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RESEARCH ARTICLE

Antimicrobial Profile of Bacterial Isolates from Patients with Acute Otitis Media in Hadhramout-Yemen

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ABSTRACT

Acute Otitis Media (AOM) becomes a challenge, especially in resource-constrained countries, because it is commonly associated with bacteria that have multiple drug resistance. This study aimed to identify bacterial agents of AOM and determine their antimicrobial patterns.

Methods: Hundred ear swabs were collected from patients who attended the outpatient clinics of Ear Nose Throat in Mukalla city, Hadhramout, Yemen, during the period from August 2021 to March 2022. The samples were diagnosed phenotypically. Then their antibiogram was evaluated by the Bauer-Kirby method.

Results: The prevalence of acute otitis media was 60%. The most common isolated bacteria were *Staphylococcus aureus* (45%), *Pseudomonas aeruginosa* (31.7%), *Proteus mirabilis* (13.3%), *Escherichia coli* (5%), *Klebsiella pneumoniae* (3.3%), and *Streptococcus pneumoniae* (1.7%). The antibiogram showed that levofloxacin was the most effective agent against all isolates, followed by ciprofloxacin with rate of 100, 63.3, 62.5, 66.7, 73.7% for *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *S. aureus, Pseudomonas mirabilis* and *Escherichia coli* respectively, while *Streptococcus pneumoniae* was resistant to it. All isolates were resistant to cephalexin, ceftazidime and aztreonam (100%), while *Streptococcus pneumoniae* was sensitive to cephalexin. Furthermore, our findings demonstrated seven resistant patterns of the isolates.

Conclusion: The isolation rate of bacteria in samples of AOM is still high and combined with a substantial resistance rate among bacterial isolates of AOM to the antibiotics under study. The high resistance rate reflects the widespread abuse of antimicrobial agents in our community, emphasizing the need for strict antimicrobial control measures and updating the empirical antimicrobial prescription guidelines.

Keywords: Antibiogram, Acute otitis media, Resistance patterns, Pathogenic bacteria.

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INTRODUCTION

Otitis media (OM), a common cause of hearing loss in children and individuals with syndromic disorders, refers to an infection affecting the middle ear or tympanic membrane. It is characterized by symptoms like ear pain and discharge.[1] Ear canal infections can manifest as either acute or chronic conditions.[2] A precise diagnosis is vital for targeted treatment, and investigative workup includes audiometry, impedance studies, microbiological analysis, and, in some cases, Auditory Brainstem Response and Computed Tomography.[3] Acute otitis media (AOM) is a public health issue that has been raised in both developing and developed nations because of their high prevalence.[4] Although ear infections are more common in young children, it can also occur in adults.[5] Children are more prone to experiencing ear infections than adults due to the incomplete maturation of their eustachian tubes.[6]

Ear infections may be caused by many pathogenic agents including viruses, fungi, or bacteria in particular *S. aureus, Streptococcus pneumoniae, Streptococcus*

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pyogenes, Haemophilus influenzae, Moraxella catarrhalis, Klebsiella spp, Pseudomonas aeruginosa, Escherichia coli, Protus mirabilis, or it may be caused by mixed microbial invasion.[7-9] Indeed, the pathogens associated with AOM have alarmingly increased resistance to many antibiotics. In light of this development, it may be necessary to reconsider the traditional antibiotic treatment for ear infections.[10,11] Bacterial AOM in childhood may be correlated with various factors, including nursing at certain ages, household smoking, household firewood consumption, the presence of siblings, pet birds, low nutritional status, and a family history of ear infections.[12,13]

Although antimicrobial resistance is a global challenge, the excessive use and abuse of antibiotics is still the primary cause of antibiotic resistance, particularly when drugs are easily accessible without medical prescriptions.[14] Western Asia is one of the Asian regions that is characterized by high antimicrobial resistance due to massive misuse and absence or weakness of antimicrobial stewardship system in this region.[15] Thus, this research intended to investigate the prevalence of bacterial species in AOM and evaluate the antimicrobial susceptibility and resistance patterns of bacteria causing otitis media.

