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RESEARCH ARTICLE OPEN ACCESS

Isolation and Identification of Pathogenic Bacteria Causing Otitis Media in Misan Governorate

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Abstract

This study examined 150 ear swab samples from patients with otitis media who consulted at Al-Sadr Teaching Hospital from January to April 2021 in Misan, Iraq. The participants were aged 14–50 years, among which the infection rate was highest in participants aged 14–22 years and lowest in those aged 40–50 years. Subsequently, bacterial isolates were identified based on their morphology in various culture media and using biochemical tests. Six bacterial species were identified, namely *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Streptococcus pneumoniae, Klebsiella pneumoniae,* and *Staphylococcus epidermidis*. *Staphylococcus aureus* had the highest infection rate (30%), whereas *Staphylococcus epidermidis* had the lowest infection rate (8.55%). When the sensitivity of each isolate to antibiotics was determined, *Escherichia coli* was the most sensitive to trimethoprim (TMP), whereas *Pseudomonas aeruginosa* was the most resistant to 75% of the tested antibiotics.

Keywords: Otitis Media, Pathogenic bacteria, Antibiotic resistance

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INTRODUCTION

Ear infection (otitis media) is a major public health issue in developing countries, with considerable infection rates and financial costs to patients, communities, and healthcare facilities. Even though it can affect adults, it is a commonly encountered ailment in children, resulting in numerous postoperative appointments in both modern and developing economies.[1] The middle ear effusion (MEE), without symptoms or signs of severe disease, is recognized in approximately 10% of children as a result of acute otitis media. Chronic OME or the absence of purulent fluid in the middle ear, in the absence of constitutional symptoms, can result to significant hearing loss and difficulties in speech, language, and social skills.[2-4,17,30] Otitis media encompasses a spectrum of conditions, including minor acute otitis media, otitis media with effusion, acute otitis media, and chronic otitis media (COM).[5] Improper treatment of OM, including imprecise diagnostic tests or misuse of antibiotics, results in inflamed otitis, frequent incisions; subsequent complications, such as RAOM and persistent middle ear effusion requiring the incorporation of a drainage tube, which frequently results in hearing loss, brain abscess, COM, and/or sepsis.[5-7] In addition, otitis externa is an inflammation of the external auditory canal, which is more prevalent in patients with eczema or diabetes mellitus.

The identification of OM is hampered by a lack of a link between clinical symptoms and the causative microorganism, as well as the pattern of medication susceptibility. Furthermore, the habitual absence of an otoscope in many health institutions, particularly in poorer countries, restricts the ability of the health personnel to make more accurate diagnoses of OM.[14] The cause and pathology of OM are complex.[8,9] Pathogenic infection of the upper respiratory tract is the most significant factor. Virus or virus antigen was identified in the middle ear of 5% to 25% of individuals with AOM.[10,11,15,16]. Bacteria can be found in approximately 40% to 70% of patients.[12,13,18,19] Moreover, a strong association was observed between the bacteria obtained from patients with middle ear OM and the major organisms found in the nasopharynx.[20,21] Bacterial ear infections frequently develop after a throat infection, flu, or other respiratory system infections in children. If the upper respiratory infection is caused by a bacterium, this may travel to the middle ear. If the upper respiratory infection is viral, such as a cold, viruses may be attracted to the habitat of the microorganism and invade the middle ear as a serious infection. Fluid accumulates behind the eardrum because of the illness.[22-24]

Prompt, fast, and successful ear infection treatments can greatly reduce the short- and long-term consequences of ear infection. Furthermore, they can enhance the lives of patients. Thus, it is critical to have the most up-to-date data on the etiology of ear infection and associated antibiotic susceptibility pattern.[25-27] Therefore, this study aimed to isolate and identify the most common bacteria causing OM in patients from the Misan governorate, Iraq, and exploring bacterial sensitivity to most commonly used antibiotics.

MATERIALS AND METHODS

Sample Collection

A total of 150 patients diagnosed with OM by specialists at (name of a clinic) in Misan governorate, Iraq from January to April 2021 was included in this study. The patients were classified according to age, ranging from 14 to 50 years. Swab sample from an irritated area was collected using a sterile cotton swab. Samples were collected on patients who had not taken antibiotics for at least one week prior to swab collection.

