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Study On Association Between Nasopharyngeal Colonizers and Middle Ear Pathogens in Chronic Suppurative Otitis Media

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Abstract

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INTRODUCTION

Ear is considered as an important sensory organ of human beings. Ear infections are a commonly encountered entity in routine clinical practice. Chronic suppurative otitis media is one of the most common chronic diseases of childhood and one of the major causes of deafness in India.[1]

Hippocrates stated that acute pain of the ear, with the continued fever, is to be dreaded, for there is danger that the man may become delirious and die.[2]

Suppurative otitis media along with its unpleasant symptoms and complications may be a catastrophe for the marvellous organ, the ear, on which much of our appreciation of life and human activity depends. It is a privilege for an otorhinolaryngologist to preserve, repair and take utmost care of the structure and function of this organ, in whatever condition it is presented. It is a challenge to prevent the progress of acute suppurative otitis media to a chronic disease especially in children.[3]

Aerobes bacteria, anaerobic bacteria, and fungi are all known potential pathogens in CSOM. Understanding of the microbiology of chronic otitis media is important for efficient and effective treatment, and prevention of complications and antibiotic resistance.[4]

The human nasopharyngeal space is wide, making it an ecological reservoir for a variety of commensals bacterial pathogens which colonise this space, which is an essential step in the development of respiratory bacterial infections like CSOM .[7]

To determine the bactériologie etiology of individual cases of otitismedia, it appears logical to culture the nasopharynx, which presumably constitutes the reservoir of middle ear pathogens 40 schwrtz

The aim of the study was to find the association between nasopharyngeal colonizers and middle ear pathogens.

OBJECTIVES OF THE STUDY

1. To isolate and identify the aerobic bacterial isolates causing chronic suppurative otitis media
2. To study the antibiotic sensitivity pattern of the bacterial isolates.
3. To study association between bacterial isolates from CSOM with colonizers in nasopharynx.

METHODOLOGY

The present study was conducted in Sri Chamarajendra Hospital, Department of Microbiology, Hassan institute of medical sciences,

Hassan from January 2018 to June 2019. One hundred and twenty patients with CSOM of all age groups and both sexes attending outpatient department and those admitted in ENT wards were selected randomly for the study based on below mentioned inclusion and exclusion criteria's.

Inclusion criteria:

- Patients with active purulent discharge in the ear for more than 2 weeks [7]
- Patients of all age groups of both sexes attending ENT OPD and admitted in ENT wards

Exclusion criteria:

- Patients on antibiotic or antifungal treatment (ear drops or systemic) within the previous two weeks
- Patients with draining ears of less than two weeks duration
- Traumatic tympanic membrane perforation
- Non co-operative patients

MATERIALS:

Study Subjects: Informed consent was taken from all the study participants. Institutional Ethical committee clearance was taken before start of the study.

Data regarding age, sex, IP/OP, type of ear discharge, laterality and clinical features were collected through a pretested questionnaire. (Annexure 1)

Sample collection:

Collection of ear swab: Ear discharge was collected under strict aseptic precautions using sterile cotton swabs with the assist of aural speculum and processed immediately in the microbiology laboratory. Two swabs were collected, one for gram staining and one for aerobic culture. [8]

Collection of nasopharyngeal swab:

With the patient in a comfortable sitting position and mouth widely opened using tongue depressor ,patient was asked to say 'Ah' to elevate the uvula and the soft palate , a sterile flexible swab(west's postnasal swab) was bent at right angle and inserted into mouth and rotated , then the swab was withdrawn and processed immediately in the laboratory.[8]

METHODS:

Direct smear examination

With one swab a thin smear was made on a clean glass slide and heat fixed and allowed to dry. Gram staining was done for the smears so made and was examined under oil immersion objective to note the various morphological types of bacteria, presence or absence of inflammatory cells and also to note the numbers of squamous epithelial cells in the sample.[8]

Aerobic culture

The second swab was used for inoculation on blood agar, nutrient agar and MacConkey agar plates. Chocolate agar plate with hemin (X factor) and nicotinamide-adenine-dinucleotide (NAD / V factor) inoculated for H.influenzae. All plates were incubated aerobically at 37°C in presence of carbon-dioxide (candle jar) and evaluated at 24 hours, 48 hours and 72 hours and discarded if there was no growth after 72 hours.

After 24hrs, 48hrs and 72 hrs of incubation the culture plates were inspected for growth and identified initially by colony characters, haemolysis on blood agar , lactose fermentation on MacConkey agar, morphology in gram staining, Catalase test, Oxidase test and motility (hanging drop) test.

