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Original Research Article

Microbiological profile of aural swab pre-operatively and cholesteatoma sac intra-operatively

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Abstract

Objectives: Ear is prone to various kinds of infections out of which chronic otitis media is the most common type. Squamosal disease of chronic otitis media is associated with cholesteatoma and is prone to secondary infections especially by aerobic bacteria. This study was done to assess culture and sensitivity of preoperative aural swab and swab from purulent material from mastoid with cholesteatoma obtained intraoperatively during surgery. Comparative study has been made.
Methods: 100 Cases of chronic superlative otitis media of squamosal type with clinical diagnosis of cholesteatoma who were admitted and underwent mastoidectomy operation in tertiary ENT care centre during the period from November 2018-September 2020 were studied. Pus obtained during aural toilet was sent for bacteriological and antibiotic sensitivity studies and intraoperatively obtained cholesteatoma matrix sent for bacteriological study.
Results: Polymicrobial floras were observed both in pre-operative and intra-operative cultures. Staphylococcus aureus and Pseudomonas aeruginosa were the most common pathogens identified.
Conclusion: This study might have a role in facilitating use of culture guided antibiotic therapy to improve postoperative outcome and also to prevent recurrences, eventually helping in giving disease free ear to the patient.

Keywords: Cholesteatoma, unsafe chronic otitis media, squamosal disease, aural swab

Introduction

Traditionally, the term chronic superlative otitis media describes chronic middle ear disease and is defined as 'chronic inflammation of middle ear and mastoid cavity, which presents with recurrent ear discharge or otorrhoea through a tympanic membrane perforation' [1]. Chronic otitis media results from long-term Eustachian tube dysfunction with a poorly aerated middle-ear space, multiple bouts of acute otitis media, persistent middle-ear infection, or other chronic inflammatory stimulus. The clinical presentation of chronic otitis media varies with the underlying severity of the infection, the host response and the time course

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over which it manifests. Chronic superlative otitis media with cholesteatoma is typically a persistent disease [2].
Cholesteatoma can cause destruction of the structures in the middle ear cleft due to its locally invasive nature. Chronic infections further enhance the osteolytic effects of cholesteatoma [3]. Proliferation of cholesteatoma is governed by extrinsic factors such as toxins or bacterial antigens combined with lytic enzymes. This explains the close relationship between aggressiveness of cholesteatoma and repeated bacterial super infections.
Cholesteatomas are classified into two types

1. Congenital.
2. Acquired.

The acquired cholesteatoma manifest after birth with a retraction pocket in the ear drum due to chronic middle ear disease. The paediatric cholesteatoma is more infectious, more aggressive and more proliferative with less favorable diagnosis [3].
Cholesteatomas remain undetected for years and can manifest as dangerously large lesions invading infratemporal structures [4]. Recurring and foul smelling otorrhea with scant and purulent discharge is the most common presentation. Hearing loss can be progressive conductive or sensorineural. Vertigo or balance dysfunction can manifest due to destruction of bones overlying the semi-circular canals [5]. Temporary or permanent facial paralysis is observed in some patients. Cholesteatomas are prone to secondary infections specifically with aerobic bacteria i.e. Pseudomonas aeruginosa, Staphylococcus aureus and Proteus species or with anaerobic infections involving Bacteroides and Peptococcus/Pepto streptococcus [6]. If these infections are not controlled with appropriate antibiotics, it can cause fatal complications such as brain/epidural abscesses, meningitis, septic cavernous sinus thrombosis, subperiosteal abscess causing acute mastoiditis [7]. In patients with otorrhea, the antibiotic steroid drops should be advised to prevent inflammation. Empirical antibiotic therapy targeted against the common infections such as Pseudomonas aeruginosa, Staphylococcus aureus or anaerobic bacteria is recommended even before the availability of culture reports [6].
Fluoroquinolones were found to be more appropriate antibiotics. Oral or systemic antibiotics are necessary when the patient is suffering from advanced infection. Surgery is the primary treatment modality with emphasis mainly on the control the disease. Two types of surgical procedures are commonly used, Canal wall up (CWU) and Canal wall down (CWD) mastoidectomy. CWU mastoidectomy removes all mastoid air cells while maintaining the integrity of contours in the ear canal [8]. CWD mastoidectomy removes the bony posterior canal wall and creates a common cavity that combines ear canal and mastoid [9]. With the advent of newer generation of antibiotics considerable importance has been attached to bacteriology of aural swab and cholesteatoma, to achieve a dry mastoid cavity. Persistent infection hampers healing leading to persistent otorrhea. Therefore, it is very important to know the bacteria involved in order to control recurrence following surgery, reduce aggressiveness of cholesteatoma and limit incidence of complications. To determine probable role of microorganisms this study was undertaken to compare the microbiological flora from aural swab obtained preoperatively and cholesteatoma obtained intraoperatively in 100 patients at tertiary care hospital. This study might have a role in facilitating use of culture guided antibiotic therapy to improve postoperative outcome and also to prevent recurrences, eventually resulting in a dry ear.

