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Study of antibiotic susceptibility pattern of the isolated organisms in otitis media

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ABSTRACT

Background: Otitis media (OM) encompasses a spectrum of inflammatory conditions affecting the middle ear, contributing significantly to health-care visits and prescriptions. Complications arising from OM frequently result in avoidable hearing loss, particularly in developing nations. **Aims and Objectives:** This study aims to ascertain the bacterial profile and antimicrobial susceptibility pattern of ear infections characterized by ear discharge complaints. **Materials and Methods:** The present study was conducted in the microbiology department of a tertiary care hospital over a 2-year period. The study involved 581 samples diagnosed with OM. Trained nurses collected pertinent patient information, while both nurses and an ENT doctor collected samples during specimen collection, utilizing an otoscope and headlight. Thorough documentation of relevant history and physical examinations accompanied the meticulous collection of ear discharge. **Results:** Culture-positive samples accounted for 96.39% (560 samples), with no growth observed in 3.61% (21 samples). Gram staining revealed 570 positive samples. Of the 581 OM samples, aerobes were isolated from 73.67% and anaerobes from 51.64%. The total isolates numbered 845, with 61.54% being aerobic and 38.46% anaerobic. Among the bacterial isolates, gram-negative bacteria slightly exceeded gram-positive bacteria, constituting 60.57% and 39.42%, respectively. **Conclusion:** In conclusion, the isolated aerobes and anaerobes shed light on the prevalent organisms in our region causing OM. The antibiotic sensitivity analysis conducted in this study emphasizes the identification of drugs suitable for the earliest treatment of OM.

Key words: Otitis media; Aerobes; Anaerobes; *Pseudomonas aeruginosa*

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INTRODUCTION

Ear infections, encompassing otitis media (OM) and otitis externa, pose a significant public health challenge in developing countries, carrying a substantial disease burden and economic impact on patients, families, and the health-care system. Particularly prevalent in children, ear infections result in frequent outpatient department visits, affecting both developed and developing nations, with a potential impact on adults as well.[1] OM comprises a group of inflammatory conditions affecting the middle ear.[2] It is a major contributor to health-care visits and prescriptions, and its complications often lead to preventable hearing loss, especially in developing countries.[1] Acute OM (AOM) and OM with effusion are the two primary types, while chronic suppurative OM (CSOM) represents another prevalent form. AOM manifests as a sudden onset of inflammatory signs and symptoms in the middle ear.[3] Conversely, CSOM is characterized by persistent ear discharges (otorrhea) through a tympanic perforation.[4]

While AOM is commonly bacterial, with *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* being usual culprits,[2] CSOM can involve aerobic (e.g., *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*) or anaerobic bacteria, such as *Bacteroides*, *Peptostreptococcus*, and

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Propionibacterium.[5] Although viruses like respiratory syncytial virus, rhinoviruses, and adenoviruses can cause AOM, this study focuses on bacterial etiologies.

The diagnosis of OM is complicated by a lack of correlation between clinical features and responsible pathogens, compounded by the routine unavailability of otoscopes in many health-care facilities, especially in developing countries. The absence of this essential tool limits health workers' ability to accurately diagnose and classify OM based on the spectrum of clinical findings required for a definitive case definition.[6]

Treatment approaches for AOM may include antibiotics but are not always necessary, as not all cases are bacterial. Observation without antibiotics is an option for selected cases based on various factors.[3] Antibiotics significantly reduce AOM episodes, but mastoidectomy and/or tympanoplasty are often necessary for a permanent CSOM cure.[4]

Various studies worldwide have identified diverse bacterial pathogens causing OM. For instance, a multinational study in the US, Israel, and Eastern Europe found *S. pneumoniae*, *H. influenzae*, and *Moraxella* in ear fluid cultures.[7] In Costa Rica, *Pseudomonas* spp., Gram-negative enterics, and *Staphylococcus* were common.[8] Studies in the Solomon Islands, India, Nepal, and Iraq reported different prevalent bacterial isolates.[9,10] Antibiotic prescribing for OM should be guided by local data due to geographical variations in etiologies and antibiotic susceptibility patterns.[11]

The impact of ear infections on public health, coupled with the economic implications for individuals and health-care systems, underscores the need for a detailed investigation into the bacterial causes and their susceptibility patterns. By elucidating the microbial profile, this study aims to support evidence-based decision-making in the prescription of antibiotics and other therapeutic interventions. Additionally, the findings may contribute to the development of region-specific guidelines, considering the geographical variability in bacterial etiologies and antibiotic resistance patterns.

