Moustafa et al | Article 8961
J Pure Appl Microbiol. 2023;17(4):2453-2466. doi: 10.22207/JPAM.17.4.43
Received: 02 September 2023 | Accepted: 11 November 2023
Published Online: 30 November 2023

P-ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Antimicrobial Susceptibility of *Klebsiella pneumoniae* Isolated from Intensive and Non-intensive Care Units Patients: A One-year Retrospective Study in a Tertiary Healthcare Hospital, Saudi Arabia

Nouran Magdy Moustafa[1,2]*, Fatma M. Mahmoud[2], Noor Wael Bu Khamsin[3], Fatima Almomen[3], Manar Alali[3], Maryam Abosbaih[3], Diyaa Mazen Khalaf[3] and Rania A. Mohamed[2]

[1]Basic Medical Science Department, College of Medicine, Dar Al Uloom University, Riyadh, Saudi Arabia.
[2]Medical Microbiology & Immunology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt.
[3]College of Medicine, Dar Al Uloom University, Riyadh, Saudi Arabia.

Abstract

Antimicrobial-resistant *Klebsiella pneumoniae(K. pneumoniae)* constitutes a major global health warning and is significantly implicated in severe infections associated with increased morbidity and mortality. As hospitalized patients in the ICU are more vulnerable to severe infections with increased cost of treatment and prolonged hospital stays, we aimed to compare antimicrobial susceptibility of *K. pneumoniae* obtained from intensive care unit (ICU) and non-intensive care unit (non-ICU) patients as well as to investigate potential impact of antimicrobial resistance on patient outcome. A retrospective, cross-sectional study conducted on ICU and non-ICU patients having *K. pneumoniae* infection during 2021 at Prince Mohammed bin Abdulaziz Hospital (PMAH) in Riyadh. Data regarding *K. pneumoniae* and their antimicrobial susceptibility, were retrieved and analyzed through R Software. 229 *K. pneumoniae* were isolated, 33.2% from ICU patients, and 66.8% from other departments. Most of the patients were males (66.8%) belonged to the older age group (62.9%). The isolates were obtained from endotracheal aspirate, sputum, blood, urine and wound samples. The ICU patients developed higher resistance to all examined antibiotics than non-ICU ($p<0.001$). More than 60% of ICU Klebsiella isolates were extended-spectrum β-lactamases (ESBL) and multidrug resistant (MDR) compared to non-ICU isolates ($p<0.001$). The most effective drugs were amikacin, imipenem, and meropenem, but their effectiveness substantially decreased against MDR strains. There was a statistically significant difference between the MDR, ESBL, and sensitive groups regarding hospital stay and mortality ($P< 0.001$). ICUs have exhibited a remarkable increase in MDR *K. pneumoniae*, which has a negative impact on patient outcomes.

Keywords: Antimicrobial Susceptibility, *K.pneumoniae*, MDR, ESBL, ICU, Hospital Stay, Mortality

***Correspondence:** nouran.m@dau.edu.sa

Citation: Moustafa NM, Mahmoud FM, Khamsin NWB, et al. Antimicrobial Susceptibility of *Klebsiella pneumoniae* Isolated from Intensive and Non-intensive Care Units Patients: A One-year Retrospective Study in a Tertiary Healthcare Hospital, Saudi Arabia. *J Pure Appl Microbiol.* 2023;17(4):2453-2466. doi: 10.22207/JPAM.17.4.43

INTRODUCTION

Since intensive care unit (ICU) patients are critically ill and frequently subjected to invasive procedures, they are more prone to severe infections caused by aggressive organisms. Furthermore, antimicrobial resistance is one of the most significant threats to ICU patients as it complicates their treatment, along with prolonged hospital stays, high costs, and severe adverse outcomes.[1] According to the World Health Organization (WHO), "AMR is one of the top 10 global public health threats facing humanity".[2] The uncontrolled use of antimicrobials, the overworked healthcare workforce, and the prolonged exposure to invasive equipments are important causes of antimicrobial resistance especially during pandemics.[3]

In the recent years, *Klebsiella pneumoniae (K. pneumoniae),* one of the major Enterobacterales, has emerged as a clinically significant pathogen because of increased antibiotic resistance and its propensity to cause serious outcomes.[4] It has been ranked the second among Gram-negative bacteria in causing hospital-acquired infections.[5] *K. pneumoniae is* recognized as the primary cause of serious nosocomial infections such as respiratory infections, urinary tract infections, soft tissue infections, bacteremia, and sepsis.[6] They are also responsible for device-associated infections in ICU patients.[7] The pathogenicity of *K. pneumoniae* is influenced by the presence of capsule, lipopolysaccharide (LPS), and other cell wall proteins that allow the organism to bind to the host cells and protect against phagocytosis.[8] Furthermore, the ability of *K. pneumonie* to form a thick layer of biofilm is considered as an important virulence factor. Biofilms permit bacteria to attach to biotic or abiotic surfaces, protect microorganisms from opsonization by antibodies, phagocytosis, and removal via the ciliary action of epithelial cells.[9] In addition, resistance to antibacterial and disinfectant agents is noticeably more among bacterial populations in biofilms than free-living planktonic cells.[10]

Multidrug resistance (MDR) is defined as "acquiring non-sensitivity to one or more agents in at least three groups of antimicrobials".[11] Multidrug"resistant gram-negative bacteria (MDR-GNB) are the leading cause of limited therapeutic options or even antimicrobial treatment failure among critically ill patients.[12] It has been revealed that indiscriminate use of antimicrobials is the main drive for these multidrug-resistant organisms' (MDROs) appearance and rapid spread.[13] Recently, *K. pneumoniae producing* extended-spectrum β-lactamases and carbapenemases have significantly spread and exhibited a high degree of multidrug resistance.[14] They become capable of hydrolyzing most antibiotics, including cephalosporins and carbapenems, which are the primary treatment options for the severe infections caused by Gram-negative bacilli.[15] According to the WHO, MDR *K. pneumoniae* pose a great public health threat and considered high-priority pathogens for research and development of novel antibiotic therapies.[16]

