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Original Article

WAR IN THE MIDDLE EAR: MICROBIOLOGY OF CHRONIC SUPPURATIVE OTITIS MEDIA WITH SPECIAL REFERENCE TO ANAEROBES AND ITS ANTIMICROBIAL SUSCEPTIBILITY PATTERN: OPTIMIZING ANTIMICROBIAL THERAPY IN A TERTIARY CARE HOSPITAL OF RURAL INDIA

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ABSTRACT

Objective: To isolate etiological organisms of Chronic Suppurative Otitis Media (CSOM), study the antimicrobial susceptibility pattern, study the risk factors and associated co-morbid conditions with CSOM, and detect biofilm production in isolated bacteria.

Methods: An ear discharge specimen was obtained from the diseased ear of the patient, using three separate sterilized swabs. One of the swabs was used for Gram staining and Aerobic culture. Antibiotic susceptibility testing was done by the Kirby-Bauer standard disc diffusion method all the g-negative isolates were screened for Extended-Spectrum Beta-Lactamase (ESBL) production and Amp C ß-lactamase production. Methicillin-Resistant Staphylococcus Aureus (MRSA) screening was done using a *Cefoxitin* disc. Biofilm production was done using the Tube method and Microtiter plate method. Anaerobic culture was inoculated into Robertson's Cooked Meat (RCM) broth. Potassium hydroxide (KOH) mount was done using 10% KOH for the presence of budding yeast cells, fungal hyphae, and spores. Tubes showing positive cultures were examined by LactoPhenol Cotton Blue (LPCB) mount. Final Candida speciation and Antifungal susceptibility testing was done using VITEK-2.

Results: A total of 500 patients were included in the study, with 62.8% being males. The most common organism isolated was *Pseudomonas aeruginosa*. Fungi accounted for 30 isolates (5%) and anaerobes were 5 isolates (1%). Gram-negative bacteria were found to be most sensitive to *Piperacillin-tazobactam* (96%) while the highest resistance was noted to *Amoxicillin-clavulanate* (65%). 100% of *Staphylococcus aureus* isolates were sensitive to *Vancomycin, Linezolid*, and *Teichoplanin*.13% of *S. aureus* were found to be *Cefoxitin* resistant (MRSA). The highest resistance in *S. aureus* isolates was noted to *Ciprofloxacin* (65%).13% of isolates of Gram-negative bacteria had ESBL production while Amp C β-Lactamase production was noted in 9% of isolates. Out of 186 isolates tested for biofilm production, 149 isolates (80%) showed biofilm production. A total of 30 fungal isolates were obtained in culture. *Aspergillus* spp. Accounted for 66.6% of fungal isolates while *Candida* spp. accounted for 33.3% of fungal isolates. The sensitivity of *Candida* isolates was tested to *Fluconazole, Itraconazole, Voriconazole*, and *Flucytosine* and they were found to be 100% sensitive to all the antifungals tested.

Conclusion: CSOM often has polymicrobial pathology and creates a war in the middle ear environment, augmented by mixed aerobic and anaerobic organisms and synergism between aerobic and anaerobic organisms. A high rate of *Clindamycin* resistance was seen in anaerobe which is noteworthy because this is a frequently utilized antimicrobial for treating anaerobic infections.

Keywords: CSOM, Anaerobic profile, Biofilm, Demographic factors, Antibiotic resistance

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INTRODUCTION

CSOM is defined as chronic inflammation of the middle ear and mastoid cavity that presents with recurrent ear discharge of more than three months duration through a perforated tympanic membrane. It is a massive health problem in developing countries and India is one of the countries with the highest CSOM prevalence (>4%) where urgent attention is needed. According to a survey, about 65-330 million people suffer from ear infections worldwide and 60% of them will develop significant hearing impairment [1].

CSOM is classically divided into) a tubo-tympanic type affecting the middle ear muco-periosteum and 2) an attico-antral type which is an active squamous disease with a growth of squamous epithelium into the middle ear cleft [2]. Recently CSOM has been modified into broader types like active mucosal, inactive mucosal, active squamous, inactive squamous and healed dimeric, tympanosclerosis, etc [3]. Unsafe CSOM is characterized by an attic cholesteatoma or a posterior-superior cholesteatoma with a history of scanty and foul-smelling ear discharge which is at times blood-stained. Cholesteatoma is a keratinizing stratified squamous epithelium accumulating within the middle ear or other pneumatised portions of the temporal bone and may get infected involving the entire middle ear cleft and can cause bone resorption possibly due to the activity of collagenase [4].

Gram-negative bacteria like *Pseudomonas aeruginosa* continue to be the most isolated organisms from cases of CSOM with maximum sensitivity for *Piperacillin-tazobactam* followed by *Meropenem*, *Piperacillin* while among the g-positive organisms, *Staphylococcus aureus* and remains the maximally isolated organism with maximum sensitivity to *Vancomycin, Linezolid,* however, the prevalence of ESBL, Carbapenemases, Amp C ß-lactamase, Metallo β-Lactamase (MBL), MRSA, Vancomycin-Resistant Staphylococcus Aureus (VRSA) is rising in this era of emerging multidrug resistance [5]. Although facultative anaerobes constitute the major chunk of causative organisms, anaerobes cannot be neglected. *Peptostreptococcus, Bacteroides species, and Fusobacterium* are the predominant anaerobic bacterial isolations from cases of CSOM. Though the anaerobes can live in complete harmony with the host and exhibit a mutually beneficial relationship with aerobic commensal, they can still become pathogenic and create a war-like environment in the middle ear when the host's defense mechanism gets impaired. There is a high tendency to see the anaerobic infection in samples showing polymicrobial appearance in Gram stain from clinical samples and this can be more virulent than those involving a single organism. Synergism between aerobic and anaerobic organisms has been recognized. Most clinical laboratories neither identify obligate anaerobes nor perform antimicrobial susceptibility studies as it is time-consuming and involves various technical skills and hence is overlooked. The frequency of antimicrobial resistance in anaerobic organisms has been increasing globally at the same pace as in aerobes and facultative anaerobes in the past few decades [6, 14].

Evaluation of the microbiological agents and their antibiotic sensitivity pattern is helpful in the initiation of specific therapy and thus minimizing its complications like skull base osteomyelitis, meningitis, etc, and the emergence of resistant strains. Due to the high incidence, long period of morbidity of CSOM, and the repeated occurrences of otorrhea during that period, patients are often prescribed empiric antibiotics in outpatient clinics resulting in bacterial resistance and ototoxicity with both topical and systemic antibiotics. Also, it is necessary that anaerobic susceptibility testing be performed and awareness of the emerging resistance among the anaerobic pathogens must be created to provide appropriate antimicrobial therapy for better patient care.