Keep the above facts in mind, the present study aims to determine the bacterial profile and antimicrobial susceptibility pattern of ear infections presenting with a complaint of ear discharge.

Aims and objectives

To determine the bacterial profile, and antimicrobial susceptibility pattern of ear infections presenting with a complaint of ear discharge.

MATERIALS AND METHODS

The present descriptive cross-sectional study was conducted in the microbiology department of a tertiary care hospital over a period of 2 years. This study incorporated a total of 581 samples diagnosed with OM. The approval of the institutional ethics committee was obtained before the beginning of the study. The primary focus was on individuals presenting with discharging ears at the E.N.T. department. Employing a consecutive sampling technique, we engaged with every patient exhibiting ear discharge throughout the study period. The decision to use ear discharge as an entry point was influenced by the potential oversight of routine otoscopic examinations. Although this approach might underestimate the prevalence of ear discharge, it was imperative to ensure inclusivity and avoid overlooking patients without obvious ear discharge.

Inclusion criteria

Comprised patients of all age categories clinically diagnosed with draining OM demonstrated a willingness to provide consent. Additionally, patients selected for the study had not undergone any form of treatment for a minimum of 7 days before sample collection.

Exclusion criteria

The patient with OM, whose ear swab and culture were not done, were excluded.

Data collection procedure

Trained nurses were responsible for gathering relevant patient information, while both nurses and an ENT doctor collected swabs during specimen collection using an otoscope and headlight. All pertinent history and physical examinations were meticulously documented. The collection of ear discharge adhered to a stringent aseptic technique, employing single-use, commercially available sterile cotton swabs to prevent surface contamination.

Samples were acquired with sterile cotton swabs, and a sterile swab was utilized to cleanse the outer portion of the discharge. A surgical soap scrub was followed by the application of 70% ethyl alcohol and a tincture of iodine for 1 min.[12] Samples were collected according to Sonnerwirth's methodology.[13] Specialized transport media with reducing agents are used to collect and maintain anaerobic specimens. These agents help to eliminate oxygen and create conditions suitable for the survival of anaerobic bacteria.

The specimens were meticulously positioned in transport vials and severed just beneath the rim, eliminating the handled portion. Those earmarked for microscopic examination were conveyed in sterile test tubes, while samples intended for aerobic culture were immersed in

Amies transport media to isolate aerobes and facultative anaerobic bacteria. Simultaneously, specimens slated for anaerobic culture were promptly introduced into thioglycollate medium for anaerobe transport. All the gathered samples were promptly sent to the laboratory for subsequent processing.

Antibiotic sensitivity testing

Antibiotic sensitivity for each isolate was determined using the Kirby Bauer disc diffusion method on freshly prepared blood agar plates, following the protocols of Mackie MacCartney, Bailley, and Scott's.[14-16] The confirmation of all isolates was carried out through the application of standard biochemical tests. The findings were analyzed following the guidelines outlined by CLSI.[17]

RESULTS

Culture-positive samples are 560 (96.39%); no growth is seen in 21 samples (3.61%). 570 samples are positive for Gram staining. Of the 581 samples of OM, total aerobes were isolated at 73.67%. Total anaerobes are isolated at 51.64%. The total isolates are 845, out of which 520 (61.54%) are aerobic isolates and 325 (38.46%) are anaerobic isolates (Table 1).

Out of the 845 strains isolated, 315 (60.57%) are Gram-negative bacilli and 205 (39.42%) are Gram-positive cocci. *P. aeruginosa* is the predominant organism, 142 (27.31%), followed by *Proteus mirabilis* 52 (10%) and *Proteus vulgaris* 38 (7.30%). The Gram-positive isolates are 205 (39.42%), with *S. aureus* 110 (21.53%) being the most common, followed by *S. Pneumoniae* 30 (5.76%) (Table 2).

All strains show maximum sensitivity to amikacin (94.88%), followed by gentamicin (89.55%), cefotaxime (87.02%), ciprofloxacin (86.18%), and the least sensitivity to penicillin (49.60%) and ampicillin (35.17%).

Gram-negative organisms are sensitive to amikacin (95.45%), followed by gentamicin, ciprofloxacin, and cefotaxime, and least sensitive to ampicillin (35.34%). Gram-positive organisms show maximum sensitivity to amikacin (94.32%), followed by gentamicin, and the least sensitivity to penicillin (49.60%) and ampicillin (34.99%) (Table 3).