The lack of effective treatment for multidrug-resistant pathogens and the resulting increase in mortality has led to an urgent need for finding a radical solution to reduce the nightmare of spread of MDR bacteria all over the world.[17] One of these important solutions is an increased focus on the preventive strategies against the transmission of these pathogens. Several organizations have established prevention plans to implement an infection prevention and control criteria in order to break the chain of transmission and halt the spread of MDR-GNB.[18] The WHO urges clinicians to implement antimicrobial stewardship as a potential solution to this substantial increase in antimicrobial resistance.[19] Antimicrobial stewardship programs have been linked to great reduction in antibiotics use, hospital stay, and medical costs with significant decrease in mortality.[20] Nevertheless, before the application of any stewardship program, surveillance of antimicrobial resistance is required to provide data on the prevalent MDROs and their antimicrobial resistance pattern.[21] Several studies have shown the importance of routine microbiological surveillance in guiding the empirical antimicrobial treatment which is essential for tackling nosocomial infections. Providing routine information on the isolates' susceptibility could offer timely evidence regarding their resistance trend rather than the traditional

methods of surveillance networks.[22] So, we aimed at this work to do surveillance study to determine antimicrobial resistance pattern of *K. pneumoniae* obtained from ICU and non-ICU patients in Prince Mohammed bin Abdulaziz Hospital in Saudi Arabia, as well as to investigate potential impact of antimicrobial resistance on patients outcomes.

METHODS

Study setting and design

A one-year retrospective, cross-sectional study was conducted from January 2021 to December 2021 at Prince Mohammed bin Abdulaziz Hospital (PMAH), one of the Ministry of Health hospitals and a major referral hospital in Riyadh with 500 beds. The hospital serves patients over the age of 12 years with diagnostic and therapeutic means of the second and third levels of internal medicine specialties and surgical acute and chronic ones.

Data collection

The demographic and clinical data regarding patients having *K. pneumoniae* infections were retrieved from the patients' medical records and microbiology laboratory information system at PMAH. For each patient, the following information was collected: age, sex, the type of sample positive with *K. pneumoniae*, the results of antimicrobial susceptibility of isolates, and the clinical outcome, including length of hospital stay and mortality.

K. pneumoniae were isolated from various microbiological samples, including (endotracheal aspirate, sputum, blood, urine, and wound) from ICU and non-ICU patients (1 per patient). In this study, we aimed to compare the antimicrobial susceptibility results of *K. pneumoniae* isolated from ICU patients to those from non-ICU departments and investigate the potential effects of antimicrobial resistance on patients' outcome.

Processing of microbiological specimens and identification of isolated organisms

The samples were processed, and the isolates were identified in accordance with the microbiology laboratory standard procedures.[23] All the media used in the isolation and identification of the pathogens were from (Oxoid, UK). All the collected specimens were inoculated onto the conventional culture media and allowed to grow for 18-24 h at 37°C. If no growth appears, incubation of the agar plates will continue for 48 hours. Based on microscopic examination and staining reactions, colonial morphology, and the VITEK 2 compact system (bioMerieux, Craponne, France), *K. pneumoniae* isolates were identified.

Antimicrobial susceptibility

Kirby-Bauer disk diffusion test was performed to determine bacterial isolates' susceptibility against antibiotics, and the results were interpreted based on the Clinical Laboratory Standards Institute (CLSI) guidelines.[24] The antibiotic discs used were obtained from Oxoid. Controls such as *P.aeruginosa* (ATCC 27853) and *E. coli* (ATCC 25922) were utilized for antimicrobial susceptibility tests.

The antibiotics with the subsequent concentrations were tested: ampicillin (10µg), Amoxicillin- Clavulanate(30µg), amikacin (30µg), ciprofloxacin (5µg), gentamycin (10µg), co-trimoxazole (25µg), cefoxitin(30µg), ceftriaxone (30µg), ceftazidime (30µg), cefepime (30µg), imipenem (10µg), meropenem(10µg), nitrofurantoin(100µg), and piperacillin-tazobactam (100/10µg) from HiMedia Laboratories, India. The zone diameters of these discs were evaluated in accordance with the latest CLSI guidelines.[24] The isolates were routinely tested for ESBL and MDR production.

- **ESBL**: A difference of 5 mm in the zone size between ceftazidime and ceftazidime+ clavulanic acid discs confirms ESBL production.[25]
- **MDR:** The Centers for Disease Control and Prevention (CDC) defines MDR isolates as acquired non-sensitivity to one or more agents in at least three groups of antimicrobials.[26]

Based on the antimicrobial susceptibility, patients were categorized into three groups: ESBL-*K. pneumoniae* group (patients with ESBL-*K. pneumoniae* infections), MDR-*K. pneumoniae* group (patients with MDR-*K. pneumoniae* infections), non-ESBL, non-MDR *K. pneumoniae* group (patients with susceptible *K. pneumoniae* Infections).

Statistical analysis

Data management & descriptive statistics

After discussing the protocol and the study objectives as well as data collection and verification, all data was fed into R Software version 3.5.2 (2018-12-20) — "Eggshell Igloo." for statistical analysis. Quantitative data were represented as mean & Standard deviation, while qualitative categorical variables were represented by numbers and percentages.

Analytical statistics

- All the comparative analysis was done using the Chi-square test or Fisher exact test.

Statistically significant results are considered if the p-value ≤ 0.05.

RESULTS

The current study aims to provide background information on the pattern of local antibiotic resistance, which is necessary for prescribing empirical antibiotic treatment against certain pathogens. This data will also be significant as a guide for implementing antimicrobial stewardship. Thus, the study will present the antimicrobial susceptibility pattern of one of the most prevalent organisms, *K. pneumoniae*, retrieved from different sites of infection among both ICU and non-ICU patients as well as the clinical sequelae during the 1-year retrospective study.

Distribution of *K. pneumoniae* isolates, among ICU and non-ICU patients, according to age, gender, and sample types

The study was conducted on 229 *K. pneumoniae* isolates collected from ICU patients (33.2%) and non-ICU patients (66.8%). More than 50% of patients belonged to the older age group (63% e"60 years), and the mean age of ICU and non-ICU patients was 55 and 65 years, respectively. The ratio of males to females was 2.04 /1. The ratio of males to females in the ICU and non-ICU departments was 2.5:1 and 1.9:1, respectively.