Materials and Methods

Recruitment of patients: Cases of chronic suppurative otitis media of squamosal type with clinical diagnosis of cholesteatoma were who were admitted and underwent mastoidectomy

operation in tertiary ENT care centre during the period from November 2018 to September 2020 were evaluated for the inclusion and exclusion criteria. A total of 100 cases (56 males and 44 females) who met these criteria were recruited for this study. The mean age of the study cohort was 28.71±16.51 yr. Informed consent was obtained from all the study subjects. The study protocol was approved by the Institutional Ethical committee.

Inclusion criteria: All patients attending ENT OPD with chronic suppurative otitis media of squamosal type

Exclusion criteria

1. Patients with chronic suppurative otitis media of mucosal type.
2. Patients with ear discharge due to otitis externa, granular myringitis, otomycosis.
3. Patients with diabetes, other immunocompromised states (HIV, patients undergoing chemoradiotherapy, clinically diagnosed immunodeficiency disorders) and patients on long duration steroid therapy for any medical/dermatological disorders.
4. Patients not willing for research.
5. Patients not fit for surgical management.

Sterilization of swabs and collection tubes: Strong glass tubes of 4x3/8 inches sterilized in autoclave were used for collecting aural swabs and cholesteatoma matrix. Swabs were prepared from wooden sticks of 5-6 inches length with cotton wool pledge wrapped around one end. The glass tube with the swab was plugged with cotton wool and autoclaved for use.

Collection of specimens

Aural swab: At the first examination, the external auditory canal was cleaned with a dry sterile swab and fresh pus was collected by using another dry sterile swab under good illumination.

Cholesteatoma matrix: During mastoid surgery, the cholesteatoma matrix exposed was collected in sterile glass tube and transported in normal saline to the laboratory for bacteriological study along with aural swab.

Microbiological studies: Processing of the specimens: In the laboratory, the swabs and cholesteatoma matrix were collected in glucose broth for enrichment and incubated for a period of 24 hrs at 37 °C. The material from glucose broth was inoculated on to culture media i.e. nutrient agar plate, blood agar plate and MacConkey agar plate. Microorganisms were identified using standard bacteriological techniques.

Antibiotic sensitivity testing: Antibiotic sensitivity pattern was determined by using appropriate antibiotic discs by disc diffusion method. The aural swab and cholesteatoma matrix specimens were processed in the similar way. The anaerobic culture was not done due to limited facility.

Statistical analysis: Student t-test was used to assess whether distribution of continuous variables between two groups is statistically different. The data is represented in the form of mean, standard deviation (SD). Fisher exact test was used for the data computed in the form of presence or absence of variable in two groups in a 2 × 2 contingency table. The output is expressed as odds ratio (OR) and 95% confidence interval (CI). All the statistics were performed using graph pad prism software. The 'p' value <0.05 was considered statistically significant.

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Results

Demographic and clinical characteristics of the study subject

The mean age of the studied cases was 28.71±16.51 yr. A total of 56 males and 44 females were studied. The left CSOM was observed in 56% cases and right CSOM was observed in 44% cases.

Table 1: Segregation of cases based on culture findings

Culture Findings	No. of subjects (%)	OR	95% CI	P-value
Aural swab and cholesteatoma culture showing same organism	18(18%)	1.98	0.86-4.53	0.15
Aural swab and cholesteatoma culture showing different organisms	34(34%)	4.64	2.14-10.05	<0.0001
Aural swab positive and cholesteatoma culture negative	23(23%)	2.69	1.21-6.00	0.02
Aural swab negative and cholesteatoma culture positive	15(15%)	1.59	0.68-3.73	0.39
Sterile culture in both aural swab and cholesteatoma	10(10%)	Ref	---	----
Total	100			

(OR: odds ratio; CI: confidence interval)

It was seen from Table 1 that bacteria were isolated in 90% of the cases. In 34% cases aural swab and cholesteatoma cultures showed different organisms, this constituted the majority. In 18% cases aural swab and cholesteatoma showed similar organism. In 23% cases only aural swab was positive for culture whereas, cholesteatoma culture showed no bacterial growth. In 15% cases only cholesteatoma culture was positive but, aural swab was negative for any bacterial growth. Out of the 100, 10% cases had no bacterial growth in both aural swab and cholesteatoma cultures.