We conducted this study with the following aims and objectives:

1. To isolate, and identify aerobic bacteria from chronic suppurative Otitis media cases and to study antibiotic susceptibility patterns of isolated aerobes and facultative anaerobes.

2. To isolate, and identify anaerobic bacteria from chronic suppurative Otitis media cases and to study antibiotic susceptibility patterns of isolated obligate anaerobes.

3. To isolate, identify fungi causing CSOM and to study the antifungal susceptibility pattern of isolated candida species.

MATERIALS AND METHODS

This prospective study was conducted in the Department of Microbiology at a tertiary care teaching hospital. A total of 500 clinically diagnosed cases of CSOM attending ENT OPD, and being admitted in ENT wards were included in the study. Ethical clearance was obtained from the Institutional Ethical Committee (letter number: AIMSR/2023/IEC/59, dated: 15th September 2023) before the study. A written consent was also obtained from the patients before they participated in the study. Subjects were explained the purpose of the study and the procedures involved.

The sample size was calculated using the Daniel formula, $n = 4\ pq/d^2$ and depending upon previous years' statistics.

Statistical analysis

The data was analyzed by using Statistical Package for Social Sciences (SPSS) version 21.0 and the prevalence of organisms was determined and expressed in percentage.

Inclusion criteria

1. Patients diagnosed as CSOM after thorough clinical evaluation i. e. the patients having ear discharge of more than 3 mo.

2. Patients who were not on antibiotic (both systemic and topical) treatment for a minimum of 24 h before sample collection.

Exclusion criteria

1. Patients suffering from CSOM who were on systemic antibiotics in the past days of presentation.

2. Patients who were on topical medications to the ear.

3. Patients having ear discharge due to some traumatic or neoplastic condition.

4. Patients having ear discharge arising from otitis externa.

Sample collection and processing

Ear discharge was obtained from the diseased ear of the patient, using three separate sterilized swabs before the instillation of any topical medication. One of the swabs was used for Gram staining and Aerobic culture and was plated on 5% sheep Blood Agar (BA), MacConkey's Agar (MA), and Chocolate Agar (CA) and incubated at 37 °C for 48 h. The plates were examined for growth and cultural characteristics and the isolated colonies were subjected to a standard set of biochemical tests for identification [7, 8]. Antibiotic susceptibility testing was done by Kirby-Bauer standard disc diffusion method on Muller-Hinton agar according to clinical and laboratory standards institute (CLSI) guidelines (M100-S24). All the g-negative isolates were screened for ESBL production by reduced zone of inhibition around Ceftazidime (30µg) and confirmed by Combined disc synergy test and were also screened for AmpC ß-lactamase by reduced susceptibility to *Cefoxitin* (30µg) with zone diameter less than 18 mm and confirmation by Amp C disc test. Isolates of *Staphylococcus aureus* were screened for MRSA by standard disc diffusion using *Cefoxitin* (30µg) disc. Detection of inducible *Clindamycin* resistance was done using a Standard disk diffusion procedure with 15µg *Erythromycin* and 2µg *Clindamycin* disks spaced 15-26 mm apart and the formation of D zone. Biofilm production among *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolates was tested by the Tube method and Microtiter plate method [9, 12].

The second swab was used for anaerobic culture and was inoculated in RCM broth and incubated at 37 °C for 48-72 h, cultures were then done from RCM on anaerobic blood agar. A *Metronidazole* disc (5 µg) was placed at the junction of the secondary and tertiary streaking areas. An anaerobic jar (Anaerobic GEN box) was used for anaerobic culture. The jars were closed and incubated at 37 °C for 72 h and thereafter, examined for the zone of inhibition around the *Metronidazole* disk. An Anaerobic Gas Pack (H_2S gas pouch) obtained from from biomerieux, which consisted of a bag containing a paper sachet filled with a black colored powder packet for Oxygen absorbing and Carbon dioxide generating agent was used. *Pseudomonas* inoculated plates were also used as an anaerobic indicator. The plates were examined for growth, and the organisms were identified by Colony characters, Gram staining, and sensitivity pattern to *metronidazole* disc. Growth around the *Metronidazole* disc was subcultured and inoculum suspensions were prepared from isolated colonies on anaerobic blood agar until a turbidity of between 2 to 3 McFarland standards was reached by using a calibrated vitek-2 densichek instrument. Identification of obligate anaerobes was further done by an automated method using a VITEK-2 ANC card [10]. The document M11-A8 was used as a reference for performing anaerobic Antimicrobial Susceptibility Testing (AST). The agar dilution method was performed using agar incorporation of different concentrations of the antimicrobial agent into Brucella agar followed by the application of a standardized number of bacterial cells to the surface of the agar plate. Plates were read after 48 h of growth by visually comparing the growths of different strains in the series, and the Minimum Inhibitory Concentration (MIC) is designated the lowest antimicrobial concentration that inhibits growth. Antimicrobial susceptibility testing was performed against *Metronidazole and Clindamycin* (HI Media Labs). MIC values were compared with the CLSI Standard for interpretation of results [10, 26] (table 1).

Table 1: Clinical laboratory standards Institute (CLSI) interpretive values for anaerobic bacteria

Antibiotic	Susceptible (µg/ml)	Intermediate (µg/ml)	Resistant (µg/ml)
Metronidazole	<8	16	>32
Clindamycin	<2	4	>8

The third swab was used for mycological culture and was inoculated onto 2 slopes of Sabouraud dextrose agar with pH 5.6 with antibiotics like *Gentamicin* to inhibit bacterial growth and incubated at 25 °C and 37 °C for 4-6 w. KOH mount was done using 10% KOH for the presence of budding yeast cells, fungal hyphae, and spores. Tubes showing positive cultures were examined for the rate of growth, texture, surface pigmentation, and pigmentation on the reverse. Microscopic examination was further done by LPCBmount and identified further based on the nature of hyphae (such as septate or aseptate, hyaline, narrow or wide), Conidiogenesis (origin, arrangement), Conidia (pigmented or hyaline, shape). A Germ Tube Test was done for presumptive identification of Candida species into Candida albicans and Non-albicansCandida. FinalCandida speciation and Antifungal susceptibility testing was done using VITEK-2 [11, 14].

