

Original article

# Prevalence and Identification of Bacterial Species Causing Antibiotic-Resistant Otitis Media in Zawia City, Libya, and Identification of Associated Risk Factors

Mabroukah Khalleefah<sup>\*1</sup>, Najat Mansour<sup>1</sup>, Ali Milad<sup>1</sup>, Suad Diab<sup>2</sup>, Lubna Elbishti<sup>3</sup>, Himedah Fheel Alboom<sup>4</sup>, Khaled Aburas<sup>5</sup>, Najat Gooma<sup>5</sup>

<sup>1</sup>Faculty of Dentistry, University of Zawia, Zawia City, Libya

<sup>2</sup>Faculty of Veterinary Medicine and Agriculture, University of Zawia, Zawia City, Libya

<sup>3</sup>Faculty of Medicine, University of Zawia, Zawia City, Libya.

<sup>4</sup>Faculty of Education, University of Zawia, Zawia City, Libya.

<sup>5</sup>Libyan Center for Medical Research, Zawia City, Libya.

**Corresponding Email.** [m.khalleefah@zu.edu.ly](mailto:m.khalleefah@zu.edu.ly)

## Abstract

Otitis media (OM) is a common infection of the middle ear, often leading to multiple visits to healthcare centers due to complications such as hearing loss and other serious infections, particularly in developing countries. Bacteria are the most common causative agents of OM. This study aimed to identify and differentiate the bacterial species causing OM in Zawia city and to determine their antimicrobial susceptibility patterns. The study was conducted at Al-Zawia Medical Laboratory from January 1, 2024, to June 1, 2024. A total of 200 individuals were included, of which 157 (78.5%) were diagnosed with middle ear infection. The results indicated a high incidence of bacterial OM in the 41-50 age group, with females being more affected than males in this group. Conversely, the 11-20 age group showed a higher infection rate in males. Of the 157 bacterial pathogens isolated, 67 (42.68%) were from male patients and 90 (57.32%) were from female patients. The most prevalent isolates were *Staphylococcus aureus* (16.5%), *Pseudomonas* sp. (14%), and *Klebsiella* sp. (9%). Antimicrobial susceptibility testing showed that Gentamycin exhibited high sensitivity against most isolates. The high rate of bacterial resistance to most tested antibiotics highlights the need to prescribe antibiotics based on local antibiogram data and bacterial sensitivity. Bacterial pathogens were identified phenotypically and culturally, and a significant variation in their susceptibility to antibiotics was observed.

**Keywords.** Prevalence, Otitis Media, Bacterial Species, Risk Factors, Antibiotic Resistance.

## Introduction

Otitis media~ (OM) is defined as inflammation of the ear mucosa and the middle ear cleft [1]. Secretions typically accompany this inflammation. In children, respiratory infections are a common cause of OM, which can lead to hearing loss and speech problems. OM is considered the second leading cause of hearing loss globally. Bacteria are the most common cause of this type of infection due to their ability to resist antibiotics [2-4]. OM represents a major public health challenge worldwide [5,6], particularly in developing countries [7,8]. Ear discharge is one of the most common signs of infection resulting from inflammation caused by certain bacterial species. Worldwide, ear infections affect approximately 330 million people, of whom about 60% suffer from hearing loss. The infection can be acute or chronic and can occur in the outer, middle, or inner parts of the ear [9]. According to the World Health Organization (WHO), 141 million people worldwide suffer from ear infections, and approximately 2.5 billion individuals worldwide will suffer from middle ear infections in one or more forms by 2050 if this type of infection is not controlled. The WHO indicates that the number of people suffering from hearing loss will reach 700 million individuals [10].

Middle ear infection is a significant health problem in children, with 60% to 80% of infections recurring in the first few years of life [11]. The WHO reports that approximately 51,000 children under the age of 5 may die each year in developing countries due to complications from otitis media [12]. Africa ranks second in terms of middle ear infection rates [13]. OM constitutes a major public health challenge in developing countries and has a significant economic impact on patients and their families [14]. This type of inflammation is widespread in developed countries, but it is more common in developing countries. The WHO has classified otitis media as a neglected disease. In most cases, otitis media occurs during childhood and persists into later stages of life.

