Attahadi Medical Journal
2026;3(3):271-277
https://doi.org/10.69667/amj.26301

OPEN ACCESS

Original article

Prevalence of Carbapenem-Resistant Gram-Negative Enterobacteriaceae among Patients in Sebha Medical Center

Mohammed Nasr[1], Khatema Moukhtar[2]*, Haleemah Ishteewi[2]

[1]Medical Laboratory Technology Department, Faculty of Medical Technology, Sebha University, Libya
[2]Zoology Department, Faculty of Science, Sebha University, Libya
Corresponding email: Moh.Abdalrhman@sebhau.edu.ly

Keywords:
Carbapenem-Resistant, Gram-Negative, Enterobacteriaceae, Patients, Sebha Medical Center.

ABSTRACT

Infections caused by multidrug-resistant (MDR) bacteria are a major cause of morbidity and mortality in hospitals. This study aimed to isolate MDR bacteria from intensive care units (ICUs) at Sabha Medical Center. A cross-sectional observational study was conducted in the period between September 2025 and March 2026 at Sabha Medical Center. A total of 128 samples were collected. Collected samples were pus swabs, urine, blood, and sputum. Identification and antimicrobial susceptibility tests were applied based on the recommendation of the Clinical and Laboratory Standards Institute. The total Gram-negative bacterial strains isolated were 59 (46%), with the predominance of Escherichia coli (45%) followed by Klebsiella pneumoniae (21%) and Acinetobacter (11%). Different antibiotics were used against these gram-negative isolates. Escherichia coli, Klebsiella pneumoniae, Acinetobacter, Proteus spp., Enterobacter spp., Pseudomonas aeruginosa and Pasteurella spp. showed a high resistance rate to the most used antibiotics and especially to carbapenems. This high prevalence of antibiotic resistance requires strict infection control, continuous monitoring, and collaborative action to monitor infection spread in hospitals.

Introduction

Patients in intensive care units (ICUs) are susceptible to nosocomial infections due to underlying diseases, device use, and prior antibiotic use. Nosocomial infections are associated with morbidity, mortality, and increased costs [1]. ICU patients are at a high risk of colonization and infection caused by multidrug-resistant organisms (MDROs). Patients with extended ICU stays have higher rates of infection by resistant organisms. MDROs, including methicillin-resistant Staphylococcus aureus, vancomycin-resistant enterococci (VRE), multi-drug resistant Acinetobacter baumannii (MRAB), and Enterobacterales that produce extended-spectrum β-lactamases (ESBL), are associated with ICU-related nosocomial infections [2]. Of these MDROs, Enterobacterales, an order of Gram-negative bacteria, harbor common pathogens for healthcare-associated infections, including pneumonia, bacteremia, and urinary tract infections.

Based on the nature of the source, antibiotics can be grouped into the following: (i) natural compounds obtained from microorganisms; (ii) semi-synthetic members that are structurally modified natural products; and (iii) synthetic products. Natural antibiotics (benzylpenicillin, cephalosporins, and gentamicin) present a critical inconvenience, high toxicity, whereas semi-synthetic (ampicillin and amikacin) and synthetic antibiotics (moxifloxacin and norfloxacin) ones exhibit an augmented therapeutic effect and a lower toxicity as compared to natural antibiotics [3].

β-lactam antibiotics are one of the most popular classes of antibiotics, having a specific signature of the presence of the β-lactam ring, with the differences between the members of the class being made by the attached side chain or additional cycles. The representatives of this class are penicillins (presenting a thiazolidine ring and a side-chain that differs for each member), cephalosporins (possessing a dihydrothiazine ring and two side chains), carbapenems (a thiazolidine ring structure slightly different from penicillins), and monobactams (with a β-lactam ring and no adjacent ring) [4].

Recently, among MDROs, carbapenem-resistant Enterobacterales (CRE) have emerged as a threat for ICU patients [5] because of limited treatment options and high mortality rates [6]; therefore, CRE outbreaks, especially in the ICU, have become a critical issue. These organisms are significant causes of healthcare-associated infections, including bloodstream infections, ventilator-associated pneumonia, urinary tract infections, surgical-site infections, and device-associated infections. Less commonly, they can cause meningitis and intra-abdominal infection [7]. A major clinical concern is the development of intrinsic and acquired multidrug resistance. The principal intrinsic mechanism is chromosomal AmpC β-lactamase, which may be inducible or derepressed and confers resistance to many β-lactams, including third-generation cephalosporins. Additionally acquired determinants, notably plasmid-mediated extended-spectrum β-lactamases and carbapenemases such as KPC and NDM, further restrict therapeutic options [8, 9]. Thus, our study aimed to isolate MDR bacteria from intensive care units (ICUs) at Sabha Medical Center and illustrate the sensitivity of Carbapenems against these isolates.