Collection and Culturing of Ear Discharge

Firstly, the outer ear was cleaned using regular saline. Then, a sample of the discharge was collected. Swabs were subsequently inoculated onto MacConkey and blood agar plates, which were incubated aerobically at 37°C for 24–48 hours, and mannitol salt agar plates, which were incubated anaerobically at 37°C for 24–48 hours.[28]

Identification of Bacteria

Bacterial colonies were initially identified based on their phenotype and cultural characteristics. Identification of the isolated bacteria was performed according to standard microbiological methods, including cultural characteristics, Gram staining; biochemical reactions, such as catalase, indole urea media, oxidase; diagnostic tests, such as motility, hemolysis, and the triple sugar iron (TSI); and the VITEK 2 Compact.[28,29]

Measurement of Bacterial Sensitivity to Antibiotics

To evaluate the antibiotic susceptibility of bacteria isolated from patients with OM, we performed the procedures developed by.[31] Briefly, Muller-Hinton agar was used for the experiment, except for *S. pneumoniae* isolates, which were grown on 5% blood agar medium. Samples were incubated at 37°C for 24 hours, and then the diameters of growth inhibition zones were measured.[33] Antibiotic discs were chosen based on the frequency of prescription in the study region and the guidelines of the Clinical Laboratory Standards Institute (CLSI) and the European Association on Antibiotic Susceptibility (EUCAST).[32,41] The following antibiotics (Oxoid, UK) were used: amoxicillin (AMC, 30µg) plus clavulanic acid, penicillin G (P-10 U), amoxicillin (AX, 25µg), and trimethoprim (TMP, 10µg).

RESULTS

Study population

A total of 150 patients (age ranging from 14 to 50 years) with OM participated in this study. Of the patients, 50 (33.3%) were aged 14–22 years, 50 (16.6%) were aged 26–30 years, and 20 (13.3%) were aged 30–35 years. The least represented group was the age range 40-50 years, with only seven participants (4.6%) (Table 1).

Table 1. Age distribution of study population

Age range (years)	Frequency (n) (n)	Percentage %
14-22	50	33.3
22-26	30	20
26-30	25	16.6
30-35	20	13.3
35-40	18	12
40-50	7	4.6

Identification of Bacteria

Our results showed that the most dominant isolate was *Staphylococcus aureus* (45/150, 30%), followed by *Pseudomonas aeruginosa* (38/150, 25.5%). *Streptococcus pneumoniae* was identified in 20 cases (13.3%), whereas *Klebsiella pneumoniae* was isolated from 18 participants (12%). *Escherichia coli* and *Staphylococcus epidermidis* were the least frequent bacteria isolated from patients, with 16 (10.6%) and 13 (8.6%), respectively Table 2 and Table 3.

Antibiotic Sensitivity Test

The following antibiotics were used in this study: amoxicillin plus clavulinic acid (AmC 30 g), penicillin G (P-10 U), amoxicillin (AX-25 g), and trimethoprim (TMP 10 g). In general, all bacteria isolated in this study showed high sensitivity to amoxicillin (100%) and a relatively high sensitivity to trimethoprim (83%). Furthermore, 67% of the isolated bacteria were resistant to penicillin, whereas 50% of the isolates were resistant to amoxicillin Table 4.

Overall, Gram-positive isolates were highly sensitive to the antimicrobials used, ranging from as low as 7.5 mm for *Streptococcus pneumoniae* sensitive to amoxicillin and as high as 12.5 mm for *Staphylococcus epidermidis* for amoxicillin and trimethoprim. *Streptococcus pneumoniae* was observed to be resistant to penicillin. For Gram-negative isolates, majority showed high resistance to almost all tested antibiotics. *Pseudomonas aeruginosa* were resistant to all tested antibiotics, except amoxicillin