Table 2: Isolates of aural swab and cholesteatoma sac cultures with similar organism

Organism identified	No. of cases (%)	OR	95% CI	P -value
Gram positive				
Staphylococcus aureus	8(44.4%)	6.40	1.12 – 36.44	0.06
Gram negative				
Pseudomonas aeruginosa	6(33.3%)	4.00	0.68 – 23.41	0.23
Proteus sps	2(11.1%)	1.00	0.13 – 8.00	1.00
Moraxella	2(11.1%)	Ref	----	----
Total	18 (99.9%)			

It was observed from Table 2 that in cases showing same organism in both cultures, 44.4% were gram positive and 55.6% were gram negative. All the Gram-positive cases showed Staphylococcus aureus infection only. Staphylococcus aureus infection found to be more prevalent with 6.4-fold increased risk compared to Moraxella infection. Among the gram-negative infections, Pseudomonas aeruginosa infection was the most common.

Table 3: Isolates showing different organisms in aural swab and cholesteatoma cultures

Aural swab culture	Cholesteatoma sac culture	No. of cases (%)	OR	95% CI	P-value
Proteus	Pseudomonas	12(35.29%)	8.72	1.78-42.90	0.006
Klebsiella	Enterococcus	4(11.76%)	2.13	0.36-12.51	0.67
Klebsiella	Pseudomonas	2(5.88%)	1.00	0.13-7.54	1.00
E. coli	Klebsiella	5(14.70%)	2.76	0.50-15.33	0.43
Proteus	Staphylococcus	4(11.76%)	2.13	0.36-12.1	0.67

Staphylococcus	Pseudomonas	5(14.70%)	2.76	0.50-15.33	0.43
Pseudomonas	Acinetobacter	2(5.88%)	1.00	0.13-7.54	1.00
Total		34			

It was seen from Table 3 that in cases exhibiting different organisms in aural swab and cholesteatoma sac cultures, Proteus infection in aural swab and Pseudomonas infection in cholesteatoma sac are the most common and associated with 8.72-folds higher incidence compared to Pseudomonas infection in aural swab and Acinetobacter infection in cholesteatoma sac.

Table 4: Isolates with aural swab positive but cholesteatoma sac negative cultures

Organism	No. of cases (%)	OR	95% CI	P-value
Gram positive				
Staphylococcus aureus	14 (60.86%)	7.39	1.89 - 28.94	0.006
Gram negative				
E. coli	05 (21.73%)	1.32	0.31 - 5.71	1.00
Proteus vulgaris	04 (17.39%)	Ref	---	--
Total	23			

As seen from Table 4 that in cases with only aural swab positive and cholesteatoma negative cultures, staphylococcus aureus infection has 7.39-folds higher incidence than Proteus vulgaris infection.

Table 5: Isolates with only cholesteatoma positive but aural swab negative cultures

Organism	No. of cases (%)	OR	95% CI	P value
Gram negative				
Pseudomonas aeruginosa	06 (40%)	9.33	0.96 – 90.94	0.08
E. coli	04 (26.66%)	5.09	0.50 – 52.29	0.33
Klebsiella pneumoniae	02 (13.33%)	2.15	0.17 – 26.67	1.00
Acinetobacter baumannii	01 (6.66%)	Ref		
Gram positive				
Staphylococcus aureus	02 (13.33%)	2.15	0.17 – 26.67	1.00
Total	15			

As observed from Table 5 that in cases with only cholesteatoma culture positive and aural swab culture negative, no statistically significance differences were observed in the incidence of infections.

