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"ANTIBIOGRAM OF DISCHARGING OTITIS MEDIA IN KARAD"

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ABSTRACT

Background: Otitis media is one among the leading causes of illness in infants and adults,which causes an inflammation in middle ear cleft without any reference to etiology or pathogenesis.Microorganisms change their growth and susceptibility based on the region and climate. Therefore, it is important to analyse microorganisms causing otitis media.

Aim: To evaluateantibiogram of the microorganisms causing otitis media in Karad.

Method: Present cross-sectional study was carried out for 176 patients with otitis media. Pus samples were collected from discharging ears. Microorganisms were isolated and susceptibility testswere performed for each isolate. Data was analysed using Chi-square test and percentages.Significance was tested at $p<0.05$ level.

Results: Among all 176 swab cultures, 162 cultures showed positive growth. Of which 62.96% belonged to acute suppurative otitis media (ASOM) and 37.04% was chronic suppurative otitis media (CSOM). Purulent (74.53%) and mucopurulent (69.70%) type of discharge was prevalent under ASOM and CSOM, respectively ($p<0.001$).*Staphylococcus aureus* (n=51) for ASOM and *Pseudomonas aeruginosa* (n=31) for CSOM were commonly observed isolates. *Pseudomonas aeruginosa* was the predominant organism followed by *Klebsiella pneumoniae* and *Staphylococcus aureus.*Ciprofloxacin was the most effective antibiotic (68.38%) in case of ASOM while Gentamicin was most effective antibiotic (69.44%) under CSOM.

Conclusion: Majority of the bacterial isolates were multidrug resistant. In this study *Pseudomonas aeruginosa* was the most dominant organism. Ciprofloxacin and Gentamicin were the most sensitive anti-drugs in ASOM and CSOM, respectively.

Keywords: Ciprofloxacin,Gentamicins, Klebsiella pneumoniae,Pseudomonas aeruginosa, Suppurative otitis media.

INTRODUCTION

Otitis media is an inflammation of the tympanic membrane and middle ear.Based on various presentations, complications associated and treatment, it is classified as acutesuppurative otitis media (ASOM), otitis media with effusion (OME) and chronic suppurative otitis media (CSOM) [1, 2]. ASOM presents with local and systemic signs and has a rapid onset. OME can occur during the resolution of ASOM when the acute inflammation has resolved but bacteria might be present [3]. While CSOM is a condition where ear discharge is lasting for 2 weeks or more through a persistent tympanic membrane perforation [4].

It is ranked fifth on the global burden of diseases and has affected 360 million people 2019[5]. It is one of the most common childhood illnesses [6].Risk factors include poor hygiene, malnutrition, overcrowding, upper respiratory tract infections, failure to breastfeed, prolonged use of a pacifier and exposure to passive smoking[4]. The most common causative bacterial species of ASOM are *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*[7]. On the other

hand *Staphylococcus aureus*, *Pseudomonas aeruginosa* and Enterobacteriaceae such as Proteus spp. and *Klebsiella pneumoniae*are the pathogens that are most commonly associated with CSOM [8]
Besides high incidence, otitis media has received attention for its issues regardingantibiotic resistance as well as ototoxicityof antibiotics [9]. The rapid emergence of resistant bacteria leads to endangering the efficacy ofantibiotics [10].Thus,it is necessary to study microbes and its drug sensitivity. Therefore,the current study aimed to assess the antibiogram of aerobic bacterial isolates in Karad, Maharashtra, India. Additionally, identification of the microorganisms associated with middle ear infections, correlation of age, sex and other predisposing factors were also studied.

METHODOLOGY

This cross-sectional study was conducted among 176 patients with a history of discharging ears at Karad(Western Maharashtra, India) from June 2016 to December 2017. Ethical clearance and a written informed consent was obtained before the commencement of the study.The inclusion criteria composed of patients presenting with a history of discharging and clinically diagnosed with acute/ chronic suppurative otitis media on examination at two tertiary care centers. Patients were not excluded on any other grounds.