WHO reports from 2015 indicate that over 5% of the world's population (approximately 328 million adults and 32 million children) suffer from conditions leading to hearing loss. The organization confirmed that the prevalence rate was high in the Africa and Asia region, while half of these problems can be prevented through primary prevention, and the rest can be treated [15]. Antibiotics have been used so widely and for so long that the infectious organisms they were designed to treat have adapted to them, making the drugs less effective [16].

Improper treatment of OM, including inaccurate diagnostic tests or misuse of antibiotics, can lead to chronic otitis media, which may result in complications such as external ear canal inflammation, brain abscesses, and sepsis. These complications are more common in patients with underlying health problems such as diabetes or eczema [17]. The increasing use of antibiotics has led to the development and spread of bacterial

resistance, which is a major concern for global health [18]. Untreated infections can lead to meningitis, and tumors of the middle ear and sinuses can also result [19].

Ear infections can be caused by bacterial, fungal, or viral infections. Bacterial infection varies depending on the source of the infection. Gram-positive bacteria often originate from the nasopharyngeal cavity. Hospital-acquired *Pseudomonas aeruginosa* also plays a role in ear and nose infections [20]. Regarding the incidence of middle ear infections, the infection rate is sometimes reported to be higher in men than in women [21]. Approximately 65 to 330 million people worldwide suffer from ear infections. This type of ear infection includes acute otitis media (AOM), chronic suppurative otitis media (CSOM), otitis media with effusion, and otitis externa. AOM remains a major public health problem worldwide. OM is associated with a reduced quality of life, and in low-income countries, recurrent OM causes more than 25,000 deaths worldwide each year due to intracranial problems [22]. OM is the second leading cause of hearing loss [23]. The WHO reported that the prevalence of chronic otitis media was 5.2% in 2004 in Southeast Asia. Although it is common globally, due to poor hygiene, poor nutrition, and overcrowding, its incidence is higher in developing countries [24].

The human ear is divided into three parts: the inner ear, the outer ear, and the middle ear. Infections are classified accordingly. The most common is otitis media, which can be classified as purulent, including acute suppurative otitis media (ASOM) and chronic suppurative otitis media (CSOM), or non-purulent, known as otitis media with effusion (OME), which refers to the presence of fluid in the middle ear showing signs of inflammation and infection. Chronic otitis media is characterized by the prolonged presence of fluid in the middle ear accompanied by infection [25].

The spread of antibiotic-resistant bacterial species that cause otitis media is a major public health problem, particularly in children and individuals with certain risk factors [18]. The study aims to identify and isolate the most common bacterial species responsible for middle ear infections. Furthermore, it seeks to assess the antimicrobial resistance and sensitivity patterns of these bacteria to various antibiotics and to identify associated risk factors, such as the frequent use of antibiotics. This study aims to improve the understanding of the causes of middle ear inflammation among residents who suffer from ear problems and visit Al-Zawia Medical Hospital.

## Materials and Methods

### Sample Collection

The study was conducted at Al-Zawia Medical Hospital in Al-Zawia city. A total of 200 swabs were collected from patients suffering from middle ear infections, pain, and related problems during the period from January 1, 2024, to June 1, 2024. Information was collected using a special form for each patient. Patients were instructed not to take any antibiotics for at least three to five days prior to swab collection. The samples were transferred to the microbiology laboratory of the Al-Zawia Hospital, as well as the laboratory of the Shorouk Clinic and the Ibn Al-Nafis laboratory in Zawia. A total of 200 cases of different ages (76 males and 124 females) were recorded in patients attending ear clinics at Al-Zawia Medical Hospital with signs and symptoms of otitis media. The number of patients with ear problems was 157, while the number of healthy samples was 43.

### Swab Culture

Swabs from patients with ear problems were cultured on the following media: Thioglycolate Broth, MacConkey agar, Blood agar, Sabouraud Dextrose Agar (Sabouraud agar), and Mannitol Salt Agar. Various diagnostic tools were also used, including Hydrogen peroxide, Human EDTA plasma, Bacitracin diagnostic disc, Optochin diagnostic disc, and API – 20E Kits for biochemical tests.