Received: *30-04-2026 -* ***Accepted:*** *28-06-2026 -* ***Published:*** *06-07-2026*

Attahadi Medical Journal
2026;3(3):271-277
https://doi.org/10.69667/amj.26301

Methods

Study design

A cross-sectional observational study was conducted in the period between September 2025 and March 2026 at Sabha Medical Center. The study was approved by the Institutional Ethics Committee. All patients were fully informed regarding the study's objectives, significance, and any potential risks involved prior to participation in this study.

Inclusion and Exclusion Criteria

In the current study, the patients included were >18 years and immunocompetent. While pediatric patients, HIV-positive patients, and patients receiving chemotherapy and radiotherapy were excluded.

Sampling

A total of 128 clinical specimens—including pus swabs, urine, blood, and sputum—were collected from ICU, FMW, MMW, FSW, and MSW patients. Specimens were inoculated onto blood agar under anaerobic conditions and MacConkey agar (No. 3) to isolate Gram-negative and other clinically relevant bacterial species. The culture plates were incubated at 37°C for 18 to 24 hours. Additionally, Mueller-Hinton agar was employed for standard antibiotic susceptibility testing, in accordance with the manufacturer's recommendations (Oxoid). All isolates were identified based on colony morphology and Gram staining. Biochemical tests were applied for Gram-negative isolates using the Analytical Profile Index (API 20E) system to assess a broad range of enzymatic activities and sugar fermentation patterns, including the metabolism of various carbohydrates, gas production, and specific enzymatic reactions.

Antimicrobial Susceptibility Testing

Based on the recommendation of the Clinical and Laboratory Standards Institute (M100 document) [10], antimicrobial susceptibility testing was performed. The diffusion method was applied to test the susceptibility to different antibiotics, as illustrated in Table 1.

Table 1: The antimicrobial agents used in the susceptibility test

S.N	Antimicrobial Agents	Symbol	Disc content
1	Levofloxacin	LVX	5µg
2	Ertapenem	ETP	10µg
3	Imipenem	IMP	10µg
4	Meropenem	MEM	10µg
5	Ciprofloxacin	CIP	5µg
6	Gentamicin	GM	10µg
7	Ceftriaxone	CRO	30µg
8	Ticarcillin/Clavulanic acid	TCC	15-10/75µg
9	Trimethoprim/Sulfamethoxazole	SXT	23.75/1.25µg
10	Cefoxitin	FOX	30µg
11	Amoxicillin/Clavulanic acid	AMC	30µg

Results

Gram staining revealed that 59 isolates (46%) out of 128 collected clinical samples were gram-negative, and the remaining (54%) were gram-positive isolates (Figure 1).

Received: *30-04-2026 -* ***Accepted:*** *28-06-2026 -* ***Published:*** *06-07-2026*

Attahadi Medical Journal
2026;3(3):271-277
https://doi.org/10.69667/amj.26301

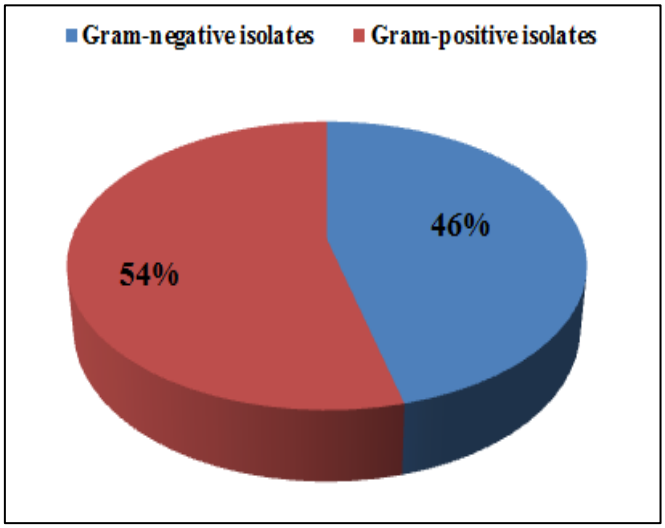

Figure 1: Distribution percentage of gram-negative and positive isolates among clinical samples.