MATERIALS AND METHODS

Study Design

The study was conducted as a cross-sectional survey from August 2021 to March 2022. One hundred samples were collected from patients aged 1 to 60 years who attended outpatient clinics at Ibn Sina Hospital, Model Hadrami Dispensary, Al-Arab Hospital, Hadhramaut Hospital, Al Burj Istishari Hospital, and Al Safwa Medical Clinic in Mukalla City, which is the capital of Hadhramout Governorate, it is about 480 kilometers east of Aden, Yemen.

Samples Collection and Inclusion Criteria

One hundred ear swabs were collected from patients clinically diagnosed with AOM by an Otolaryngologist during the study period. The collected specimens were transferred to the Central Public Health Laboratory in Al Mukalla City for processing. Informed consents were distributed before starting sample collection to inform the participants about the study's processes steps, and to assure them that the collected data would be protected and used only for research purposes. Furthermore, patients who had taken antibiotics within two weeks before sample collection were excluded from enrollment.

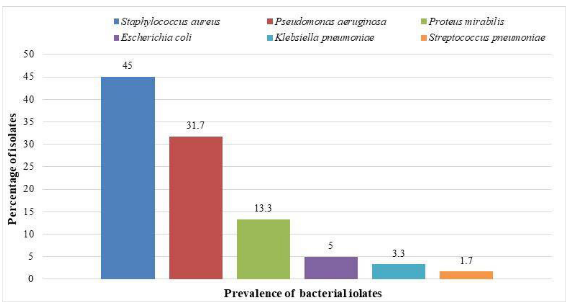

Figure 1: Prevalence of bacterial isolates among AOM in Al Mukalla City

Bacterial Isolation and Identification

Samples were processed according to the Cheesbrough protocol for the examination of pus swabs.[16] The inoculated Blood Agar and MacConkey's Agar were incubated aerobically, and one blood agar was incubated under microaerophilic conditions of carbon dioxide for 24 hours at 37°C. The isolated colonies were initially diagnosed based on culture characteristics gram stain, and confirmed by biochemical tests.

Antimicrobial Activity

The antibiotic sensitivity test was done by Bauer-Kirby technique on Muller Hinton Agar. During investigation fourteenth antibacterial agents were tested including aztreonam, ceftazidime, amikacin, ciprofloxacin, ampicillin, levofloxacin, erythromycin, azithromycin, cephalexin, vancomycin, cefotaxime, amoxicillin-clavulanic, ofloxacin, and cefadroxil. The susceptibility of bacterial isolates for antibiotic discs was determined after 24 hours at suitable conditions, and then the results were assessed following the guidelines of the Clinical and Laboratory Standards Institute, 2021.[17]

Statistical Analysis

The data underwent cleaning, with groups being categorized, and descriptive statistics were conducted using Statistical Package for the Social Sciences (SPSS) version 24.

RESULTS

The prevalence of acute bacterial otitis media among the total cohort was 60%, the prevalence of positive bacterial culture among male group 51%, while it was 69.4% among the female cohort (Table 1).

The highest isolation rate of bacterial isolates among the patients with AOM was observed for *Staphylococcus aureus* (45%), followed by *Pseudomonas aeruginosa* (31.7%), *Proteus mirabilis* (13.3%), *Escherichia coli* (5%), and *Klebsiella pneumoniae* (3.3%). The lowest isolation rate (1.7%) was recorded for *Streptococcus pneumoniae* (Figure 1).

Unfortunately, *S. aureus* exhibited total resistance against aztreonam, cefadroxil, cephalexin, ceftazidime,

Table 1: The prevalence of bacterial acute otitis media among males and females

Gender	*Negative*	*Positive*	*Total*
Males	25	26	51
Females	15	34	49
Total	40	60	100

and erythromycin. Furthermore, *S. aureus* showed resistance rate surpassing 70% to ampicillin, amoxicillin-clavulanic, cefotaxime, and vancomycin. as well, amikacin, azithromycin, and ofloxacin had rate over 50% for this microbe. Additionally, ciprofloxacin and levofloxacin displayed good activity against coagulase-positive staphylococci (Table 2).

