

Bacterial Etiology of Otitis Media and Their Antibiotic Resistance in Thi-Qar Province

Sahira Abd Alhasan Helal¹ and Rahman Laibi Chelab¹

Email : sahrera.abd.helal@utq.edu.com

^{1,2} Biology Department, College of Education for Pure Science, Thi-Qar University, Thi-Qar, Iraq

Abstract

Otitis media continues to be a major clinical challenge, especially in developing communities with rising bacterial resistance to standard treatments. Despite the clinical importance of the disease, there is still a clear scarcity of new research that has addressed the changing bacterial patterns in Thi-Qar Governorate. Based on the above, the initiative emerged to conduct a study, diagnosis, and evaluation of bacterial isolates from patients with otitis media within Thi-Qar Governorate and to test their antibiotic sensitivity. 110 samples were collected from the E.N.T. unit at Al-Haboub General Hospital and Al-Rifa'a General Hospital and returned to the basic and advanced diagnostic teams, such as Al-Manwal, Vaitk_2, and IPE. The results showed that the largest percentage in the samples was 87% positive for bacterial infection, while 23% were negative for other infections, such as viruses, fungi, and other psychological diseases. The demographic study also showed that the infection among people did not differ reliably according to gender, while on the other hand, the highest rates were recorded among adults at the expense of young people. This is attributed to the environmental, living, and health conditions of adults within this governorate. The microbiological examination showed five common species, namely *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella* spp., *Proteus* spp., and *Providencia* spp. Importantly, *P. aeruginosa* was identified as a key pathogen with a concerning level of multidrug resistance. The paper emphasizes the importance of evidence-based treatment in the context of antimicrobial resistance, a growing problem, and the importance of accurate laboratory diagnosis in guiding therapeutic decisions.

Keywords: Otitis media, antibiotic resistance, Thi-Qar Province, Bacterial Identification.

الخلاصة:

ظهرت هذه المبادرة لإجراء دراسة وتشخيص وتقييم للعزلات البكتيرية من مرضى التهاب الأذن الوسطى في محافظة ذي قار، واختبار حساسيتها للمضادات الحيوية. جُمعت 110 عينات من وحدة الأنف والأذن والحنجرة في مستشفى الحبوب العام ومستشفى الرفاع العام، وأعيدت إلى فرق التشخيص الأساسية والمتقدمة، مثل فرق المنوال، وفريق فيتك 2، وفريق علم الأمراض الجلدية والتناسلية. أظهرت النتائج أن النسبة الأكبر من العينات كانت إيجابية للعدوى البكتيرية (87%)، بينما كانت 23% منها سلبية لأنواع أخرى من العدوى، مثل الفيروسات والفطريات والأمراض النفسية. كما أظهرت الدراسة الديموغرافية أن معدل الإصابة بين الأفراد لم يختلف بشكل ملحوظ بين الجنسين، بينما سُجلت أعلى المعدلات بين البالغين على حساب الشباب. يُعزى ذلك إلى الظروف البيئية والمعيشية والصحية للبالغين في هذه المحافظة. أظهر الفحص الميكروبيولوجي وجود خمسة أنواع شائعة، هي: الزائفة الزنجارية، والمكورات العنقودية الذهبية، وأنواع الكلبسيلا، وأنواع البروتيس، وأنواع البروفيدنسيا. ومن الجدير بالذكر أن الزائفة الزنجارية تم تحديدها كعامل ممرض رئيسي ذي مستوى مثير للقلق من مقاومة المضادات الحيوية المتعددة. تؤكد هذه الورقة البحثية على أهمية العلاج القائم على الأدلة في سياق مقاومة المضادات الحيوية، وهي مشكلة متفاقمة، وعلى أهمية التشخيص المختبري الدقيق في توجيه القرارات العلاجية.