Procedure

Sample collection: External ear of the subject was cleaned with a sterile wet cotton swab and simultaneously the type of discharge was noted. Auditory speculum was carefully inserted in the ear. With the help ofa thin sterile cotton swab, pus was collected adjacent to the tympanic membrane which was introduced into sterile culture tubes.
The requisite sample size was calculated using the following formula:

$$n = \frac{p\,(1-p)\,Z^2}{d^2}$$

where "n" is the required sample size, "p" is the percentage occurrence of a state or condition (proportion or prevalence), "d" is the percentage maximum error required, "z" is the value corresponding to level of confidence required. Prevalence of *Klebsiella pneumoniae* isolates found in otitis media wastaken as 9.42% from previous studies with 95% confidence level and 5% error [11].
Sample processing: To collect the discharge, three swabs were prepared for the following purpose:
First swab used for gram staining and aerobic culture. Through gram staining typical morphology, gram nature of bacteria was observed. For aerobic culture plain blood agar, MacConkey's agar and chocolate agar was used for inoculation.The culture was incubated aerobically at 37^0C, overnight. Aerobic organisms were identified according to standard methods (Mackie & McCartney)[12].Isolated organisms were also tested for their sensitivity against various antibiotics according to the Kirby-Bauer method by Clinical and Laboratory Standards Institute (CLSI) standards on Muller Hinton agar and on blood agar depending on the gram nature of isolated organism. Available control organism strains such as *E.coli* ATCC 25922,*Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883 were used.
Second swab was made for direct examination of fungal elements. Direct examination (potassium hydroxidepreparation)was carried out to observe – yeast cells, pseudohyphae, and hyphae.
Third swab was processed for the examination of fungal culture. Two sets of Sabourauds Dextrose Agar (SDA) were inoculated with specimen (one was incubated at 37^0C and other at room temperature). It was examined daily for 1st week and then twice a week during next 3 weeks for the growth of any yeast or mycelial fungus.[13] Lacto phenol cotton blue preparations followed by slide culture was done to identify species, however for cultures indicative of *Candida albicans*, Germ tube test was also done.

Statistical analysis

Categorical data was analyzed by usingR 3.6.3 software and Excel and expressed in percentages.Chi-square test was performed to see the association between two categorical varaibles.$P \leq 0.05$ at 95% confidence interval was considered to be statistically significant.

RESULTS

Table 1: Analysis of isolates in otitis media cases

	OTITIS MEDIA	**ASOM**		**CSOM**	
Total number of patients	176	106	60.23%	70	39.77%
Swabs showing positi growth	162	102	62.96%	60	37.04%
Culture Sterile	14	4	28.57%	10	71.43%
Age distribution					
0 – 20 years	82	66	62.26%	16	22.86%
21 – 40 years	73	40	37.73%	33	47.14%
> 40 years	21	0	0	21	30%
Sex distribution					
Male	128	76	59.38%	52	40.63%
Female	48	30	62.50%	18	37.50%
Ear involvement					
Unilateral	139	94	67.63%	45	32.37%
Bilateral	37	12	32.43%	25	67.57%

ASOM, acute suppurative otitis media; CSOM, chronic suppurative otitis media
Data is presented as numbers and frequency (%)

In this study there were a total 176 cases, of which 162 were positive for growth (ASOM: 62.96% and CSOM: 37.04%). Majority of the patients were male (128 subjects). Maximum patients had unilateral discharge (139 patients) (**Table 1**).

Table 2: Type, Duration and Signs/symptoms among Otitis media

Parameters	**ASOM [n (%)]**	**CSOM [n (%)]**
Type of discharge		
Purulent	79 (77.45%)	9 (8.57%)
Mucopurulent	21 (20.59%)	46 (76.67%)
Mucoid	4 (3.77%)	7 (10%)
Blood mixed	02 (1.96%)	-
Blackish debris	-	04 (6.67%)
Duration		
<1 week	38(35.85%)	6(8.57%)
1 weeks to 6 weeks	48(45.28%)	21(30.00%)
6 weeks to 3 months	20(18.87%)	8(11.43%)
3 months to 6 months	-	25(35.71%)

6 months to 1 year	-	6(8.57%)
1 year and above	-	4(5.71%)
Signs and Symptoms		
Deafness	26 (25.49%)	58 (96.69%)
Fullness or blocking sensation ofEar	32 (31.37%)	18 (30%)
Otalgia	86 (84.31%)	42 (70%)
Pulsatile discharge	52 (50.98%)	-
Tonsillitis	12 (11.76%)	14 (23.33%)
External otitis	-	12 (20%)
Itching	-	9 (15%)
Giddiness and tinnitus	-	16 (26.67%)
Febrileness	48 (47.06%)	-

ASOM, acute suppurativeotitis media; CSOM, chronic suppurative otitis media

Table 2 depicts that the maximum discharge underASOM and CSOM was purulent (77.45%) and mucopurulent (76.67%) respectively. The peak of discharge was observed at 1 week to 6 weeks (ASOM: 45.28%) and 3 months to 6 months for (CSOM: 35.71%). Otalgia (84.31%) was the most prevalent symptom in ASOM while Deafness (96.69%) was more frequent in CSOM. Presence of *Candida albicans* was seen in only one case out of 102 culture positive ASOMCases, whereas the same fungal isolate was noted in 3 cases out of 60 culture positive cases of CSOM. *Aspergillus fumigatus* was seen in 5 cases while only 1 belonged to *Aspergillus flavus*.