Table 2: Antibiotic susceptibility test for *S. aureus*

Isolates (No.)	*Antibiotics*	*No. of Sensitive isolates (%)*	*No. of Moderate isolates (%)*	*No. of Resistant isolates (%)*
S. aureus (27)	Amikacin	(14.8) 4	(25.9) 7	(59.3) 16
	Oflaxacin	(18.5) 5	(22.2) 6	(59.3) 16
	Ciprofloxacin	(66.7) 18	(22.2) 6	(11.1) 3
	Levofloxacin	(70.3) 19	(18.5) 5	(11.1) 3
	Vancomycin	(14.8) 4	(14.8) 4	(70.4) 19
	Azithromycin	(25.9) 7	(14.8) 4	(59.3) 16
	Cefotaxime	(7.4) 2	(11.1) 3	(81.5) 22
	Cefadroxil	0	0	(100) 27
	Amoxicillin-Clavulanic	(14.8) 4	(11.1) 3	(74.1) 20
	Ampicillin	(11.1) 3	(11.1) 3	(77.8) 21
	Aztreonam	0	0	(100) 27
	Cephalexin	0	0	(100) 27
	Erythromycin	0	0	(100) 27
	Ceftazidime	0	0	(100) 27

Table 3: Antibiotic susceptibility test for *Pseudomonas aeruginosa* isolates

Isolates (No.)	*Antibiotics*	*No. of Sensitive isolates (%)*	*No. of Moderate Isolates (%)*	*No. of Resistant Isolates (%)*
Pseudomonas aeruginosa (19)	Amikacin	(36.8)7	(15.8) 3	(47.4)9
	Ampicillin	0	(21.1) 4	(78.9)15
	Azithromycin	(36.8) 7	(21.1) 4	(42.1)8
	Amoxicillin-Clavulanic	0	(15.8) 3	(84.2)16
	Aztreonam	0	(21.1) 4	(78.9)15
	Ceftazidime	0	0	(100)19
	Cephalexin	0	0	(100)19
	Cefadroxil	0	(15.8) 3	(84.2)16
	Ciprofloxacin	(73.7) 14	(21.1) 4	(5.3)1
	Cefotaxime	(15.8) 3	0	(84.2)16
	Erythromycin	0	(21.1) 4	(78.9)15
	Levofloxacin	(98.9) 15	(21.1) 4	0
	Oflaxacin	(31.6) 6	(21.1) 4	(47.4)9

Pseudomonas aeruginosa presented absolute resistance against ceftazidime and cephalexin. Moreover, *Pseudomonas aeruginosa* displayed a resistance rate exceeding 70% for amoxicillin-clavulanic, ampicillin, aztreonam, cefadroxil, cefotaxime, and erythromycin. While those isolates showed the same resistance rate (47%) for amikacin and ofloxacin. On the other hand ciprofloxacin had the least resistance against *Pseudomonas aeruginosa*, and no resistant strains were observed against levofloxacin (Table 3).

Proteus mirabilis presented full resistance against amoxicillin-clavulanic, ampicillin, aztreonam, ceftazidime, cephalexin, and cefotaxime. Furthermore, they outlined resistance rates exceeding 70% for cefadroxil and erythromycin, while these isolates had a resistance rate of 62.5% for oflaxacin. In contrast, ciprofloxacin showed good sensitivity against *Proteus mirabilis*, followed by levofloxacin (Table 4).

Unfortunately, *Klebsiella pneumonia* exhibited full resistance for eight out of thirteen tested antibiotics (61.5%),

Table 4: Antibiotic susceptibility test for *Proteus mirabilis* isolates

Isolates (No.)	*Antibiotics*	*No. of Sensitive Isolates (%)*	*No. of Moderate Isolates (%)*	*No. of Resistant Isolates (%)*
Proteus mirabilis (8)	Amikacin	(12.5)1	(25) 2	(62.5) 5
	Ampicillin	0	0	(100) 8
	Azithromycin	(25)2	0	(75) 6
	Amoxicillin-Clavulanic	0	0	(100) 8
	Aztreonam	0	0	(100) 8
	Ceftazidime	0	0	(100) 8
	Cephalexin	0	0	(100) 8
	Cefadroxil	(12.5) 1	(12.5) 1	(75) 6
	Ciprofloxacin	(62.5) 5	(25) 2	(12.5) 1
	Cefotaxime	0	0	(100) 8
	Erythromycin	0	(12.5) 1	(87.5) 7
	Levofloxacin	(25) 2	(62.5) 5	(12.5) 1
	Oflaxacin	(37.5) 3	0	(62.5) 5