الكلمات المفتاحية: التهاب الأذن الوسطى، مقاومة المضادات الحيوية، محافظة ذي قار، تحديد البكتيريا

Introduction

Otitis Media is a middle ear effusion behind an intact tympanic membrane and does not cause acute symptoms such as acute otitis media [1,5,7]. Middle ear effusion (MEE) results in impairment of transduction of sound waves in the middle ear and leads to conductive hearing loss. Recurrent hearing loss may affect language, behavior, and academic achievement [6,8,33]. By the age of 10, 80% of children have otitis media with effusion (OME). OME can develop following a viral infection or an acute otitis media (AOM) when the inflammation resolves, but middle ear effusion (MEE)

remains [9,34]. All children develop effusion after acute otitis media. OME is a risk factor for AOM, showing its association [10, 11, 35].

Chronic suppurative otitis media (CSOM) is defined as a chronic inflammation of the middle ear and mastoid cavity with recurrent ear discharge through a perforation of the tympanic membrane or a ventilation tube [12]. CSOM can lead to conductive auditory loss as well as damage to the ossicular chain of the middle ear. It also increases the risk of intracranial problems and persistent sensorineural hearing loss [12,31]. This condition is more common in low- and middle-income countries [1-3].

Since the publication of a seminal review on OM over a decade ago [13], global progress has been made in OM prevention through pneumococcal conjugate vaccination and management with new guidelines that stress accurate diagnosis and judicious use of antibiotics.

Materials and Methods.

Sample Collection and Handling:

Samples were collected from Al-Rifai General Hospital, Al-Haboubi General Hospital, and Some clinics in the Thi-Qar governorate. The 110 samples for otitis media were collected using stringent safety measures because they were infectious. MediaTranslate gowns were used. This included disinfecting the site of infection, collecting samples using the sterile transport medium swab, and culturing them on nutrient agar, MacConkey agar, and blood agar. The samples were incubated at 37°C for 24-48 hours at Pure Sciences Laboratories, College of Education, University of Thi-Qar. Bacterial growth was monitored by culture media. and bacterial species were purified by repeated culture to obtain a single coliform [14].

Bacterial Isolation and Identification:

Bacterial isolates linked with otitis media were isolated and identified using a stepwise laboratory technique. Clinical materials were plated onto blood agar, MacConkey agar, and nutrient agar to optimise bacterial recovery and assess initial colonial morphology. Sub-culturing of pure colonies on selective and differential media, such as Mannitol Salt Agar for isolation and differentiation of staphylococci and Cetrimide Agar for selective isolation of *Pseudomonas aeruginosa*, was useful in initial differentiation of bacterial species on the basis of their cultural and biochemical

characteristics.[4]

Preliminary identification was based on colony shape and Gram staining. Conventional biochemical characterisation was based on catalase, oxidase, coagulase, indole, urease, citrate utilisation, motility, and carbohydrate fermentation assays. The methods used in this work enabled the identification of the mostly isolated bacterial pathogens. These included *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus epidermidis*, and *Providencia stuartii*. [17,30] To increase the accuracy, bacterial identification was done using the VITEK-2 Compact automated identification system based on biochemical profiling. In addition, the API (Analytical Profile Index) system was employed as a confirmatory identification tool to augment the standard laboratory findings and to improve the accuracy of species-level identification.

[15-16]

Antibiotic Susceptibility Testing

Antimicrobial susceptibility profiles of bacterial isolates from otitis media patients were determined by the Kirby–Bauer disc diffusion method on Mueller–Hinton agar (MHA) according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [13,37]. Before testing, fresh bacterial colonies were emulsified in sterile normal saline, and the turbidity of each suspension was adjusted to the 0.5 McFarland standard. A standardised inoculum was spread evenly over the entire surface of Mueller–Hinton agar plates with a sterile cotton swab to obtain a confluent bacterial lawn. Then, antibiotic discs were aseptically placed on the inoculated agar surface at sufficient distances to avoid merging of inhibition zones. The inoculated plates were incubated aerobically at $35 \pm 2^\circ\text{C}$ for 16–18 h, and the diameters of inhibition zones were measured in millimetres. The results were interpreted and classified as Susceptible (S),

Intermediate (I), or Resistant (R) according to the current CLSI interpretive criteria.[38]