Screening the effect of different antibiotics on gram negative isolates revealed that 38 isolate (64.40%) out of the 59 gram-negative isolate are resistant, while only 21 isolates (35.59%) were sensitive (figure 2). *Escherichia coli* showed the highest resistance (70.80%) to Ceftriaxone, Ceftazidime and Meropenem. Klebsiella spp. Showed the highest resistance rate to Amoxicillin and Ceftazidime (73.30%). Acinetobacter spp and Proteus spp possess high resistance rate to Amoxicillin (71.4% and 80%, respectively) and to Ceftriaxone (71.4% and 80%, respectively). Pseudomonas spp was the most resistant species by being resistant to most antibiotics. Seven gram negative species (*Escherichia coli, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa, Proteus mirabilis, Enterobacter and Pasteurella sp.*) are resistant to all members of carbapenem class of antibiotics. Only *Citrobacter* possess sensitivity to carbapenem family (table 2, figure 3).

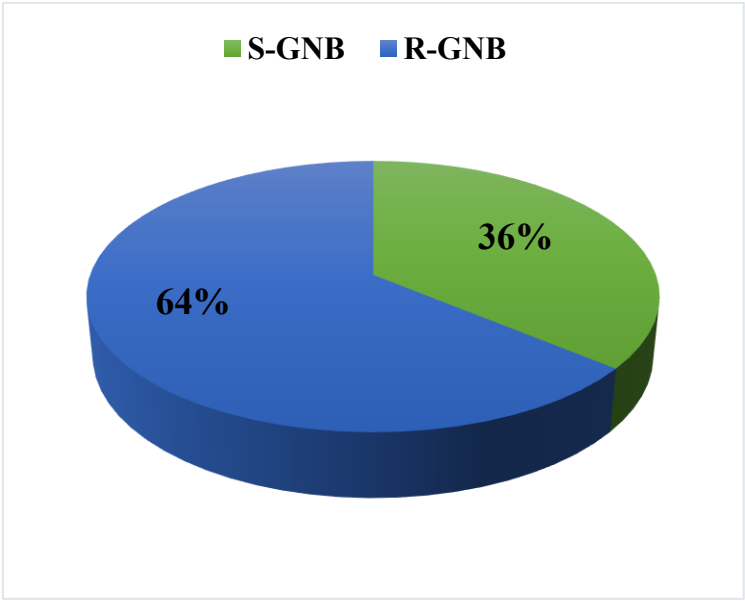

Figure 2: Distribution percentage of resistant and sensitive Gram-negative isolates

Table 2: Resistance pattern of gram-negative bacterial species (59) against different classes of antibiotics

Antibiotics	E coli (n=24)	Klebsiella spp (n=15)	Acinetobacter spp (n=7)	Proteus spp (n = 5)	Enterobacter spp (n=4)	Pseudomonas spp (n =2)	Citrobacter (n = 1)	Pasteurella (n =1)
Ciprofloxacin	54.16%	53.30%	57.10%	40%	25%	0%	100%	100%
Levofloxacin	50%	33.30%	28.50%	40%	50%	0%	0%	100%
piperacillin/ Tazobactam	50%	53.30%	57.10%	20%	50%	50%	100%	100%

Received: 30-04-2026 - **Accepted**: 28-06-2026 - **Published**: 06-07-2026

Attahadi Medical Journal
2026;3(3):271-277
https://doi.org/10.69667/amj.26301

Amoxicillin	62.50%	73.30%	71.40%	80%	100%	100%	100%	100%
Amikacin	12.50%	26.60%	42.80%	0%	25%	100%	0%	0%
Aztreonam	37.50%	53.30%	57.10%	0%	50%	100%	0%	0%
Cefoxitin	45.80%	53.30%	71.`40%	20%	75%	100%	100%	100%
Ceftriaxone	70.80%	66.70%	71.40%	80%	50%	100%	100%	100%
Ceftazidime	70.80%	73.30%	57.14%	60%	50%	100%	0%	0%
Gentamycin	45.80%	33.30%	42.80%	40%	25%	0%	100%	100%
Nitrofuran	20.80%	60%	57.10%	40%	25%	100%	0%	0%
Trimethoprim	58.30%	40%	14.20%	60%	50%	50%	0%	0%
Imipenem	62.50%	40%	57.10%	20%	50%	0%	0%	100%
Meropenem	70.80%	53.30%	57.10%	60%	100%	100%	0%	100%
Ertapenem	66.67%	53.30%	57.10%	80%	25%	50%	0%	100%