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Antibiotic sensitivity patterns in aural swab

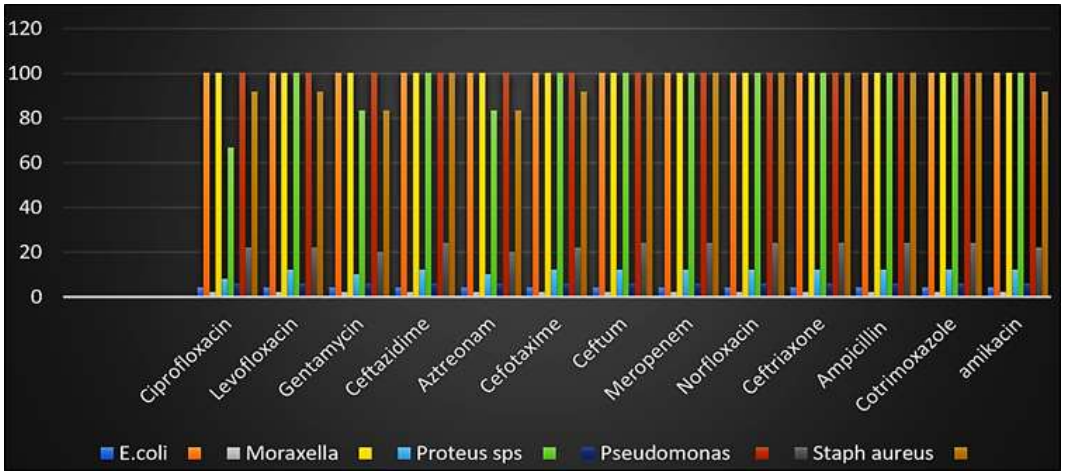

Fig 1: Antibiogram of isolates of aural swab culture showing percentage sensitivity

As observed from Fig. 1 that in the aural swab isolates, E. coli, Moraxella, Pseudomonas aeruginosa were found to be sensitive for the twelve antibiotics tested. A total of 7.4% isolates of staphylococcus aeruginosa were resistant to ciprofloxacin, levofloxacin, cefotaxime and amikacin. A total of 14.8% isolates of staphylococcus aureus were resistant to Gentamycin and Aztreonam. A total of 33.33% isolates of Proteus species were resistant to ciprofloxacin. A total of 16.67% isolates of Proteus species were resistant to gentamycin and aztreonam. A total of 33.33% Klebsiella pneumonia are resistant to gentamycin and cefotaxime. A total of 16.67% Klebsiella pneumonia are resistant to ampicillin and amikacin. All the Klebsiella isolates were resistant to cotrimoxazole and aztreonam.

Antibiotic sensitivity patterns in cholesteatoma swab

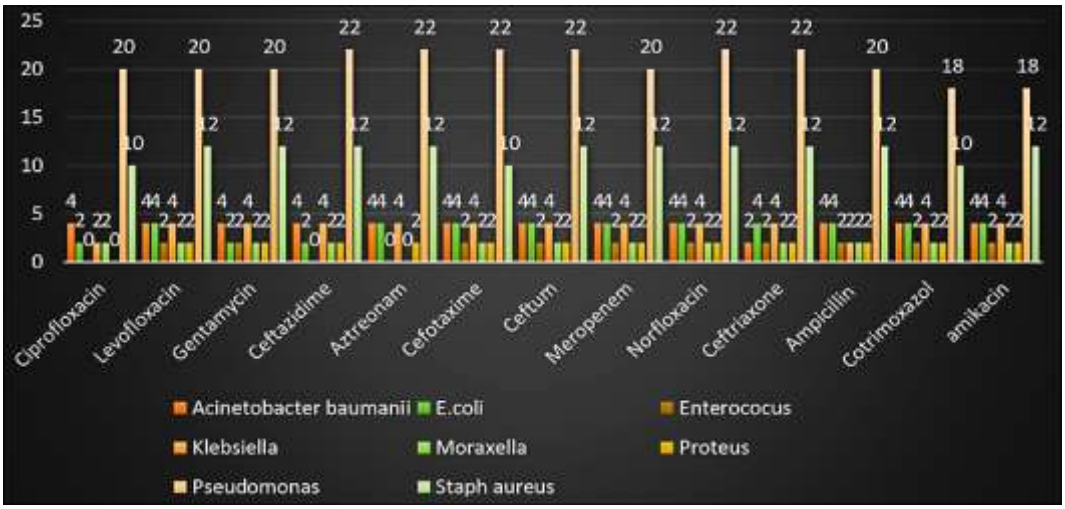

Fig 2: Antibiogram of cholesteatoma culture

As seen from Fig. 2 that in cholesteatoma culture isolates, *Acinetobacter baumannii* showed resistance to ceftriaxone in 33.37% cases. A total of 50% isolates of Escherichia coli showed resistance to ciprofloxacin, gentamycin and ceftazidime. All enterococcus isolates showed resistance to ciprofloxacin, ceftazidime, aztreonam. A total of 42.85% Klebsiella isolates showed resistance to ciprofloxacin and ampicillin. Moraxella species showed resistance to aztreonam. Proteus species showed resistance to ciprofloxacin. A total of 6.4% Pseudomonas

isolates were resistant to ciprofloxacin, levofloxacin, meropenem and ampicillin. A total of 12.91% Pseudomonas isolates were resistant to Amikacin. A total of 14.29% Staphylococcus aureus isolates were resistant to ciprofloxacin, cefotaxime.