Most isolates were obtained from endotracheal aspirate samples (28.4%). The ICU isolates were retrieved mostly from endotracheal aspirates, sputum, and blood samples (52.6%, 19.7 %, and 14.5%, respectively), and few were from urine & wound samples (28.8% and 28.1%, respectively), while the remainder were endotracheal aspirate, blood, and sputum samples (16.3%,14.4%,12.2%, respectively) (Table 1).

Antimicrobial susceptibility of *K. pneumoniae* isolates

Isolates obtained from ICU patients were more resistant to all antibiotics than those isolated from non-ICU patients. The rate of ampicillin resistance was the highest, with 100% resistance among ICU *Klebsiella* isolates and 98.0% among non-ICU isolates without any statistically significant difference (p=0.553). Then, ceftriaxone showed significantly higher resistance, 82.9% among ICU isolates vs. 51.0% among

Table 1. Comparative analysis for baseline patients' characteristics & sample types of ICU vs. non-ICU patients

Baseline characteristics		ICU isolates N= 76 (33.2%)	Non-ICU isolates N= 153 (66.8%)	Total N= 229 (100%)
Age	Mean ± SD	55.0 ± 17.8	56.0 ± 16.8	55.7 ±17.1
	(<60)	35(%46)	50 (32.7%)	85(37.1%)
	(>60)	41(%54)	103 (67.3%)	144(62.9%)
Gender	Female	22 (20.3%)	54 (35.3%)	76 (33.2%)
	Male	54(71.1%)	99(64.7%)	153(66.8%)
Sample type	Endotracheal aspirate	40 (52.6%)	25 (16.3%)	65(28.4%)
	Sputum	15 (19.7%)	19 (12.4%)	34(14.8%)
	Blood	11 (14.5%)	22 (14.4%)	33(14.4%)
	Urine	6 (7.9%)	43 (28.1%)	49(21.4%)
	Wound	4 (5.2%)	44 (28.8%)	48(21%)

*Data are represented as count (%)

non -ICU isolates (p<0.001), and ceftazidime showed significantly higher resistance, 80.3% among ICU isolates vs. 49.0% among non -ICU isolates (p<0.001). Resistance to trimethoprim-sulphamethoxazole was significantly higher among ICU isolates, 77.6% versus 41.4% among non-ICU isolates (p<0.001). Amoxacillin-clavulanate showed a significantly higher resistance 69.7% rate vs. 30.1% in non-ICU (p<0.001). It was found that 67.1 % of ICU isolates were resistant to cefoxitin, compared to 26.8% of non-ICU isolates(p<0.001). ICU isolates were significantly more resistant to cefepime than non-ICU isolates, 64.5% vs. 35.3%, respectively (p<0.001). Ciprofloxacin, gentamycin,

Table 2. Comparative analysis for different antimicrobial susceptibility of ICU vs. non-ICU klebsiella isolates

Antibiotics susceptibility		ICU isolates N= 76 (33.2%)	Non- ICU isolates N= 153 (66.8%)	Total N= 229 (100%)	P value
AK (Amikacin)	Resistant	43 (56.6)	32 (20.9)	75 (32.8)	<0.001
	Sensitive	32 (42.1)	121 (79.1)	153 (66.8)	
	Intermediate	1 (1.3)	0 (0.0)	1 (0.4)	
AMC (Amoxicillin Clavulanate)	Resistant	53 (69.7)	46 (30.1)	99 (43.2)	<0.001
	Sensitive	18 (23.7)	98 (64.1)	116 (50.7)	
	Intermediate	5 (6.6)	9 (5.9)	14 (6.1)	
AMP (Ampicillin)	Resistant	76 (100.0)	150 (98.0)	226 (98.7)	0.553
	Sensitive	0 (0.0)	3 (2.0)	3 (1.3)	
FEP (Cefepime)	Resistant	49 (64.5)	54 (35.3)	103 (45.0)	<0.001
	Sensitive	27 (35.5)	98 (64.1)	125 (54.6)	
	Intermediate	0 (0.0)	1 (0.7)	1 (0.4)	
FOX (Cefoxitin)	Resistant	51 (67.1)	41 (26.8)	92 (40.2)	<0.001
	Sensitive	25 (32.9)	111 (72.5)	136 (59.4)	
	Intermediate	0 (0.0)	1 (0.7)	1 (0.4)	
CAZ (Ceftazidime)	Resistant	61 (80.3)	75 (49.0)	136 (59.4)	<0.001
	Sensitive	13 (17.1)	76 (49.7)	89 (38.9)	
	Intermediate	2 (2.6)	2 (1.3)	4 (1.7)	
CRO (Ceftriaxone)	Resistant	63 (82.9)	78 (51.0)	141 (61.6)	<0.001
	Sensitive	13 (17.1)	74 (48.4)	87 (38.0)	
	Intermediate	0 (0.0)	1 (0.7)	1 (0.4)	
CiP (Ciprofloxacin)	Resistant	50 (65.8)	39 (25.5)	89 (38.9)	<0.001
	Sensitive	26 (34.2)	114 (74.5)	140 (61.1)	
GN (Gentamycin)	Resistant	50 (65.8)	40 (26.1)	90 (39.3)	<0.001
	Sensitive	26 (34.2)	113 (73.9)	139 (60.7)	
IMP (Impenem)	Resistant	46 (60.5)	34 (22.2)	80 (34.9)	<0.001
	Sensitive	30 (39.5)	119 (77.8)	149 (65.1)	
MEM (Meropenem)	Resistant	46 (60.5)	33 (21.6)	79 (34.5)	<0.001
	Sensitive	30 (39.5)	118 (77.1)	148 (64.6)	
	Intermediate	0 (0.0)	2 (1.3)	2 (0.9)	
NF (Nitrofurantoin)	Resistant	50 (65.8)	45 (29.4)	95 (41.5)	<0.001
	Sensitive	15 (19.7)	78 (51.0)	93 (40.6)	
	Intermediate	11 (14.5)	30 (19.6)	41 (17.9)	
TZP (Pipracillin-Tazobactam)	Resistant	47 (61.8)	35 (22.9)	82 (35.8)	<0.001
	Sensitive	26 (34.2)	106 (69.3)	132 (57.6)	
	Intermediate	3 (3.9)	12 (7.8)	15 (6.6)	
SXT (Sulpha-Trimethoprim)	Resistant	59 (77.6)	63 (41.4)	122 (53.5)	<0.001
	Sensitive	17 (22.4)	89 (58.6)	106 (46.5)	

*Data are represented as count (%)

and nitrofurantoin showed 65.8% resistance among ICU isolates vs. 25.5%, 26.1%, and 29.4%, respectively, among non -ICU isolates (p<0.001). Piperacillin-tazobactam, imipenem, meropenem, and amikacin have a lower level of resistance (61.8%, 60.5%, 60.5%, 56.6% in ICU vs. 22.9%, 22.2%, 21.6 %, and 20.9% in non-ICU) (p<0.001) (Table 2) (Figure 1).