RESULTS

A total of 500 patients were included in the study, with 62.8% of the patients being males while 37.2% of patients were females. The highest incidence of CSOM was found to be among patients in the age group of 20-29 y (32.8%) while the lowest incidence was found in patients ≥ 60 y of age (2.8%) (table 2). 400 patients (80%) were outpatients while 100 patients (20%) were inpatients.

Table 2: Age and sex-wise distribution of patients (n=500)

Age (y)	Males	Females	Total	Percentage	p-value
<10	57	27	84	16.8%	<0.0001
10-19	52	32	84	16.8%	0.0021
20-29	100	64	164	32.8%	0.0001
30-39	42	33	75	15%	0.1430
40-49	40	19	59	11.8%	<0.0001
50-59	13	7	20	4%	0.0610
≥60	10	4	14	2.8%	0.0291
Total	314	186	500	100%	<0.0001

The most common presenting complaint was ear discharge for more than 3 mo in 93% of cases followed by decreased hearing in 66% (fig. 1). Upper respiratory tract infection was noted in 33% of cases and allergic history was noted in 30% of cases. 26% of cases had a history of Modified Radical Mastoidectomy with tympanoplasty (table 3). Tonsillitis and Deviation of Nasal Septum (DNS) were noted in 20% of patients each. Tuberculosis and adenoids were seen in 4% and 2% of cases respectively (fig. 1, table 3).

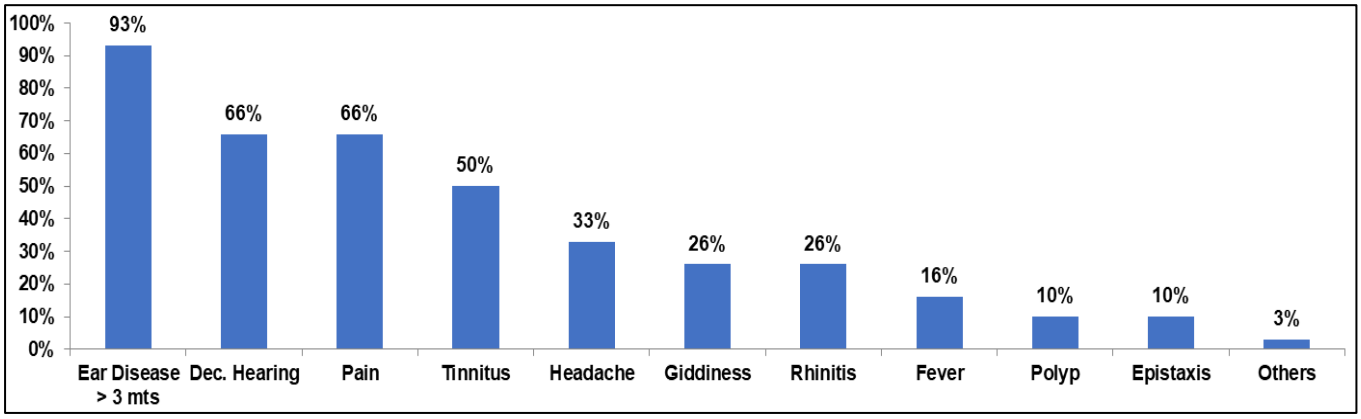


Fig. 1: Distribution of presenting complaints

Table 3: Diseases associated with CSOM among patients

Associated features	Number of cases	Percentage (%)
URTI	165	33%
Allergy	150	30%
P/H OF modified radical mastoidectomy	130	26%
Tonsillitis	100	20%
DNS	100	20%
Tuberculosis	20	4%
Adenoids	10	2%

In this study, 550 isolates obtained 355 isolates were obtained in pure growth, 150 isolates (75 samples) were in mixed growth while about 45 isolates were commensals like diptheroids.310gram-negative isolates (56.4%) were obtained which outnumbered g-positive (aerobic and anaerobic) isolates160 (30%). The most common organism isolated was *Pseudomonas aeruginosa* 200 isolates (39%) followed by *Staphylococcus aureus*110 isolates (21%). Fungi accounted for 30 isolates (5%) and anaerobes were 5 isolates (1%). In mixed culture growth, the most common isolates were *P. aeruginosa and K. pneumoniae* (2.8%) followed by *P. aeruginosa* and *E. coli* (2.6%). Attico-antral type of CSOM was found in 150 cases (30%) while 350 cases (70%) were diagnosed with a tubo-tympanic type of CSOM (table 4,5,6 fig. 3-5).

Table 4: Culture results from patients with ear discharge (n=500)

Type of organism	Total cases	Total isolates	Percentage
Pure growth	355	355	71%
Mixed growth	75	150(75*2)	15%
No growth	25	0	5%
Commensals (Diptheroids)	45	45	9%
Total	500	550	100%

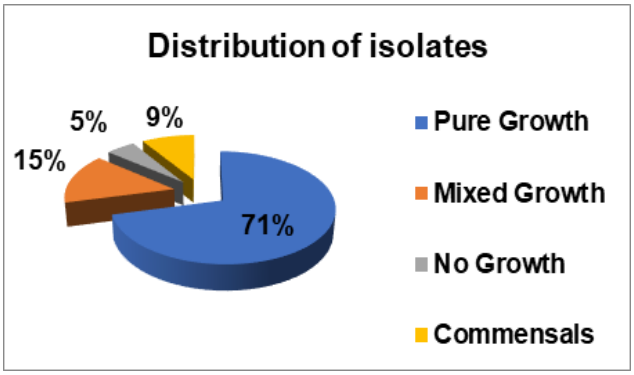


Fig. 3: Distribution of isolates (n=500)

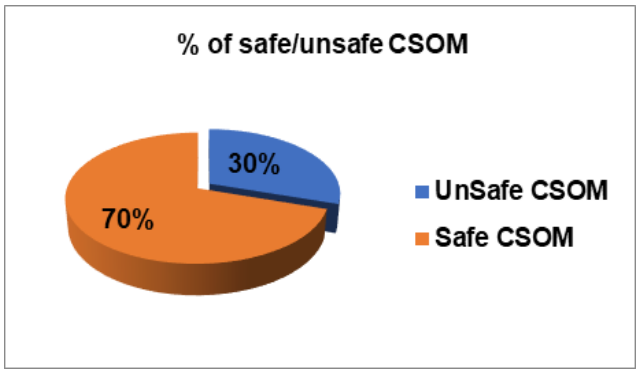


Fig. 4: Type of CSOM cases (n=500)