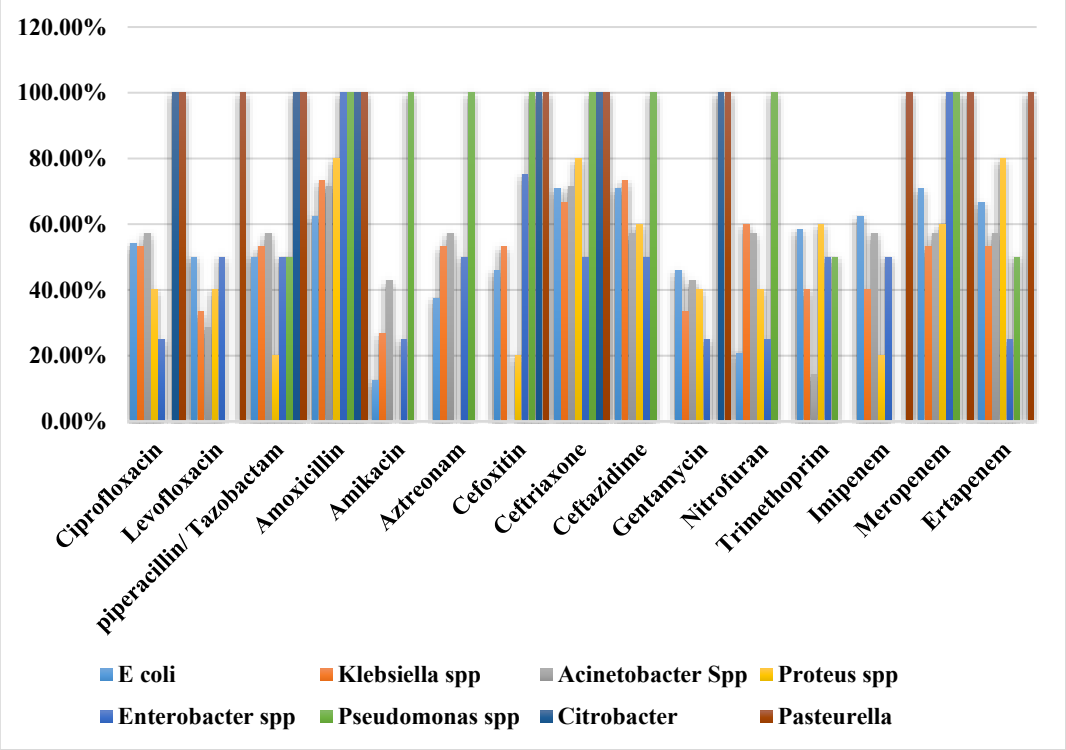

Figure 3: Resistance pattern of Gram-negative bacterial species (59) against different classes of antibiotics

The resistant Gram-negative isolates were classified into 7 species, as illustrated in Figure 4. The highest distributed species was Escherichia coli (45%) followed by Klebsiella pneumoniae (21%), Acinetobacter (11%), Proteus (11%), Enterobacter species (5%), Pseudomonas aeruginosa (5%), and Pasteurella (3%).

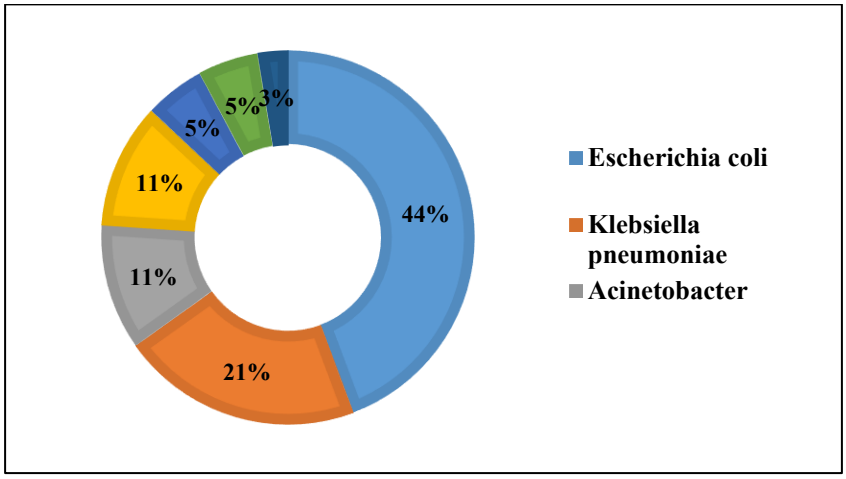

Figure 4: The percentage of the 7 detected resistant Gram-negative isolates.