Prevalence of ESBL, MDR, and sensitive groups among ICU *Klebsiella* isolates vs. non-ICU

MDR *K. pneumoniae* were found in 60.5% of ICU isolates but only in 21.6% of non-ICU isolates (P<0.001). ICU klebsiella isolates had a significantly lower prevalence of ESBL (15.8%) than non-ICU isolates (30.1%) (P<0.001). About half of the *K. pneumoniae* isolates (48.4%) obtained from non-ICU patients were significantly susceptible and did not show either ESBL or MDR resistance, while only 23.7% of ICU ones were susceptible (p<0.001) (Table 3) (Figure 2).

Imipenem, meropenem, and amikacin exhibited higher efficacy among ESBL strains in both ICU and non-ICU patients (100% for ICU), (97.8%,95.8%,95.8% for non-ICU) followed by cefepime, cefoxitin, and ciprofloxacin (83.3% in ICU) (84.4, 84.8 and 56.5%for non-ICU). While among MDR strains, the efficacy of these agents was nearly lost except for amikacin and sulpha-trimethoprim, which retained very little efficacy at 6.5% and 4.3% among ICU patients and 9.1% and 12.1% among non-ICU patients, respectively.

Table 3. Comparative analysis of ESBL, MDR and sensitive groups among ICU vs. non-ICU isolates

Klebsiella isolates		ICU isolates N= 76 (33.2%)	Non-ICU isolates N= 153 (66.8%)	Total N= 229 (100%)	P value
Resistance	ESBL	12 (15.8%)	46 (30.1%)	58(25.3%)	<0.001
	MDR	46 (60.5%)	33 (21.6%)	79(34.5%)	
	Sensitive	18 (23.7%)	74 (48.4%)	92(40.2%)	

*Data are represented as count (%)

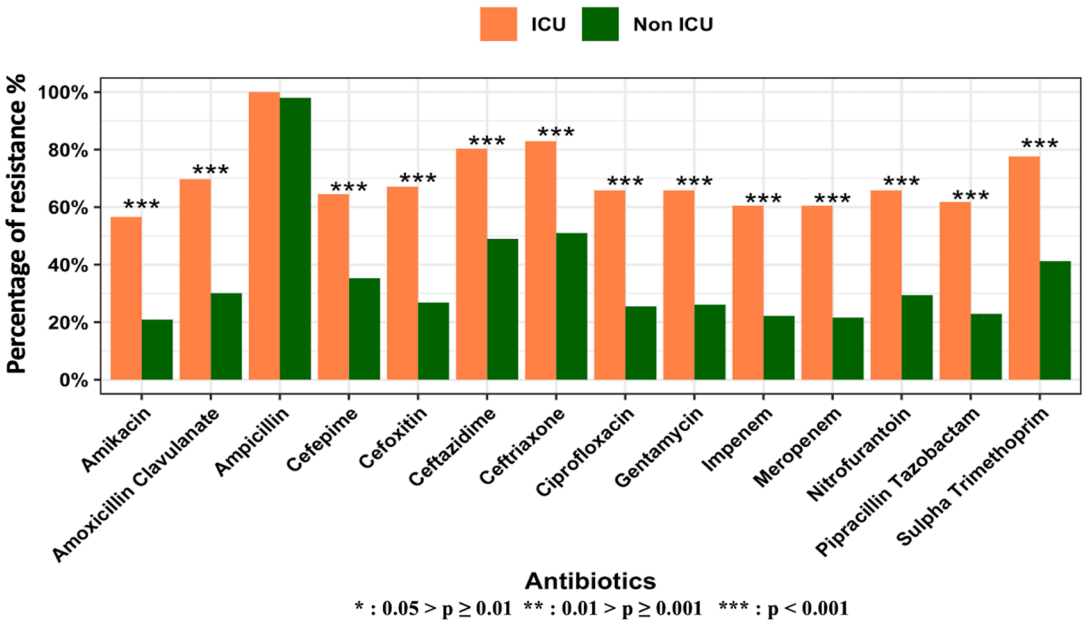

Figure 1. Antimicrobial susceptibility of Klebsiella isolates in ICU and non-ICU

Table 4. Comparative analysis for different antibiotics susceptibility of ESBL & MDR klebsiella isolates from ICU vs. non-ICU

Antibiotics susceptibility	Susceptibility		Total	P value**
	ICU isolates	Non-ICU isolates		
ESBL Isolates	(12)	(46)	(58)	
AK (Amikacin)	12 (100.0)	44 (95.7)	56 (96.6)	0.9
AMC (Amoxicillin Clavulanate)	5 (41.7)	30 (65.2)	35 (60.3)	0.35
AMP (Ampicillin)	0 (0.0)	0 (0.0)	0 (0.0)	--
FEP (Cefepime)	10 (83.3)	26 (56.5)	36 (62.1)	0.29
FOX (Cefoxitin)	10 (83.3)	39 (84.8)	49 (84.5)	0.96
CAZ (Ceftazidime)	2 (16.7)	5 (10.9)	7 (12.1)	0.61
CRO (Ceftriaxone)	1 (8.3)	6 (13.0)	7 (12.1)	0.68
CiP (Ciprofloxacin)	10 (83.3)	39 (84.8)	49 (84.5)	0.96
GN (Gentamycin)	11 (91.7)	38 (82.6)	49 (84.5)	0.76
IMP (Impenem)	12 (100.0)	45 (97.8)	57 (98.3)	0.95
MEM (Meropenem)	12 (100.0)	44 (95.7)	56 (96.6)	0.89
NF (Nitrofurantoin)	5 (41.7)	21 (45.7)	26 (44.8)	0.85
TZP (Pipracillin Tazobactam)	9 (75.0)	33 (71.7)	42 (72.4)	0.91
SXT (Sulpha Trimethoprim)	5 (41.7)	21 (45.7)	26 (44.8)	0.85
MDR Isolates	(46)	(33)	(79)	
AK (Amikacin)	3 (6.5)	3 (9.1)	6 (7.6)	0.68
AMC (Amoxicillin Clavulanate)	0 (0.0)	0 (0.0)	0 (0.0)	--
AMP (Ampicillin)	0 (0.0)	0 (0.0)	0 (0.0)	--
FEP (Cefepime)	0 (0.0)	0 (0.0)	0 (0.0)	--
FOX (Cefoxitin)	0 (0.0)	0 (0.0)	0 (0.0)	--
CAZ (Ceftazidime)	0 (0.0)	0 (0.0)	0 (0.0)	--
CRO (Ceftriaxone)	0 (0.0)	0 (0.0)	0 (0.0)	--
CiP (Ciprofloxacin)	0 (0.0)	1 (3.0)	1 (1.3)	0.24
GN (Gentamycin)	1 (2.2)	1 (3.0)	2 (2.5)	0.81
IMP (Impenem)	1 (2.2)	0 (0.0)	1 (1.3)	0.397
MEM (Meropenem)	1 (2.2)	0 (0.0)	1 (1.3)	0.397
NF (Nitrofurantoin)	0 (0.0)	0 (0.0)	0 (0.0)	--
TZP (Pipracillin Tazobactam)	0 (0.0)	0 (0.0)	0 (0.0)	--
SXT (Sulpha Trimethoprim)	2 (4.3)	4 (12.1)	6 (7.6)	0.22