The preliminary identification of potential pathogens, later confirmed up to species level by standard biochemical tests and special tests which includes Indole test, Methyl red test, Voges Proskauer test, Citrate utilization test, Urease test, Triple sugar iron test, O/F test, Coagulase (Tube coagulase and Slide coagulase) test, Sugar fermentation for glucose, lactose, sucrose, mannose, mannitol, xylose, maltose, arabinose fermentation test, amino acid decarboxylation test for arginine, lysine and ornithine.(Annexure 5).[8]

Antibiotic sensitivity test is done by using Kirby-Bauer disc diffusion method on Mueller Hinton agar plate as per CLSI (2018). In this method commercially available filter paper discs in 6mm diameter charged with the various concentrations of the drugs were used.

Inoculum preparation ; 3-4 similar colonies were touched with loop for gram negative bacteria's and 6-8 similar colonies for gram positive bacteria's and inoculated into nutrient broth and incubated for 4-6 hours. Inoculum preparation for fastidious is done using suspension of bacterial growth in saline.

Volume 4, Issue 4; July-August 2021; Page No 1192-1205

The turbidity of the broth with inoculums is adjusted to 0.5 McFarland standards. Lawn culture was done on Mueller Hinton agar plate using sterile swabs. Sensitivity for H.influenzae was done on 5% blood agar with factor V (NAD) and for S.pneumoniae on 5% sheep blood agar. After drying the plate at 37^0c for 30 minutes, antibiotic discs (6 per 90mm plate) are applied with sterile forceps. After 16-18 hours of incubation(24hours for H.influenzae) in presence of 5% CO2, the degree of sensitivity determined by measuring the zones of inhibition of growth around the discs. Growth is inhibited around discs containing antimicrobials to which the bacterium is susceptible but not around those to which it is resistant.[9]

S. aureus ATCC 25923, E. coli ATCC 25922 and P. aeruginosa 25873 were used for internal quality control of antibiotic susceptibility testing.

Detection of MRSA: Cefoxitin disc diffusion method.

All strains were tested with 30 µg Cefoxitin discs (Hi-Media) on Mueller–Hinton agar plates. For each strain, a bacterial suspension adjusted to 0.5 McFarland was used as Inoculum. The zone of inhibition was determined after 16–18 h incubation at 35 ^{0}C. Zone size was interpreted according to CLSI (2018) criteria: Strains of S. aureus having zone of inhibition of ≤21 mm was considered MRSA.[9]

Detection of HLAR: All strains were tested with High content Gentamicin (120µg) discs (Hi-Media) on Mueller–Hinton agar plates. For each strain, a bacterial suspension adjusted to 0.5 McFarland was used. The zone of inhibition was determined after 16–18 h incubation at 37 ^{0}C. Zone size was interpreted according to CLSI (2018) criteria: Strains of Enterococcus having zone of inhibition of ≤10 mm was considered HLAR.[9]

Detection of ESBL: ESBL detection by double disc synergy test:

Screening test done using Ceftazidime 30µg. If found resistant with zone size <22mm, confirmatory test was done by placing Ceftazidime disc and Ceftazidime/ Clavulanic acid 30µg/10µg at a distance of 15mm .A 5mm enhanced zone with CAC disc compared to CAZ was confirmatory of ESBL producer.[9]

Detection AmpC beta-lactamase: Isolates with zone diameters less than 18 mm with 30-µg Cefoxitin disk were selected for confirmation of AmpC production.

Confirmation done by AmpC disk test: MHA plate was inoculated with ATCC E.coli strain, later AmpC disk was rehydrated with 20 µl of saline, and test organism applied to it. A 30 µg Cefoxitin disk is placed on MHA plate. Next AmpC disk is placed almost touching the Cefoxitin disk and incubated overnight at 35°C. Plate with an indentation or a flattening of the zone of inhibition is considered AmpC positive.[22]

Detection of Metallo-beta-lactamase*:* If the zone of Imipenem was reduced to 16-20 mm or less or heaping occurred, we tested the isolate for MBL production. Double Disc synergy test using EDTA were used for detection of MBL. An enhanced zone with EDTA disc was considered MBL.[9]

RESULTS

This study was conducted in Sri Chamarajendra Hospital, Department of Microbiology, HIMS, Hassan. One hundred and twenty patients with CSOM of all age groups and both sexes attending outpatient department and those admitted in ENT wards were selected randomly for the study.