From the 581 samples, anaerobes were isolated from 300 samples (51.64%). The strains isolated are 325; *Prevotella melaninogenica* is the predominant isolate, 99 (30.46%), followed by *Peptostreptococcus magnus* 84 (25.85%), *Peptostreptococcus assacharolyticus* 45 (13.85%), and others (Table 4).

The anaerobes are seen to be most sensitive to vancomycin (97.23%), followed by metronidazole (95.73%), amikacin (90.34%), gentamicin (89.10%), and least sensitive to penicillin (34.38%) (Table 5).

Table 1: Distribution of microorganisms from total samples of otitis media

Distribution	Samples	Percentage (n=581)
Culture-positive samples	560	96.39
Total samples isolating aerobes	428	73.67
Total samples isolating anaerobes	300	51.64
Samples isolating only aerobes	275	47.33
Samples isolating only anaerobes	147	25.30
Mixed growth samples (aerobes+anaerobes)	153	26.33
Total aerobic isolates	520	
Total anaerobic isolates	325	

Table 2: Total aerobes

	Organisms	No. of strains isolated	Percentage
A	Gram-negative bacilli	315	60.57
1.	*Pseudomonas aeruginosa*	142	27.31
2.	*Proteus mirabilis*	52	10.0
3.	*Proteus vulgaris*	38	7.31
4.	*Escherichia coli*	38	7.31
5.	*Klebsiella species*	36	6.92
6.	*Citrobacter freundii*	9	1.73
B	Gram-positive cocci	205	39.42
1.	*Staphylococcus aureus*	110	21.15
3.	*Streptococcus pneumoniae*	30	5.77
4.	*Streptococcus pyogenes*	20	3.85
5.	*Streptococcus viridians*	11	2.12
	Total (A + B)	520	100

DISCUSSION

In the present study, the rate of bacterial isolation stood at 96.39%, surpassing the figures documented in earlier studies by Worku and Bekele at 52.1%,[18] Hailu et al., at 80.4%,[19] and Argaw-Denboba et al., at 83.6%.[20] However, it was lower than the findings reported by Wasihun and Zemene, which recorded a rate of 98.2%.[21]

In the present study, from the total bacterial isolates, Gram-negative bacteria (60.57%) were slightly higher than Gram-positive bacteria (39.42%), which is in agreement with previous studies done by Wasihun and Zemene (56%),[21] Hailu et al., (58.8%),[19] Argaw-Denboba et al., A (75.8%),[20] and Worku and Bekele (79.5%).[18]

Isolates of ear specimens and their sensitivity to various antibiotics

P. aeruginosa

In the present study, the isolates of *P. aeruginosa* are sensitive to amikacin (94.6%), ciprofloxacin (90.54%), followed

Table 3: Antibiotic sensitivity of aerobes

Organisms	No of strains	Percentage of sensitive strains										
		A	G	Cf	Ce	Co	Ak	Ox	E	P	Pb	Cb
Gram-negative organisms												
Pseudomonas aeruginosa	142	40.27	89.60	90.54	61.48	50.13	94.6	-	-	-	85.40	46.62
Proteus mirabilis	52	41.1	93.15	83.56	93.45	64.38	94.52	-	-	-	-	-
Proteus vulgaris	38	35.3	84.11	84.31	92.15	76.47	96.08	-	-	-	-	-
Escherichia coli	38	51.51	80.90	91.81	87.87	63.63	93.93	-	-	-	-	-
Klebsiella species	36	10.58	87.09	77.41	87.09	14.51	93.54	-	-	-	-	-
Citrobacter freundii	9	33.33	100	77.77	95.77	66.66	100	-	-	-	-	-
Total	315	35.34	89.14	84.23	86.30	55.96	95.45	-	-	-	85.40	46.62
Gram-positive organisms												
Staphylococcus aureus	110	31.81	84.54	90	91.81	52.72	89.63	68.18	80	62.72	-	-
Streptococcus pneumoniae	30	46.66	83.33	93.33	96.67	46.67	96.76	46.67	100	56.67	-	-
Streptococcus pyogenes	20	42.85	100	100	85.71	57.14	100	85.71	85.71	71.42	-	-
Streptococcus viridians	11	36.36	90.90	81.81	90.90	81.81	90.90	81.89	81.81	36.36	-	-
Total	205	34.99	89.95	88.13	87.74	55.83	94.32	65.09	88.07	49.60	-	-
Overall total	520	35.17	89.55	86.18	87.02	55.90	94.88	65.09	88.07	49.60	85.40	46.62

A: Ampicillin, G: Gentamicin, Cf: Cefotaxime, Co: Cotrimoxazole, Ak: Amikacin, Ox: Oxacillin, E: Erythromycin, P: Penicillin, Pb: Polymyxin B, Cb: Carbenicillin