Table 2. Diagnostic criteria of isolated bacteria

Bacterial Isolates	Gram Stain	Catalase	S.C.	Indol	Motility	Ozidase	TSI	Haemolysis
Staphylococcus aureus	+ve	+ve	+ve	-ve	+ve	-ve	/	β
Streptococcus pneumoniae	+ve	+ve	+ve	-ve	+ve	-ve	/	β
Pseudomonas aeruginosa	-ve	+ve	+ve	-ve	V	+ve	R/R	β
Staphylococcus epidermidis	+ve	+ve	+ve	-ve	V	-ve	/	y
Escherichia coli	-ve	+ve	-ve	+ve	V	-ve	Y/Y	y
Klebsiella pneumoniae	-ve	+ve	+ve	-ve	-ve	-ve	Y/Y/	

+ve - gram positive; -ve - gram negative; V - Variable; R - Red; Y Yellow; B - Beta; Y Gama

(12.5 mm), followed by *Escherichia coli* that reacted as 50% resistant to amoxicillin and penicillin, while showed 50 % sensitivity for amoxicillin (13.5mm) and for trimethoprim with 15 mm inhibition zone measure (Fig. 1).

DISCUSSION

Middle ear inflammation associated with OM is a common childhood and adult disease.[34] This study enrolled 150 patients from the Misan governorate, Iraq, who were screened to determine the causative bacteria of OM and their susceptibility to several commonly used antibiotics. Additionally, we investigated the prevalence of OM across the examined age groups. The findings indicated that women (66%) had a higher prevalence of OM than men (44 %). This observation is consistent with[35,36] who reported that females (66.1%) were more affected by OM than males (33.9 %). Several studies have demonstrated an equal prevalence of AOM in men and women, while numerous previous studies have indicated an increased incidence in boys. This could be a reflection of the environmental conditions (in-depth, recommended study) associated with the onset of OM symptoms.

Table 3. Bacterial types isolated from patients with OM

Bacterial isolates	Number	Percentage %
Staphylococcus aureus	45	30
Streptococcus pneumoniae	20	13.3
Pseudomomas aeruginosa	38	25.3
Staphylococcus epidermidis	13	8.6
Escherichia coli	16	10.6
Klebsiella pneumoniae	18	12

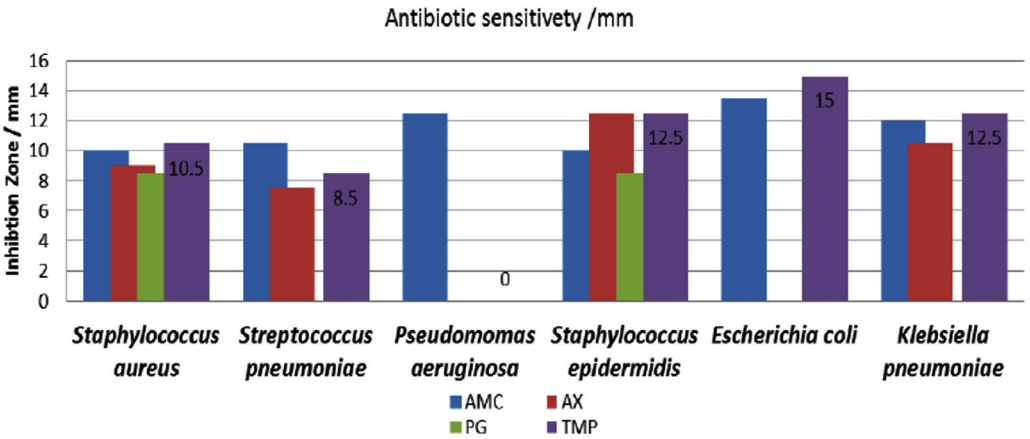

Fig. 1. Antibiotic sensitivity.

Table 4. Antibiotic resistance

	Antibiotic Resistance (mm)			
	AMC	AX	PG	TMP
*Staphylococcus aureus**	10	9	8.5	10.5
*Streptococcus pneumoniae**	10.5	7.5	R	8.5
*Pseudomonas aeruginosa***	12.5	R	R	R
*Staphylococcus epidermidis**	10	12.5	8.5	12.5
*Escherichia coli***	13.5	R	R	15
*Klebsiella pneumoniae***	12	10.5	R	12.5
Sensitivity percent %	100	50	33	83
Resistance percent %	0	50	67	17