Moreover, susceptibility to imipenem and meropenem decreased dramatically to 2.2% among ICU patients and 0% among non-ICU patients (Table 4).

Clinical outcome of patients

Regarding the clinical outcome, the length of hospital stay was significantly higher (IQR:16.0 days) among the patients with MDR -*K. pneumoniae* infection compared to patients with ESBL-*K. pneumoniae* infection (IQR: 11.0 days) and patients with susceptible strains (IQR:8.5 days) (P<0.001) (Figure 3). The correlation of mortality with antimicrobial resistance showed that,out of the 20 deaths (8.7%), 13 occurred among MDR patients, five among the ESBL group, and two among patients with susceptible strains with clinically significant difference (p= 0.004) (Table 5) (Figure 4).

DISCUSSION

K. pneumoniae is one of the main pathogens in the hospitals that causes various nosocomial infections with MDR pattern. ESBL producing *strains* are of major concern globally. In the United States, Carbapenem-resistant klebsiella have increased from 1.6 to 10.4% during the period from 2001 till 2011, and have provoked

the worldwide attention due to challenging treatment.[27]

In the current study, most *K. pneumoniae* isolates were retrieved from elderly male patients. This finding could be due to the predominantly male population in this hospital. Infections generally occur in males more than females because of the variations in the levels of sex steroids and differences in sex chromosome-linked genes.[28] Increased occurrence of infection