Table 4: Prevalence of different anaerobic bacteria

No.	Organisms	No. of samples	%
Gram-negative anaerobes			
1.	*Prevotella melaninogenica*	99	30.46
2.	*Fusobacterium nucleatum*	40	12.31
3.	*Bacteroides fragilis*	32	9.85
4.	*Veillonella*	4	1.23
Gram-positive anaerobes			
1.	*Peptostreptococcus magnus*	84	25.85
2.	*Peptostreptococcus assacharolyticus*	45	13.85
3.	*Peptostreptococcus anaerobius*	15	4.62
4.	*Gram-positive nonsporing anaerobic bacilli*	4	1.23
5.	*Clostridium species*	1	0.31
6.	*Propionibacterium acnes*	1	0.31
	Total	325	100

by gentamicin (89.60%), polymxyin B. (85.40%), and carbenicillin (46.62%). Maji et al., reported that *Pseudomonas* was most sensitive to amikacin at 100%, followed by gentamicin at 86%, cefotaxime at 85.4%, and ciprofloxacin at 46.6%.[22] A study by Mansoor et al., showed *Pseudomonas* was sensitive to amikacin, cefoparazone, and ciprofloxacin.[23]

S. aureus

In the present study, *S. aureus* shows maximum sensitivity to cefotaxime (91.81%), ciprofloxacin (90%), amikacin (89.63%), followed by amikacin (89.63%), gentamicin (84.54%), and the least sensitivity to ampicillin (31.81%). Similar findings were reported by Maji et al., amikacin (100%), Gentamicin (87.5%), cefotaxime (80.3%), ciprofloxacin (64.3%), and the least sensitivity to ampicillin (7.1%).[22]

The leading isolated bacteria in this study was *P. aeruginosa* (27.31%), followed by *P. mirabilis* (10%) and *P. vulgaris* (7.30%). Similar findings identified *P. aeruginosa* as the main isolate, followed by *S. aureus* and *Proteus sppt.* Were reported by Fatima G.[24]

In this study, a good overall antimicrobial susceptibility pattern (>80%) was seen for amikacin, gentamicin, and ciprofloxacin, which is also in line with other studies conducted.[18-20,25,26] Many of the isolates showed high levels of sensitivity to amikacin, which is consistent with other reports.[11,18]

Proteus species

Isolates of Proteus are sensitive to amikacin (94.52%), cefotaxime (93.45%), gentamicin (93.15%), ciprofloxacin (83.56%), followed by cotrimoxazole (64.38%), and ampicillin (41.1%). Whereas other studies done by Patel et al., reported Gentamycin is only 85.5% effective,[27] whereas previous studies by Zaida and Abdulla showed Gentamycin being the drug of choice.[28]

E. coli

The isolates of *E. coli* are sensitive to amikacin (93.93%), ciprofloxacin (91.81%), followed by cefotaxime (87.87%), gentamicin (80.90%), cotrimoxazole (63.63%), and ampicillin (51.51%). *E. coli* were sensitive to amikacin, ciprofloxacin, and piperacillin. A study by Iqbal et al., showed that *E. coli* were resistant to Ciprofloxacin.[29]

Klebsiella species

Isolates of *Klebsiella* species are sensitive to amikacin (93.54%), followed by cefotaxime (87.09%), gentamicin (87.09%), ciprofloxacin (77.41%), cotrimoxazole (14.51%), and only 10.58% of isolates are sensitive to ampicillin. Whereas another study done by Patel et al., reported being sensitive to ciprofloxacin.[27]

Table 5: Antimicrobial susceptibility of anaerobic isolates

Name of organisms	Strains	M	G	V	Ak	Ca	E	P	K	N	Cl
Prevotella melaninogenica	99	100	97.97	98.98	100	100	81.81	37.37	47.47	39.39	67.67
Peptostreptococcus magnus	84	79.76	53.57	88.09	85.71	77.38	75	35.71	44.05	39.29	51.19
Peptostreptococcus assacharolyticus	45	88.88	84.44	93.93	91.91	93.93	84.44	44.44	68.88	64.64	30.68
Fusobacterium nucleatum	40	95	92.5	97.5	100	92.5	92.5	55	75	76	70
Bacteroides fragilis	32	93.75	87.5	93.75	87.5	87.5	87.5	62.5	68.75	75	68.75
Peptostreptococcus anaerobius	15	100	100	100	88.24	94.11	88.24	58.82	58.82	64.71	88.24
Veillonella	4	100	75	100	75	100	75	25	50	50	75
Gram-positive nonsporing bacilli	4	100	100	100	75	75	75	25	50	75	75
Clostridium species	1	100	100	100	100	0.0	100	0.0	0.0	0.0	100
Propioniobacterium acnes	1	100	100	100	100	100	0.0	0.0	0.0	0.0	0.0
Total	325	95.73	89.10	97.23	90.34	82.04	75.95	34.38	46.28	48.40	62.65

