ARTICLE / INVESTIGACIÓN

Bacteriological study and its antibiotics susceptibility pattern of Otitis Media in Iraqi patients

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Abstract: Otitis media is an acute upper respiratory tract infection-related inflammation of the middle ear and tympanic membrane, frequently affecting children. Typically, a subsequent bacterial infection complicates a viral infection, which ultimately causes the condition. The study aims to study the function of bacterial ear infections and its causes, as well as their resistance to medications, which was the focus of this investigation. The first axis of the research was the identification of bacterial isolates using recognized diagnostic tools, and the second axis was determining the antibiotic's resistance and sensitivity. Patients with otitis media were gathered from Al-Hakim General Hospital and Al-Sadr city hospital in Al-Najaf city between November 2020 and April 2021 for 100 clinical samples. More than 80 samples were found to be infected with bacteria. Bacterial strains found in this investigation are (30) isolates of *Pseudomonas aeruginosa*, (20) isolates of *Klebsiella spp*, (20) isolates of *Proteus spp*, (15) isolates of *Staphylococcus aureus*, (8) isolates *Escherichia coli* and (7) isolates *Enterococcus fecalies*. As part of this research, the disk diffusion method was used to assess how sensitive the test was. The results showed that *Pseudomonas aeruginosa* was resistant to most antibiotics, particularly the penicillin family, cephalosporin, and trimethoprim, with the existence of isolates resistant to meropenem. The investigation results varied for the quinolone, aminoglycoside, and macrolide families. *Klebsiella spp.* were tested for antibiotic sensitivity and found to be resistant to most antibiotics, particularly those in the penicillin family, cephalosporins, and trimethoprim. Some quinolones, aminoglycosides, and macrolides are also resistant. *Proteus spp* were resistant to most antibiotics, particularly the penicillin family (except for augmentin, which had some sensitive isolates) and cephalosporin (except for cefdinir and cefepime) had some susceptible isolates) and trimethoprim, in addition to the presence of isolates resistant to meropenem. There is a discrepancy in the examination results for the quinolone family. The aminoglycoside family is also highly resistant. *S. aureus* isolates were resistant to penicillin (except for augmentin, which some isolates were responsive to), trimethoprim, and quinolones, with the presence of isolates resistant to vancomycin. The macrolide class (azithromycin) also has a significant resistance level. *Escherichia coli* is susceptible to meropenem, imipenem, and certain cephalosporin generations. Augmentin, cefepime, cephalothin, meropenem, imipenem, and azithromycin were ineffective against *Enterococcus fecal*. The conclusion is that *Pseudomonas spp* has a role in ear infections and the germs *Klebsiella spp., Proteus spp., Staphylococcus aureus, Escherichia coli*, and *Enterococcus fecalies*. Penicillin and cephalosporin resistance was seen in the majority of the identified isolates. The existence of isolates of Proteus and Pseudomonas species resistant to meropenem. Vancomycin-resistant strains of *Staphylococcus aureus* isolates are present.

Key words: Otitis media, Resistance antibiotic, *S.aureus, P.aerginosa*.

Introduction

Otitis Media (OM) is an inflammatory condition that affects the aperture in the middle ear, whether or not the tympanic membrane is healthy. The complex etiology and pathophysiology of otitis media include genetics, infections, allergies, the environment, social and racial factors, and eustachian tube dysfunction[1]. Acute and chronic otitis media are equally prevalent conditions. Otitis media affects around 16 percent of the Nepalese population over five. Nearly two-thirds of these incidents involve elementary or middle school-aged children, most of whom originate from low-income households; the spread of antibiotic-resistant bacteria was a worldwide public health problem because OM, its etiological negotiators, and its antibiotic susceptibility array were found early on[2]. Among the most frequent bacteria found in the middle ear of patients with AOM, you'll discover *S. pneumoniae, H. influenzae, M. catarrhalis*, and *S. aureus*[3]. Ear discomfort is the most common symptom of acute otitis media; additional symptoms include fever and decreased hearing during sickness, the skin above the ear inflamed, ears dripping with pus, the patient being irritable, and diarrhea (in infants). Because an upper respiratory tract infection (URTI) generally precedes an episode of otitis media, symptoms such as a cough and nasal discharge are familiar. A sensation of fullness in the ear is also possible. Ear discharge can be caused by perforation of the eardrum in acute otitis media, tympanostomy tube otorrhea, and acute otitis externa[4]. Thus, this research aims to identify the bacteria that cause the disease and determine their susceptibility to medications.