Received: 30-04-2026 - **Accepted**: 28-06-2026 - **Published**: 06-07-2026

Attahadi Medical Journal
2026;3(3):271-277
https://doi.org/10.69667/amj.26301

The distribution of each species of the resistant gram-negative isolates in the different collected clinical samples (blood, urine, Sputum and wound) was represented in Table 3 and Figure 5. Escherichia coli and Proteus were highly recorded in urine (82.35% and 75%, respectively), while Klebsiella pneumonia and Acinetobacter were highly recorded in Sputum (50% and 75%, respectively). Enterobacter species was detected only in pus samples and Pasteurella was recorded only in urine sample. The presence of resistant species in blood was the lowest by detecting only 3 species Escherichia coli, Klebsiella pneumonia and Pseudomonas aeruginosa (Table 3 and Figure 5).

Table 3: Distribution of the 7 resistant gram-negative bacterial species among different clinical samples

Organisms	Sputum	Blood	Urine	Wound (pus)
Escherichia coli (n=17)	1(5.88%)	1(5.88%)	14(82.35%)	1(5.88%)
Klebsiella pneumoniae (n=8)	4(50%)	1(12.5%)	2(25%)	1(12.5%)
Acinetobacter (n=4)	3(75%)	-	-	1(25%)
Proteus (n = 4)	-	-	3(75%)	1(25%)
Enterobacter species (n=2)	-	-	-	2(100%)
Pseudomonas aeruginosa (n =2)	-	1(50%)	1(50%)	-
Pasteurella (n =1)	-	-	1(100%)	-

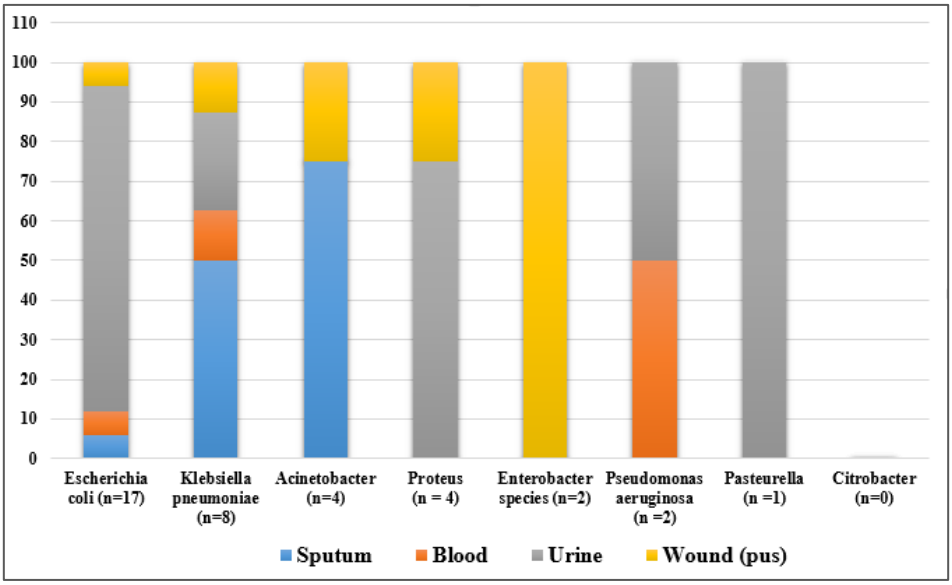

Figure 5: Distribution of the 7 resistant gram negative bacterial species among different clinical samples.

Discussion

Patients in intensive care units (ICU) are more susceptible to nosocomial pathogens, especially multidrug-resistant (MDR) bacteria. Hospital outbreaks can result from the spread of bacteria from patients, health care workers, and ICU medical equipment and vice versa, causing cross-infection and contamination [11]. Gram-negative bacteria such as *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Acinetobacter baumannii* are major pathogens associated with hospital-acquired infections [12].