Eight antimicrobial agents from different classes of antimicrobials were chosen to give a broad overview of the susceptibility profiles of the recovered isolates. They included the aminoglycosides Amikacin (AK) and Gentamicin (CN), which inhibit bacterial protein synthesis by binding to the 30S ribosomal subunit; Ciprofloxacin (CIP), a fluoroquinolone that inhibits DNA gyrase and topoisomerase IV; Amoxicillin (AMX), a β -lactam antibiotic that inhibits bacterial cell wall synthesis; Erythromycin (E) and Azithromycin (AZM), macrolides that inhibit protein synthesis by binding to the 50S ribosomal subunit; Clindamycin (DA), a lincosamide that inhibits protein synthesis by binding to the 50S ribosomal subunit; and Vancomycin (VA), a glycopeptide antibiotic that inhibits bacterial cell wall synthesis by preventing peptidoglycan formation. Therapeutic relevance and routine clinical use in the management of bacterial infections associated with otitis media were the criteria for the selection of these antimicrobial

agents, and the study provides a comprehensive evaluation of resistance and susceptibility patterns among the bacterial isolates recovered.[32]

Results:

Among these, a high rate of positive bacterial growth was recorded at 79.09% ($n = 87$), whereas the remaining 20.91% ($n = 23$) of the samples showed no bacterial growth (negative growth).

The gender distribution among the positive isolates ($n = 87$) was very similar, with males having infection rates of 50.57% ($n = 44$) and females 49.43% ($n = 43$). As for age groups, patients were divided into two main cohorts. The paediatric group (children), including patients from birth (1 day old) to 17 years of age, comprised 16.09% ($n = 14$) of all positive growth samples. In contrast, the adult group, which ranged in age from 17 to 85 years, had the highest prevalence of infection with 83.91% ($n = 73$) of the positive cases.

Table (1): Demographic Distribution of Bacterial Isolates from Patients in Thi-Qar Province According to Growth Pattern, Gender, and Age Groups

Variable	Category	Number	Percentage
Growth Pattern	Growth	87	79.09%
	Non growth	23	20.91%
	Total	110	100%
Gender	Male	44	50.57%
	Female	43	49.43%
	Total	87	100%
Age Group	Adults	73	83.91%
	Children	14	16.09%
	Total	87	100%

In this study, a total of 87 bacterial isolates were identified in the present study from patients diagnosed with otitis media. As shown in Table 2, the predominant bacterial species was

Pseudomonas aeruginosa, with 43 isolates (49.4%), which indicates its major role in the aetiology of otitis media in the studied population. The second most common organism

was *Staphylococcus aureus* with 27 isolates (31.0%), followed by *Proteus mirabilis* with 9 isolates (10.3%). *Staphylococcus epidermidis* was isolated in 6 isolates (6.9%), and *Klebsiella pneumoniae* and *Providencia spp.* were isolated

once each (1.15%). In the present study, gram-negative bacteria, particularly *P. aeruginosa*, were the predominant bacterial pathogens isolated.

Table (2): Bacterial Infection Rates Among Isolated Samples

Bacteria	Number	Percentage
<i>Pseudomonas aeruginosa</i>	43	49.4%
<i>Staphylococcus aureus</i>	27	31%
<i>Proteus mirabilis</i>	9	10.3%
<i>Klebsiella pneumoniae</i>	1	1.14%
<i>Providencia spp</i>	1	1.14%
<i>Staphylococcus epidermidis</i>	6	6.8%
Total	87	100%