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Materials and methods

Specimens' collection

Patients suffering from otitis media at Al-Hakim General Hospital and Al-Sadder Medical City in the governorate of Al-Najaf gave 100 clinical specimens from November 2020 to April 2021. Using a sample of an ear infection.

Specimens Culture and biochemical test

After collecting the samples using swabs, they were cultured on the commonly used media (Nutrient, Mannitol salt, Blood, and MacConkey) based on isolation and initial diagnosis. And then adopting the biochemical tests from them, IMVIC test, catalase test, coagulase and oxidase, to differentiate between genus and species. In addition to using VITEK-2 Compact System to confirm the diagnosis[5,6].

Identification Using VITEK-2 Compact System

These two kinds of bacteria are identified using GP and GN cards, made up of pure colonies taken from the culture medium and suspended in a solution before being loaded onto a card. It relies on biochemical testing for this method[7].

Antimicrobial Activity

The antibiotic sensitivity test is done by using different types and many antibiotic disks with known concentrations on Muller Hinton medium, reading the result after 24 hours, and determining the diameter of the inhibition zone by comparing the result with CLSI[8].

Results and discussion

Isolation of pathogenic bacteria and sensitivity test

One hundred clinical specimens were gathered from patients over the trial period from November 2020 to April 2021. Swabs from the ears. The results showed 80 (80%) samples that contained bacterial growth with 100 isolates because mix growth, while 20 (20%) samples had no bacterial growth, as shown in table (1).

This study was conducted on isolates of Pseudomonas aeruginosa isolated from middle ear infections to test for sensitivity consistent with[9] they were resistant to most antibiotics, especially the penicillin family, cephalosporin, and trimethoprim, in addition to the presence of isolates resistant to meropenem. There is a difference in the examination results for the families of quinolones, aminoglycosides, and macrolides, as shown in table (2). The transposons present in Pseudomonas aeruginosa modify aminoglycoside enzymes to produce resistance. Infections brought on by microorganisms are treated using a variety of classes/groups of aminoglycoside antibiotics. Examples of antibiotics include gentamicin, streptomycin, amikacin, neomycin, kanamycin, and gentamicin. Three different types of enzyme conformational alterations that result in drug resistance have been identified by prior research. One of these is aminoglycoside phosphoryl transferase (APH) phosphorylation. Because of lipopolysaccharide, a variety of exocompounds are impermeable to Gram-negative bacteria. These include aminoglycoside nucleotidyl transferase (ANT) adenylation and aminoglycoside acetyltransferase (AAC) acetylation (LPS). Each component of LPS has a covalent bond, including the lipid A, oligosaccharide core, and O antigen. LPS adheres to cell membranes with the help of the phosphorylated glucosamine disaccharide in the hydrophobic lipid A region. The outer membrane channel-forming protein (OMC), the resistance nodulation division (RND), and the membrane fusion protein, which joins the first two proteins through the periplasm, comprise the drug efflux system in bacteria[10].

During this study, which was conducted on isolates of *Klebsiella spp.* isolated from middle ear infections to test for sensitivity, they were resistant to most antibiotics, especially the penicillin family, cephalosporin, and trimethoprim. Some types of quinolone, aminoglycosides, and macrolides also give resistance, as shown in table (3).

Pneumonia is brought on by the bacterium *K. pneumoniae*. Infections that are not resistant to medication can be treated with antibiotics. Because few medicines are effective against *K. pneumoniae* infections, they might be challenging to treat. Testing in a microbiology laboratory is necessary to ascertain which antibiotics will be beneficial in treating the disease in such situations. Treatments for *Klebsiella pneumonia* that are more specific include imipenem/cilastatin, quinolones, and aztreonam. To treat multidrug-resistant urinary tract infections brought on by Klebsiella species, amikacin and meropenem have been recommended[11].