*Gram positive bacteria **Gram negative bacteria

Age is a significant factor in determining the percentage of OM infections. In the present study, the highest infected age group was 14–22 (33.3 %), followed by 22–26 (33.3 %) (20%). A low distribution of OM (4.6 %) was discovered in adults aged 40–50. Children are evidently more susceptible to OM due to their susceptibility to upper respiratory tract infections.[37] reported that majority of their subjects were aged of 0–15 years, with 0–5 years accounting for 67.5%. This finding is consistent with the reasons children are more likely to contract OM than adults: children have a more vulnerable immune system and must fight off multiple infectious pathogens. Furthermore, the eustachian tube is a relatively small passageway. A child's ear is shorter and more angular than that of an adult, making it more susceptible to blockage. Additionally, infection with adenoids may contribute to pathogen transmission into the eustachian tube.[38] Other studies discovered that OM was the most prevalent disease in the first two years of children.[39,40]

Majority of the bacteria isolated in this study were Gram-positive (50 %), and the most common isolate was *Staphylococcus aureus* (30 %). *Pseudomonas aeruginosa* represented the majority with 25.3 percent. Similar findings were observed by Roland and Storman,[42] who observed that *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the most dominant bacterial pathogens in OM, and[43-46,55] who reported that *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the most common bacteria among patients with chronic OM.

S. pneumoniae represented 16% of infections isolated from Brazilian participants with severe otitis media, whereas *S. aureus* was responsible for only 1%.[48] *S. pneumoniae* was the most frequently isolated pathogen from the middle ear of children with otitis media drug resistance with (39.69%), whereas *S. aureus* was recovered from 16.03 % of the participants.[47] Geographical heterogeneity may be responsible for the differences in the distribution of bacteria.[49]

This study discovered that amoxicillin plus clavulanic acid (AmC 30 g) was 100% effective against most bacterial isolates from patients with OM, whereas trimethoprim TMP (10 g) was 83% effective. Amoxicillin is primarily bactericidal as a β-lactam antibiotic. It inhibits the third and final stages of bacterial cell wall synthesis by preferentially binding to a specific PBP found in the bacterial cell wall. As is the case with all β-lactam antibiotics, inhibition PBP-mediated cell wall synthesis results in cell lysis.[50,51] Clavulanic acid is a β-lactamase inhibitor with negligible antimicrobial activity, and it acts as an effective "suicide" suppressor of a range of infectious β-lactamases mediated by plasmids and chromosomes.[52] *Pseudomonas aeruginosa* exhibited the highest resistance to 75% of antibiotics tested but showed good sensitivity to amoxicillin. *Escherichia coli* was identified as the most susceptible isolate to trimethoprim (15 mm) and amoxicillin (13.5 mm). Antibiotic resistance is increasing worldwide as a result of antimicrobial misappropriation and overuse.[53]

A number of pediatricians and infectious disease organizations have recently launched a program to limit antibiotic use for competitive reasons.[54,56] The Centers for Disease Prevention and U.S. Centers for Disease Control collaborated to review the prudent use of antimicrobial agents in infectious agents and recommend measures for potential pathogens, including those causing AOM.[57,59] These guidelines emphasize the importance of limiting the use of antimicrobial agents. If these guidelines are adopted, the selective pressure caused by widespread antiviral use may be alleviated. Antimicrobial resistance is a significant challenge to international public health, and such guidelines may help reduce the therapeutic failures caused by drug-resistant bacteria.[58]

CONCLUSION

The findings of this study shed light on pathogenic bacteria that caused OM in patients from Misan, Iraq, and highlighted the phenomenon of antibiotic resistance, which has captured the interest of biological scientists, particularly microbiologists, in recent years. The most frequently isolated organism was *Staphylococcus aureus*, followed by *Pseudomonas aeruginosa*. Antibiotics, such as amoxicillin and trimethoprim, were the most efficient against most of the isolates. We firmly advocate for a nationwide antimicrobial surveillance to ensure appropriate antibiotic recommendations and rigorous adherence to antibiotic use policies and

prevent the spread of drug-resistant bacteria and related consequences throughout the country, particularly in Misan.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript

ETHICS STATEMENT

Permission to conduct this study was issued by the Health institutional; Al-Sadr teaching hospital and the Swabbing from patients was carried out by a public health technician.

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