The dishes were inoculated according to the streaking method. Plates were incubated at 37°C for 18 to 24 hours. Growing colonies were diagnosed using cultural and phenotypic characteristics and biochemical tests for species [26]. The samples were inoculated in Thioglycolate Broth. All inoculated samples were then subcultured on MacConkey agar, Blood agar, and Sabouraud agar. The inoculated plates were examined and further processed for organism identification. Blood plates were checked for the type of hemolysis. The Catalase test was performed for the separation of *Staphylococcus* and *Streptococcus*. Sabouraud agar plates were checked for the growth of fungi. MacConkey agar plates were checked for the growth of Gram-negative bacilli. Biochemical tests (API- 20E KITS) were performed for further identification of organisms. Finally, susceptibility testing was done on Mueller-Hinton agar by adjusting the bacterial suspension against the 0.5 McFarland standard using a VITEK Densicheck [26].

### Antibiotic Sensitivity Testing

To estimate the sensitivity of isolated bacteria to antibiotics, the Kirby-Bauer disk diffusion method was used. A total of 25 types of antibiotics manufactured by a Turkish company were used in this test on Mueller-Hinton agar. The plates were incubated at 37°C for 18 to 24 hours, where a zone of inhibition of bacterial growth began to appear around the disks containing the active antibiotic due to the diffusion of the antibiotic from the filter paper into the agar. The zone of inhibition was measured in millimeters using a transparent

ruler. Bacterial isolates were classified as Sensitive (S), Resistant (R), or Intermediate (I) according to the standards of the National Committee for Clinical Laboratory Standards NCCLS.,1997 [27].

### Statistical Analysis

All statistical analysis was undertaken using the Statistical Package for the Social Sciences (SPSS) software for biostatistical analyses.

### Ethical Approval

The study protocol was reviewed and approved by the Ethics Committee of the Faculty of Dentistry, University of Zawia (Approval No.25). Written informed consent was obtained from all adult participants, and from the parents or legal guardians of participants under the age of 18. All procedures were performed in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

### Results

Table 1 demonstrated significant gender disparities among the 200 otitis media patients in Zawia City. The cohort consisted of 124 females (62%) and 76 males (38%). The notably higher female representation suggests potential correlations between gender and infection rates.

**Table 1. Frequency Distribution of Gender and Percentage of Infection in All Studied Samples**

Gender	Frequency	Percentage
Female	124	62%
Male	76	38%
Total	200	100%

Data presented in Table 2 showed that *Candida albicans* recorded the highest abundance with 39 isolates (19.5%) of the total samples, followed by bacteria *Staphylococcus aureus* with 33 isolates (16.5%). *A. baumannii*, *Staphylococcus epidermidis*, and *Streptococcus* sp. recorded the lowest abundance with 1 isolate each (0.5%) of the total examined samples.

**Table 2. Distribution of Otitis Media Pathogens and Percentage of Infection**

Isolate otitis media	Frequency	Percentage (%)
<i>Staphylococcus aureus</i>	33	16.5%
<i>Pseudomonas aeruginosa</i>	28	14%
<i>Klebsiella</i> sp	18	9%
<i>Candida albicans</i>	39	19.5%
<i>Escherichia coli</i>	16	8%
<i>Streptococcus pyogenes</i>	9	4.5%
<i>Streptococcus agalactiae</i>	6	3%
<i>Enterococcus faecalis</i>	3	1.5%
<i>Protus</i> sp	2	1%
<i>Acinetobacter baumannii</i>	1	0.5%
<i>Staphylococcus epidermidis</i>	1	0.5%
<i>Streptococcus</i> sp	1	0.5%
Total	157	100%

Table 3 illustrates age-specific infection rates. Females (62%) are more affected by otitis media than males (38%). Infection rates peak in the 41-50 age group (17%) and decline thereafter. Females show higher infection rates in the 21-30 (67.74%) and 41-50 (64.71%) age groups, while males have higher infection rates in the 0-10 (51.6%) age group.

Table 4 presents the distribution of bacterial agents responsible for otitis media in relation to gender. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella* sp. were the most common bacterial genera in both genders. Females had higher rates of *Pseudomonas aeruginosa*. (18.9% of female isolates) and *Streptococcus pyogenes* (7.8% of female isolates) infections. Males had higher rates of *Staphylococcus aureus* (25.4% of male isolates) and *Escherichia coli* (9% of male isolates) infections. Ten bacterial genera were identified, highlighting the complexity of the otitis media microflora. Female prevalence (57.3% of total infections) was consistent with the overall sample distribution.

