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Study of anaerobic bacteria and their antibiotic susceptibility pattern in chronic suppurative otitis media

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INTRODUCTION: Hearing is one of the most important senses of man which provides him with the ability to perceive the world and also helps him in communication.Chronic suppurative otitis media (CSOM),a tremendous health predicament since time immemorial, is a chronic inflammation of the middle ear cleft which may lead to serious sequelae and complications. The wide spread use of antibiotics has precipitated the emergence of multiple resistant strains of bacteria which can produce both primary and postoperative infections.

MATERIALS AND METHODS: This is a Prospective studyconducted at Department of Microbiology,Tertiary care teaching hospitalover a period of 1 year with200 samples were collected from patients attendingat out Patient department of ENT included in the study.After cleaning the external ear with alcohol, ear discharge is collected aided by a Bull lamp and aural speculum with absorbable sterile cotton swabs under strict aseptic precaution.

RESULTS: Analysis of these cases of CSOM in both sexes and age groups as shown in Table - 1: revealed that the majority of cases of CSOM were in the age 11-20yrs in both sexes .The overall incidence of CSOM was found to be more in males 117 than in females 83 and this predominance was noted in all the age groups.The details of anaerobic organisms isolated in csom cases.Bacteroidsfragilis is the predominant organism followed by peptosteptococcus.All anaerobes were 100% sensitive to Metronidazole.

CONCLUSION: Out of 200 samples 14 anaerobic organisms were isolated of which *Bacteroidesfragilis* 4(28.6%) were predominant anaerobes followed by *Peptostreptococcus species 3(21.4%).*This sudy is designed to have the knowledge of the pathogens and antibiotic sensitivity pattern responsible for CSOM and choosing suitable antibiotics according to susceptibility tests should guide the management of disease treatment and reduce the recurrence and complications of CSOM.

Keywords: Anaerobic Microbial Flora, Chronic Suppurative Otitis Media, Antibiotics

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INTRODUCTION

Hearing is one of the most important senses of man which provides him with the ability to perceive the world and also helps him in communication.Chronic suppurative otitis media (CSOM),a tremendous health predicament since time immemorial, is a chronic inflammation of the middle ear cleft which may lead to serious sequelae and complications[1].It is the most common infection of the ear characterised by persistant or recurrent purulent discharge from the middle ear through a persistant non intact tympanic membrane[2].

CSOM is always secondary to upper respiratory tract infection,organisms reach the middle ear via the Eustachian tube[3].Infection can spread from middle-ear to vital structures such as mastiod, facial nerve, labyrinth, lateral sinus, meninges and brain leading to mastoid abscess, facial nerve paralysis ,deafness, lateral sinus thrombosis ,meningitis and intracranial abscess[4]. Of all the complications, hearing loss associated with chronic ear discharge is nearly always significant, reported in 50% of cases and tending to be more severe then those reported in other types of otitis media[5].

The infection may occur during the first 6 years of a child's life, with a peak around 2 years. The point in time when ASOM becomes CSOM is still controversial. Generally, patients with tympanic perforations who continue to discharge mucoid material for periods from 6 weeks to 3 months, despite medical treatment, are recognized as CSOM cases. The disease affects 65–330 million people worldwide, mainly in developing countries[6]. It has been estimated that there are 31 million new cases of CSOM per year, with 22.6 % in children less than 5 years old (Monasta et al., 2012). According to the World Health Organization (WHO) estimates of 2015 over 5% of the world's populations (328 million adults and 32 million children) have disabling hearing loss. The highest prevalence is found in the Asia Pacific, South Asia, and Sub-Saharan African regions[7].

The wide spread use of antibiotics has precipitated the emergence of multiple resistant strains of bacteria which can produce both primary and postoperative infections.[2] The indiscriminate, haphazard and half-hearted use of antibiotics and poor follow-up of patients have resulted in persistence of low grade infections[8].

Often, the primary care physicians are usually the first to see these patients and mostly rely on empirical antibiotic therapy and only refer to the otolaryngologist when their treatments fail. Due to its recurrent nature and the development of resistant pathogenic organisms, control of infection poses a greatest therapeutic challenge. The challenges of resistance have even been compounded by the activities of quacks in this part of the country where they engage in uninformed administration of antibiotics to these patients. These days, it is rare for an otolaryngologist to encounter bacterial flora of a chronic discharging ear that has not already been modified by previous antibiotic therapy with some of them returning sterile cultures[9].