Details of isolation	**Total number of swabs studied**				**p**
	Ear swabs	**(%)**	**Nasopharynx**	**(%)**	
Positive cultures	98	81.66%	79	65.84%	0.005313
Negative cultures	22	18.34%	41	34.16%	
Total	120	100%	120	100%	

Table 1: Results of culture positivity of CSOM cases studied

Volume 4, Issue 4; July-August 2021; Page No 1192-1205

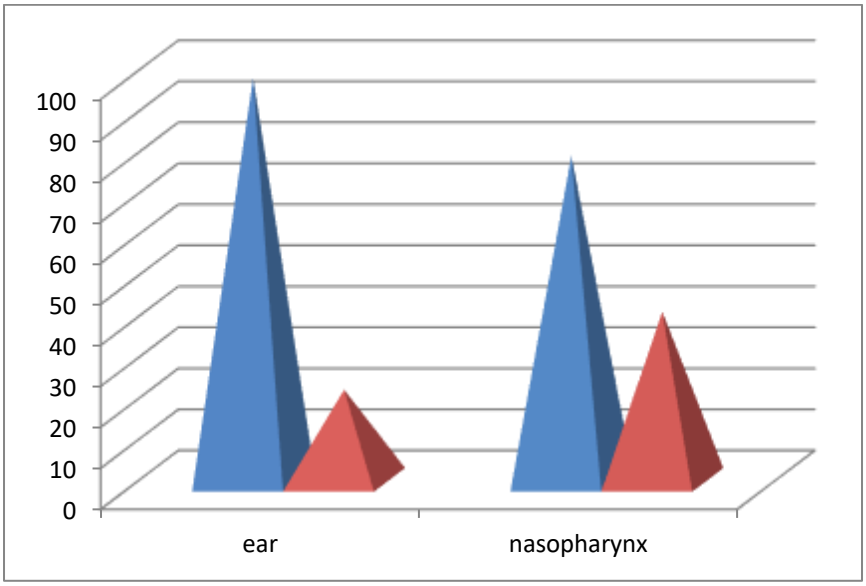

Figure 1: Schematic distribution of culture positivity

Note: This shows that the isolates obtained from ear swabs and nasopharyngeal swabs have significant statistical difference.

Organisms	**Total number of strains and Percentage excluding known commensals**				**p**
	Ear	**(%)**	**Nasopharynx**	**(%)**	
Monomicrobial	78	79.59 %	78	97.5%	
Polymicrobial	20	20.40%	02	2.5%	
Total	98	100%	80	100%	0.000305

Table2: Incidence of pure and mixed cultures

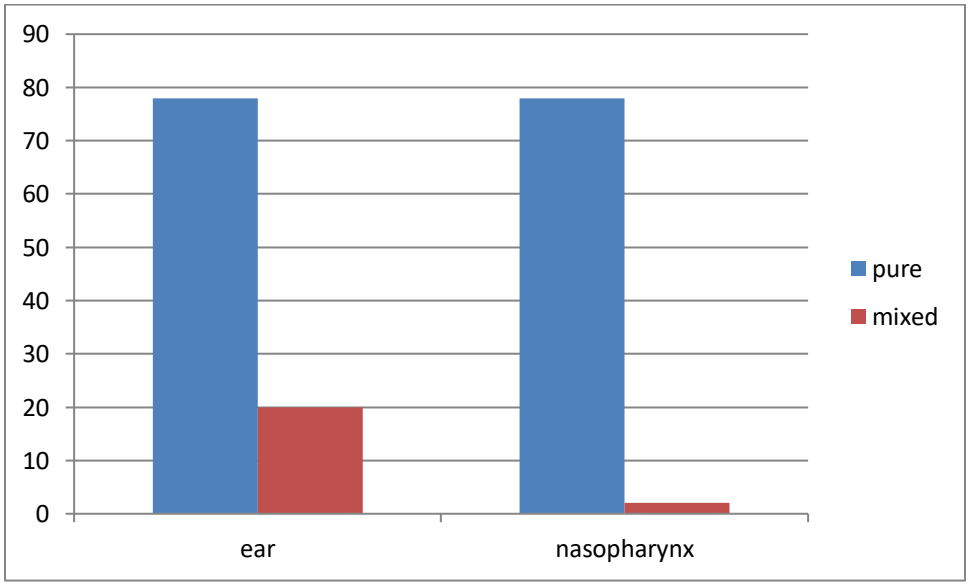

Figure 2: Schematic distribution of pure and mixed cultures

Volume 4, Issue 4; July-August 2021; Page No 1192-1205

Note: Monomicrobial etiology was found to be 78 (79.51%) and 78 (97.5%) in ear and nasopharynx. Polymicrobial was 20 (20.49%) and 02 (2.5%) respectively which is statistically significant.