**Table 3. Distribution of Bacteria Isolated from the Study Participants with Otitis Media by Age and Gender**

Age group (years)	Male	Female	Total infection	Percentage (%)
0-10	16	15	31	15.5%
11-20	15	13	28	14%
21-30	10	21	31	15.5%
31-40	9	19	28	14%
41-50	12	22	34	17%
51-60	8	14	22	11%
<61	6	20	26	13%
Total	76	124	200	100%

**Table 4. Distribution of Bacterial Agents of Otitis Media in Relation to Gender**

Sex	Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa	Klebsiella sp	Streptococcus pyogenes	Enterococcus faecalis	Streptococcus agalactiae	Protus sp	Acinetobacter baumannii	Staphylococcus epidermidis	Streptococcus sp	Candida albicans	Total	Percentage %
Male	17	6	11	10	2	2	1	0	1	1	1	15	67	42.68 %
Female	16	10	17	8	7	1	5	2	0	0	0	24	90	57.32 %
Total	33	16	28	18	9	3	6	2	1	1	1	39	157	100%

Table 5 presents the in vitro antibiotic susceptibility profiles of the most prevalent bacterial pathogens isolated from otitis media cases. Among the 33 isolates of *Staphylococcus aureus*, the highest sensitivity was observed against Gentamycin (CN; 72.7%), followed by Amikacin (AK; 51.5%), Imipenem (IPM; 57.5%), and Augmentin (AMC; 60.6%). Notably, resistance was common to Azithromycin (AZM; 15% sensitive) and Clarithromycin (CLR; 15% sensitive). For *Escherichia coli* (n=16), Gentamycin demonstrated 100% susceptibility. High sensitivity was also noted for Amikacin (81.2%) and Imipenem (81.2%), whereas Amoxicillin (AMX; 6.25%) and Fusidic acid (FA; 6.2%) showed poor activity.

*Pseudomonas aeruginosa* (n=28) exhibited the greatest sensitivity to Gentamycin (85.7%) and Imipenem (67.85%). Moderate susceptibility was observed for Amikacin, Ciprofloxacin, and Ceftriaxone (each 53.5%), whereas resistance was notable against Meropenem (MEM; 17.8%) and Cefotaxime (FOX; 17.8%). *Klebsiella* spp. (n=18) were most susceptible to Imipenem (88.8%) and Gentamycin (83.3%). Sensitivity to Amikacin and Cefazidime was moderate (61.1% each). Low susceptibility was recorded for Amoxicillin (0%) and Meropenem (16.6%). *Streptococcus pyogenes* (n=9) showed limited susceptibility across tested antibiotics, with the highest sensitivity to Gentamycin (55.5%) and Tetracycline (TE; 55.5%). No susceptibility was observed for Augmentin, Nitrofurantoin, or Fusidic acid.

Overall, Gentamycin consistently demonstrated the highest efficacy across all major bacterial isolates, suggesting its potential utility as an empirical therapeutic option in the local setting, pending culture and sensitivity results.

**Table 5. Antibiogram Used Against Bacterial Isolates (Sensitivity Percentage)**

Antibiotics	<i>Staphylococcus aureus</i> (n=33)	<i>Escherichia coli</i> (n=16)	<i>Pseudomonas aeruginosa</i> (n= 28)	<i>Klebsiella</i> sp (n=18)	<i>Streptococcus pyogenes</i> (n= 9)
Amikacin (AK)	17 (51.5%)	13 (81.2%)	15 (53.5%)	11 (61.1%)	4 (44.4%)
Amoxicillin (AMX)	9 (27.2%)	1 (6.25%)	8 (28.5%)	0	1 (11.1%)
Augmentin (AMC)	20 (60.6%)	11 (68.7%)	12 (42.8%)	10 (55.5%)	0
Azithromycin (AZM)	5 (15%)	3 (18.7%)	—	—	—
Cefotaxime (CTX)	18 (54.5%)	10 (62.5%)	14 (50%)	9 (47.3%)	—
Cefotaxime (FOX)	7 (21.2%)	10 (62.5%)	5 (17.8%)	4 (22.2%)	—
Bactrim (SXT)	4 (12%)	7 (43.7%)	5 (17.8%)	4 (22.2%)	—

