Original article

Antibiotic Resistance Patterns of Enterobacteriaceae Isolated from Tobruk Medical Centre

Rema Tahir[1], Amane Amhamed[1], Manal Mahjoob[2]

[1]Department of Zoology, Faculty of Science, University of Tobruk, Tobruk, Libya
[2]Tobruk Medical Centre, Libya
Corresponding email. rema.karaiam@tu.edu.ly

Abstract
Antimicrobial resistance poses a major threat to human health around the world. Resistance of the Enterobacteriaceae to antibiotics, especially of the β-lactam type, is increasingly dominated by the mobilization of continuously expressed single genes that encode efficient drug-modifying enzymes. This study from Tobruk Medical Centre characterized resistance patterns in Enterobacteriaceae across different antimicrobial classes. Enterobacteriaceae from hospital samples were isolated on blood and MacConkey agar. Stool samples were cultured on S.S. agar, Sorbitol MacConkey agar, and Mueller-Hinton broth for swab inoculation. To determine antimicrobial susceptibility patterns, the multidrug resistance disc diffusion method was used. The findings revealed that Escherichia coli was the most prevalent bacterial isolate, followed by Klebsiella spp., while lower prevalence rates were observed for other organisms, such as Proteus and Enterobacter. This study demonstrated a markedly high resistance rate to cephalosporins, which showed the highest percentages among both inpatient and outpatient isolates, with significantly greater resistance observed in inpatient settings. Carbapenems exhibited moderate resistance levels compared with other antimicrobial classes, whereas aminoglycosides showed substantially increased resistance among inpatient isolates. Quinolones also demonstrated relatively high resistance rates in both patient groups. Analysis based on specimen type indicated that blood and abscess samples exhibited the highest antimicrobial resistance rates, particularly against cephalosporins, while carbapenems and aminoglycosides displayed comparatively lower resistance in certain specimen categories. Furthermore, Klebsiella spp. isolates demonstrated elevated resistance to ceftriaxone and ampicillin compared with other bacterial isolates. These findings highlight the growing prevalence of antimicrobial-resistant bacterial strains, especially in hospital environments, emphasizing the urgent need for strengthened infection prevention and control strategies, implementation of antimicrobial stewardship programs, and continuous surveillance of resistance patterns to improve treatment outcomes and limit the future spread of resistant pathogens.
Keywords. Bacteria, Drug Resistance, Gram-negative Clinical isolates.

Introduction

Antimicrobial resistance has risen worldwide and contributes to severe mortality in settings with limited diagnostic and healthcare [1]. The worldwide emergence of multi-drug resistant (MDR) E. coli and Klebsiella is a growing public health issue [2]. Also, this phenomenon is a great danger to inpatients, especially those hospitalized, which can lead to resistant infections and consequently a high mortality rate [3]. Once effective drugs that held promise in 1940 for eradicating infectious diseases around the world are no longer effective, as antimicrobial resistance is gradually becoming more common [4].

Antimicrobial resistance is the most pressing healthcare issue, as it correlates with longer hospital stays, increased mortality, and greater healthcare costs [5,6]. The Enterobacteriaceae family, which includes key members like Escherichia coli, Klebsiella pneumoniae, and Salmonella spp., is implicated in the dissemination of resistance genes (such as the mcr-1 gene) [7]. This bacterial group includes clinically important pathogens such as Klebsiella spp. and Proteus spp., which are commonly associated with urinary tract infections, bloodstream infections, and gastrointestinal infections. The irrational use of antimicrobial agents in both hospital and community settings has accelerated the emergence and spread of resistant bacterial strains, thereby reducing the effectiveness of commonly prescribed antibiotics [8].

Accordingly, this study aimed to evaluate the antimicrobial resistance patterns among Enterobacteriaceae isolates obtained from different clinical specimens, compare resistance rates between inpatient departments (IPD) and outpatient departments (OPD), analyze the distribution of predominant bacterial isolates, and identify the antibiotics exhibiting the highest and lowest resistance rates.

Methods

Study Design and Population

This cross-sectional study was conducted from January 2024 to September 2025, focusing on samples received at the microbiology unit from which microorganisms of the Enterobacteriaceae family were isolated.

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Sample Collection

The clinical specimens included in this study were urine, stool, blood, upper and lower respiratory tract samples, wounds, urethral swabs, and others, submitted for bacterial cultures in the microbiology unit of Tobruk Medical Center. The samples were collected aseptically at hospital wards and sent to the laboratory.