Organisms	**Frequency in Ear**	**%**	**Frequency in Nasopharynx**	**%**	**Common in both (no)**
Staphylococcous aureus	32	28.82	13	16.4	10
Pseudomonas aeruginosa	22	19.81	08	10.1	06
Klebsiella pnemoniae	16	14.41	08	10.1	07
Proteus vulgaris	05	4.5	02	2.53	02
Proteus mirabilis	03	2.7	00	0.0	00
Moraxella catarrhalis	08	7.20	12	15.18	07
Haemophilus influenzae	06	5.4	08	10.1	05
Escherichia coli	06	5.4	00	0.0	00
Streptococcus pneumoniae	05	4.5	12	15.18	04
Acinetobacter baumanii	04	3.6	03	3.79	02
Enterococci faecalis	02	1.8	03	3.79	01
Citrobacter freundii	02	1.8	00	0	00

Table 3: Distribution of isolates in ear and nasopharynx

Staphylococcus aureus was the predominant organism in ear discharge followed by Pseudomonas aeruginosa , Klebsiella pneumoniae , Proteus spp, and Moraxella catarrhalis .Similarly in nasopharyngeal isolates maximum growth of staphylococcus aureus was seen followed by Moraxella catarrhalis, Klebsiella pneumoniae, Pseudomonas aeruginosa ,Hemophilus influenza and Streptococcus pneumoniae

Volume 4, Issue 4; July-August 2021; Page No 1192-1205

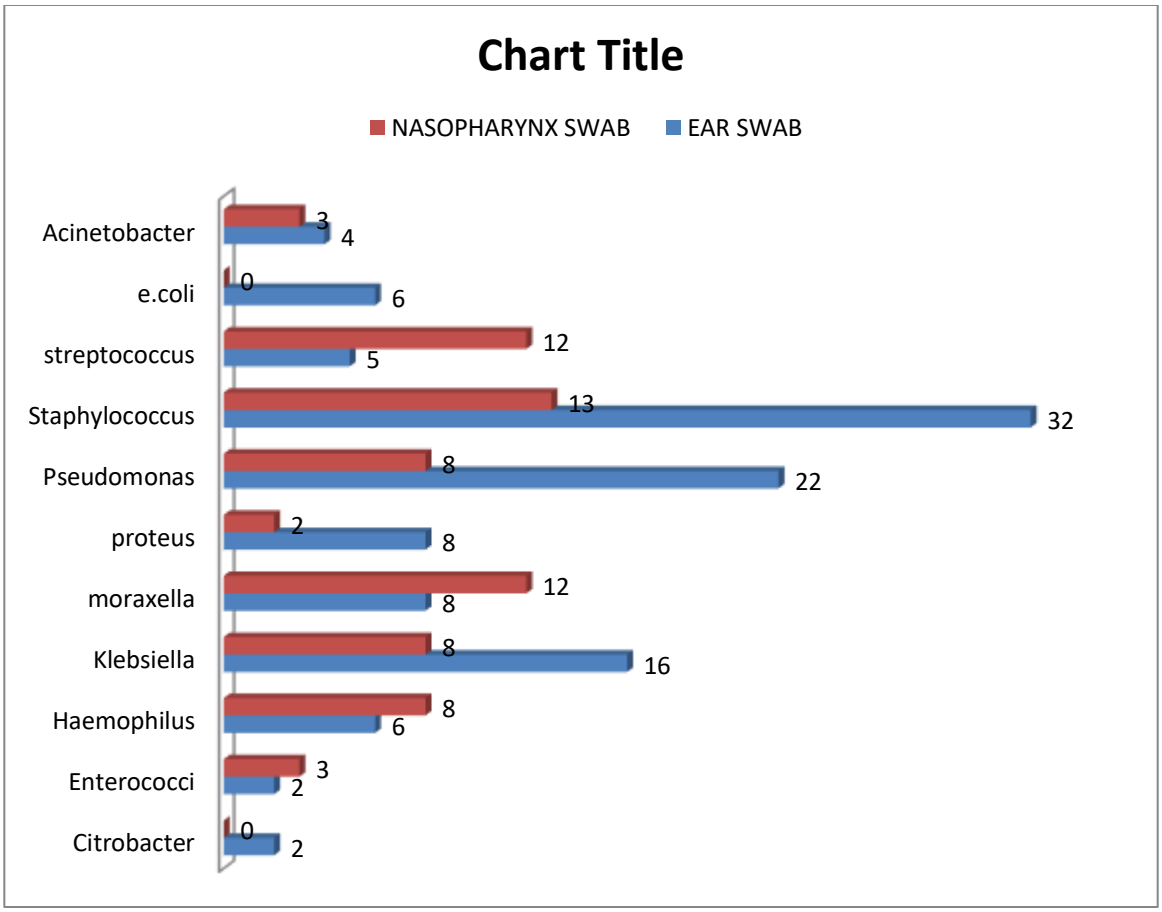

Figure 3: Schematic distribution of isolates

Chi-Square Tests					
			NASO PHARYNX SWAB		**P**
			PRESENT	**ABSENT**	
EAR SWAB	PRESENT	Count	66	31	<0.001
		%	78.6%	34.4%	
	ABSENT	Count	18	59	
		%	21.4%	65.6%	

Statistically significant association was found between middle ear pathogens and nasopharyngeal colonizers.