Table 6: Follow-up of cholesteatoma cases following CWD and CWU procedure

Total number of canal wall down procedures	
Total number of canal wall up procedures	36 (36%)
Follow up in canal wall down cases	
3 months to 1 year follow up	52(81.25%)
2 yrs follow up cases	34 (53.13%)
Follow up in canal wall up cases	
3 months to 1 year follow up	28(77.78%)
2 yrs follow up	14 (38.89%)
Complications	15
Discharging ear	8 (5 CWD + 3 CWU)
Recurrence and recidivism	7 (1 CWD + 6 CWU)

It was observed from Table 6 that 64 cases were managed by canal wall down procedures and 36 cases were managed by canal wall up procedure. Among the patients who underwent canal wall down procedures 52 patients (81.25%) were followed from3months to 1 year postoperatively and only 34(53.13%) patients came for follow up till 2 years with the rest of them lost to follow up. Among the patients who underwent canal wall up procedures 28 cases (77.78%) were followed up from 3 months to 1 year postoperatively while 14 cases (38.89%) were followed till end of 2 years. Among all the patients, 15 cases developed long term complications. 8 cases (5 canal wall down patients and 3 canal wall up patients) had a discharging ear and 7 cases (1 canal wall down and 6 canal wall up) had recurrence and recidivism. No statistically significant differences were observed in CWU vs. CWD procedures in postsurgical complications related to discharging ear (OR: 1.07, 95% CI: 0.24 – 4.78, p=1.00). The recurrence and recidivism were higher in CWU than CWD (OR: 12.6, 95% CI: 1.45-109.39, p=0.02). The first year and second year follow-ups in CWU vs. CWD showed no statistically significant differences.

Discussion

The current study explored the microbiological profiles of aural swab pre-operatively and cholesteatoma sac intra-operatively. Our study is in agreement with the study of Mustafa *et al*. (2008) [10] in demonstrating slight increase in the incidence of cholesteatoma in males compared to females (56:44 vs. 55:45) and covering the wide age group of patients (6 - 85 yrs vs. 7 - 73 yr). The age-specific incidence of cholesteatoma increased in the second decade of life in our study. A large-scale study of 5850 cholesteatoma cases reported a peak incidence date rate at the age of 9 yrs (Djurhuus *et al*., 2015) [11]. Infectious aetiology was observed in 90% of cholesteatoma cases with the hallmark of presence of polymicrobial flora consistent with the existing literature.

Among the Gram-positive bacteria, Staphylococcus aureus infection was the most predominant. Among the Gram-negative bacteria, Pseudomonas aeruginosa infection was the most predominant followed by Proteus species, Escherichia coli, Klebsiella pneumonia and Moraxella species. This corroborated with an earlier study of 150 cholesteatoma patients showing the highest incidence of Pseudomonas aeruginosa, Staphylococcus aureus, Proteus mirabilis, Escherichia coli and Klebsiella pneumonia among the aerobic flora (Ricciardiello *et al*., 2009) [6]. In another study of 250 patients with CSOM also increased predominance of Pseudomonas aeruginosa and Staphylococcus aureus was reported (Sharma *et al*., 2004) [12]. In a study from China, Proteus, Staphylococcus aureus, Pseudomonas aeruginosa and

coagulase negative staphylococci were the top most pathogens identified in cholesteatoma (Xu *et al*., 2020) [13].

Minami *et al*., (2017) [14] demonstrated the presence of diverse microbial communities in the human middle ear such as Proteobacteria, Actinobacteria, Firmicutes and Bacteroides using the 16S rRNA sequencing. The middle ear microbiome was found to be altered in the CSOM patients with active inflammation with increased prevalence of Firmicutes and lower prevalence of Proteobacteria. This is consistent with our study showing higher prevalence of Staphylococcus aureus that belongs to the phylum Firmicutes.