During this study, which was conducted on isolated Proteus spp isolated from middle ear infections to test for

Types	Number (total 100 isolates)
Pseudomonas aeruginosa	30
Klebsiella spp	20
Proteus spp	20
Staphylococcus aureus	15
Escherichia coli	8
Enterococcus fecalies	7

Table 1. Illustrates the different kinds and numbers of otitis media-specific bacteria.

Synthesis of Cell wall		Synthesis of DNA	
AMOXICILLIN	Resistance		Sensitive
PIPERACILLN	Resistance		Variable
AUGMENTIN	Resistance		Variable
PIPERACILLIN	Resistance	NORFLOXACIN	Variable
	Resistance	NALIDIXIC	Resistance
IMIPENEM	Sensitive	Metabolism of Folic acid	
MEROPENEM	Variable		Resistance
CEFTRIAXONE	Resistance	Synthesis of Protein	
CEFIXIME	Resistance		Variable
	Variable	AMIKACIN	Sensitive
CEFDINIR	Resistance	GENTAMYCIN	Sensitive
CEFEPIME	Variable	TOBRAMYCIN	Resistance
CEPHALOTHIN	Resistance	DOXYCYCLINE	Resistance

R: Resistance S: Sensitivity H: Hight

Table 2. Susceptibility test to Pseudomonas aeruginosa isolated from otitis media,

Synthesis of Cell wall		Synthesis of DNA	
AMOXICILLIN	Resistance	**CIPROFLOXACIN (quinolone)**	Sensitive
BENZYLPENI-	Resistance	**LEVOFLOXACIN**	**Variable**
AUGMENTIN	Resistance	**MOXIFLOXACIN**	**Variable**
PIPERACILLIN	Resistance	**NORFLOXACIN**	**Variable**
CARBENICIL-	Resistance	**NALIDIXIC ACID**	Resistance
IMIPENEM	Resistance	**Metabolism of Folic acid**	
MEROPENEM	Resistance	**TRIMETHOPRIM**	Resistance
CEFTRIAXONE	Resistance	**Synthesis of Protein**	
CEFIXIME	Resistance	**AZIYHROMYCN (macrolide)**	Resistance
	Resistance	**AMIKACIN (aminoglycoside)**	**Variable**
CEFDINIR	Resistance	**GENTAMYCIN**	Resistance
CEFEPIME	Resistance	**TOBRAMYCIN**	Sensitive
CEPHA-	Resistance	**DOXYCYCLINE (tetracycline)**	Sensitive

R: Resistance S: Sensitivity H: Hight

Table 3. Susceptibility test to *Klebsiella spp.* Isolated from otitis media.

sensitivity, they were resistant to most antibiotics, especially the penicillin family (except for augmentin, some isolates were sensitive) and cephalosporin (except for cefdinir and cefepime were sensitive) and trimethoprim in addition to the presence of isolates resistant to meropenem this result agree with[12], table 4 displays the resistance rates for the carbapenem community, which comprises imipenem and meropenem (effective -lactam antibiotics), at 5.9 %. There is a discrepancy in the testing results for the quinolone family. The aminoglycoside family also has a significant level of resistance. Extended-spectrum -lactamases (ESBLs), the AmpC-type cephalosporins, and infrequently carbapenemases, are produced by multidrug-resistant (MDR) strains of *P. mirabilis*, and their frequency in some contexts is instead high[13].

This study, which was conducted on isolates of Staphylococcus aureus isolated from otitis media, agrees with[14,15] demonstrate *Staphylococcus aureus* isolates from tonsillitis to test for sensitivity and disagree with[16] including high resistance to ceftriaxone and erythromycin, they were resistant to penicillin (except for augmentin some isolates were sensitive) and trimethoprim and quinolone, in addition to the presence of some isolates that resistant to vancomycin. There is also a high resistance to the macrolide family(azithromycin), as shown in table (5).

Glycopeptides like vancomycin are bactericidal because they bind to the D-ala-D-ala terminus of the peptidoglycan precursor Lipid II and block peptidoglycan synthesis[17]. Vancomycin is effective against a wide spectrum of gram-positive infections because Gram-positive bacteria, such as *S. aureus*[18], frequently retain the D-Ala-D-Ala terminus.