Table 5: Antibiotic susceptibility test for *Klebsiella pneumonia* isolates

Isolates (No.)	*Antibiotics*	*No. of Sensitive Isolates (%)*	*No. of Moderate isolates (%)*	*No. of Resistant isolates (%)*
Klebsiella pneumonia (2)	Amikacin	0	(50) 1	(50) 1
	Ampicillin	0	1(50)	1(50)
	Azithromycin	(50) 1	0	(50) 1
	Amoxicillin-Clavulanic	0	0	(100) 2
	Aztreonam	0	0	(100) 2
	Cephalexin	0	0	(100) 2
	Ceftazidime	0	0	(100) 2
	Cefadroxil	0	0	(100) 2
	Ciprofloxacin	(100) 2	0	0
	Cefotaxime	0	0	(100) 2
	Erythromycin	0	0	(100) 2
	Levofloxacin	(50) 1	0	(50) 1
	Oflaxacin	0	0	(100) 2

including amoxicillin-clavulanic, aztreonam, cefadroxil, cefotaxime, cephalexin, ceftazidime, erythromycin, and ofloxacin. *Klebsiella pneumonia* had a resistance rate of 50% for amikacin, ampicillin, azithromycin, and levofloxacin. These isolates showed the highest sensitivity with ciprofloxacin (Table 5).

Escherichia coli expressed full resistance for nine out of thirteen tested antibiotics (69.2%), including amikacin, ampicillin, amoxicillin-clavulanic, aztreonam, ceftazidime, cephalexin, cefadroxil, cefotaxime, and erythromycin. Moreover, they had resistance rates of 66.7 and 33.3% for azithromycin and levofloxacin, respectively. Similarly, *Escherichia coli* showed sensitivity to ciprofloxacin, like *Klebsiella pneumonia* (Table 6).

The *Streptococcus pneumoniae* isolate exhibited absolute resistance to aztreonam, ceftazidime, cefadroxil,

Table 6: Antibiotic susceptibility test for *Escherichia coli* isolates

Isolates (no.)	*Antibiotics*	*No. of sensitive isolates (%)*	*No. of moderate isolates (%)*	*No. of resistant isolates (%)*
Escherichia coli (3)	Amikacin	0	0	(100) 3
	Ampicillin	0	0	(100) 3
	Azithromycin	(33.3) 1	0	(66.7) 2
	Amoxicillin-Clavulanic	0	0	(100) 3
	Aztreonam	0	0	(100) 3
	Ceftazidime	0	0	(100) 3
	Cephalexin	0	0	(100) 3
	Cefadroxil	0	0	(100) 3
	Ciprofloxacin	(33.3) 1	(66.7)2	0
	Cefotaxime	0	0	(100) 3
	Erythromycin	0	0	(100) 3
	Levofloxacin	(33.3) 1	(33.3)1	(33.3) 1
	Oflaxacin	(33.3) 1	0	(66.7) 2

Table 7: Antibiotic susceptibility test for *Streptococcus pneumoniae* isolate

Isolates (No.)	*Antibiotics*	*No. of sensitive isolates (%)*	*No. of moderate isolates (%)*	*No. of resistant isolates (%)*
Streptococcus pneumonia (1)	Amikacin	0	(100) 1	0
	Ampicillin	0	(100) 1	0
	Azithromycin	0	(100) 1	0
	Amoxicillin-Clavulanic	(100) 1	0	0
	Aztreonam	0	0	(100) 1
	Ceftazidime	0	0	(100) 1
	Cephalexin	0	(100) 1	0
	Cefadroxil	0	0	(100) 1
	Ciprofloxacin	0	(100) 1	0
	Cefotaxime	0	0	(100) 1
	Erythromycin	0	0	(100) 1
	Levofloxacin	(100) 1	0	0
	Oflaxacin	0	(100) 1	0
	Vancomycin	0	0	(100) 1