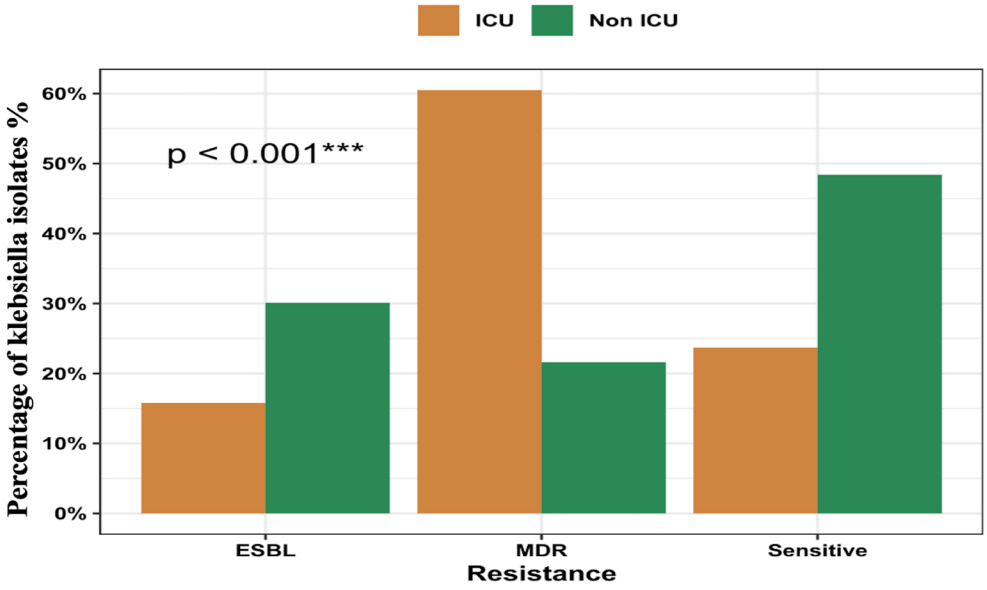

Figure 2. Pattern of resistance among ICU and non-ICU Klebsiella isolates

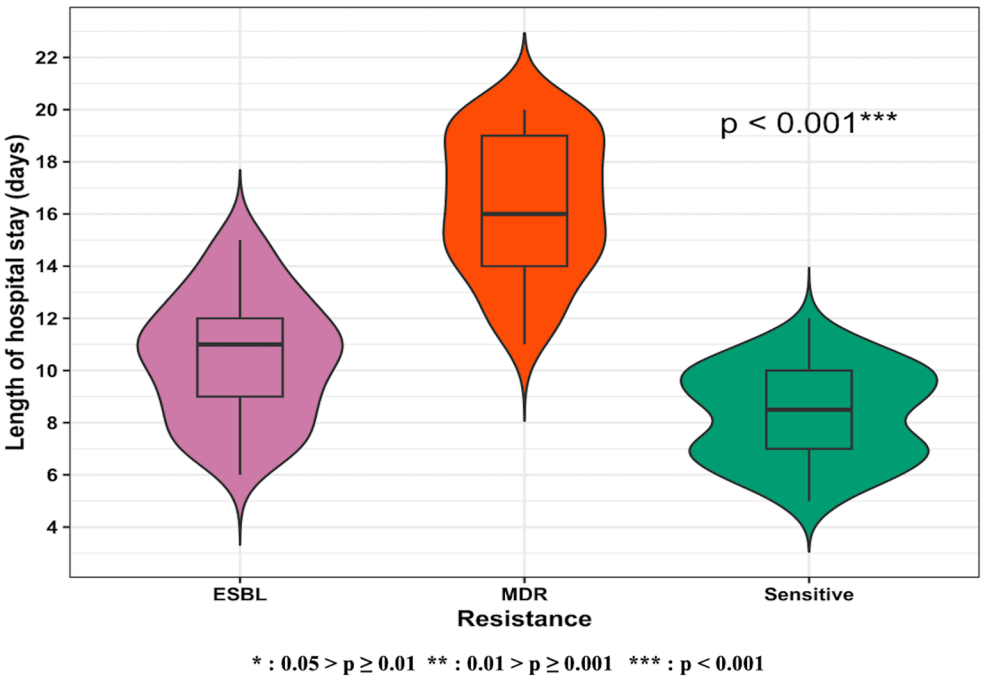

Figure 3. Relationship between resistance pattern of klebsiella isolates and length of hospital stay

in the elderly could be referred to defective immune system and defense mechanisms, ICU admission,invasive mechanical ventilation, and frequent exposure to antimicrobials. Comorbidities as diabetes mellitus,cardiovascular diseases and malignancy are also considered risk factors.[29] In several studies, similar to our results, *K. pneumoniae* and other gram-negative bacteria predominated in elderly male patients.[30,31] Moreover, most isolates, especially those from ICU, were isolated from respiratory and blood samples, while those from non-ICU were retrieved from urine and wound. This could be due to the prevalence of invasive devices as ventilators and central venous catheters and their associated infections in ICU rather than non-ICU. Similarly, other studies revealed the predominance of respiratory tract infections in the ICU.[32] The study by Rao *et al.*, also showed the highest isolation rate of *K. pneumoniae* from sputum (51.85%), followed by blood samples (11.11%).[33] Contrary to our study, *K. pneumoniae* were primarily isolated from blood, urine,[11] and ear discharge in other studies.[34] In the present study, we investigated the antimicrobial susceptibility of *K. pneumoniae* isolated from ICU and non-ICU patients. As previously reported

Table 5. Clinical outcome of patients having klebsiella pneumoniae infection

	ESBL N= 58 (25.3%)	MDR N= 79 (34.5%)	Sensitive N= 92 (40.2%)	Total N= 229 (100%)	P value
Median length of hospital stays in days (IQR)	11.0 (9.0 to 12.0)	16.0 (14.0 to 19.0)	8.5 (7.0 to 10.0)	11.0 (8.0 to 15.0)	<0.001
No of discharged N (%)	53 (91.4%)	66 (83.5%)	90 (97.8%)	209 (91.3%)	0.004
No of deaths N (%)	5 (8.6%)	13 (16.5%)	2 (2.2%)	20 (8.7%)	

*Data are represented as median (IQR:interquartile range) or count (%)

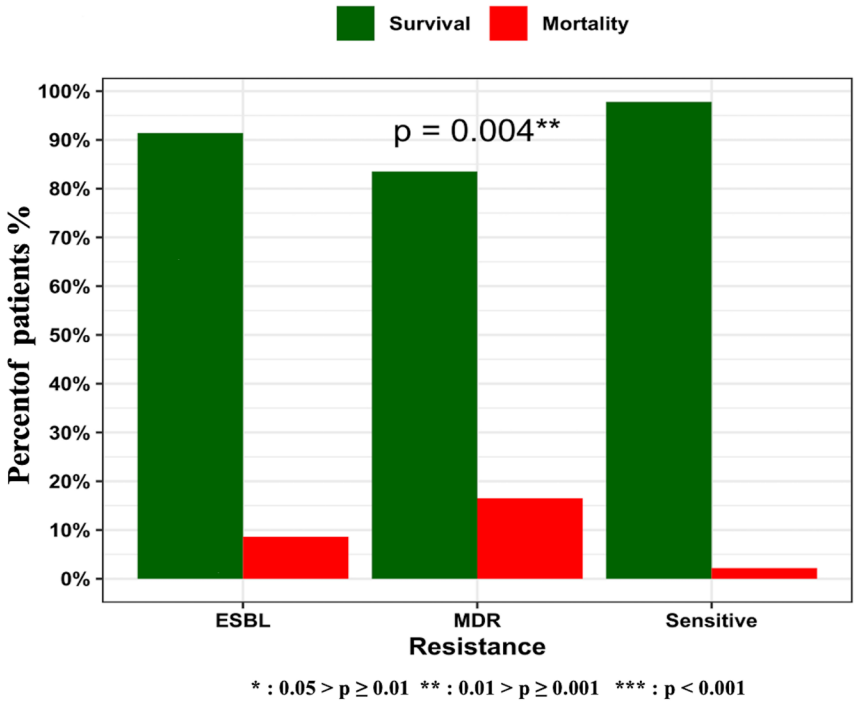

Figure 4. Mortality and survival among patients having klebsiella infection

in different studies,[35] antimicrobial resistance was higher among isolates obtained from ICU compared to non-ICU patients. This finding can be attributed to increased usage of these agents and lack of adherence to infection control measures in the ICU department.[36] *Klebsiella spp*. isolated from hospitals have different resistance mechanisms, such as ESBLs, AmpC, and carbapenemases, which render the isolates resistant to certain penicillins, cephalosporins.[37] and carbapenems.[38] In our study, in ICU, higher resistance rates were detected toward ampicillin (100%),3rd generation cephalosporin (82.9%,80.3%), and trimethoprim-sulphamethoxazole (77.6%). In comparison, lower rates were observed toward amoxicillin-clavulanate (69.7%), 2nd, and 4th-generation cephalosporins (67.1%,64.5%), quinolones (65.8%), aminoglycosides (65.8%), piperacillin-tazobactam (61.8%) and carbapenems with resistance rates (60.5%). Higher resistance to the commonly used antibiotics is challenging and could adversely affect outcomes. Failure of treatment and the need to save an effective alternative safer drug continues to be an urgent need. Our results align with Wani *et al.*, who reported the highest resistance among ICU patients toward ampicillin (%95) followed by third and fourth generations cephalosporins.[39] A previous study in KSA also reported higher resistance of *K. pneumoniae* isolates obtained from ICU toward cephalosporins and trimethoprim/sulfamethoxazole (60%).[31] These studies also reported lower resistance toward quinolones, aminoglycosides, piperacillin-tazobactam and carbapenems similar to our results. In addition, a study done by Sader *et al.*, similarly observed higher resistance among ICU patients toward third generation cephalosporins but it differs from our results that it shows also higher resistance towards carbapenems.[40] On the contrary, the study done by Ghenea *et al.* showed the highest resistance of *Klebsiella* strains in the ICU toward amoxicillin/clavulanate (85.31%), and 1st generation cephalosporins, cefazoline (78.90%) but in consistence with our results, the resistance to quinolones, ciprofloxacin (30.57%), and meropenem (27.78%) was lower.[34] Another study revealed distinct susceptibility profiles of *K. pneumoniae* isolated from ICU patients with higher resistance toward aztreonam, ticarcillin-clavulanic acid, ciprofloxacin, and levofloxacin while relatively lower resistance was detected toward colistin and tigecycline.[41] The susceptibility profile may differ according to the geographic area, antimicrobial agents used, and adherence to infection control standards and antimicrobial stewardship.