Isolation and Identification of Enterobacteriaceae

The received samples were inoculated immediately on blood agar and MacConkey agar. Muller-Hinton broth was used for swab inoculation. Stool samples were inoculated into S.S. agar, Sorbitol MacConkey agar, and Selenite broth, then incubated aerobically at 37°C overnight. After incubation, the isolated microorganisms were identified by the morphological growth of colonies, fermentation type, Gram staining, and biochemical tests.

Antimicrobial Susceptibility Testing

The identified Enterobacteriaceae colonies were suspended in sterile saline to a 0.5 McFarland standard and inoculated onto Muller-Hinton agar. This disc diffusion method was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The zone diameters were measured (in mm) and interpreted as Sensitive, Intermediate, or Resistant according to the latest CLSI standards.

The antimicrobial agents used belonged to the following classes: penicillins, cephalosporins, carbapenems, aminoglycosides, and quinolones. The concentrations of the antibiotic discs were: Ampicillin (10 µg), Ampicillin-sulbactam (10/10 µg), Amoxicillin-clavulanic acid (20/10 µg), Ceftazidime (30 µg), Cefepime (30 µg), Ceftriaxone (30 µg), Cefotaxime (30 µg), Amikacin (30 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), Imipenem (10 µg), Meropenem (10 µg), Ertapenem (10 µg), and Trimethoprim-sulfamethoxazole (1.25/23.75 µg).

Results

A total of 496 bacterial isolates were obtained from clinical samples. The most prevalent organism was Escherichia coli, accounting for 219 isolates (44.2%), followed by Klebsiella spp. with 105 isolates (21.2%). Other significant Enterobacterales included Proteus spp. (23 isolates), Enterobacter spp. (12 isolates), and Citrobacter spp. (11 isolates). A complete breakdown of the species isolated is presented in (Table 1).

Table 1. Distribution of bacterial isolates

Species	Total number of isolates
Escherichia coli	219
Klebsiella spp	105
Proteus spp	23
Enterobacter spp	12
Citrobacter spp	11
Serratia spp	10
Pantoea spp	8
Salmonella spp	8
Shigella spp	2
Morganella spp	1
Other non-enterobacterial	97

The antimicrobial resistance profiles varied among the three most common bacterial species. Notably, resistance to ampicillin was high across all groups, with Klebsiella spp. showing the highest rate at 93.3%. Resistance to carbapenems was observed but varied significantly; Proteus spp. showed 0% resistance to imipenem and ertapenem, while Klebsiella spp. exhibited resistance rates of approximately 40% for all three carbapenems tested. High levels of resistance to fluoroquinolones were also observed, particularly in Klebsiella spp. (52.8% for ciprofloxacin). Detailed resistance percentages are shown in (Table 2).

Table 2. Resistance percentages by species

Antibiotics	E. coli (n=219)	Klebsiella spp (n=105)	Proteus spp (n=23)
Ampicillin	81.2%	93.3%	88%
Ceftriaxone	64%	70%	64%
Ceftazidime	53.4%	66.3%	56%
Cefepime	43.5%	63.1%	32%
Imipenem	9.7%	40.9%	0%
Meropenem	10.1%	40%	4.1%
Ertapenem	12%	39%	0%
Amikacin	5.1%	32.6%	4.1%

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Antibiotics	E. coli (n=219)	Klebsiella spp (n=105)	Proteus spp (n=23)
Gentamicin	19.7%	40.3%	29.1%
Ciprofloxacin	40.6%	52.8%	32%
Levofloxacin	29.3%	40%	32%
Trimethoprim-Sulfamethoxazole	48.4%	51.1%	71.4%

A comparison of resistance rates between outpatient and inpatient departments revealed that inpatients had consistently higher rates of resistance for most antimicrobial classes. The most pronounced difference was observed for aminoglycosides, where resistance in inpatients (46.5%) was more than five times higher than in outpatients (8.2%). Resistance to cephalosporins and carbapenems was also notably higher in the inpatient setting (Table 3).

Table 3. Resistance by the patient department

Enterobacterial class	OPD	IPD
Cephalosporins	32.5%	57.6%
Carbapenems	12.6%	27.8%
Aminoglycosides	8.2%	46.5%
Quinolones	32.5%	31.2%

Resistance rates also varied considerably depending on the specimen source. Isolates from blood specimens exhibited the highest resistance rates to cephalosporins (71.4%) and carbapenems (47.6%). In contrast, isolates from stool samples showed the lowest resistance rates, particularly to carbapenems (4.1%) and quinolones (5.5%). High-level quinolone resistance was noted in isolates from abscesses (44.8%). The data for each specimen type is presented in (Table 4).