The frequency of MDR among different microorganisms is different. In the recent study, 46% of the detected bacteria were gram-negative and 54% are gram- positive and the frequency of the resistant gram-negative bacteria (64%) is higher than that of sensitive gram-negative bacteria (36%). The majority of the gram-negative bacteria were MDR isolates with a rate of 64%. The rate of MDR bacteria was 57% in critically ill elderly Egyptian patients [13] and 36.6% in cerebrospinal fluid in [14] study. In a study conducted at Tobruk Medical Center, Libya, researchers reported a resistance rate of 23.5% [15]. The fact that the resistance rate in our study exceeded the 60% threshold, compared with the previous reported rate, clearly highlights the magnitude of the epidemiological challenge in the study area.

The current study showed the high prevalence of MDR *Enterobacteriaceae*; mainly *Escherichia coli* (45%), *Klebsiella pneumoniae* (21%), and *Acinetobacter* (11%). In Sudan, *K. pneumoniae was* reported as the highest prevalent (80%) MDR bacterium according to [16]. In Libya, *K. pneumonia* showed the highest rates of MDRs (91%) [17]. Ibrahim et al. [18] study showed the predominance of *A. baumannii* (44%) followed by *K. pneumoniae* (40%), and *P. aeruginosa* (5.3%) at intensive

Received: *30-04-2026 -* ***Accepted:*** *28-06-2026 -* ***Published:*** *06-07-2026*

care units at Tripoli University Hospital. *E. coli* and *K. pneumonia* were the predominant pathogens and this was consistent with a previous study in Libya [19].

More than 40% of Enterobacterales were Extended-Spectrum β-Lactamase (ESBL) producers. This high prevalence likely results from extensive use of cephalosporins and fluoroquinolones without adequate antimicrobial stewardship oversight, promoting horizontal spread of ESBL genes [19]. ESBL bacteria possess resistance against β-lactam antibiotics, including cephalosporins and carbapenems.

In the current study, all the detected isolates (*Escherichia coli, Klebsiella pneumonia, Acinetobacter, Proteus spp., Enterobacter spp., Pseudomonas aeruginosa and Pasteurella spp*.) showed high resistance level to carbapenem family (Imipenem, Meropenem and Ertapenem). In [19] study, Carbapenem resistance reached 42% in *A. baumannii* and 18% in *K. pneumonia*. Our results are in agreement with the previous studies of [20, 21]. The high prevalence of ESBL- and carbapenem-resistant pathogens compromises empiric therapy, increases preventable deaths and strains healthcare systems in low- and middle-income settings [22].

Conclusion

Our study shows the high prevalence of multi-drug resistance gram negative bacteria in Libya. This high prevalence is associated with increased mortality, prolonged hospitalization, and frequent inappropriate empiric therapy. There is an urgent need to tolerate treatment using antibiotics, and infection control programs should be applied to improve outcomes.