Knowledge of local micro-organism pattern and their antibiotic sensitivity is essential for effective and low cost treatment.This study would assist clinicians and microbiologists to formulate guidelines for antimicrobial sensitivityof the pathogens and treatment modalities for the disease which in turn would help reduce the morbidity and mortality.

Aims and Objective:To isolate an identify anaerobic bacteria involved in the causation of Chronic suppurative otitis media and to study antimicrobial sensitivity pattern of the isolated organisms.

MATERIALS AND METHODS

This is a Prospective studyconducted at Department of Microbiology,Tertiary care teaching hospital over a periodof 1 year with200 samples were collected from patients attending at out Patient department of the ENT Department included in the study.

INCLUSION CRITERIA:

1. Patients with ear discharge for more than 3 months.
2. Patients should not be on antibiotics in the past 7 days.
3. Patients irrespective of age and sex are included.

EXCLUSION CRITERIA:

Patients on antibiotics are excluded.

SPECIMEN COLLECTION AND PROCESSING

Specimen: Ear discharge (Pus).

Sample collection: After cleaning the external ear with alcohol, ear discharge is collected aided by a Bull lamp and aural speculum with absorbable sterile cotton swabs under strict aseptic precaution.

Sample processing: swab were collected from each patient .

Swab kept in to the Robertson's cooked meat medium for anaerobic culture.

ANAEROBIC CULTURE

Specimen : The swab placed in RCM is overlaid with sterile liquid paraffin or paraffin wax for anaerobic atmosphere .Presence of anaerobic organisms is indicated by displacement of paraffin wax due to production of H_2S.

Direct smear examination: The swab after inoculating on to anaerobic blood agar plates , smear is made on a clean glass slide, air dried, heat fixed and stained by Jensen's modification of Grams stain. Smear is examined under oil immersion for morphology of bacteria and presence of polymorpho nuclear lymphocytes.

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Culture : The swab from RCM which showed abundant gas and turbidity are inoculated on to Anaerobic blood agar plate supplemented with sheep blood (5%) and hemin (5mcg/ml) &Vit K (1mg/ml).The antibiotic disc of Metronidazole (5mcg)is placed in well and Kanamycin (10mcg), Colistin (10mcg) & Vancomycin (5mcg) discs are placed on streak lines with the help of a sterile forceps on the plate which also serve as antibiotic resistance tests (Wadsworth anaerobic bacteriology manual 3rd edition).

The jar is tightly sealed with the help of metal clips present on the jar. Slight heat is produced in the jar indicating the starting of anaerobiasis.

The plates are incubated at 37^0C observed for growth after 48hrs with minimum exposure to oxygen .If there is no growth then reincubated. If growth is present ,then inhibition zone around the antibiotic discs are looked for. Since all anaerobes are sensitive to Metronidazole and all other organisms universally resistant to it. A zone of inhibition around the Metronidazole disc indicates the presence of anaerobes. The specimens did not show zone of inhibition around Metronidazole are reported as negative for anaerobes after 48hrs incubation .The plates which showed sensitivity to Metronidazole are further processed. For isolated colonies direct plating from the edge of the inhibition onto a fresh anaerobic blood agar plate and incubated for another 48 hrs in anaerobic jar.

Anaerobes are usually present in mixed culture either as multiple anaerobes or with facultative aerobes (Wadsworth Anaerobic bacteriology manual). Each colony type is subcultured into thioglycollate broth supplemented with Vit K(0.1mcg/ml), Hemin (5mcg/ml) and Sodiumbicarbonate(1mg/ml).

Presumptive identification based on antibiotic discs

The inoculum from thioglycolate broth is streaked on to a fresh anaerobic blood agar plate.
Metronidazole (5mcg), Kanamycin (10mcg), Colistin (10mcg), Vancomycin (10mcg), Nitrate discs are placed on the streak lines with the help of sterile forceps .

After anaerobic incubation for 48 hrs the zones of inhibition are looked.
Any zone of size equal to or more than 10mm is taken as sensitive. (Wadsworth Anaerobic Bacteriology manual).