Table 5: Organisms isolated from CSOM cases (n=475)

Type of organism	Total cases	Percentage of cases	Total isolates (Pure and mixed)
Pseudomonas aeruginosa	182	39%	200
Staphylococcus aureus	96	21%	110
Coagulase-Negative Staphylococci (CONS)	39	8%	50
Acinetobacter baumannii	9	6%	35
Klebsiella spp.	20	4%	25
E. Coli	15	3%	20
Proteus spp.	10	2%	15
Enterobacter spp.	5	1%	10
Citrobacter spp.	5	1%	5
Candida Spp.	8	2%	10
Aspergillus Spp.	16	3%	20
Anaerobes (Peptostreptococcus)	5	1%	5
Commensals(Diptheroids)	45	9%	45
Total	475	100 %	550

Table 6: Micro-organisms isolated in pure culture (n=355)

Type of organism (pure culture)	Total isolates (pure culture)	Percentage (pure culture)
Pseudomonas aeruginosa	172	48.45%
Staphylococcus aureus	86	24.22%
CONS	35	9.87%
Acinetobacter baumannii	12	3.38%
Klebsiella Spp.	7	1.97%
E. Coli	7	1.97%
Proteus spp.	5	1.4%
Enterobacter Spp.	5	1.4%
Citrobacter Spp.	4	1.13%
Candida spp.	6	1.7%
Aspergillus Spp.	11	3.09%
Anaerobe (Peptostreptococcus)	5	1.4%
Total	355	100%

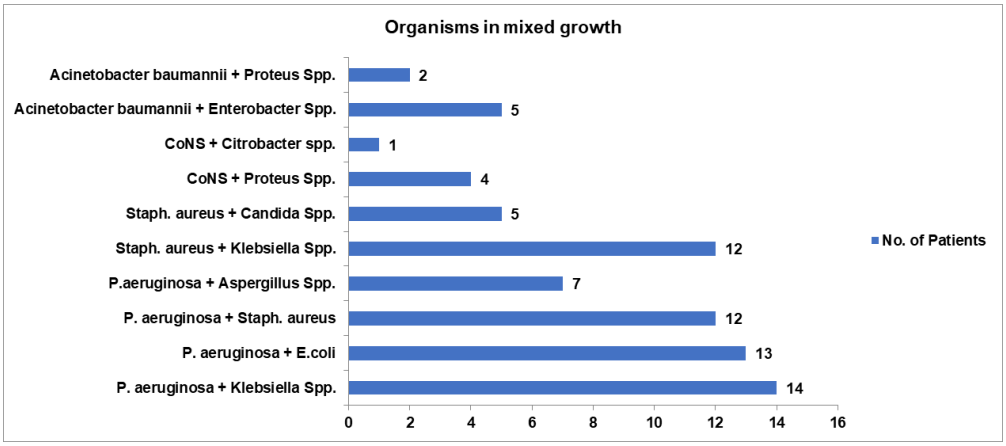


Fig. 5: Organisms seen in mixed culture (n=150)

Antibiotic susceptibility pattern in bacterial isolates

Gram-negative bacteria were found to be most sensitive to *Piperacillin-tazobactam* (96%) followed by *Meropenem* (94%) and *Cefoperazone-Sulbactam* (91%) while the highest resistance was noted to *Amoxicillin-clavulanate* (65%). Among *Pseudomonas aeruginosa* isolates, the highest sensitivity was noted for *Piperacillin-tazobactam* (97%) followed by *Meropenem*(95%), *Cefoperazone* (93%), and *Piperacillin* (93%) while only 68% isolates of *P. aeruginosa* were found to be sensitive to *Ciprofloxacin.*100% of *Staphylococcus aureus* isolates were found to be sensitive to *Vancomycin, Linezolid*, and *Teicoplanin*. 87% of *S. aureus* isolates were *Cefoxitin* sensitive (MSSA) and 13% of S. aureus isolates were found to be *Cefoxitin* resistant (MRSA). The highest resistance in S. aureus isolates was noted to *Ciprofloxacin* (65%) followed by *Ampicillin* (63%) and *Amoxicillin-Clavulanate* (54%). Among *CONS* isolates, 100% were sensitive to *Linezolid* and *Vancomycin* each, 94% were sensitive to *Teichoplanin* and 88% were sensitive to *Amikacin* while 92% isolates were resistant to *Penicillin*, 65% were resistant to *Amoxicillin-Clavulanate* and 58% were resistant to *Ciprofloxacin.* Among the isolates from unsafe CSOM, the highest sensitivity was noted for *Piperacillin-Tazobactam* (96%) while 95% of isolates from unsafe CSOM were resistant to *Amoxicillin-clavulanate.*13% isolates of Gram-negative bacteria had ESBL production while Amp C β-Lactamase production was noted in 9% isolates (fig. 6-8).

Biofilm production in pseudomonas aeruginosa and staphylococcus aureus

Out of 186 isolates tested for biofilm production, 149 isolates (80%) showed biofilm production. 50% of biofilm-producing isolates were moderately positive, 47% were weakly positive and 3% were strongly positive.

Out of the moderately positive and strongly positive biofilm producers, 90%(132) isolates were from unsafe CSOM out of which 107(80%) were *Pseudomonas aeruginosa* while 25 (20%) were *Staphylococcus aureus.* 87%(22) isolates of *Staphylococcus aureus* were MRSA while 13% (3) were MSSA. All these patients with unsafe CSOM had a history of recurrent CSOM and were on antibiotic therapy for many years (mean time on antibiotic treatment-8 y). 60 patients (84.5%) with unsafe CSOM with detection of moderately positive or strongly positive biofilm production had undergone Modified Radical Mastoidectomy (MRM) with type 2 tympanoplasty in the past.