For example, in VRSA and enterococci, unique operons compose genetic regulatory systems that code for diverse antibiotic resistance factors, and six resistance patterns have been found (called "VanA through VanG"). When an organism develops resistance to vancomycin, it transforms the precursors of the cell wall dipeptides into molecules that have a lower affinity for the antibiotic, such as D-alanyl-D-lactate (for VanA, VanB, and VanD subtypes) and/or D-alanyl-D-serine (for the same subtypes) (VanC, VanE, and VanG). An operon on the Tn1546 genetic element that was isolated from a vancomycin-resistant Enterobacteriaceae (VRSA) strain is responsible for VRSA resistance. Co-infections with VRE have been found in every single case as of this writing[19].

As for the study that was conducted in the axis of sensitivity examination of *Escherichia coli*, it was shown that it was resistant and variable to the type of antibiotics used

Synthesis of Cell wall		**Synthesis of DNA**	
AMOXICILLIN	Resistance	**CIPROFLOXACIN**	**Variable**
BENZYLPENICILLIN	Resistance	**LEVOFLOXACIN**	**Variable**
AUGMENTIN	**Variable**	**MOXIFLOXACIN**	**Variable**
PIPERACILLIN	Resistance	**NORFLOXACIN**	**Variable**
CARPENCILLIN	Resistance	**NALIDIXIC ACID**	Resistance
AMPICLOIC	Resistance	**Synthesis of RNA**	
TICARCILLIN	Resistance	**RIFAMPICIN**	Resistance
IMIPENEM	Sensitive	**Metabolism of Folic acid**	
MEROPENEM	**Variable**	**TRIMETHOPRIM**	Resistance
CEFTRIAXONE	Resistance	**Synthesis of Protein**	
CEFIXIME	Resistance	**AZITHROMYCIN**	Resistance
CEFTAZIDIME	Resistance	**AMIKACIN**	**Variable**
CEFDINER	Sensitive	**GENTAMYCIN**	Resistance
CEFPODOXIME	Resistance	**TOBRAMYCIN**	Resistance
CEFEPIME	Sensitive	**TIGECYCLIN**	Resistance
CEPHALOTHIN	Resistance	**DOXYCYCLINE**	Resistance

R: Resistance S: Sensitivity H: Hight

Table 4. Susceptibility test to *Proteus spp* isolated from otitis media.

Synthesis of Cell wall		Synthesis of DNA	
AMOXICILLIN	Resistance	**COPROFLOXACIN**	Resistance
BENZYLPENICILLIN	Resistance	**LEVOFLOXACIN**	Resistance
AUGMENTIN	**Variable**	**MOXIFLOXACIN**	**Variable**
PIPERACILLIN	Resistance	**NORFLOXACIN**	Resistance
CARBENICILLIN	Resistance	**NALIDIXIC ACID**	Resistance
VANCOMYCIN	**Variable**		
IMIPENEM	Sensitive	**Metabolism of Folic acid**	
MEROPENEM	Sensitive	**TRIMETHOPRIM**	Resistance
CEFTRIAXONE	Sensitive	**Synthesis of Protein**	
CEFIXIME	Resistance	**AZITHROMYCIN**	Resistance
CEFTAZIDIME	Resistance	**TIGECYCLINE**	Sensitive
CEFDINIR	Sensitive	**TETRACYCLINE**	Resistance
CEFPODOXIME	Resistance	**DOXYCYCLINE**	**Variable**
CEFEPIME	Sensitive	**CLINDAMYCIN**	Sensitive
CEPHALOTHIN	Sensitive	**LINEZOLID**	Sensitive

R: Resistance S: Sensitivity H: Hight

Table 5. Susceptibility test to *Staphylococcus aureus* isolated from otitis media.

according to the above table. Although Ampicillin, Cefazolin, and Trimethoprim/Sulfamethoxazole are more resistant to *E. coli* than Meropenem, Imipenem, and some generations of cephalosporin, this is in agreement with[19] that *E. coli* is highly sensitive to Amikacin, Tigecycline, Gentamycin, Ciprofloxacin, Levofloxacin, Nitrofurantoin, Imipenem, Meropenem.

Regarding the transmission of antibiotic-resistance genes, conjugation is generally considered the most likely mode of transmission because many of these genes are linked to plasmids or transposons. Bacterial plasmids have spread the ESBL and carbapenemase genes, which significantly impact human health, among other resistant genes. ESBL genes can be transferred from *E. coli* through conjugation, and this may explain why there is such a high prevalence of ESBL-producing *E. coli* found through excretion[21].