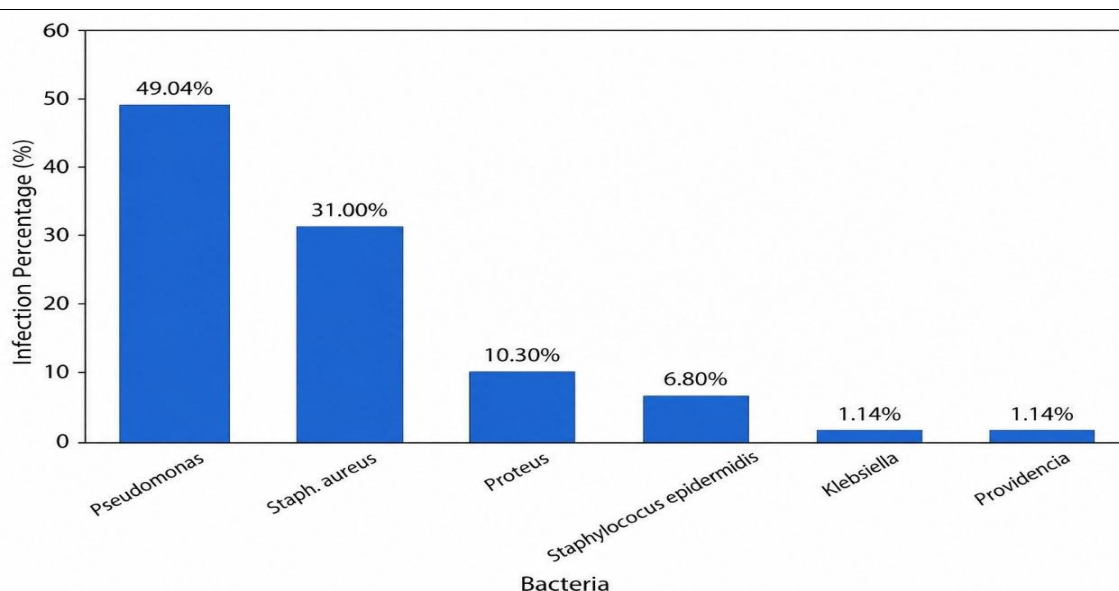


Figure (1): Bacterial Infection Distribution for all positive samples.

Antibiotic Sensitivity Patterns

Figure 2 and Table 3 show the bacterial isolates that are sensitive and resistant to the antibiotics used in the study. The measurement of the inhibition zone around the antibiotic tablets was adopted and compared with the standard tables according to the CLSI.[38] The most commonly used antibiotics in the treatment of otitis media were AK, CN, E, AMX, GN, VA, AT, and DA. The results of the current study showed that *P. aeruginosa* is the most resistant to all antibiotics and is sensitive only to AK. The study results also indicated that *Proteus mirabilis* bacteria are resistant to both CIP and AMX. E and DA and

have high sensitivity to AK, VA, AT, and CN. The study results also showed that *Klebsiella pneumoniae* bacteria are resistant to VA, E, and DA and have high sensitivity to AT, CIP, CN, and AK. The results also showed that *S. aureus* bacteria are resistant to the antibiotics VA, CN, and DN and highly sensitive only to AT, CIP, AK, and AMX. The results indicated that *Providencia stuartii* bacteria were resistant to VA, E, and DA; moderately sensitive to AMX and AT; and highly sensitive to CIP, CN, and AK. The problem of antimicrobial resistance in Thi-Qar Province may be linked to the abuse and overuse of antibiotics [18,30].