Bacterial biofilm formation was reported in 81.3% cholesteatoma cases suggesting the possibility of keratinized matrix of cholesteatoma serving as the ideal substrate for the biofilm colonization and survival (Galli *et al*., 2016) [15]. This in turn collaborates with our findings showing infectious etiology in 90% of cholesteatoma cases. Otopathogenic Pseudomonas aeruginosa (OPPA) strains were reported to exhibit increased adherence to human keratinocytes compared to a laboratory strain of Pseudomonas due to mucoid characteristics (Wang *et al*., 2005). This is consistent with increased incidence of Pseudomonas aeruginosa in cholesteatoma cases. Pseudomonas aeruginosa infection was reported to increase the size of cholesteatoma in an experimental model (Jung *et al*., 2011) [16].

In the current study, 75 of 100 (75%) preoperative aural swab cultures were positive for the bacterial species. The absence of bacteria in 25% of the cases might be attributable to treatment with the antibiotics before the culture and sensitivity analysis.

Of the 25 aural swabs preoperatively negative for bacteria, 15 were positive for pathogenic bacteria in the cholesteatoma sac culture (intra operatively) suggesting that preoperative culture negativity cannot rule out the infectious aetiology. Among the cultured pathogens, Staphylococcus aureus infection is the most common in pre-operative cultures. The percentage of cases showing different organisms in pre-operative and intra-operative cultures is more than the percentage of cases showing same organisms in the current study, which is consistent with an earlier study (Ahn *et al*., 2012) [17].

A long-term study of 1191 isolates derived from CSOM revealed Staphylococcus aureus infection as the most common (Kim *et al*., 2017) [18]. Ciprofloxacin-resistant Pseudomonas aeruginosa infection increased over time (Kim *et al*., 2017) [18]. In the current study also, two out of 22 (9.09%) isolates showed ciprofloxacin resistant Pseudomonas aeruginosa infection. Our study is in agreement with Madana *et al*., (2011) [19] in reporting high sensitivity of Pseudomonas and Proteus to ceftazidime (100%) and 84-86% sensitivity of Staphylococcus to ciprofloxacin. The higher antibiotic resistance in elderly cases could be due to increased use of over the counter antibiotics.

Our study reported high incidence of recidivism in CWU than CWD, which is substantiated by a meta-analysis in which six studies showed higher recidivism in CWU than CWD (16.7-61.0% vs. 0-13.2%) (Kerckhoffs *et al*., 2016) [20]. In a study from Denmark, age <15yr, stapes erosion and incus erosion and cholesteatoma localised to the mastoid were identified independent prognostic factors for recidivism (Britze *et al*., 2017) [21]. This is corroborating with our study that showed bad prognosis in younger patients.

The major strengths of our study are:

i) Examination of pre-operative and intra-operative cultures to rule out infectious etiology of cholesteatoma.
ii) Antibiotic sensitivity profiling in both specimens across broad range of antibiotics.
iii) Follow-up of cholesteatoma cases for a period of two years to monitor prognosis and recidivism.

In the current study, we have examined the aerobic bacteria profiling and anaerobic bacteria

flora were not evaluated. Future studies are warranted using scanning electron microscopy and 16S rRNA sequencing to corroborate these findings further. However, the existing literature is substantiating our observations.

Conclusions

The study concluded that an upsurge in the incidence of cholesteatoma was observed in the second decade of life. Gender differences in the incidence of cholesteatoma are not statistically significant. Polymicrobial floras were observed both pre-operative and intra-operative cultures. Staphylococcus aureus and Pseudomonas aeruginosa are the most common pathogens identified in the pre-operative and intra-operative cultures. Pre-operative and intra-operative cultures showing different organisms are higher than those showing same organism. Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli exhibited resistance to aminoglycosides. Enterococcus and Escherichia coli showed resistance to cephalosporins. The antibiotic resistance for ciprofloxacin and ceftazidime is higher in elderly subjects compared to younger subjects.

- No gender differences were observed in antibiotic resistance profiling. Poor prognosis was observed in subjects under the age of <20 yr compared to the adults due to surgical limitations, recidivism, presence of microflora in ear canal and increased propensity of infections. The recurrence and recidivism rates are higher in CWU than CWD. However, no significant differences were observed in the post-surgical complications related to discharging ear in CWU and CWD. Thus, the knowledge of pathogens and their sensitivity in aural swab and cholesteatoma will help in choosing the appropriate antibiotics during the post-operative period which will prevent recurrences and recidivism and will eventually lead to a healthy ear. Further studies are warranted using scanning electron microscopy and 16S rRNA sequencing to find out bacterial genome that might be present in cholesteatomas thereby improve our understanding of role of middle ear microbiota in the pathogenesis of chronic inflammatory disease.

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Written and Informed consent: Written and informed consent was taken.

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