Table 8: Antibiotic resistance patterns (APR) for bacterial isolates

Bacterial isolated	*Number of isolates*	*Antibiotics*	*Number of antibiotics*	*Antibiogram patterns (APR)*
S. aureus, P. aeruginosa, S. pneumonia, E. coli, P. mirabilis, K. pneumonia	6	CFR/AT/E/ CAZ/VA/CTX	6	R1
S. aureus, P. mirabilis, K. pneumonia, E. coli, P. aeruginosa	5	COX/AMC	2	R2
S. aureus, P. mirabilis, P. aeruginosa, K. pneumonia	4	AMP	1	R3
S. aureus, P. mirabilis, K. pneumonia, E. coli	4	AZM/OF	2	R4
P. mirabilis, S. aureus, K. pneumonia, P. aeruginosa	4	COX/AMC/ AMP	3	R5
P. mirabilis, S. aureus, K. pneumonia	3	AMP/AZM/OF	3	R6
S. aureus, P. mirabilis, E. coli	3	AZM/AK/OF	3	R7

cefotaxime, erythromycin, and vancomycin. However, it showed either sensitivity or moderate sensitivity to other antibiotics tested in this study (Table 7).

The current investigation showed a discrepancy in antimicrobial susceptibility. It was found that levofloxacin and ciprofloxacin were the most effective antibiotics for all the isolated bacteria. The effectiveness rates of levofloxacin and ciprofloxacin were (70.3, 66.7%) for *S. aureus*, (98.9, 73.7%) for *Pseudomonas aeruginosa*, (25, 62.5%) for *Proteus mirabilis*, (50, 100%) for *Klebsiellas pneumonia*, (33.3, 33.3%) for *Escherichia coli*, as well as the sensitivity of *Streptococcus pneumonia* was (0–100%), respectively (Tables 2-7).

Furthermore, our data pointed out that the most resistant antibiotic among all bacterial isolates (100%) was antibiotic ceftazidime, followed by cephalexin, which had 100% resistance rate among all isolates except for *Streptococcus pneumonia* isolate. Aztreonam also exhibited resistance among all isolates (100%) except for *Pseudomonas aeruginosa*. The least resistant rate for all the isolates was demonstrated for amikacin, except for *Escherichia coli,* which showed 100% resistance to this agent (Table 2-7).

Throughout the investigative journey, seven patterns of antimicrobial resistance were elucidated. The first pattern (R1) shows that all AOM bacterial isolates were resistant to the six antibiotics intended in this survey. The second pattern (R2) has resistance to 5 AOM bacterial isolates that comprise *S. aureus, P. mirabilis, K. pneumonia, E. coli and P. aeruginosa*. Among the R2, only two antibiotics (Cephalexin and Amoxicillin-Clavulanic) exhibited resistance for the five bacterial isolates. While in the patterns (R3, R4, R5) there were 4 isolates which resistant to 1, 2, and 3 antibiotics, respectively. Moreover, in patterns (R6, R7), three isolates exhibited resistance to antibiotics. In pattern (R6), resistance was found against *S. aureus, K. pneumonia,* and *P. mirabilis* for ampicillin, azithromycin, and oflaxacin. Whereas in pattern (R7), the isolated *S. aureus, P. mirabilis* and *E. coli* showed resistance to azithromycin, amikacin, and oflaxacin (Table 8).

AMC - Amoxicillin-Clavulanic, AMP – Ampicillin, AT - Aztreonam, AZM - Azithromycin, CAZ - Ceftazidime, CFR - Ceftriaxone, COX - Cefuroxime, CTX – Cefotaxime, E - Erythromycin, OF - Ofloxacin, VA – Vancomycin, R= (APR).