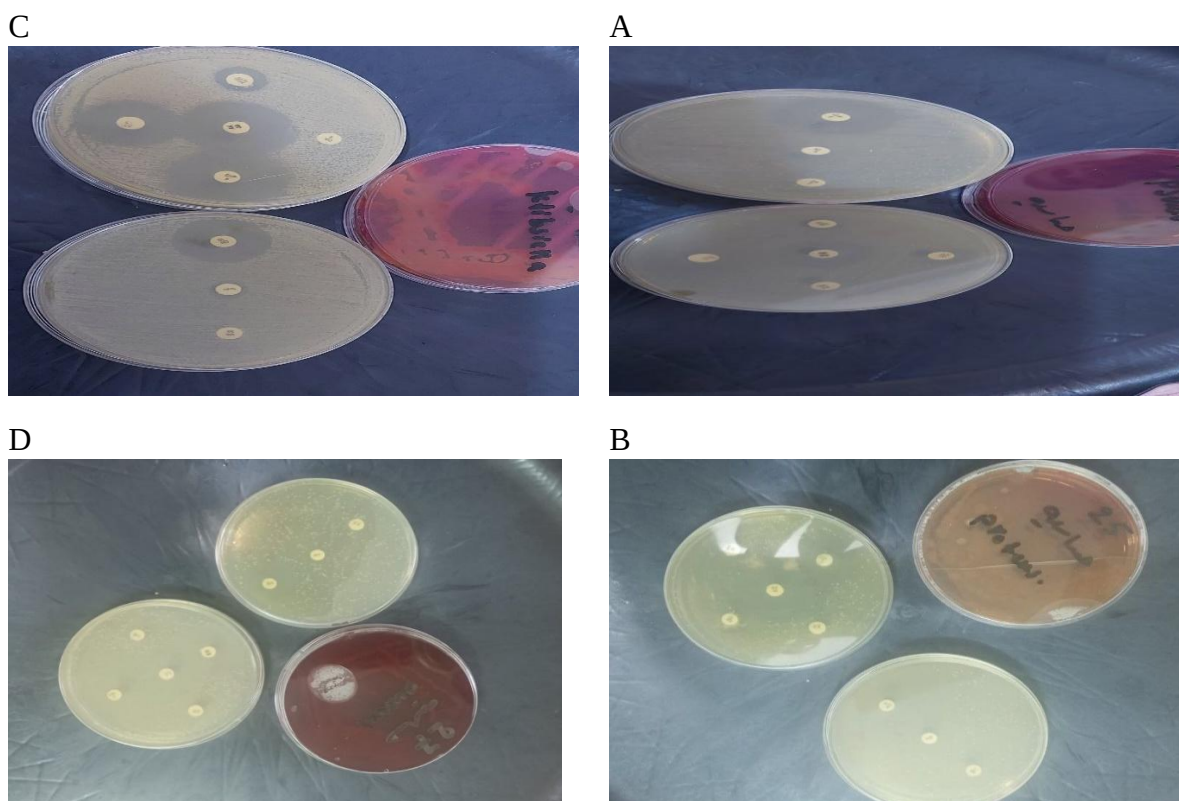


Figure (2): Measurement of Antibiotic Sensitivity for Bacterial Isolates by Disk Diffusion Technique (A) *P. aeruginosa*, (B) *P. mirabilis*, (C) *K. pneumoniae*. (D). *S. aureus*.

Table (3): Antibiotic Susceptibility Patterns of Bacterial Isolates from Otitis Media Patients in Thi-Qar Province

Bacteria	VA	AT	CIP	CN	DA	AK	AMX	E
<i>Pseudomonas aeruginosa</i>	R	R	S	R	R	S	R	R
<i>Staphylococcus aureus</i>	S	S	S	S	S	S	S	S
<i>Proteus mirabilis</i>	S	S	R	S	R	S	R	S
<i>Klebsiella pneumonia</i>	R	S	S	S	R	S	S	R
<i>Providencia spp.</i>	R	I	S	S	R	S	I	R

Discussion

The current study revealed a specific ratio: the number of males (50.57%) was slightly higher than that of females (49.43%). These findings are consistent with other studies that found no statistically significant differences between the sexes in susceptibility to common bacterial diseases [18-19]. The statistics suggest that gender may not represent a significant risk factor for infection. Several behavioral and environmental factors may have a marginal influence on the likelihood of infection. The age distribution showed a higher number of isolates among adults in the (17–85) year age group (83.91%) compared to children in the (1–17) year age group (16.09%), which is consistent with studies showing that adults are more exposed to environmental and occupational risk factors, in addition to differences in healthcare-seeking behaviors

[20-23]. However, other studies show a higher incidence rate among children, depending on the type of disease and the study methodology. The isolates showed significant variation in patterns of antibiotic susceptibility (Table 3). This illustrates the complexity of otitis media and the multiple ways in which these microbes may evade treatment. Furthermore, most of the antibiotics included in the study showed resistance against the bacterial isolates in this study.