ANTIBIOTICS	**S.aureus (32)**	**%**	**Enterococci (02)**	**%**	**S.pneumoniae (05)**	**%**
P	01	3.1	01	50	05	100
CX	25	78.12	02	100	05	100
E	07	21.87	01	50	04	80

Volume 4, Issue 4; July-August 2021; Page No 1192-1205

CD	20	62.5	02	100	04	80
COT	20	62.5	02	100	02	40
GEN	28	87.5	01	50	04	80
CIP	16	50	01	50	03	66.6
VA	32	100	02	100	05	100
DOX	18	56.2	02	100	05	100
LZ	27	84.3	02	100	05	100
LE	28	87.5	02	100	04	80
PTZ	17	53.12	01	50	05	100
AMX	04	12.5	00	00	05	100
AMC	06	18.75	00	00	05	100
CTR	27	84.3	01	50	05	100
AK	28	87.5	02	100	05	100

Table 4: Antibiotic sensitivity pattern of gram positive organisms isolated in ear discharge

All the Streptococcus spp. isolates were 100 % sensitive to all the drugs tested as per the guidelines. S. aureus were sensitive Linezolid, Vancomycin and Clindamycin.

Antibiotic	**Klebsiella (16)**		**Proteus (08)**		**Acinetobacter (04)**		**Citrobacter (02)**		**H.influenzae (06)**		**E.coli (06)**		**Moraxella (08)**	
AMX	01	6.2	04	50	00	00	00	00	05	83.3	02	33.3	03	37.5
AMC	04	25	06	75	00	00	00	00	05	83.3	02	33.3	04	50
AK	11	68.7	03	37.5	02	50	01	50	04	66.6	05	83.3	06	75
GEN	09	56.2	03	37.5	03	75	01	50	02	33.3	03	50	05	62.5
CIP	07	43.75	05	62.5	00	00	01	50	05	83.3	04	66.6	04	50
CTR	04	25	07	87.5	00	00	00	00	04	66.6	04	66.6	03	37.5
CAZ	04	25	05	62.5	01	25	01	50	04	66.6	01	16.6	04	50
CAC	11	68.7	08	100	01	25	01	50	05	83.3	05	83.3	06	75
CX	09	56.2	07	87.5	02	50	02	100	05	83.3	05	83.3	06	75
PTZ	07	43.7	05	62.5	02	50	01	50	05	83.3	04	66.6	05	62.5
COT	08	50	03	37.5	02	50	01	50	06	100	04	66.6	05	62.5
CL	16	100	NT	00	04	100	02	100	NT	00	06	100	08	100
IPM	14	87.5	07	87.5	03	75	02	100	06	100	05	83.3	08	100
LE	13	81.25	07	87.5	03	75	01	50	05	83.3	05	83.3	07	87.5

Table 5: Antibiotic sensitivity pattern of gram-negative organisms isolated in ear discharge

Volume 4, Issue 4; July-August 2021; Page No 1192-1205

Resistance was highest with Ampicillin followed by Cefoxitin and Cotrimoxazole. Gram negative isolates were highly sensitive to Colistin followed by Imipenem, amikacin, and Levofloxacin

ANTIBIOTICS	Pseudomonas (24)	%
AMX	01	4.16
AMC	02	8.33
AK	17	17.83
GEN	11	45.83
CIP	18	75
CTR	13	54.16
CAZ	13	54.16
CAC	20	83.83
CX	22	91.66
PTZ	20	83.83
COT	15	62.5
CL	24	100
IPM	22	91.66
LE	20	83.83
TOB	20	83.83
AT	19	79.16

Table 6: Antibiotic sensitivity pattern of pseudomonas isolated in ear discharge

ANTIBIOTICS	S.aureus (13)	%	Enterococci (03)	%	S.pneumoniae (12)	%
P	13	100	02	66.6	12	100
CX	10	76.92	03	100	12	100
E	10	76.92	02	66.6	10	83.33
CD	09	69.23	02	66.6	10	83.33
COT	09	69.23	02	66.6	09	75
GEN	08	61.53	02	66.6	08	66.6
CIP	05	38.46	02	66.6	09	75
VA	13	100	03	100	12	100
DOX	09	69.23	02	66.6	12	100

Volume 4, Issue 4; July-August 2021; Page No 1192-1205

LZ	13	100	03	100	12	100
LE	12	92.30	03	100	12	100
PTZ	12	92.30	03	100	12	100
AMX	13	100	02	66.6	12	100
AMC	13	100	02	66.6	12	100

Table 7: Antibiotic sensitivity pattern of gram-positive organisms isolated in nasopharynx