DISCUSSION

This study demonstrated a high prevalence of bacterial agents among Yemen patients of AOM, our findings were slightly in line with a recent study conducted in Dhamar city in 2020.[18] In contrast, another Yemeni study found that the occurrence rate of bacteria pathogens among chronic suppurative otitis media patients was higher than our rate.[19] In point of fact, the isolation rates of bacterial pathogens associated with otitis media were common in the third world. They differed from study to another depending on many factors like the patient's age, in Iraq, Agha and colleagues, as well as Almayali and colleagues, figured out that the rate ranged from 80 to 85.8% inpatient.[20,21] In comparison, other study conducted in Northwest Ethiopia pointed out the rate at 76.7%.[9] On the other hand, Al-Ofairi and colleagues demonstrated that the prevalence of AOM in Yemeni children in Ibb City was 30% during the period from 2013 to 2018 in Ibb City.[22]

The highest percentage of bacterial isolates was *S. aureus*. This finding was in line with data of Al-Khamesy and his collaborators among Yemeni patients in Sana'a City,[19] as well as with findings of Tadesse and colleagues along with Getaneh and associates among Southern and Northwest Ethiopian patients.[6,9] The prevalence of gram-negative bacterial isolates remains higher than that of gram-positive strains in causing AOM in our investigation. This aligns with the recent findings of Al-Khamesy and his team.[19] However, it contrasts with the findings of Al-Ofairi and his group, which may be related to differences in the ages of the participants.[22]

The second commonest isolate in our sample was *Pseudomonas aeruginosa*, this result was in agreement with Al-Khamesy and colleagues.[19] The third isolated one was *Proteus mirabilis*, then followed by *Escherichia coli*, *Klebsiella pneumonia*, which were identified as significant pathogens implicated in AOM in our investigation, as mentioned in many previous researches.[6,9,19,23] Our study demonstrated that the least isolated pathogen was *Streptococcus pneumoniae,* this rate in line was pointed out in the study of Agha and his associates, as well as Draman and colleagues.[20,23]

This survey documented that Levofloxacin and Ciprofloxacin were the most effective antibiotics in all bacterial isolates. This finding was not consistent with previous studies conducted in Yemen, where these discrepancies may be related to many factors, such as the prescription rate of the two antibiotics, self-medications, and sample size.[24,25] Furthermore, ceftazidime and aztreonam were demonstrated as the most resistant antibiotics among all bacterial isolates except for *Pseudomonas aeruginosa,* where some strains were sensitive for aztreonam. This result was not in consistent with the previous data from Yemen.[19,25] Additionally, it was found that there was considerable resistance against ceftazidime in clinical field,[26] as well as the role of Metallo-beta-lactamases in the resistance of ceftazidime and aztreonam.[27] Moreover, azithromycin and Amikacin were the least antibiotic-resistant of among the isolates, except for *Escherichia coli* that gave absolute resistance to amikacin, these two antibiotics have substantial resistance in clinical practice.[25,26]

The current study illustrated seven antibacterial resistance patterns, which reflect the massive antibiotic use in Yemeni community.[28] The first pattern (R1) revealed that all bacterial isolates were resistant to six antibiotics out of the tested antibacterial agents. This finding highlights a worsening trend of multiple resistance, particularly evident in low-constrained resource countries.[29] It was noted in R2 that five isolates exhibited resistance to two antibiotics, namely Cephalexin and Amoxicillin-Clavulanic. The data concerning cephalexin indicated lower efficacy than the study conducted by Al-Khamesy and colleagues in patients with chronic ear infections in Sana'a city.[19] While Amoxicillin-Clavulanic efficacy in our study was higher than the finding of Badulla and associates about the resistance among patients of Aden City.[25] Similarly, our data found notable variability in antibiotic resistance patterns among the isolates in patterns R3, R4, R5, R6, and R7. These discrepancies address multidrug resistance in this context, as occurred in many regions of Yemen and other third-world areas.[19, 20, 25,29-33]

CONCLUSION

The prevalence of bacterial isolation in among Yemeni Acute Otitis Media (AOM) Patients remains notably elevated, aggravated by a significant resistance rate among the bacterial isolates towards the empirically prescribed antibiotics. This heightened resistance underscores the pervasive misuse and overuse of antimicrobial agents within our community. In light of this concerning trend, there is a compelling need for the implementation of stringent antimicrobial control measures. These measures should be complemented by a proactive approach towards updating and reinforcing the empirical antimicrobial prescription guidelines. By doing so, we can address the root causes of antibiotic resistance, promote responsible antimicrobial use, and ensure more effective treatment strategies for AOM to protect society from such challenges.

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