Libyan Med J. 2025;17(4):378-382
https://doi.org/10.69667/lmj.2517403
Libyan Medical Journal
https://lmj.ly/index.php/ojs/index eISSN: 2079-1224

Original article

Bacterial Etiology and Antimicrobial Resistance in Ear Infections: A Cross-Sectional Study from Tripoli, Libya

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Keywords:
Ear Infection, Bacterial Etiologies, Antibiotic Susceptibility, Tripoli, Libya.

ABSTRACT

This cross-sectional study included 100 patients aged 18 to 60 from a teaching hospital in Tripoli, Libya, with an ear infection. Following normal bacteriological procedures, sterile ear swabs were used to collect middle ear secretions from study participants for culture and antimicrobial susceptibility testing. A total of eight bacterial species and one fungal species were recovered. The microbiological analysis of the clinical isolates demonstrated a predominance of Gram-negative bacteria (48.0%), followed by Gram-positive bacteria (34.0%) and fungal isolates (14.0%), while 4.0% of samples showed no microbial growth. Among Gram-negative organisms, Pseudomonas aeruginosa was the most frequently recovered pathogen, accounting for 28.0% of all isolates. Gram-positive bacteria were primarily represented by Staphylococcus epidermidis (16.0%) and Staphylococcus aureus (11.0%). Candida albicans was the only fungal pathogen identified in this cohort. Cefotaxime showed 100% resistance in Pseudomonas and Klebsiella spp., while gentamicin (100% efficacy in Gram-positive cocci; 85.7% in Pseudomonas) and amikacin (100% in Klebsiella; 92.9% in Pseudomonas) were the most effective agents, underscoring the urgent need for targeted antibiotic selection. Gram-negative bacteria, Gram-positive bacteria, and fungi in samples were the leading cause of ear infections, and they were generally susceptible to most of the antibiotics tested. Amikacin, cefepime, gentamicin, ciprofloxacin, and meropenem could be valuable in the empirical management of ear infections.

Introduction

The ear is a vital sensory organ responsible for both hearing and maintaining balance. Anatomically, it is divided into three main sections: the outer ear, middle ear, and inner ear. Among these, the outer and middle ear are particularly vulnerable to infections [1]. Ear infections, especially otitis media, are a significant health concern that affects individuals of all ages, especially young children. This condition involves inflammation of the middle ear and can lead to severe complications, including hearing loss and negative impacts on cognitive development, particularly in children from disadvantaged backgrounds. The high prevalence of ear infections is closely linked to various socio-economic factors, such as limited access to healthcare, malnutrition, poor living conditions, and inadequate preventive health measures like vaccinations [2,3].

Even though fungi and viruses can both cause ear infections, bacterial pathogens are the main causes of these infections, with notable contributors including Staphylococcus aureus, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*. These bacteria can enter the middle ear through multiple routes, including dysfunction of the Eustachian tube or direct extension from nearby anatomical structures. The complex interaction between the host's immune defenses and the virulence factors of bacteria is crucial in influencing the clinical outcomes associated with ear infections [2,4].

Understanding the dynamics of bacterial ear infections involves not only identifying common bacterial strains but also recognizing how various resistance mechanisms can hinder effective treatment options. The global misuse of antibiotics contributes to increasing rates of antimicrobial resistance, a concerning trend that complicates management strategies for widespread conditions like otitis media [5]. Raising awareness about the causes of otitis media is essential for developing effective public health interventions aimed at reducing incidence rates and improving management strategies within affected communities [2,3].

The objective of this study was to isolate and identify the bacterial pathogens associated with otitis media infections and to evaluate their antibiotic resistance profiles. Such data are critical for guiding physicians in the selection of appropriate empirical therapy and for informing the development of effective therapeutic protocols, particularly considering the growing challenge of antimicrobial resistance.

Received: *12-08-2025* - ***Accepted***: *11-10-2025* - ***Published***: *19-10-2025*

Methods

Study design and area

A Cross-sectional study was conducted at Teaching Tripoli Hospital, Libya, over six months from January to June 2025. Clinical ear swab samples were collected from patients presenting with symptoms indicative of otitis media or external ear infections.