BIOCHEMICAL TESTS

Catalase test: A drop of 10% H_2O_2 is taken on a clean glass slide and the colony to be tested was taken with a sterile wooden applicator stick and dipped in it .instantanoeus development of bubbles indicates a positive reaction .

Nitrate reduction test : Nitrate discs after incubation for 48hrs on Columbia agar with the growth were removed and placed in a clean petri dish and 1 drop of reagent A and B were added

.Development of pink colour indicated that nitrate was reduced .The discs showing no colour change after 2-3 min were investigated for presence of unreduced nitrate by sprinkling small amount of zinc dust on it .Development of red colour was indicative of a true negative reaction while, no colour change was presumptive indication of reduction beyond nitrates.

Spot indole test: Discs impregnated with Indole spot reagent (1% cinnamaldehyde) is smeared with a test culture colony with the help of sterile wooden applicator loop. Development of blue or blue green colour within 30 seconds indicates a positive , while development of pink colour indicates negative reaction .

Esculin hydrolysis test: Discs impregnated with 2mg of esculin were placed on the medium inoculated with the organism and incubated. After 48hrs results are read by adding 2 drops of 10% solution of ferric chloride to the disc. Development of black colour around the disc or underside of the plate within 60 min at room temperature indicates positive reaction .

Carbohydrate fermentation tests: From the thioglycolate broth , the subculture is taken with sterile swab and lawn culture is done on the Colombia agar plate supplemented with Vitamin K (15%) and Hemin (0.5%). Carbohydrate discs are placed near the circumference of each plate and the plates are incubated in Gaspak jar.

After 48hrs of incubation the plates are opened and looked for increased growth on and around the disc indicating the utilization of that particular sugar.3 drops of aqueous bromothymol blue (0.2%) the colour of which has been stabilised by adding 0.1% NaOH to each disc .Yellow colour indicates a positive reaction while blue green colour indicates negative reaction .

ANTIBIOTIC SUSCEPTIBILITY TESTING OF ANAEROBES

Antimicrobial therapy of anaerobes is determined empirically as their isolation and identification is difficult as well as they are slow growers ,results will be obtained in an additional 2-4 days .Antibiotic susceptibility testing is similar to that of aerobes. Agar diffusion method is used, based on tentative zone diameter interpretation is done .(Wadsworth Anaerobic Bacteriology manual).

RESULTS

Two hundred patients attending the ENT Depertment of Hospital with the clinical diagnosis of CSOM with or without complications were taken as the study group. Ear swabs were collected and processed .

TABLE: 1 AGE AND SEX PREVALENCE OF CSOM CASES

AGE	MALES	%	FEMALES	%	TOTAL	%
<1yr	2	1.70	-	-	2	1
1-10yrs	20	17.09	18	21.68	38	19
11-20yrs	48	41.02	41	16.86	89	44.5

21-30yrs	20	17.09	11	13.25	31	15.5
31-40yrs	14	11.96	07	8.43	21	10.5
41-50yrs	09	7.69	06	7.22	15	7.5
51-60yrs	03	2.56	-		3	1.5
>60yrs	01	0.85			01	0.5
TOTAL	117		83		200	

Analysis of these cases of CSOM in both sexes and age groups as shown in Table -1: revealed that the majority of cases of CSOM were in the age 11-20yrs in both sexes .The overall incidence of CSOM was found to be more in males 117 than in females 83 and this predominance was noted in all the age groups.

TABLE -2 DETAILS OF ANAEROBIC BACTERIA ISOLATED FROM CSOM CASES

ORGANISM	MONOMICROBIAL	POLYMICROBIAL	NUMBER	PERCENTAGE
Peptococcusmagnus	1	1	2	14.2
Peptococcusasaccharolyticus	1	-	1	7.2
Peptostreptococcus	2	1	3	21.4
Propionibacterium acnes	1	1	2	14.3
Bacteroidesfragilis	2	2	4	28.6
Bacteroidesmelaninogenicus	1	1	2	14.3
TOTAL	8	6	14	100

Table -2 shows the details of anaerobic organisms isolated in csom cases .Bacteroidsfragilis is the predominant organism followed by peptosteptococcus.

TABLE -3 ANTIBIOTIC SENSITIVITY TESTING OF ANAEROBES

Organism	**Number**	**Vancomycin**	**Kanamycin**	**Metronidazole**	**Colistin**
Bacteroidessps	6	-	-	6	-
Peptococcus	3	3	2	3	-
Peptostreptococcus	3	2	2	3	-
Propionobacterium	2	2	2	2	-
Total	14	-	-	14	-

All anaerobes were 100% sensitive to Metronidazole.

