattern of pseudomonas was surprisingly not coinciding with the results of many studies and showed sensitivity for only few antibiotics which appears to be the warning sign for all the otololaryngologists treating COM in and around Hisar Haryana. Pseudomonas showing more resistance even to fluroquinolone group of antibiotic like lomifloxacin advocates us to ensure optimal and judicious use of antibiotic in treatment of COM. The reasons for the increase in resistance with pseudomonas open a new area for future research. Moreover the most common bacterial isolate in cholesteatoma patient was pseudomonas giving a new insight about resistance to antibiotic playing a crucial role in development of cholesteatoma.

The antibiotic sensitivity pattern revealed that *S. aureus* was found highly sensitive to as many as 10 antibiotics with 100% sensitivity for LZ-Linzeolid followed by TEI-Teicoplanin (99.06%), TOB- Tobramycin (98.11%), VA-Vancomycin (98.11%), LE-Levofloxacin (97.17%), AK-Amikacin (97.17%), PIT-Piperacillin-Tazobactam (97.17%), IP- Imepenem (97.17%), G- Gentamicin (96.23%), Doxycyclin (95.28%), CFS- Cefoperazon/Tazobactum (92.45%), CPT- Cefepime /Tazobactum (92.45%), MRP-Meropenam (80%) *S. aureus* displayed high resistance against A-Ampicillin 91.51% and LOM-Lomifloxacin (76.42%) (Figure 6). Our results differ from the study by Harvinder Kumar and Sonia Seth which showed cephalosporins (100%) and amoxicillin clavulanic acid (100%) were the most effective antibiotics against *staphylococcus aureus* [18]. Results of our study for *S. aureus* best coincided with Vijay Kumar Poorey [28], Pooja Thakur et al. [22], and Arvind N, Pavan Chand and Vishrutha [29]. Staphylococcus species sensitivity was higher with vancomycin, linezolid, and teicoplaninin which was the study reported on bacteriological profile of chronic suppurative otitis media in a rural tertiary care hospital [30].

Although the best modality of treatment for COM with or without cholesteatoma is surgery, that is modified radical mastecdectomy (canal wall up or canal wall down) [31] but the role of antibiotics and knowledge about the bacterial isolates responsible for development of COM will help the treating otololaryngologists for choosing rationale antibiotic prior to, during and immediately after surgery for complete eradication of infection and cure from disease, in the coming future.

Conclusion

Pseudomonas aeruginosa was found to be the most common bacterial isolatein the ear swabs of patients of COM with or without cholesteatoma. *Staphylococcus aureus* was the second most common bacterial isolate and was the only gram positive isolate found in the ear specimens.

The most sensitive drug for patients of COM with or without cholesteatoma remained MRP – Meropenam, PIT- Piperacillin-Tazobactam, IPM- Imepenem for both *staphylococcus aureus* and pseudomonas while lomifloxacin was the highly resistant drug. The rise in resistance with pseudomonas remains the matter of high concern.

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