Patient Selection and Sample Collection

A total of 100 patients participated in the study, including 60 males and 40 females, aged between 18 and 60 years. Verbal informed consent was obtained from all participants before sampling. The selection of participants for this investigation into bacterial infections among ear patients utilized distinct inclusion and exclusion criteria to ensure both the significance and reliability of the results. The inclusion criteria primarily focused on individuals exhibiting symptoms indicative of ear infections, such as ear pain, hearing impairment, or discharge from the ear. A diverse age range was considered to provide a comprehensive perspective on bacterial infections across various demographics. Notably, those who had previously received antibiotic treatment were included to evaluate how prior medication might influence patterns of bacterial resistance.
Conversely, the exclusion criteria eliminated individuals with conditions that could distort results or impede accurate diagnosis. This included patients who had been administered antibiotics within the month leading up to sample collection, as well as those suffering from chronic ear diseases or other significant comorbidities that could affect infection outcomes. Additionally, individuals who were immunocompromised or had undergone recent ear surgery were also omitted. This careful selection process aimed to reduce variability in patient backgrounds and treatment histories, thereby enhancing the credibility of the study's findings regarding bacterial profiles and resistance to antimicrobials.
Ear swab specimens were obtained under aseptic conditions using sterile cotton swabs. The external ear canal was disinfected with benzalkonium chloride solution (1:1000) to minimize contamination. In cases of tympanic membrane perforation with purulent discharge, samples were aspirated using a sterile suction device by an otolaryngology specialist to ensure the integrity and accuracy of specimen collection. This approach aimed to accurately determine the prevalence of specific bacterial strains responsible for ear infections while effectively evaluating their resistance patterns [2].

Culture and Identification Procedures

Ear discharge specimens were aseptically collected using sterile cotton swabs in accordance with standard operating procedures to prevent contamination. Samples were placed in transport media, properly labeled, and promptly delivered to the microbiology laboratory for processing. Each specimen was inoculated onto MacConkey agar, blood agar, mannitol salt agar, and nutrient agar plates (Oxoid, UK) and incubated aerobically at 37 °C for 24 hours. To control the swarming phenomenon of *Proteus* species, mixed colonies were subcultured onto MacConkey agar. Preliminary identification of pure bacterial isolates was based on colony morphology, Gram stain characteristics, pigment production, hemolytic patterns on blood agar, and standard biochemical assays, including catalase and indole tests. Final identification of bacterial species was confirmed using conventional microbiological techniques as outlined by Cheesbrough [6].

Antibiotic Susceptibility Testing

The antimicrobial susceptibility of the bacterial isolates was assessed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar plates, performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2024). The antibiotic panel tested included amoxicillin, ceftriaxone, erythromycin, ciprofloxacin, gentamicin, tetracycline, and ampicillin. Following incubation at 37 °C for 18–24 hours, zones of inhibition were measured in millimeters and interpreted as susceptible (S), intermediate (I), or resistant (R) based on CLSI-established breakpoints. This standardized method ensures reproducibility and accuracy in determining resistance patterns among clinical bacterial isolates [7].

Processing of data

Statistical Products and Services Software (SPSS), version 25, was used for analysis after the data were imported into Microsoft Excel 2019.

Results

The present study included 100 patients presenting with symptoms of ear infection at a teaching hospital in Tripoli, Libya. The age of participants ranged from 18 to 60 years. Of these, 60 were males (60%) and 40 were females (40%), yielding a male-to-female ratio of 1.5:1. This distribution demonstrates a predominance of male patients within the study cohort.

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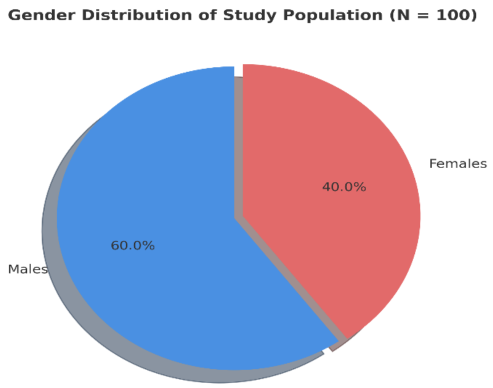

Figure1. Percentage distribution of bacterial isolates according to patient sex

The analysis of 100 clinical isolates reveals a distinct and clinically significant microbiological profile. Gram-negative bacteria were the predominant group, accounting for 48.0% of all isolates, followed by Gram-positive bacteria (34.0%) and fungal isolates (14.0%). While 4% of samples showed no microbial growth.
Among the Gram-negative pathogens, Pseudomonas aeruginosa was the most frequently isolated organism overall (28.0%), followed by Klebsiella pneumoniae (9.0%), Escherichia coli (6.0%), and Enterobacter spp. (5.0%). Gram-positive bacteria accounted for 34% of isolates, with Staphylococcus epidermidis (16%) and Staphylococcus aureus (11%) representing the leading contributors, and Streptococcus pneumoniae (7.0%) further underscores the diversity of Gram-positive threats.
In addition, fungal isolates constituted 14%, with Candida albicans as the only fungal pathogen identified, while 4% of samples showed no microbial growth. The distribution of isolates is presented in (Table 1).