Table 3: Analysis of isolates among positive ASOM and CSOM cases in pure and mixed form

	ASOM [N (%)]			**CSOM [n (%)]**		
Organism	**Mono bacteria**	**Poly bacteria**	**Total No. of Isolates**	**Mono bacterial**	**Poly Bacterial**	**Total No. of isolates**
Staphylococcus aureus	34 (40.96)	17 (48.57)	51	9 (18.00%	8 (22.86%	17
Klebsiella pneumonia	31 (37.35)	11 (31.43)	42	15(30.91%	1 (2.86%)	16
Pseudomonas	8(9.64)	3 (8.57)	11	25 (50.00%	6(17.14%)	31
E.coli	5(6.25)	2 (5.71)	7	1 (2.00%)	5 (14.29%	6
Citrobacter spp.	1 (1.25)	0 (0)	1			
Streptococcous Pneumoniae	2 (2.41)	- (-)	2			
Streptococcous Pyogenes	2 (2.41)	1 (2.86)	3			

Candida (yeast)		1 (2.86)	1		3 (8.57%)	3
Diptheroids					1 (2.86%)	1
Micrococcus					1 (2.86%)	1
Aspergillus spp					6 (17.14%	6
Total	83 (81.34%)	35 (34.31%)	118	50 (61.73%)	31 (38.27%	81

ASOM, acute suppurative otitis media; CSOM, chronic suppurative otitis media

Table 3 shows thatout of 102 culture, positive ASOM cases 83 (81.37%) were in pure form and 35 (34.31%) were in mixed form. On the other hand, out of 60 culture positive CSOM cases, 50 (61.73%) were in pure form and 31 (38.27%) were in mixed form.

Table 4: Antibiogram of 117 aerobic isolates from 106 culture positive ASOM cases

	Total No. of stra	Resi stan to al	AK	AZK	AG	AM	CN	CX	PG	GM	PC	PR	RP	RC	CF
Staphylococcus Aureus	51	4	30	7	24	8	22	6	1	31	28	6	21	42	18
Klebsiella pneumoniae	42	2	21	31	13	3	4	9	6	9	22	32	6	21	22
Pseudomonas aeruginosa	11	6	8	1	1	3	4	5	2	11	8	7	9	10	11
E.coli	7		6	2	2	1	4	2	0	5	4	2	3	5	3
Citrobacter koseri.	1		1	0	1	1	1	1	1	1	1	1	1	1	1
StreptococcousPn moniae	2		1	1	1	0	0	1	0	1	0	1	1	1	1
Streptococcous Pyogenes	3		2	2	2	0	0	2	1	2	0	2	0	0	2
Total	117	-	69	44	44	16	35	26	11	60	63	51	41	80	58
Percentage	-	-	58.9	37.61	37.61	13.68	29.91	22.22	9.40	51.28	53.85	43.59	35.04	68.38	49.57

ASOM, acute suppurative otitis media; AK, Amikacin; AM, Ampicillin; AG, Augmentin; AZK, Azithromycin; CF, Cephotaxime; CN, Carbencillin; CSOM, chronic suppurative otitis media; CX, Cloxacillin; GM, Gentamycin; PC, Piperacillin; PG, Penicillin; PR, Cephalexin; RC, Ciprofloxacin; RP, Ceftriaxione.