Our study revealed that the most effective antibiotic toward *Klebsiella isolates* was amikacin, which has a susceptibility rate of 42.1% and 79.1% among ICU and non-ICU patients, respectively. Imipenem and meropenem were also effective, with a susceptibility rate of 39.5% among ICU patients and 77.8, and 77.1 among non-ICU patients, respectively. In consistence with our results, Kumari *et al.* also informed that amikacin antibiotic is the most effective, with a sensitivity rate of 69.5%.[42] Leelarasamee *et al.* also considered amikacin as an effective drug and reported 43% resistance rate.[43] In addition to amikacin, other agents, including meropenem-vaborbactam, ceftazidime-avibactam, and colistin, were observed by Sader *et al.*, to be also effective against *K. pneumoniae* isolated from ICU and non-ICU patients.[40]

An increased number of MDR bacteria in the ICU is referred to several contributing factors including excessive use of antibiotics, prolonged ICU stay, use of invasive medical devices, comorbidities and lack of hygienic measures and isolation precautions. This causes a problem in patients' treatment, necessitating adherence to rigorous infection control procedures in healthcare settings and warranting the proper usage of antimicrobials.[44] In the current study, it was observed that MDR *K. pneumoniae* isolates were higher among ICU patients (60.5%) compared to non-ICU (21.6%). While higher prevalence of ESBL was noticed in non-ICU departments (30.1% in non-ICU vs. 15.8% in ICU) .The increased use of cephalosporins, which promote the emergence of ESBL-producing isolates, may account for the higher prevalence of ESBL in non-ICU departments.[45] Analogous to us, Sader *et al.*, reported the isolation of resistant phenotypes, such as MDR *K. pneumoniae,* which have very few treatment choices, more common from ICU rather than non-ICU patients.[46] However, Altaf Bandy and Bilal Tantry reported the isolation of 39.3%,13% MDR, and ESBL *K. pneumoniae*, respectively, with considerably higher rates from clinical specimens of intensive care units ($p < 0.01$)

in comparison to the samples obtained from non-ICU departments.[47] The prevalence of MDR or ESBL depends on different factors, such as the regional guidelines for antimicrobial prescription and use, self-administration of antibiotics, and infection control guidelines.[48] Carbapenems are the last-resort antibiotics for the treatment of Gram-negative ESBLs and the main defense against MDR Gram-negative infections. However, our study results proved that carbapenem resistance among MDR isolates is very high and alarming. Imipenem and meropenem reported effectiveness against ESBL strains in the current study in both ICU and non-ICU (100%,100%, 97.8%, and 95.8%, respectively), while their efficacy toward MDR strains decreased significantly in ICU and totally lost among non-ICU (2.2%, 2.2%, 0.0%, and 0.0%, respectively). This higher resistance toward carbapenems among MDR strains suggest carbapenemase production by the bacteria.[47] Hawkey *et al.*, declared that there is an increasing fear of the rise in carbapenem resistance among MDR-GNB.[49] Furthermore, Indrajith *et al.* reported that 58% of MDR *K. pneumoniae* were carbapenem-resistant.[50] The global rise in Carbapenem- resistant strains is a public health threat that aroused worldwide attention, because of the restricted therapy.It is linked to increased rate of mortality and poor outcomes regardless of the chosen antibiotic therapy.[51] Routine surveillance system in hospitals is very important for judicious detection of carbapenem susceptibility and the empirical therapy to control these infections. In addition, the use of new antimicrobials, such as ceftazidime-avibactam, meropenem-vaborbactam, tigecycline, and colistin, should be approached against MDR strains.[52,53] The current study revealed a significant association between MDR, ESBL production by *K. pneumoniae* and poor patient outcome including increased mortality, and hospital stay ($p<0.001$). This finding can be explained by the delayed or inappropriate use of the proper antibiotics. Comorbid conditions (like pneumonia, diabetes mellitus, renal disease), immunocompromised state and use of invasive devices could also affect patient outcome.[54] In consistence with our study, Siwakoti *et al.* also reported a significant association between MDR GNB infection and hospital stay and mortality.[55] A study done by Ben-David D *et al.*[56] also reported higher mortality among patients with carbapenem-resistant *K. pneumoniae* than those with carbapenem susceptible klebsiella infection (48% vs.17%). Cosgrove *et al.*[57] also found a significant association between the resistance of Enterobacter spp. toward third generation cephalosporins and patient mortality (Relative risk, 5.02). Contrary to our findings, other studies[58,59] reported a non-significant association between antimicrobial resistance and patient outcome.

CONCLUSION

There is a significant increase in ESBL and MDR producing *K. pneumoniae* in hospital settings, particularly ICUs. The prevalence of resistance in ICUs more than any other hospital department, can be linked to increased burden of co-morbidity in ICU patients, the use of immunosuppressive medications, the uncontrolled use of antibiotics like prolonged use of broad-spectrum ones, lack of strict adherence to infection control measures as poor compliance to hand hygiene and isolation precautions especially during pandemics. Carbapenems are the main line of treatment against ESBL strains, but more effective agents for carbapenem resistant strains such as colistin, tigecycline, and Ceftazidime/avibactam should also be considered to save patients life. Alternative strategies that could control MDR *K. pneumoniae* including phytochemicals and bacterial metabolites such as biosurfactants are also enabling further research. Hospital-acquired infections caused by resistant pathogens leads to increased patient mortality, prolonged hospitalization, and extra costs for medical care with serious economic burden. The situation is alarming and the health authorities should provide unlimited attention to decrease the risk of nosocomial infections. Hospitals should implement the proposals of CDC and WHO to decrease nosocomial infections.