Table 1. Frequency and percentage of microorganisms isolated from patients with Ear Infection (N = 100)

Bacterial Isolates	Frequency	Percentage
Gram-positive bacteria		
Streptococcus pneumoniae	7	7.0%
Staphylococcus epidermidis	16	16.0%
Staphylococcus aureus	11	11.0%
Total	34	34%
Gram-negative bacteria		
Pseudomonas aeruginosa	28	28.0%
Klebsiella pneumoniae	9	9.0%
Enterobacter spp.	5	5.0%
Escherichia coli	6	6.0%
Total	48	48.0%
No growth	4	4.0%
Fungal Isolates		
Candida albicans	14	14.0%

The analysis included just bacterial isolates (organisms enumerated in the susceptibility table: *Streptococcus pneumoniae.* (n = 7), *Staphylococcus aureus* (n = 11), *Staphylococcus epidermidis* (n = 16), *Pseudomonas spp.* (n = 28), *Klebsiella pneumoniae* (n = 9), *Enterobacter spp.* (n = 5), *Escherichia coli* (n = 6). Total bacterial isolates = 82 (sum of the species above). Fungal isolates: *Candida albicans* and “no growth” samples were excluded from these antibiotic susceptibility calculations.

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Table 2. Susceptibility and resistance summary by antibiotic (bacterial isolates, N = 82)

Antibiotic	Susceptible (n)	Susceptible (%)	Resistant (n)	Resistant (%)
Chloramphenicol	48	58.5%	34	41.5%
Gentamicin	71	86.6%	11	13.4%
Amikacin	63	76.8%	19	23.2%
Ciprofloxacin	58	70.7%	24	29.3%
Imipenem	67	81.7%	15	18.3%
Ceftriaxone	58	70.7%	24	29.3%
Cefotaxime	9	11.0%	73	89.0%
Augmentin	45	54.9%	37	45.1%

Antibiotic susceptibility testing of 82 bacterial isolates from hospital-derived ear samples showed variable activity among commonly used antimicrobials: (Gentamicin and imipenem) had the highest in vitro effectiveness (susceptibility rates of 86.6% and 81.7%, respectively), while (cefotaxime) exhibited the highest resistance (89.0% resistant).
The results emphasize the need for targeted antibiotic selection guided by local susceptibility patterns and suggest that routine reliance on (cefotaxime or augmentin) for empiric coverage of these isolates may be inappropriate.

Table 3. The percentage of resistant isolates for each antibiotic, organized by bacterial species

Bacterial Species (Total n)	CRO	CTX	AMC	GEN	AMK	CIP	CHL	IMP
Gram-positive bacteria								
Streptococcus spp. (7)	14.3%	85.7%	14.3%	0.0%	28.6%	28.6%	14.3%	14.3%
Staphylococcus aureus (11)	18.2%	72.7%	9.1%	0.0%	27.3%	27.3%	27.3%	18.2%
Staphylococcus epidermidis (16)	6.3%	81.3%	12.5%	0.0%	25.0%	0.0%	25.0%	6.3%
Gram-negative bacteria								
Pseudomonas spp. (28)	57.1%	100%	71.4%	14.3%	7.1%	21.4%	50.0%	10.7%
Klebsiella spp. (9)	22.2%	100%	66.7%	11.1%	0.0%	77.8%	66.7%	22.2%
Enterobacter spp. (5)	40.0%	80.0%	60.0%	40.0%	60.0%	20.0%	40.0%	60.0%
Escherichia coli (6)	0.0%	83.3%	66.7%	66.7%	83.3%	83.3%	66.7%	50.0%

Antibiotic Key: CRO, Ceftriaxone; CTX, Cefotaxime; AMC, Amoxicillin-clavulanate (Augmentin); GEN, Gentamicin; AMK, Amikacin; CIP, Ciprofloxacin; CHL, Chloramphenicol; IMP, Imipenem.