Table 5: Antibiogram of 72 aerobic isolates from 60 culture positive CSOM cases

	Tota	Resist nat to a	AK	AZK	AG	CN	CX	PG	GM	PC	PR	RP	RC	CF	AM
Pseudomonas aeruginosa	31	12	18	8	15	10	9	11	21	15	14	16	16	9	12

Klebsiella Pneumoniae	16	8	9	6	10	12	6	8	10	3	-	9	8	7	8
Staphylococci Aureus	17	1	10	8	12	6	12	4	13	16	21	7	5	8	5
E. coli	6	-	3	2	1	1	1	-	4	1	1	3	2	1	1
Diptheroids	1	-	1	1	1	-	-	-	1	-	1	1	-	1	1
Micrococcus	1	-	1	1	-	-	-	-	1	-	1	-	1	1	-
Total	72	-	42	26	39	29	28	23	50	35	38	36	32	27	27
Percentage		-	55.26	34.21	51.32	38.16	36.84	30.26	65.79	46.05	50.00	47.37	42.11	35.53	35.53

ASOM, acute suppurative otitis media; AK, Amikacin; AM, Ampicillin; AG, Augmentin; AZK, Azithromycin; CF, Cephotaxime; CN, Carbencillin; CSOM, chronic suppurative otitis media; CX, Cloxacillin; GM, Gentamycin; PC, Piperacillin; PG, Penicillin; PR, Cephalexin; RC, Ciprofloxacin; RP, Ceftriaxione.

Tables 4 and 5 shows sensitivity pattern of various aerobic isolates from ASOM and CSOM cases respectively. From these observations, *Pseudomonas aeruginosa* was the most resistant organism.Further, organisms such as *Staphylococcus aureus* and *Klebsiellapneumonia* were also resistant.

Table 6: Sensitive pattern of aerobic isolates against antibiotics in ASOM and CSOM cases

	ASOM		**CSOM**	
Antibiotics	**No. of Sensitive Strains**	**% of sensitive strains**	**No. of Sensitive strains**	**% of sensitive strains**
RC (05µm)	80	68.38	32	44.44
AK (30µgm)	69	58.97	42	58.33
PC (100 µgm)	63	53.85	35	48.61
GM (10 µgm)	60	51.28	50	69.44
CF (30 µgm)	58	49.57	27	37.50
PR (30 µgm)	51	43.59	38	52.78
AZK (20 µgm)	44	37.61	26	36.11
AG (20 µgm)	44	37.61	39	54.17
RP (30 µgm)	41	35.04	36	50.00
CN (20 µgm)	35	29.91	29	40.28
CX (10 µgm)	26	22.22	28	38.89
AM (30 µgm)	16	13.68	27	37.50
PG (10 µgm)	11	9.4	23	31.94

ASOM, acute suppurative otitis media; CSOM, chronic suppurative otitis media, AK, Amikacin; AM, Ampicillin; AG, Augmentin; AZK, Azithromycin; CF, Cephotaxime; CN, Carbencillin; CX,

Cloxacillin; GM, Gentamycin; PC, Piperacillin; PG, Penicillin; PR, Cephalexin; RC, Ciprofloxacin; RP, Ceftriaxione.

In ASOM study, Ciprofloxacin was the mosteffective antibiotic (68.38%), followed by Amikacin (58.97%), Piperacillin (53.85%) and Gentamicin (51.28%). Ampicillin (13.68%) and Penicillin (9.4%) were among the least sensitive antibiotics. Under CSOM cases,Gentamicin was the most sensitive antibiotic (69.44%), followed by Amikacin (58.33%), Augmentin (54.17%) and Ceftriaxone (52.78%). Azithromycin (36.11%) and Penicillin (31.94%)were least sensitive (**Table 6**)

DISCUSSION

Otitis media is directly related to the colonization rate of the nasopharynx by the bacteria. Some of these bacteria are resistant to some of the drugs [2].Elucidation of common microorganisms associated with otitis media and determining their antibiotic sensitivity patterns are ineluctable for appropriate patient management. Thus, this study focused on analysing the antibiogram of aerobic bacterial isolates.

The study consisted of 176 subjects, of which 162 showed positive for growth. ASMO (62.96%) was significantly observed. In regard to age specific distribution, ASOM cases were predominantly observed among the 0-20 years age group, whereas CSOM was well noted across all ages but was the most among 21-40 years old. Akinjogunla*et al.*on evaluating the prevalence of acute otitis media reported that age group ≤ 10 years constituted the majority of cases 84 (30.9%) while the age group ≤ 61 accounted for the lowest prevalence [13].similarly, in the current study none of the ASOM cases belonged to the > 41 years age group. Jitendra and Kumar on studying the antibiogram of CSOM at a tertiary care facility in Jaipur found that CSOM mainly affected the 11-20 years (32%) age group as well as ages 21-30 years (21%), in consonance with this study [14]