Antibiotics	**Klebsiella (08)**		**Pseudomonas (08)**		**Acinetobacter (02)**		**Proteus (02)**		**H.influenzae (08)**		**E.coli (03)**		**Moraxella (12)**	
AMX	01	12.5	01	12.5	00	00	00	00	07	87.5	01	33.3	11	91.6
AMC	04	50	03	37.5	00	00	00	00	08	100	02	66.6	12	100
AK	07	87.5	06	75	01	50	02	100	07	87.5	03	100	10	83.3
GEN	05	62.5	05	62.5	00	00	01	50	05	62.5	02	66.6	09	75
CIP	05	62.5	06	75	00	00	01	50	06	75	02	66.6	08	66.6
CTR	06	75	07	87.5	01	50	02	100	07	87.5	02	66.6	09	75
CAZ	06	75	06	75	01	00	02	100	07	87.5	02	66.6	11	91.6
CAC	07	87.5	07	87.5	02	100	02	100	07	87.5	03	100	12	100
CX	07	87.5	07	87.5	02	100	02	100	07	87.5	02	66.6	11	91.6
PTZ	07	87.5	07	87.5	02	100	01	50	07	87.5	02	66.6	10	83.3
COT	04	50	05	62.5	00	00	02	100	06	75	02	66.6	08	66.6
CL	08	100	08	100	02	100	NA	NA	08	100	03	100	12	100
IPM	08	100	08	100	02	100	02	100	08	100	03	100	12	100
LE	08	100	07	87.5	02	100	02	100	08	100	03	100	11	91.6

Table 8: Antibiotic sensitivity pattern of gram-negative organisms isolated in nasopharynx

Volume 4, Issue 4; July-August 2021; Page No 1192-1205

Resistance markers	S.aureus	Klebsiella	E.coli	Pseudomonas
MRSA	07/32 (21.8%)	--	--	--
HLAR	01/02(50%)	--	--	--
ESBL	--	07(29.1%)	04(20.8%)	04(20.8%)
AmpC	--	02(18.2%)	01(15.4%)	02(18.2%)
Carbapenamase	--	02(33.3%)	01(16.6%)	02(33.3%)

Table 9: Frequencies of resistance markers with percentage

Overall ESBL rate was 24/74 (36.5%), AmpC rate was 15/74(20.2%), and Carbapenamase was 06/74(08.1%).

DISCUSSION

In the present study an attempt is made to know the aerobic bacteriological profile of CSOM, with antimicrobial susceptibility testing of the bacterial isolates, and association of isolates from CSOM cases with nasopharyngeal colonizers.

Culture results of cases studied

In the present study 98(81.667%) specimens were positive and 22(18.333%) were negative for the culture. Similar observations was seen in studies done by Chauhan J et al. (2019)[20] and Khatoon et al. (2015)[21]. But the culture results are variable with other workers. Prakash M et al. (2013) got 93.75% positive cultures and 6.25% negative cultures. This could be due to the difference in the patient population studied and geographical variations.

Negative cultures can be attributed to CSOM because of fungal and anaerobic bacterial etiology.

Incidence of pure and mixed cultures

In the present study monomicrobial etiology was found in 79.591% and polymicrobial etiology in 20.409% of cases. My study is correlated with Majumder et al. (2019)[10] and Gopi et al. (2016)[01]. But Yousuf A et al. (2012)[22] and Chirwa M et al. (2015)[4] found equal incidence of mixed and pure culture. Availability and use of topical and systemic broad-spectrum antibiotics in the period before consultation was probably responsible for the lower incidence of mixed infection in our study.

Aerobic bacteriological profile in CSOM cases.

In present study among 120 cases of CSOM, Staphylococcus aureus was the predominant organism 32 (34.23%) followed by Pseudomonas aeruginosa 22 (19.81%), Klebsiella pneumoniae 16 (14.41%), Proteus spp. 08(7.20%), Moraxella catarrhalis 08(7.20%), Hemophilus influenzae 06 (05.40%), Enterococcus faecalis 8 (5.48%), E. coli 06 (5.40%), Streptococcus pneumoniae 05(4.50%), Acinetobacter baumanii 4 (3.60%).

The frequency of Staphylococcus aureus in the middle ear infections can be attributed to their ubiquitous nature and high carriage of resistant strains in the external auditory canal and upper respiratory tract.

However workers like Sudhindra et al. (2014)[6], Chauhan et al. (2019)[20], Nagraj M.et al. (2018) [17], Serry et al. (2017)[11], Khatoon et al. (2015)[21]have found Staphylococcus aureus as the second most common organism causing CSOM.

The next predominant organism in the present study was Pseudomonas aeruginosa 22 (19.81%). My study is correlated with Chauhan et al. (2019)[20] and Nagraj M.et al. (2018)[17]. However some workers like Sudhindra et al. (2014)[6] and Yousuf A et al.. (2012)[22] have found Pseudomonas spp. as the predominant organism causing CSOM.