The results revealed 100% resistance to cefotaxime among *Pseudomonas* spp. and *Klebsiella spp.*, confirming its lack of efficacy as an empirical treatment option. High resistance rates were also observed in Gram-negative bacilli, with *E. coli* showing resistance to amikacin (83.3%), ciprofloxacin (83.3%), and amoxicillin-clavulanate (66.7%), while *Klebsiella spp.* Displayed 77.8% resistance to ciprofloxacin and *Enterobacter* spp. Exhibited 60% resistance to imipenem. In contrast, the most consistently effective antibiotics were gentamicin (100% efficacy against Gram-positive cocci; 85.7% susceptibility in *Pseudomonas*; 88.9% in *Klebsiella*), amikacin (92.9% susceptibility in *Pseudomonas*; 100% in *Klebsiella*), and imipenem (maintaining 50% susceptibility in *E. coli* and 40% in *Enterobacter* spp.). Overall, Gram-positive cocci were more susceptible, particularly to gentamicin (100%) and augmentin, with *Staphylococcus epidermidis* showing 100% susceptibility to ciprofloxacin.

Discussion

The current study of 100 clinical isolates from hospital settings in Tripoli demonstrates a concerning predominance of Gram-negative bacteria (48.0%), followed by Gram-positive bacteria (34.0%) and fungal isolates (14.0%), with *Pseudomonas aeruginosa* accounting for 28.0% of all isolates. This pattern is consistent with findings in recent studies that identify *Pseudomonas aeruginosa* as a leading pathogen in hospital-acquired otic infections [8]. The high occurrence of *Staphylococcus epidermidis* and *Staphylococcus aureus*

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among Gram-positive isolates aligns with global data showing that these cocci continue to contribute significantly to ear and surface-derived infections (Prevalence of bacterial ear infections and multidrug resistance [9].

The antibiotic susceptibility profile reveals extreme resistance to cefotaxime, with *Pseudomonas* and *Klebsiella* spp. Showing 100% resistance in the current series. Recent literature also indicates rapidly increasing resistance among *P. aeruginosa* to cephalosporins and other β-lactams [10]. Gram-negative rods such as *E. coli* manifested high resistance to multiple agents, including amikacin and ciprofloxacin, a trend similarly reported in chronic suppurative otitis media (CSOM) studies (Antimicrobial Resistance Patterns in *Pseudomonas aeruginosa* Isolated from CSOM [11].

In contrast, aminoglycosides (gentamicin, amikacin) and imipenem retained higher efficacy, particularly against Gram-positive cocci and certain Gram-negative isolates. This correlates with previous work showing gentamicin remains effective in many otic pathogens, albeit with concerns about toxicity and the emergence of resistance [12]. However, the noted imipenem resistance in *Enterobacter* spp. in this dataset highlights an emerging threat, similar to those observed in other regional surveillance studies (Karachi CSOM data), where carbapenem resistance is now being documented in Gram-negative otic isolates [11].

These combined findings suggest that empirical use of cefotaxime is no longer advisable in this setting, especially against *Pseudomonas* and *Klebsiella*. Efficient treatment should rely more on agents with demonstrated preserved activity, alongside ongoing surveillance, stewardship interventions, and possibly local antibiogram-guided prescribing practices.

Conclusion

This study delineates a critical microbiological profile of hospital-derived ear infections in Tripoli, Libya, characterized by a significant preponderance of Gram-negative bacteria (48.0%), with Pseudomonas aeruginosa emerging as the single most prevalent pathogen (28.0%). The accompanying antimicrobial susceptibility pattern reveals a challenging clinical environment, marked by profound resistance to commonly used agents. Most notably, universal resistance to cefotaxime (100%) was observed in key pathogens like Pseudomonas and Klebsiella spp., effectively invalidating its use for empirical therapy. Conversely, aminoglycosides (gentamicin and amikacin) and carbapenems (imipenem) demonstrated sustained, though not absolute, efficacy, positioning them as more reliable therapeutic options. Collectively, these findings underscore an alarming trend toward multidrug resistance among Gram-negative isolates. This necessitates an urgent paradigm shift in clinical management.

Conflict of interest. Nil

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Received*: 12-08-2025 -* ***Accepted****: 11-10-2025 -* ***Published****: 19-10-2025*