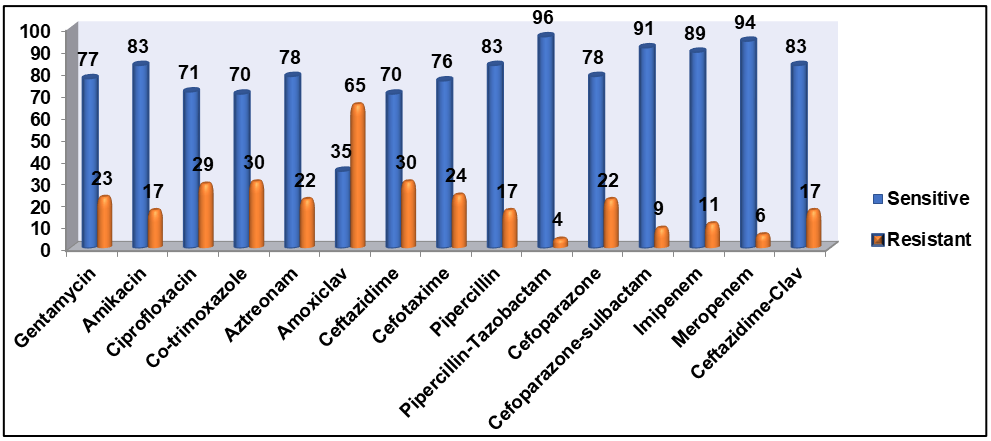


Fig. 6: Antibiotic susceptibility pattern of g-negative bacteria (n=310)

Table 7: Antibiotic sensitivity pattern of anaerobic isolates obtained from CSOM cases (n=5)

Antibiotic	Range noted(ug/ml)	MIC_{50}(ug/ml)	MIC_{90}(ug/ml)	%Resistance
Metronidazole	0.25-4	0.5	2	0%
Clindamycin	<0.25->128	16	>128	40%

Peptostreptococcus showed 100% sensitivity to Metronidazole while Clindamycin was sensitive in 40% of isolates. Table 7, fig. 9 depicts the MIC range.

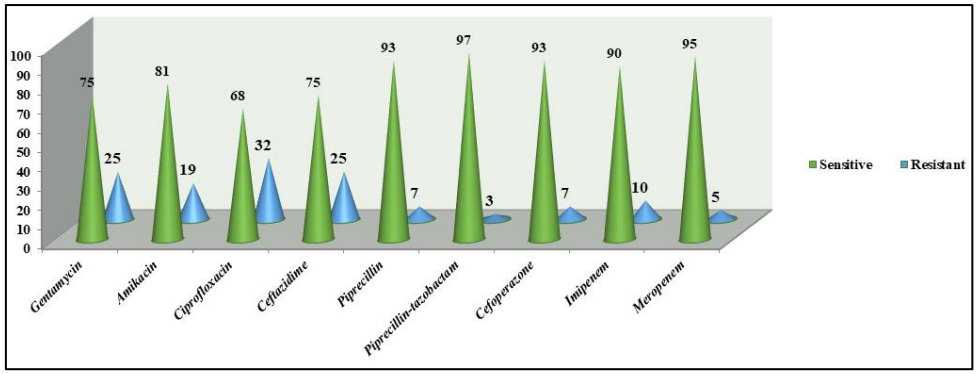


Fig. 7: Antibiotic sensitivity pattern of pseudomonas aeruginosa (n=200)

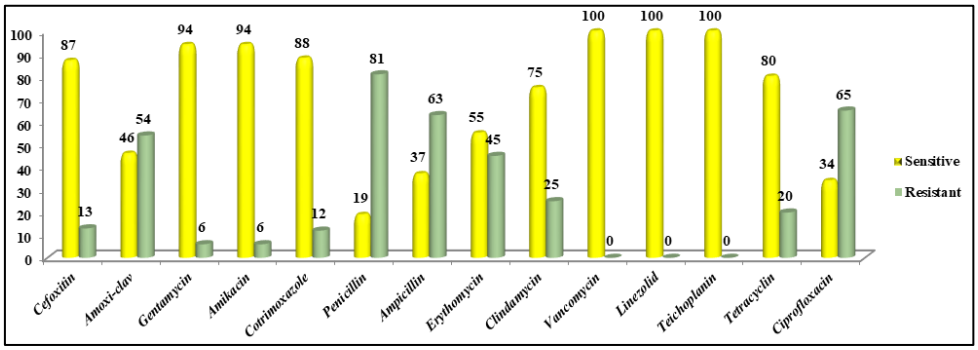
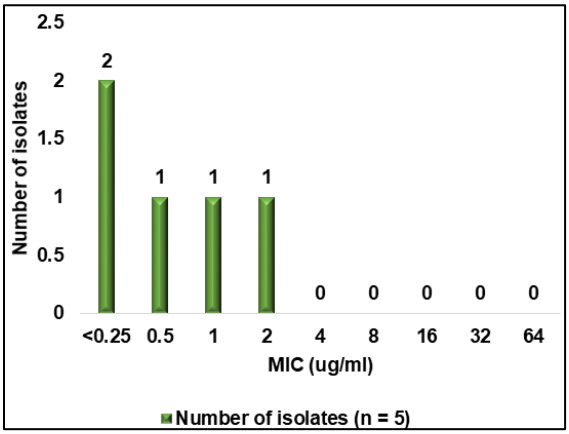
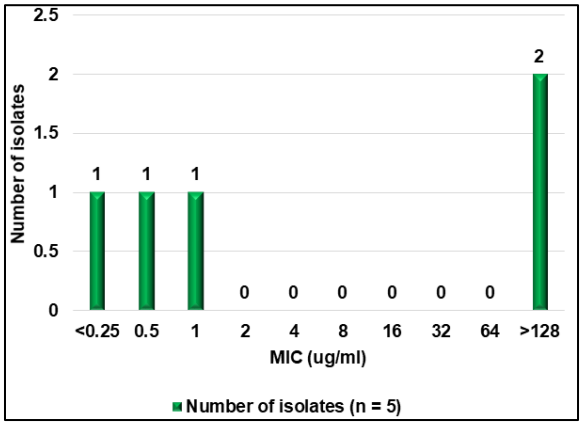


Fig. 8: Antibiotic sensitivity pattern of staphylococcus aureus (n=110)



MIC values of *Metronidazole*



MIC values of *Clindamycin*

Fig. 9: MIC values of *metronidazole and clindamycin*

Fungal isolates

A total of 30 fungal isolates were obtained in culture. *Aspergillus Sp*. Accounted for 66.6% of fungal isolates while *Candida Sp.* Accounted for 33.3% of fungal isolates. Among *Aspergillus spp*., *Aspergillus Niger* accounted for 43.3%, *Aspergillus flavus* 16.7% and *Aspergillus fumigatus*6.6%. Among Candida Sp*., Candida albicans* accounted for 26.7% and *Candida tropicalis* 6.7% (table 8).

Table 8: Distribution of fungal isolates (n=30)

Fungal isolate	Number	Percentage
Aspergillusniger	13	43.3%
Aspergillus flavus	5	16.7%
Aspergillus fumigatus	2	6.6%
Candida albicans	8	26.7%
Candida tropicalis	2	6.7%
Total	30	100%

Antifungal sensitivity pattern

The sensitivity of Candida isolates was tested via VITEK-2 to Fluconazole, Itraconazole, Voriconazole, and Flucytosine and they were found to be 100% sensitive to all the antifungals tested.