Table 4. Resistance by specimen type

Antimicrobial class	Blood	Urine	Abscess	Stool
Cephalosporins	71.4%	43%	62.5%	58%
Carbapenems	47.6%	13.8%	38.8%	4.1%
Aminoglycosides	25%	7.7%	32%	-
Quinolones	25%	31.8%	44.8%	5.5%

Discussion

In this study, we analyzed antimicrobial resistance among Enterobacteria isolates from Tobruk Medical Center. The most widespread organism was E. coli (44.2%), followed by Klebsiella spp. (21.2%). This is consistent with global and regional reports identifying these species as the dominant Enterobacteria in clinical settings [9]. Resistance to ampicillin exceeded 80% across all major isolates, reaching 93.3% in Klebsiella spp. This renders ampicillin therapeutically useless in this setting. Similarly, resistance to third-generation cephalosporins (ceftriaxone) ranged from 64% to 70% [10], suggesting widespread circulation of extended-spectrum beta-lactamase (ESBL) producing strains. However, the slightly lower resistance to cefepime (32–63%) indicates that this fourth-generation cephalosporin may retain some activity.

On the other hand, the most concerning finding is the high carbapenem resistance observed, particularly in Klebsiella spp. (40.9%, 40%, and 39%). This is considered one of the highest rates in the region. In comparison, a multicenter study in Tripoli reported carbapenem resistance in Klebsiella spp. between 15% and 20% [11]. The detection of such high-level carbapenem resistance in Tobruk Medical Center strongly suggests the presence of carbapenemase-producing Enterobacteriaceae (CPE), expressing enzymes such as NDM, KPC, or OXA-48-like enzymes. Notably, Proteus spp. showed 0% resistance to imipenem and ertapenem, which is consistent with intrinsic reduced permeability or different resistance mechanisms characteristic of this genus [12].

While amikacin remained an effective agent against E. coli (5.1% resistance) and Proteus spp. (4.1%), Its activity against Klebsiella spp. was compromised (32.6% resistance). Gentamicin resistance ranged from 19.7% (E. coli) to 40.3% (Klebsiella). Ciprofloxacin resistance exceeded 40% in both E. coli (40.6%) and Klebsiella (52.8%). These findings mirror trends observed across the MENA region and indicate that empirical therapy with these agents is no longer reliable [13].

There is a clear difference between the resistance profiles of hospitalized patients (inpatients) and outpatients. Inpatients showed more than a five-fold higher resistance rate to aminoglycosides (46.5% vs 8.2%) and significantly higher resistance to cephalosporins (57.6% vs 32.5%) and carbapenems (27.8% vs 12.6%) [14]. This variation could be due to several factors: greater antibiotic selection pressure within hospitals, longer duration of hospitalization, invasive procedures, and nosocomial transmission of resistant clones [15].

Isolates from blood specimens exhibited the highest resistance rates to cephalosporins (71.4%) and carbapenems (47.6%). This is clinically critical because bloodstream infections require prompt empirical therapy; high resistance in this group increases the risk of inappropriate initial antibiotic selection and subsequent mortality [16]. In contrast, stool isolates showed the lowest resistance rates, particularly to carbapenems (4.1%) and quinolones (5.5%), likely reflecting community-acquired rather than healthcare-associated strains.

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Conclusion

This study revealed a critical level of antimicrobial resistance among Enterobacteriaceae isolates at Tobruk Medical Center, with E. coli and Klebsiella spp. being the predominant organisms. The frighteningly high resistance to ampicillin (>80%), third-generation cephalosporins (64–70%), and carbapenems (up to 40.9% in Klebsiella) indicates the widespread presence of ESBL- and carbapenemase-producing strains, posing a serious threat to empirical therapy and infection control. Significantly, carbapenem resistance in Tobruk is notably higher than previously reported in other Libyan regions, suggesting an urgent need for local surveillance and stewardship. While amikacin and cefepime retain some activity against certain species, their reliability is compromised in others, particularly Klebsiella. The marked difference in resistance between inpatient and outpatient isolates highlights the impact of nosocomial transmission and antibiotic selection pressure, while the elevated resistance in bloodstream isolates underscores the clinical urgency for rapid diagnostics and targeted therapy. In conclusion, these findings demand immediate implementation of antimicrobial stewardship programs, enhanced infection control measures, and routine surveillance to curb the spread of multidrug-resistant Enterobacteriaceae in Tobruk Medical Center and across Libya.

Conflict of interest. Nil

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