References

1. Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, Moreno R, Lipman J, Gomersall C, Sakr Y, Reinhart K. International study of the prevalence and outcomes of infection in intensive care units. Jama. 2009 Dec 2;302(21):2323-9.
2. Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA, Fridkin SK. Antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. Infection Control & Hospital Epidemiology. 2008 Nov;29(11):996-1011.
3. Oloke JK. Activity pattern of natural and synthetic antibacterial agents among hospital isolates. Microbios. 2000 Jan 1;102(403):175-81.
4. James CW, Gurk-Turner C. Cross-reactivity of beta-lactam antibiotics. InBaylor University Medical Center Proceedings 2001 Jan 1 (Vol. 14, No. 1, pp. 106-107). Taylor & Francis.
5. Guh AY, Bulens SN, Mu Y, Jacob JT, Reno J, Scott J, Wilson LE, Vaeth E, Lynfield R, Shaw KM, Vagnone PM. Epidemiology of carbapenem-resistant Enterobacteriaceae in 7 US communities, 2012-2013. Jama. 2015 Oct 13;314(14):1479-87.
6. Doi Y. Treatment options for carbapenem-resistant gram-negative bacterial infections. Clinical Infectious Diseases. 2019 Nov 13;69(Supplement_7):S565-75.
7. Tobin EH, Zahra F. Nosocomial infections. InStatPearls [Internet] 2025 Aug 2. StatPearls Publishing.
8. Husna A, Rahman MM, Badruzzaman AT, Sikder MH, Islam MR, Rahman MT, Alam J, Ashour HM. Extended-spectrum β-lactamases (ESBL): challenges and opportunities. Biomedicines. 2023 Oct 30;11(11):2937.
9. Zeng Z, Wei Y, Ye C, Jiang Y, Feng C, Guo T, Song M, Ding Y, Zhan P, Liu J. Carbapenem-resistant Enterobacter cloacae complex in Southwest China: molecular characteristics and risk factors caused by NDM producers. Infection and Drug Resistance. 2024 Dec 31:1643-52.
10. Weinstein MP, Limbago B, Patel J, Mathers A, Campeau S, Mazzulli T, Eliopoulos G, Patel R, Galas M, Richter S. M100 Performance Standards for Antimicrobial Susceptibility Testing. Clinical and Laboratory Standards Institute; Wayne, PA, USA: 2018
11. Galvin S, Dolan A, Cahill O, Daniels S, Humphreys H. Microbial monitoring of the hospital environment: why and how?. Journal of Hospital Infection. 2012 Nov 1;82(3):143-51.
12. Atia A. Antibiotic resistance in Libya and the prevalence of antibiotic self-medication: A review. Razi Medical Journal. 2025 Mar 22:2-6
13. Hassan MA, Abd El-Aziz S, Elbadry HM, El-Aassar SA, Tamer TM. Prevalence, antimicrobial resistance profile, and characterization of multi-drug resistant bacteria from various infected wounds in North Egypt. Saudi Journal of Biological Sciences. 2022 Apr 1;29(4):2978-88.
14. Abdelkader MM, Aboshanab KM, El-Ashry MA, Aboulwafa MM. Prevalence of MDR pathogens of bacterial meningitis in Egypt and new synergistic antibiotic combinations. PloS one. 2017 Feb 16;12(2):e0171349.
15. Fathi S, Hassan H, Alfageih L. Prevalence of carbapenem-resistant Enterobacteriaceae isolated from clinical samples in Medical Tobruk Center. Razi Medical Journal. 2025 Jul 21:126-36.
16. Osman EA, Yokoyama M, Altayb HN, Cantillon D, Wille J, Seifert H, Higgins PG, Al-Hassan L. Klebsiella pneumonia in Sudan: multidrug resistance, polyclonal dissemination, and virulence. Antibiotics. 2023 Jan 21;12(2):233.
17. Elramalli A, Almshawt N, Ahmed MO. Current problematic and emergence of carbapenemase-producing bacteria: a brief report from a Libyan hospital. The Pan African Medical Journal. 2017 Mar 29;26:180.

***Received**: 30-04-2026 - **Accepted**: 28-06-2026 - **Published**: 06-07-2026*

18. Ibrahim K, Thwood D, ELgheriani H, Salem M, Elgadiym Z, Zaghdani A, Alhudiri I, Habibi A, Elfahem A, Belaid S, Ermithi O. Prevalence of multi-drug resistant bacteria in intensive care units at Tripoli University Hospital, Tripoli, Libya. Libyan Journal of Medicine. 2024 Dec 31;19(1):2348235.
19. Alwashaish MM. Clinical outcomes and risk factors associated with bloodstream infections caused by multidrug-resistant Gram-negative bacteria in hospitalized patients: a prospective cohort study in Libya. Journal of Medical Microbiology. 2025 Nov 27;74(11):002101.
20. Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant Enterobacteriaceae: the impact and evolution of a global menace. The Journal of infectious diseases. 2017 Feb 15;215(suppl_1):S28-36.
21. Grundmann H, Glasner C, Albiger B, Aanensen DM, Tomlinson CT, Andrasević AT, Cantón R, Carmeli Y, Friedrich AW, Giske CG, Glupczynski Y. Occurrence of carbapenemase-producing Klebsiella pneumoniae and Escherichia coli in the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE): a prospective, multinational study. The Lancet infectious diseases. 2017 Feb 1;17(2):153-63.
22. Allegranzi B, Pittet D. Healthcare-associated infection in developing countries: simple solutions to meet complex challenges. Infection Control & Hospital Epidemiology. 2007 Dec;28(12):1323-7.

Received*: 30-04-2026 -* ***Accepted****: 28-06-2026 -* ***Published****: 06-07-2026*