DISCUSSION

This prospective study, which was conducted on diagnosed cases of CSOM, included a total of 500 patients and 550isolates were obtained. CSOM was found to be more common among males than females with males accounting for 62.8% of cases. This may reflect the overall male predominance of infections due to behavioral differences between males and females.

Analysis of age distribution in our study showed peak incidence of CSOM in the third decade of life with 32.8% cases in the age group of 20-29 y which may be related to socioeconomic factors, lifestyle, and swimming in infected water. The findings of our study correlated with a study done byShreshtha *et al.* [15], Prakash *et al.* [6], however had reported peak incidence in the first decade of life. Children tend to have a higher predisposition to ear infections than adults because of the anatomy of the Eustachian tube. Other factors that predispose children to CSOM include attending childcare centers, having a mother who reported a history of purulent ear discharge, having smokers in the household, a high burden of upper respiratory tract infections, and racial factors as stated by Koch *et al.* [16].

The most common presenting complaint in our study was ear discharge for more than 3 mo (93% cases), followed by decreased hearing (66%). Some of the risk factors noted in our study were upper respiratory tract infection noted in 33% of cases and allergic history noted in 30% of cases. 26% of cases had a history of modified radical mastoidectomy with tympanoplasty.

In the present study, the majority of patients had tubo-tympanic type of CSOM (70%). This is in concordance with the previous study by Poorey *et al.* and Sharma *et al.* [17].

Analysis of the bacterial profile of the present study showed that Gram-negative bacilli (56.4%) outnumbered Gram-positive bacteria (30%) which is similar to the study by Malkappa *et al.* [18]. In contrast, Prakash *et al.* documented *Staphylococcus aureus* to be the predominant isolate comprising 48.69% of the total isolates followed by *Pseudomonas aeruginosa* [6]. These studies depict that the microbial profile varies between different regions, based on patient population and geographical distribution. *Pseudomonas aeruginosa* was the most isolated organism among cases of unsafe CSOM (51.3%) followed by *Staphylococcus aureus* (27.3%). *Pseudomonas aeruginosa*, the predominant cause of CSOM in the tropical region usually does not inhabit the upper respiratory tract, its presence in the middle ear cannot be ascribed to an invasion through the Eustachian tube and it is considered to gain access to the middle ear via a defect in tympanic membrane. Prakash *et al.* reported coliforms including *Klebsiella pneumoniae* and *Escherichia coli* in 9.42% and 7.33% of cases respectively [6]. Frequent isolation of coliforms like *E. coli* and *Klebsiella* indicates that individuals are at high risk of acquiring infection when they are exposed to water contaminated with fecal flora while bathing or in activities like swimming. *Peptostreptococcus* was the anaerobe isolated and accounted for 3.4% of the total isolates isolated in unsafe type of CSOM. *Peptostreptococcus* remain a significant anaerobic pathogen with reported rates of isolation ranging from 6-7%. However, a study from India found anaerobic etiology in 10.2% of cases. A review study found a minimum of 1.8% and a maximum of 22.4% isolation of anaerobes. The reason of this wide variation may be due to sampling error, prior administration of antibiotics, or differences in the timing of sampling during disease as stated by Aroop Mohanty *et al.* [27]. Anaerobes accounted for 29.41% of the isolates in a study conducted by Prakash *et al.* [6]. In the present study, fungi were isolated among 30 patients. Among the fungal isolates, Aspergillus species accounted for 66.7% of isolates and Candida species accounted for 33.3% of isolates. Among *Aspergillus sp., Aspergillus niger* was the most common isolate (43.3%) followed by *Aspergillus flavus* (16.7%) and *Aspergillus fumigatus* (6.7%). Candida albicans was isolated in 8 cases (26.7%) and Candida tropicalis in 2 cases (6.7%). In a study from Haryana, India, fungal etiology was found in 15% of cases, out of which 60% were *Candida* species 40% were *Aspergillus* species 74.2% of *Aspergillus* species, and 19.3% of *Candida* species among the fungal isolates were reported by Gupta *et al.* [19].

In the present study, Gram-negative bacteria showed the highest sensitivity to *Piperacillin-tazobactam* (96%) followed by *Meropenem* (94%) and *Cefoperazone-sulbactam* (91%). 70% of isolates of Gram-negative bacteria showed sensitivity to *Ceftazidime* whereas 83% showed sensitivity to *Ceftazidime-clavulanate*. 83% of isolates of Gram-negative bacteria were sensitive to *Amikacin* while 71%showed sensitivity to *Ciprofloxacin*. Antibiotic susceptibility pattern of *P. aeruginosa* revealed 97% of the isolates were sensitive to *Piperacillin-tazobactam*, 95% were sensitive to *Meropenem*, 93% isolates were sensitive to *Piperacillin*, 93% to *Cefoperazone*, 90% to *Imipenem*, 81% to *Amikacin* and 68% to *Ciprofloxacin*. The sensitivity pattern of *Pseudomonas aeruginosa* correlated with a study done by Lee *et al.* [20]. Aminoglycosides are bactericidal antibiotics that are frequently used because of their activity against Gram-negative bacteria. In the present study majority of the Gram-negative isolates including *Pseudomonas aeruginosa* isolates were found to be more sensitive to *Amikacin* than *Gentamicin*. Fluoroquinolones inhibit the bacterial DNA gyrase, or the topoisomerase II and they have a broad range of activity against *Pseudomonas aeruginosa.* In the present study, 71% of Gram-negative isolates and 68% of *P. aeruginosa* were found to be sensitive to *Ciprofloxacin* which may be due to the frequent use of topical and intravenous formulations of fluoroquinolones and is similar to the sensitivity reported by Kumar S *et al. Ceftazidime i*s the most frequently prescribed third-generation cephalosporin that has an extended Gram-negative spectrum [4]. However, resistance to *Ceftazidime* is increasing, complicating the management of patients with such isolates. In the present study, 70% of isolates of Gram-negative bacteria were sensitive to *Ceftazidime* which is in concordance with the study conducted by Mahajan *et al.* who reported 84% sensitivity to *Ceftazidime* in Gram-negative bacteria [21]. *Ceftazidime* resistance is mainly mediated by the production of β-lactamases such as ESBL, MBL, and occasionally AmpC-β-lactamases. In the present study out of 310 isolates of Gram-negative bacteria, 13% were found to be positive for ESBL production, and 9% of the Gram-negative isolates were observed to be Amp C