M: Metronidazole, V: Vancomycin, Ak: Amikacin, Ca: Ceftazidime, E: Erythromycin, G: Gentamicin, P: Penicillin, K: Kanamycin, N: Neomycin, Cl: Colistin

Streptococcus pneumoniae

Isolates of *S. pneumoniae* are seen as sensitive to erythromycin (100%), amikacin, cefotaxime (96.67%) each, ciprofloxacin (93.33%), penicillin (56.67%), and ampicillin, cotrimoxazole, and oxacillin (46.67%) each. Whereas another study done by Kombade et al., reported that *S. pneumoniae* exhibited sensitivity to penicillin, levofloxacin, vancomycin, and linezolid.[30]

In our study, *P. aeruginosa* emerged as the most prevalent organism responsible for acute ear infections, constituting 27.3% of the cases. Interestingly, our findings diverge from reports in other African countries where *S. aureus* and *S. pyogenes* were identified as the predominant isolates.[31] This discrepancy may be attributed to variations in geographic location, the prevalence of respiratory infections, the extent of pneumococcal conjugate vaccine coverage, and the potential overuse of antimicrobials. The latter factor could have resulted in the elimination of sensitive organisms, creating a favorable environment for drug-resistant strains to dominate.

The anaerobes show the highest sensitivity to vancomycin (97.23%), metronidazole (95.73%), amikacin (90.34%), gentamicin (89.10%), and least sensitivity to penicillin (34.38%). Loy, in his study, found that both aerobes and anaerobes were sensitive to gentamicin (82.6%), neomycin (67.8%), and chloramphenicol (62.8%).[32] Maji et al., in their study, found the most effective antibiotic for anaerobes was cefoperazone sodium, and for aerobes was amikacin.[22]

As a consequence of the indiscriminate utilization of antibiotics, many microorganisms, such as Pseudomonas, tend to develop resistance. Furthermore, the administration of antibiotics without prior determination of sensitivity by practitioners contributes to this issue and should therefore be discouraged. In instances where organisms exhibit resistance to standard antibiotics, it becomes necessary to transition to more potent antibiotic treatments for OM.

The impact of the study's results is significant in several aspects. First, by identifying the bacterial profile and antimicrobial susceptibility patterns in ear infections, the study provides crucial information for health-care practitioners. This knowledge can guide the selection of appropriate antibiotics for the treatment of OM, contributing to more effective and targeted therapeutic interventions. Moreover, understanding the prevalence of specific organisms causing OM in the region can inform public health strategies. It enables the development of tailored preventive measures and interventions to address the root causes of these infections, potentially reducing the overall burden of OM in the community.

The study's findings also have implications for antibiotic stewardship. With insights into the resistance patterns of bacteria causing ear infections, health-care professionals can make more informed decisions about antibiotic prescriptions. This, in turn, may help mitigate the growing concern about antibiotic resistance, promoting the responsible use of these medications. In addition, the study sheds light on the regional variations in bacterial isolates, emphasizing the importance of considering local factors in health-care decision-making.

Limitations of the study

The geographic specificity, limited sample size, and focus on a single-center setting may restrict the generalizability of findings. The two-year study duration and specific antibiotic sensitivity testing may not fully capture temporal variations and the complete spectrum of antimicrobial susceptibility. Despite these limitations, the study offers a foundation for understanding ear infections in the studied context, emphasizing the importance of considering these constraints in the broader application of its findings.

CONCLUSION

In conclusion, the aerobes and anaerobes that were isolated reveal the prevalence of organisms in our region that cause

OM. The antibiotic sensitivity analysis conducted in this study specifically focuses on identifying drugs suitable for the earliest treatment of OM. This approach aims to prevent the onset of further complications and disabilities associated with the condition.

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UAL- Concept and design of the study, prepared first draft of manuscript, reviewed the literature, preparation of manuscript and revision of the manuscript; **SLA-** Statistical analysis, interpreted the results; reviewed the literature and manuscript preparation; **AL-** Concept, statistical analysis and interpretation, preparation of manuscript and revision of the manuscript.

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