P. aeruginosa. “This phenotype is consistent with its innate and acquired defense mechanisms, such as production of strong

biofilms, activation of efflux pumps, and limiting outer membrane permeability. The sensitivity and resistance of the bacterial isolates to the antibiotics used are shown in Table 3, and the mechanism of action of these antibiotics involves the inhibition of protein synthesis by repeated binding of the 30S ribosomal subunit responsible for protein coordination. This results in incorrect readings of the mRNA, as we observe with the antibiotic AK, a member of the aminoglycoside class. CIP is a fluoroquinolone-class antibiotic that acts on DNA. It inhibits its enzymatic activity and inhibits DNA replication. AMX is a beta-lactam penicillin-type antibiotic that targets the cell wall, preventing the formation of beta-glycans (peptidoglycans) in the wall, which causes the cell to break down. Antibiotic CN is also an aminoglycoside. It binds the small 30S ribosomal subunit and inhibits protein synthesis. This causes errors in protein translation. The antibiotic VA also affects the cell wall. It inhibits cell wall synthesis by stabilizing peptidoglycan synthesis. The antibiotic AT also acts on the cell wall by binding to penicillin-binding proteins and inhibiting cell wall synthesis. Finally, the antibiotic DA is also a lincosamide; it binds to the 50S ribosomal subunit, inhibits protein synthesis, and prevents the elongation of the protein chain. The difference in susceptibility and resistance in the bacterial groups in the study samples is due to the wrong or excessive use of antibiotics or not following the dosage

schedule. This shows the importance of strict diagnosis of the samples taken from patients with otitis media to confirm their susceptibility and resistance status. The ribosomal subunit inhibits protein synthesis and prevents the elongation of the protein chain. The differences in susceptibility and resistance among bacterial groups in the study samples are due to improper or excessive antibiotic use or failure to follow the dosage schedule. This shows the importance of strict diagnosis of the samples taken from patients with otitis media to confirm their susceptibility and resistance status, but some. This phenotype is consistent with its innate and acquired defensive mechanisms, such as the development of strong biofilms, activation of efflux pumps, and low outer membrane permeability.[36] *Staphylococcus aureus* remained susceptible to a variety of medicines, in particular aminoglycosides and fluoroquinolones; isolates were resistant to them [24-25]. This difference is probably due to the occurrence of methicillin-sensitive (MSSA) and methicillin-resistant (MRSA) populations in the samples and, therefore, the need for rigorous confirmation procedures. Moreover, *Proteus mirabilis* and *Klebsiella pneumoniae* showed different susceptibility patterns. probability of beta-lactam resistance, particularly amoxicillin [26-27]. The prevalent mechanism of resistance is the release of beta-lactamase enzymes [25,28,29].

Conclusions

Pseudomonas aeruginosa and *Staphylococcus aureus* are the most common pathogens in cases of otitis media in Thi-Qar Governorate. The isolated bacterial strains showed a high prevalence of antimicrobial resistance. Different bacterial species have different resistance patterns, so we need good diagnostics. Antibiotic sensitivity testing is an important step in the choice of the right

treatment.” Unrestricted use of antibiotics is a major driver in the development of bacterial resistance.

6. DECLARATIONS

6.1. Study Limitations

More samples need to be examined from different small hospitals and villages. In addition, wide-spectrum antibiotics need to be tested on these bacteria.

6.2. Acknowledgements

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6.3. Funding source

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6.4. Competing Interests

The authors hereby declare that they have no competing financial, professional, or personal interests that could have influenced the design, execution, analysis, interpretation, or reporting of this research. All authors confirm complete independence from any commercial entities or funding sources that might have a potential interest in the outcomes of this study.

6.5. Open Access

Additional datasets and methods available from the corresponding author upon reasonable request.

6.6. Use of AI

The authors declare that artificial intelligence tools were utilized to assist in the translation and

linguistic enhancement of the manuscript, which was originally written in Arabic. AI was employed exclusively to improve the grammatical quality, clarity, and fluency of the English text without altering the scientific content, presented data, or interpretations of results. All statistical analyses, conclusions, and intellectual contributions remain the sole responsibility of the authors. The authors thoroughly reviewed the final manuscript to ensure the accuracy and integrity of all scientific content.

7. HUMAN AND ANIMAL-RELATED STUDIES

6.1. Ethical Approval

All the experiments got approved by the ethical approval committee under EAC/20902 on 10/01/2025.

7.2. Informed Consent

Consent was obtained from all patients participating in the study.

7.3. Author Contributions Sahira Abd Alhasan Helal and Rahman Laibi Chelab conceptualized and designed the study. Both authors analyzed the results and contributed to manuscript preparation and revision.

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