producers in our study. This is in concordance with the studies of Aggarwal *et al.* who documented ESBL production of about 21% [22]. ESBL-producing organisms are frequently resistant to other classes of antibiotics, including aminoglycosides and fluoroquinolones due to the coexistence of genes encoding drug resistance to other antibiotics on the plasmids which encode ESBL. The second most isolated organism in our study was *Staphylococcus aureus* comprising 110 (22%) of the isolates.87% of *Staphylococcus aureus* were found to be MSSA whereas 13% were found to be MRSA.100% isolates of *Staphylococcus aureus* were sensitive to *Vancomycin, Linezolid, and Teicoplanin.* 65% of *Staphylococcus aureus* isolates were resistant to *Ciprofloxacin*, 63% were resistant to *Ampicillin* and 54% were resistant to *Amoxicillin-Clavulanate*. This indicates a high level of resistance among *Staphylococcus aureus* for β-Lactams and Fluoroquinolones. In a study done by Prakash *et al.* out of 181 isolates, 48% of isolates were *Staphylococcus aureus*, and all were found to be methicillin-sensitive [6].

In our study, *Escherichia coli* showed 80% sensitivity to *Amikacin* and 20% sensitivity to *ciprofloxacin*. The sensitivity of *Proteus mirabilis* was good with *Amikacin* and *Gentamicin*. *Klebsiella pneumoniae* showed 85% sensitivity with *Amikacin* and 57% sensitivity with both *Ciprofloxacin* and *Gentamicin*. Among NFGNB isolates, 100% sensitivity was noted *in Amikacin, Gentamycin, Ciprofloxacin*, and *Piperacillin-Tazobactam* while 89% of isolates were sensitive to *Piperacillin.*

In the present study, all 5 isolates of Pe*ptostreptococcus* showed 100% sensitivity to *Metronidazole* while *Clindamycin* was sensitive in 40% of isolates. However, few studies have shown varying rates of *Metronidazole* resistance. Padmaja *et al.* 25 reported resistance rates of 5.5% against *Metronidazole* among *Peptostreptococcus spp*. *Clindamycin*, a lincosamide antibiotic has a good spectrum of activity against most of the Gram-positive anaerobic organisms and our study showed higher rates of resistance of 40% to *Clindamycin*. There are reports of emerging resistance against *Clindamycin* among anaerobes as stated by Padmaja *et al.* and Brook *et al.* [23, 26].

In our study, we tested 186 isolates for biofilm production out of which 149 isolates (80%) showed biofilm production. 50% of biofilm-producing isolates were moderately positive, 47% were weakly positive and 3% were strongly positive. Out of the moderately positive and strongly positive biofilm producers,(132) 90% of isolates were from unsafe CSOM out of which 107(80%) were *Pseudomonas aeruginosa* while 25 (20%) were *Staphylococcus aureus.* 87% (22) isolates of *Staphylococcus aureus* were MRSA while 13% (3) were MSSA. Host immune responses to planktonic species have been relatively well characterized and how they modulate anti-bacterial effector mechanisms when organized in this protective milieu as stated by Hank *et al.* [24]. Biofilms act as non-selective physical barriers that obstruct antibiotic diffusion and hinder the cellular and humoral immune. They have a characteristic physiology and architecture that form the basis of biofilm resistance to many antibiotics and mechanisms of host defense as stated by Stewart *et al.* [12]. Bacteria may communicate with each other through diffusible molecules within the milieu of a biofilm through a mechanism known as quorum sensing as per Li and Tian. [25]An epidemiological knowledge of the prevalence of biofilm production in CSOM is necessary as biofilm production may account for the recurrence of CSOM after treatment, failure of surgery, and resistance to antibiotic therapy. All the patients with unsafe CSOM in our study had a history of recurrent CSOM and were on antibiotic therapy for many years (mean time on antibiotic treatment – 8 yrs.). 60 patients (84.5%) with unsafe CSOM with detection of moderately positive or strongly positive biofilm production had undergone MRM with type 2 tympanoplasty in the past.

The study had a few limitations. We could not perform the antifungal susceptibility testing for molds in our study. Also, the antibiotic sensitivity profile of anaerobes could not be evaluated more extensively.

CONCLUSION

CSOM is generally treated by oral medications with Fluoroquinolone antibiotic drops, the absence of oral formulations of *Cephalosporins* and *Carbapenems* which limited the use of these antibiotics in patients with CSOM otorrhea in a past era, resulted in less resistance however there has been a recent trend in increasing resistance to beta-lactams due to production of ESBL and Amp C beta-lactamases. Isolation rate of MRSA has also seen an upward trend. The emergence of multi-drug-resistant organisms may complicate patient management. The formation of biofilm by certain bacteria may explain recurrence and resistance patterns in unsafe CSOM and post-surgical procedures like modified radical mastoidectomy with tympanoplasty creating surfaces for biofilm to develop. CSOM often has polymicrobial pathology creating war in the middle ear environment, augmentation of pathology being created by mixed aerobic and anaerobic organisms, and synergism between aerobic and anaerobic organisms has already been recognized. A high rate of *Clindamycin* resistance was seen in anaerobes which is noteworthy because this is a frequently utilized antimicrobial for treating anaerobic infections. Periodic surveillance of the antimicrobial susceptibility profile of bacteria is the need of the hour to guide empiric antibiotic therapy and to formulate local antibiotic policy.

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AUTHORS CONTRIBUTIONS

Dr Priya Bhat collected the data performed analysis of samples and prepared the manuscript. Dr Upasana Bhumbla supervised the methodology of the study and was involved in the analysis of the data. Dr Jasleen Kaur was involved in the preparation of the manuscript and data analysis. All the authors approved the final manuscript.

CONFLICT OF INTERESTS

The authors have no conflicts of interest regarding this investigation.