Ceftazidime (CAZ)	9 (27%)	10 (62.5%)	9 (32.14%)	11(61.1%)	—
Ceftriaxone (CRO)	16 (48.4%)	10 (62.5%)	15 (53.5%)	9 (47.3%)	—
Ciprofloxacin (CIP)	15 (45.4%)	7 (43.7%)	15 (53.5%)	9 (47.3%)	1 (11.11%)
Clarithromycin (CLR)	5 (15%)	3 (18.7%)	—	—	—
Imipenem (IPM)	19 (57.5%)	13 (81.2%)	19 (67.85%)	16 (88.8%)	—
Gentamycin (CN)	24 (72.7%)	16 (100%)	24 (85.7%)	15 (83.3%)	5(55.5%)
Meropenem (MEM)	9 (27.2%)	3 (18.7%)	5 (17.8%)	3 (16.6%)	4(44.4%)
Nitrofurantoin (F)	16 (48.4%)	6 (37.5%)	8 (28.5%)	9 (47.3%)	0
Nalidixicacin (NA)	11 (33.3%)	7 (43.7%)	11 (39.28%)	8 (44.4%)	4(44.4%)
Norfloxacin (NOR)	14 (42.4%)	8 (50%)	9 (32.14%)	9 (47.3%)	—
Fusidic acid (FA)	13 (39.3%)	1 (6.2%)	7 (25%)	1 (55.5%)	—
Oxacillin (OX)	14 (42.4%)	3 (18.7%)	7 (25%)	1(55.5%)	—
Tetracycline (TE)	16 (48.4%)	6 (37.5%)	8 (28.5%)	6 (33.3%)	5 (55.5%)
Tobramycin (TOB)	17 (51.5%)	12 (75%)	14 (50%)	9 (47.3%)	1 (11.1%)
Vancomycin (VA)	—	—	1 (3.57%)	—	—

## Discussion

Otitis media is a common problem worldwide and affects people of all ages. It is a cause of hearing loss in all developing and developed countries. It can lead to serious complications such as meningitis, brain abscesses, and hearing loss. In this study, we find that otitis media problems are more common in women than in men. This may be attributed to higher healthcare-seeking behavior among women compared to men [28], which indicates that ear infections are more common in women than in men. Bacteria reach the middle ear in adults through the Eustachian tube. After that, infection occurs, and some cells accumulate and form a yellowish-white fluid in the middle ear. The microbes infect the middle and outer ear. In this study, it was found that bacteria are one of the causes of middle ear infection, as most patients were aged 41-50 years. It is in agreement with [29,30,31] but disagrees with the study of [19] where the prevalence of otitis was in people aged 10-14 years.

In this study, *Staphylococcus aureus* was the most common isolate among other species and the cause of otitis media in general. This is consistent with other studies [29,30,31,32] followed by *Pseudomonas aeruginosa*, but it differs from studies conducted in some developed countries where the results of these studies on the following microbes, *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* were dominant [33]. The remaining bacterial isolates were *Escherichia coli*, *Klebsiella* sp, *Streptococcus pyogenes*, *Enterococcus faecalis*, *Streptococcus agalactiae*, *Protus* sp, *Acinetobacter baumannii*, *S. epidermidis*, and to a lesser extent, *Staphylococcus* sp. The most common fungal species was *Candida albicans*, and this is consistent with [34]. Gentamicin showed high susceptibility rates against all bacterial pathogens. What was the cause of the middle ear infection? Ear infections are among the most common diseases that lead to increased prescription of antibiotics, and this is one of the reasons why pathogenic bacteria become resistant to antibiotics [30]. Bacteria were resistant to antibiotics to some extent [35,36].

## Conclusion

An overall prevalence of 78.5% of otitis media was observed among patients attending Al-Zawi Medical Hospital, Zawia city, Libya. *S. aureus*, *Pseudomonas* sp, and *Klebsiella* sp. were the predominant isolates causing otitis media. Antibiotic susceptibility rates were generally moderate. Otitis media is a microbial infection that affects the middle ear, especially the area directly behind the eardrum. It often occurs as a result of a blockage of the Eustachian tube, as mucus, pus, and microbes accumulate behind the eardrum, causing pain. The dominant bacteria causing otitis media were *S. aureus*, followed by *Pseudomonas aeruginosa*, common in women (57.32%). As for the most affected age group (41-50 years), it has been found that some antibiotics are effective. Gentamicin showed the highest in vitro efficacy against most isolates in this study, suggesting it could be a potent empirical choice pending local antibiogram data. In addition, one should not use cleaning agents and keep the ear dry when using water. Although the use of antibiotics reduces ear infections and infection problems in general, their long-term effect is not known.

**Conflict of interest.** Nil

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