The other organisms isolated in the present study are Moraxella catarrhalis 08(7.20%) Hemophilus influenza 06(05.40%), Enterococcus faecalis 8(5.48%), Acinetobacter baumanii 4(3.60%) and Streptococcus pneumoniae 05(4.50%), These findings are correlated with Nia et al. (2011) and Khatoon et al.(2015).

Volume 4, Issue 4; July-August 2021; Page No 1192-1205

The organisms like Pseudomonas, Proteus spp, E. coli, Acinetobacter spp and Klebsiella spp, are considered mostly as secondary invaders from external auditory canal which gains access to the middle ear via a defect in tympanic membrane resulting from an acute episode of otitis media. Presence of organisms like Pseudomonas and Acinetobacter and presence of Multidrug resistant Gram-negative bacilli in CSOM cases indicates that, those patients would be frequent visitors of hospital.

Susceptibility of Gram-positive bacterial isolates to selected antimicrobial agents

Antibiotic sensitivity was carried out for all the isolates by Kirby-Bauer disc diffusion method. In the present study S.aureus showed maximum susceptibility to Vancomycin (100%), Linezolid (84.3%), Ceftriaxone (84.3%) and least susceptibility to Amoxicillin (12.5%) and Erythromycin (21.8%). Ciprofloxacin was 54.2% susceptible and Doxycycline was 56.2% susceptible. Cefoxitin showed 78.9% susceptibility, hence MRSA isolates were 21.9% .Similar observations was seen in studies done by Khatoon et al. (2015)[21] and Serry et al. (2017)[11]. But higher rates of MRSA was found in Majumder et al. (2019)[10]. It is also observed that the most commonly used drug ciprofloxacin is exhibiting increasing resistance.

In a study done by Dhirendra et al. (2016) [23], Clindamycin showed 85% susceptibility to S. Aureus, but in our study more resistance was observed.

Streptococcus pneumoniae showed maximum susceptibility to Penicillin (100%), Vancomycin (100%) and least susceptibility to Cotrimoxazole. Kazeem et al. (2017)[10] reported 50% resistance to tetracycline's. Enterococci showed 100% sensitivity to Vancomycin, Linezolid, High level Gentamycin and Levofloxacin. Amoxicillin was the least susceptible antibiotic. Similar results were observed in studies done by Kazeem et al. (2017)[29] and Devi et al. (2015).[28]

Susceptibility of Gram-negative bacterial isolates to selected antimicrobial agents

Among Gram negative organisms, highest susceptibility was shown by Colistin (100%) and Imipenem (91.9%) followed by Amikacin (68.7%), Ciprofloxacin (58.2%) and least susceptibility to Amoxicillin (12.5%) and Amoxiclav(18.2%).This was correlated with Gopi et al.[1], Prakash et al. (2013) and Khatoon et al. (2015)[21].

In pseudomonas aeruginosa showed maximum sensitivity to Colistin (100%), Imipenem (91.6%), Tobramycin (83.3%) and least was shown to Gentamycin (45.8%) and Amoxicillin (4.1%). In a study done by Sharma et al. Pseudomonas showed maximum sensitivity to Amikacin (82.3%) and Ciprofloxacin (76.5%).

Klebsiella showed maximum sensitivity to Colistin (100%) and Imipenem (92.4%) and least to Amoxicillin (12.5%). E.coli was highly susceptible to Colistin (100%) and Imipenem (83.4%) and least susceptible to Ceftazidime (20%). In Pseudomonas Colistin (100%), Imipenem (91.6%) and Tobramycin (83.3%) showed maximum sensitivity and least was shown by Gentamycin (45.8%) and Amoxicillin (4.1%). Study by Nia et al.[24] showed high sensitivity to Ciprofloxacin (95%) and relative sensitivity to Gentamicin (85%). H.influenzae showed maximum sensitivity to Imipenem and Levofloxacin.

The results of culture and sensitivity pattern vary from place to place and time to time. This may be because of various reasons like changes in prevalence of particular organisms, environmental variations, changes in the antibiotic prescription pattern etc. Therefore, culture and susceptibility testing for CSOM in a population/ geographical area is of paramount importance for appropriate antimicrobial therapy of CSOM.

In this study, most of the isolates were found to be resistant to regularly used cell wall inhibitors like penicillin group of drugs and cephalosporins. MRSA was detected in (29.1%) S.aureus. ESBL and AmpC were detected in 36.5%- and 20.2%-Gram negative bacteria respectively. MBL was detected in 8.1%.