REFERENCES

1. Vuppala R, Burugula K, RB. Middle ear and nasopharyngeal bacterial flora and their sensitivity in active stage of mucosal disease otitis media: a comparison. Asian J Pharm Clin Res. 2016 Sep 1;9(5):235-8. doi: 10.22159/ajpcr.2016.v9i5.13366.
2. Moorthy PN, Lingaiah J, Katari S, Nakirakanti A. Clinical application of a microbiological study on chronic suppurative otitis media. Int J Otolaryngol Head Neck Surg. 2013;2(6):290-4. doi: 10.4236/ijohns.2013.26060.
3. MP, KL, SA, GN S. Bacteriological profile and their antibiotic susceptibility pattern of cases of chronic suppurative otitis media. Asian J Pharm Clin Res. 2013 Aug 1;6(7):210-2.
4. Kumar S, Sharma R, Saxena A, Pandey A, Gautam P, Taneja V. Bacterial flora of infected unsafe CSOM. Indian J Otol. 2012;18(4):208-11. doi: 10.4103/0971-7749.104800.
5. Mittal R, Lisi CV, Gerring R, Mittal J, Mathee K, Narasimhan G. Current concepts in the pathogenesis and treatment of chronic suppurative otitis media. J Med Microbiol. 2015 Oct;64(10):1103-16. doi: 10.1099/jmm.0.000155, PMID 26248613.
6. Prakash R, Juyal D, Negi V, Pal S, Adekhandi S, Sharma M. Microbiology of chronic suppurative otitis media in a tertiary care setup of Uttarakhand State India. N Am J Med Sci. 2013 Apr;5(4):282-7. doi: 10.4103/1947-2714.110436, PMID 23724403.
7. Forbes BA, Sahm DF, Alice AS. Bailey and scott's diagnostic microbiology. 15th ed: Mosby Elsevier; 2007. p. 254-64.
8. Winn W JR, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P. Colour atlas and textbook of diagnostic microbiology. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2006. p. 623-71.
9. Stepanovic S, Vukovic D, Dakic I, Savic B, Svabic Vlahovic M. A modified microtiter plate test for quantification of staphylococcal biofilm formation. J Microbiol Methods. 2000 Apr;40(2):175-9. doi: 10.1016/s0167-7012(00)00122-6, PMID 10699673.
10. Song Y, Liu C, Finegold SM. Development of a flow chart for identification of gram positive anaerobic cocci in the clinical laboratory. J Clin Microbiol. 2007 Feb;45(2):512-6. doi: 10.1128/JCM.01872-06, PMID 17135439.

11. Chander J. Chapter 20. Textbook of medical mycology. 4th ed. India: Jaypee Brothers (Pvt. Ltd.); 2018. p. 408-21.
12. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. Lancet. 2001 Jul 14;358(9276):135-8. doi: 10.1016/s0140-6736(01)05321-1, PMID 11463434.
13. Hiremath B, Mudhol RS, Vagrali MA. Bacteriological profile and antimicrobial susceptibility pattern in chronic suppurative otitis media: a 1-year cross sectional study. Indian J Otolaryngol Head Neck Surg. 2019 Nov;71 Suppl 2:1221-6. doi: 10.1007/s12070-018-1279-6, PMID 31750155.
14. Arikan S. Current status of antifungal susceptibility testing methods. Med Mycol. 2007 Nov;45(7):569-87. doi: 10.1080/13693780701436794, PMID 17885947.
15. Shrestha D, Thapa P, Bhandari YB. Types of pathology and ossicular status in atticoantral disease undergoing mastoidectomy at Bir Hospital. J Coll Med Sci Nepal. 2010;6(4):26-30. doi: 10.3126/jcmsn.v6i4.6722.
16. Koch A, Homoe P, Pipper C, Hjuler T, Melbye M. Chronic suppurative otitis media in a birth cohort of children in greenland: population based study of incidence and risk factors. Pediatr Infect Dis J. 2011 Jan;30(1):25-9. doi: 10.1097/INF.0b013e3181efaa11, PMID 20700079.
17. Poorey VK, Lyer A. Study of bacterial flora in CSOM and its clinical significance. Indian J Otolaryngol Head Neck Surg. 2002 Apr;54(2):91-5. doi: 10.1007/BF02968724, PMID 23119866.
18. Malkappa SK, Kondapaneni S, Surpam RB, Chakraverti TK. Study of aerobic bacterial isolates and their antibiotic susceptibility pattern in chronic suppurative otitis media. Indian J Otol. 2012;18(3):136-9. doi: 10.4103/0971-7749.103440.
19. Sen Gupta RP, Kacker SK. Otomycosis. Indian J Med Sci. 1978 Jan-Feb;32(1-2):5-7. PMID 153329.
20. Lee SK, Lee MS, Jung SY, Byun JY, Park MS, Yeo SG. Antimicrobial resistance of pseudomonas aeruginosa from otorrhea of chronic suppurative otitis media patients. Otolaryngol Head Neck Surg. 2010 Oct;143(4):500-5. doi: 10.1016/j.otohns.2010.06.906, PMID 20869558.
21. Mahajan RK, Agarwal S, Jeram H, Vashishtha RC. Antimicrobial susceptibility pattern of bacterial isolates in patients of chronic suppurative otitis media in a tertiary care hospital in India. Int J Res Med Sci. 2018;6(11):3705-9. doi: 10.18203/2320-6012.ijrms20184434.
22. Aggarwal R, Chaudhary U, Bala K. Detection of extended spectrum beta-lactamase in pseudomonas aeruginosa. Indian J Pathol Microbiol. 2008 Apr-Jun;51(2):222-4. doi: 10.4103/0377-4929.41693, PMID 18603687.
23. Shenoy PA, Lobo S, Shetty S, Vishwanath S, Chawla K. Antimicrobial susceptibility profile of clinical isolates of peptostreptococcus anaerobius. J Pure Appl Microbiol. 2018;12(3):1239-45. doi: 10.22207/JPAM.12.3.25.
24. Hanke ML, Kielian T. Deciphering mechanisms of staphylococcal biofilm evasion of host immunity. Front Cell Infect Microbiol. 2012;2:62. doi: 10.3389/fcimb.2012.00062, PMID 22919653.
25. LI YH, Tian X. Quorum sensing and bacterial social interactions in biofilms. Sensors (Basel). 2012;12(3):2519-38. doi: 10.3390/s120302519, PMID 22736963.
26. Brook I, Wexler HM, Goldstein EJ. Antianaerobic antimicrobials: spectrum and susceptibility testing. Clin Microbiol Rev. 2013;26(3):526-46. doi: 10.1128/CMR.00086-12, PMID 23824372.
27. Gupta P, Varshney S, Kumar SK, Mohanty A, Jha MK. Chronic suppurative otitis media: a microbiological review of 20 Y. Indian J Otol. 2020;26(2):59-67. doi: 10.4103/indianjotol.INDIANJOTOL_141_19.