Enterococcus fecalis isolated from ear infections were completely sensitive to augmentin, cefepime, cephalothin, meropenem, imipenem, and azithromycin. As shown in table (7), *E. faecalis* is frequently resistant to various antimicrobial medications, including aminoglycosides, aztreonam, and quinolones[22]. Numerous drug-resistance genes found on the chromosome or plasmid[23] act as a conduit for the resistance.

Conclusions

Pseudomonas spp has a role in ear infections. In addition to the presence of bacteria *Klebsiella spp, Proteus spp, S. aureus, E. coli* and *Enterococcus fecalies*.The majority of the identified isolates were cephalosporin and penicillin-resistant. The existence of isolates of Proteus and Pseudomonas species that are meropenem-resistant. The presence of vancomycin-resistant *Staphylococcus aureus* isolates.

Author Contributions

Conceptualization, E.J.B. and I.A.A.; methodology, E.J.B. and I.A.A.; software,E.J.B.; validation, A.M.N., I.A.A and I.A.A.; formal analysis, I.A.A.; investigation, E.J.B.; resources, L.H.A.. and I.A.A.; data curation, I.A.A and E.J.B.; writing—original draft preparation, E.J.B.; writ-ing—review and editing, E.J.B. and I.A.A.; visualization, I.A.A.; supervision, A.M.N.; project administration, I.A.A.; funding acquisition, L.H.A., I.A.A and AMN. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

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Synthesis of Cell wall		Synthesis of DNA	
AMOXICILLIN	Resistance	CIPROFLOXACIN	Resistance
BENZYLPENICILLIN	Resistance	LEVOFLOXACIN	Resistance
AUGMENTIN	Resistance	MOXIFLOXACIN	Variable
PIPERACILLIN	Resistance	NORFLOXACIN	Variable
CARVENCILLIN	Resistance	NALIDIXIC ACID	Variable
AMPICLOXIC	Resistance	Synthesis of RNA	
TICARCILLIN	Resistance	RIFAMPICIN	Resistance
IMIPENEM	Sensitive	Metabolism of Folic acid	
MEROPENEM	Sensitive	TRIMETHOPRIM	Resistance
CEFOTAXIME	Sensitive	TRIMETHOPRIM/	Resistance
CEFTRIAXONE	Sensitive	Synthesis of Protein	
CEFIXIME	Resistance	AZITHROMYCIN	Resistance
CEFTAZIDIME	Resistance	AMIKACIN	Variable
CEFDINIR	Resistance	GENTAMYCIN	Variable
CEFPODOXIME	Resistance	TOBRAMYCIN	Variable
CEFEPIME	Sensitive	TIGECYCLINE	Variable
CEPHALOTHIN	Resistance	DOXYCYCLINE	Variable

R: Resistance S: Sensitivity H: Hight

Table 6. Susceptibility test to *Escherichia coli* isolated from otitis media.

Conflicts of Interest

The authors declare no conflict of interest.

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Cell wall synthesis		DNA synthesis	
Amoxicillin	R	Ciprofloxacin (quinolone)	R
Benzylpenicillin	R	Levofloxacin	Variable
Augmentin	HS	Moxifloxacin	Variable
Piperacillin	R	Norfloxacin	Variable
Carbenicillin	R	Nalidixic acid	R
Ampicloxic	R	RNA Synthesis	
Ticarcillin	R	Rifampicin	R
Imipenem	HS	Folic acid metabolism	
Meropenem	HS	Trimethoprim	R
Cefotaxime	R	Trimethoprim with sulfonamide	R
Ceftriaxone	Variable	Protein synthesis	
Cefixime	R	Azithromycin (macrolide)	H.S
Ceftazidime	R	Amikacin (aminoglycoside)	Variable
Cefdinir	R	Gentamycin	R
Cefpodoxime	R	Tobramycin	Variable
Cefepime	HS	Tigecycline (tetracycline)	Variable
Cephalothin	HS	Doxycycline	Variable

R: Resistance S: Sensitivity H: Hight

Table 7. Sensitivity test to *Enterococcus fecalies* isolated from otitis media.

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