However, In study done by Khatoon et al.[21], MRSA and HLAR were detected in 9(29%) S. aureus and 1(50%) Enterococcus faecalis. ESBL and AmpC were detected in 11(18.3%) and 12(20%) Gram negative bacteria respectively. MBL producer was not detected in Gram negative bacteria.

Prevalence of ESBL, AmpC β-lactamase and MRSA were found to be 48.9 %, 20.4 %, and 27.5 % respectively in a study done by Sasirekha (2013).

Volume 4, Issue 4; July-August 2021; Page No 1192-1205

Higher rates of resistance markers were seen in study done by Chellaiah et al. (2014). They got 56.6 % MRSA. 67.3% of Enterobacteriaceae were ESBL producers, 6.1% were AmpC producers and 27.2% of *Pseudomonas aeruginosa* were MBL producers.

Ibrahim et al. in 2019 found the frequency of ESBL and AmpC β-lactamase producers to be 27% and 101 32.5%, respectively.

This indicates that, the incidence of various resistance markers is increasing which reflects the increasing level of resistance in the community. Increased prevalence of the resistance markers like MRSA, ESBL etc may be because of ineffective implementation of Infection control and antibiotic policies. This could be also because of improved reporting of the resistance markers with routine testing.

In today's age, where there is increasing concern regarding antimicrobial resistance and the increasing rate of MRSA, HLAR, ESBL and AmpC is disheartening.

The early knowledge of bacterial isolates in CSOM cases aids in giving a probable chance of upcoming complications and better prognosis. Hence timely management of CSOM cases with proper culture and sensitivity report helps in getting better outcome in CSOM patients.

Correlation between nasopharyngeal colonizers and ear swab isolates.

In the present study we collected swabs from both ear and nasopharynx of the same patient from all 120 cases.

We analysed the pattern of isolates obtained in both the swabs and tried to find their association with regard to CSOM.

Similar microorganisms were obtained in both nasopharynx and ear discharge in 57.12% (58) cases which is statistically significant.

This is in correlation with studies done by Chang J et al. who got 26.5% (18/68) association.[30] But Sonawale et al. (2018)[31] and Schwartz et al. (2015)[32]showed higher association rates of 72% and 77.97% respectively. The variations in the different studies could be because of difference in methodologies and interpretations.

Revai et al. (2009)[33], Xu et al. (2012)[34] and Faden et al. (2019)[35] have considered only the colonization rates of nasopharynx and have concluded Hemophilus influenza, Streptococcus pneumonia and Moraxella catarrhalis as common nasopharyngeal colonizers. But comparative studies of the same with ear discharge in CSOM cases are limited.

The pattern of colonization in nasopharyngeal isolates in our study were similar when compared to the above-mentioned studies. We also could find an association between these colonizers and middle ear pathogens in CSOM cases. Therefore, early screening of potential pathogens may be helpful in prediction of possible pathogens and there by prevention of complications in CSOM.

The maximum association was seen in Staphylococcus aureus (10) followed by Moraxella (08), Klebsiella (08) and Hemophilus (05) and least with Enterococci (01). Similar results was obtained by Chang et al.[30] who got 18 (26.5%) association with S.aureus. But a different result was obtained by Afolabi et al. (2015)[7] who found association with organisms like P. aeruginosa, K. pneumoniae and E. Faecalis and no association between S. aureus, P. mirabilis and E. Coli. This differences in the association may be because of changes in prevalence of organisms in different geographical regions.

CONCLUSION

The aerobic bacteriological study of CSOM showed Staphylococcus aureus as the most common causative agent followed by Pseudomonas aeruginosa, Klebsiella pneumoniae and Proteus mirabilis. The overall isolation rate of MDR gram positive and gram-negative organisms were found to be high. This may be due to frequent visit of patients to hospital. Hence the rate can be reduced significantly if we could also focus on hospital infection control.

The antibiotic susceptibility testing to gram positive and gram-negative isolates showed maximum sensitivity to expensive and higher class of drugs like to Vancomycin, Linezolid, Colistin and Imipenem. The high degree of resistance rate is observed to the most commonly used antibiotics like Ciprofloxacin, Gentamycin etc in present study. This may be due to the irrational use and over the counter availability of antibiotics. To prevent development of drug resistance, prescription of antibiotics should always be

Volume 4, Issue 4; July-August 2021; Page No 1192-1205

guided by culture and sensitivity reports and escalation or de-escalation of dosage following empirical therapy done accordingly based sensitivity report.

In our study we observed a correlation between nasopharyngeal colonizers and middle ear pathogens, hence prompt evaluation of nasopharyngeal colonizers may help in the prediction of potential pathogens leading to CSOM. As variable results were obtained in different studies, we suggest further research in this regard.

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Volume 4, Issue 4; July-August 2021; Page No 1192-1205

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Volume 4, Issue 4; July-August 2021; Page No 1192-1205