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DISCUSSION

CSOM is a condition of the middle ear that is characterized by persistent or recurrent discharge through a chronic perforation of the tympanic membrane.Due to perforation of the tympanic membrane, microorganisms may gain entry to the middle ear via the external ear. It is a destructive and persistent disease with reversible sequelae and can proceed to serious intra and extra cranial complications.Such complications were very common in the preantibiotic era .Though such serious complications are low at present,still some patients have complications ranging from persistent otorrhea, mastoiditis, labyrinthitisand facial nerve palsy. Even though the complications are rare, treatment should be started early and effectively to avoid and reduce the chances of complications.

The therapeutic use of antibiotics is usually started empirically prior to the results of microbiological culture. Selection of any antibiotic is influenced by by its efficacy, resistance of bacteria, safety, risk of toxicity and cost. Knowledge of the local microorganism pattern and their antibiotic sensitivity is essential to allow effective and cost-saving treatment.

In the present study an attempt is made to know the etiology of CSOM, with antimicrobial susceptibility testing of the isolates.Despite the advances in medical care and antimicrobials, CSOM still remains the most common cause of hearing loss in children and adults.Emergence of antimicrobial resistance makes it further more difficult for the otologists to reduce the risk of complications.

CSOM can be unilateral or bilateral.Unilateral can be right or left ear .In the present study unilateral infection was predominant. Left ear was affected (51%) and the right ear was affected in (43.5%).Bilateral involvement was seen only in 5.5% cases. These findings correlate with the studies by Laxmipathi&Bhaskaran (1965) who found unilateral predominance[10].

CSOM associated with complications were granulations in 3 case (1.5%), mastoiditis in 3 case (1.5%), facial palsy in 2 case (1%) and vertigo and vomiting in 2(1%). Brook Itzhak (1997) also found these same complication in his study.[11]

In the present study 14 specimens were positive and 186 were negative for anaerobic culture. The culture results are variable with other studies.

Anaerobic culture was done in present study using Gaspack and anaerobic jar. Total 14 strains were isolated from 200 cases *Bacteroidsfragilis* was the most predominant anaerobe with 4(28.6%) followed by *Peptostreptococcus* 3 (21.4%), *Peptococcussps* 3 (21.4%), *B.melaninogenicus* 2(14.2%) and *Propionobacterium*2 (14.3%).

Many studies revealed anaerobes as pathogens in their studies. RajathPrakash et al (2013) isolated anaerobes in their studies[12].*Bacteroides species* play a major role as pathogen in CSOM.In the present study 6 *Bacteroides species* were isolated.Ologe FE et al (2012) reported *Bacteroids species* in their studies. [13]

Chiwra et al (2015) in their study anaerobes were isolated in 33.1% of the total samples most commonly *Bacteroides spp*., followed by *Peptostreptococcus spp*. and *Clostridium spp.*[15]*Bacteroidesspp, Porphyromonas, Prevotella* were common isolates in the studies done by AHC Rajat Prakash et al (2013)[12].*Fusobacterium* was also isolated n th studies of Brook (2004)[11].Rajat Prakash (2013) reported *Clostridium species* as the predominant organism.But in present study *Clostridium species* is not isolated [14].*Prevotella species* was isolated in the studies of Jere M et al[15].Antimicrobial sensitivity testing of anaerobes was done for preliminary identification. All anaerobes were 100% sensitive to Metronidazole while *Bacteroides species* were sensitive to Colistin and Kanamycin.

CONCLUSION

Out of 200 samples 14 anaerobic organisms were isolated of which *Bacteroidesfragilis* 4(28.6%) were predominant anaerobes followed by *Peptostreptococcus species 3(21.4%).*

This sudy is designed to have the knowledge of the pathogens and antibiotic sensitivity pattern responsible for CSOM and choosing suitable antibiotics according to susceptibility tests should guide the management of disease treatment and reduce the recurrence and complications of CSOM. This study has been done in a Hospital, it has adopted conventional, cost effective yet efficient methods for the isolation of pathogens and their sensitivity patterns of anaerobes which will help in executing effective treatment modalities

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