This study revealed that otitis media wasprevalent in males (ASOM: 59.38%; CSOM: 40.63%). Study by Agrawal *et al.* showed that CSOM was higher in males 53.6% while studies by Hassan *et al.* showed majority of otits media cases in females (52.7%). This variation is because otitis media is not gender specific[15, 16]

Ear involvementand duration of discharge showed notable variation across ASOM and CSOM cases, with the peak of discharge being 1 week to 6 weeks for ASOM and 3 months to 6 months for CSOM. Jakribettu*et al.* noted unilateral discharge in 86.6% of ASOM cases, which albeit higher, is similar to the study finding of 67.63% [17]. However the CSOM cases in the current study predominantly demonstrated bilateral discharge (67.57%), in dissonance with previous studies reporting of bilateral CSOM prevalence of 11% and 21.12% [14,18].This deviation be on account of lower sample sizes considered in the previous studies, stage of otitis media, and tendency of contralateral otitis media to present as a bilateral condition[19].In this study, Purulent (77.45%) and Mucopurulent discharge (76.67%) were commonly noticed in ASOM and CSOM cases, respectively. Gorems*et al.* reported that majority had Purulent (65.32%) and watery (14.45%) discharge[20]. Purulent discharge indicates the presence of infection; bloody discharge may follow trauma, while mucoid discharge indicates a perforation of the tympanic membrane [21]. Deafness was the most common symptom observed (96.69%). This was in alignment with Madana*et al.* (86%) [22]. Conductive hearing loss caused by impaired transduction of sound waves in the middle ear due to the presence of middle ear effusion (MEE) or constant or recurrent discharge of ear. [23].

Monobacteria (ASOM: 81.34%; CSOM: 61.73%) was mostly observed in this study than polybacteria. This was in accordance with Wasihun *et al*. 60.5% [24].In this study *Pseudomonas Aeruginosa* was the most dominant speciesthat was resistant to all the antibiotics in both the cases.*Klebsiella Pneumoniae* and *Staphylococci Aureus* were the other leading cause of otitis media. Under ASOM and CSOM,*Staphylococcusaureus*and *Pseudomonas aeruginosa* were the most leading strains isolated respectively. Agarwal *et al.* reported *Staphylococcus* species, *Pseudomonas aeruginosa,*and*Klebsiella pneumonia* to be the cause of otitis media.[15]Wasihun *et al*.found that *Staphylococcus aureus*, *P. mirabilis* and *P. aeruginosa* were the most dominant isolates [24].Such

difference in results is attributed to the difference in the patient population studied and geographical variations of organisms [25].Incidence of 10 fungal isolates in culture positive otitis media cases are comparable to the findings of Patel et al., who isolated 13 fungal strains from 140 samples. However, Patel et al. noted Aspergillus niger to be as the most common fungal strain, as compared to the more common observance of Aspergillus fumigatus species in this study[26]. A review by Gupta et al. on CSOM focused studies, found that the most common fungal isolates were Aspergillus species, and followed by the Candida species, which is in congruence to the present findings [27].

In ASOM,Ciprofloxacin was the most effective antibiotic followed by, Amikacin, Piperacillin and Gentamicin. On other hand in CSMO, Gentamicin was most effective followed by Amikacin, Augmentin,and Ceftriaxone.Penicillin and Ampicillin was found less susceptible against all isolated organisms. Study by Raakhee *et al.* showed Ciprofloxacin as the highest susceptible antibiotic (91.52%), followed by Gentamicin (89.6%) and Chloramphenicol (59.53%) [18].High drug resistance is due to excessive misuse of antibiotics and inappropriate prescribing habits and an over-zealous desire to treat every infection. This study limits in isolating anaerobic bacteria which are also the causative agents for otitis media.The strength of this study lies in its resolve to study both ASOM and CSOM in patients of all ages, as previous studies on ASOM have been limited to children and have considered small sample sizes. Furthermore, the study includes a comprehensive list of pertinent antibiotics in testing the sensitivity of microbial isolates. Further analysing and comparison of the antibiogram by including OME and Otitis media without discharge is recommended.

CONCLUSION

This study concluded that ASMO was more common than CSMO in this geographical region. Ciprofloxacin and Gentamicin were effective antibiotics in ASMO and CSMO, respectively. *Pseudomonas aeruginosa* was the predominant organism and was resistant to all the antibiotics. Majority of microorganisms were resistant to multi drugs.

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