Strengths and limitations

The current study is the first work done in the region to compare the antibiogram of one of the most common bacteria in healthcare settings (*K. pneumoniae*) between ICU and non-ICU.

However, being a single-center study that does not reflect the whole region is the main limitation. The lack of information about the history of improper use of empirical antibiotics could also be considered.

Recommendations

The inclusion of other Gram-negative and Gram-positive bacteria is compulsory to provide a comprehensive picture of antibiotic resistance. A study of the underlying molecular basis of antimicrobial resistance of MDR-*K. pneumoniae* should also be done.

ACKNOWLEDGMENTS

None.

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

This work was funded by the Deanship of Postgraduate and Scientific Research, Dar Al-Uloom University, Riyadh, Saudi Arabia.

DATA AVAILABILITY

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

ETHICS STATEMENT

The study was approved by the Ethical Committee of the Faculty of Medicine Dar Al Uloom University and Prince Mohammed bin Abdelaziz Hospital in Riyadh (IRB No: Pro21110003, 8th February 2022).

REFERENCES

1. MacVane SH. Antimicrobial Resistance in the Intensive Care Unit: A Focus on Gram-Negative Bacterial Infections. *J Intensive Care Med.* 2017;32(1):25-37. doi: 10.1177/0885066615619895
2. World Health Organization (WHO). Antimicrobial Resistance. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance; 2020.
3. Gaspar GG, Tamasco G, Abichabki N, et al. Nosocomial Outbreak of Extensively Drug-Resistant (Polymyxin B and Carbapenem) *Klebsiella pneumoniae* in a Collapsed University Hospital Due to COVID-19 Pandemic. *Antibiot (Basel).* 2022;11(6):814. doi: 10.3390/antibiotics11060814
4. Lima AM, de Melo ME, Alves LC, Brayner FA, Lopes ACS. Investigation of class 1 integrons in *Klebsiella pneumoniae* clinical and microbiota isolates belonging to different phylogenetic groups in Recife, State of Pernambuco. *Rev Soc Bras Med Trop.* 2014;47(2):165-169. doi: 10.1590/0037-8682-0021-2014
5. Abdelsalam MF, Abdalla MS, El-Abhar HS. Prospective, comparative clinical study between high-dose colistin monotherapy and colistin-meropenem combination therapy for treatment of hospital-acquired pneumonia and ventilator-associated pneumonia caused by multidrug- resistant Klebsiella pneumoniae. *J Glob Antimicrob Resist.* 2018;15:127-135. doi: 10.1016/j.jgar.2018.07.003
6. Kotb S, Lyman M, Ismail G, et al. Epidemiology of Carbapenem-resistant *Enterobacteriaceae* in Egyptian intensive care units using National Healthcare-associated Infections Surveillance Data, 2011-2017. *Antimicrob Resist Infect Control.* 2020;9(1):2. doi: 10.1186/s13756-019-0639-7
7. Sommerstein R, Damonti L, Marschall J, et al. Distribution of pathogens and antimicrobial resistance in ICU-bloodstream infections during hospitalization: a nationwide surveillance study. *Sci Rep.* 2021; 19;11(1):16876. doi: 10.1038/s41598-021-95873-z
8. Tomasz CzekajMarcin Ciszewski. *Klebsiella pneumoniae* NDM - new emerging superbacteria. *Med Rodz.* 118 23-27.
9. Guerra ME, Destro G, Vieira B, et al. *Klebsiella pneumoniae* biofilms and their role in disease pathogenesis. *Front Cell Infect Microbiol.* 2022;12:877995. doi: 10.3389/fcimb.2022.877995
10. Kang JS, Yi J, Ko MK, Lee SO, Lee JE, Kim KH. Prevalence and Risk Factors of Carbapenem-resistant *Enterobacteriaceae* Acquisition in an Emergency Intensive Care Unit in a Tertiary Hospital in Korea: a Case-Control Study. *J Korean Med Sci.* 2019;34(18):e140. doi: 10.3346/jkms.2019.34.e140
11. Fahim NAE. Prevalence and antimicrobial susceptibility profile of multidrug-resistant bacteria among intensive care units patients at Ain Shams University Hospitals in Egypt-a retrospective study. *J Egypt Public Health Assoc.* 2021;96(1):7. doi: 10.1186/s42506-020-00065-8
12. Fernandez-Martinez NF, Carcel-Fernandez S, De la Fuente-Martos C, et al. Risk factors for multidrug-resistant gram-negative bacteria carriage upon admission to the intensive care unit. *Int J Environ Res Public Health.* 2022;19(3):1039. doi: 10.3390/ijerph19031039
13. Canton R, Gijon D, Ruiz-Garbajosa P. Antimicrobial resistance in ICUs: an update in the light of the COVID-19 pandemic. *Curr Opin Crit Care.* 2020;26(5):433-441. doi: 10.1097/MCC.0000000000000755
14. Becker L, Fuchs S, Pfeifer Y, et al. Whole Genome Sequence Analysis of CTX-M-15 Producing *Klebsiella*

Isolates Allowed Dissecting a Polyclonal Outbreak Scenario. *Front Microbiol.* 2018;9:322. doi: 10.3389/fmicb.2018.00322

15. Reyes J, Aguilar AC, Caicedo A. Carbapenem-Resistant *Klebsiella pneumoniae*: Microbiology Key Points for Clinical Practice. *Int J Gen Med.* 2019;12:437-446. doi: 10.2147/IJGM.S214305
16. World Health Organization. News release (Internet). 2017. https://www.who.int/news-room/detail/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed
17. Stewardson AJ, Marimuthu K, Sengupta S, et al. Effect of carbapenem resistance on outcomes of bloodstream infection caused by *Enterobacteriaceae* in low-income and middle-income countries (PANORAMA): a multinational prospective cohort study. *Lancet Infect Dis.* 2019;19(6):601-610. doi: 10.1016/S1473-3099(18)30792-8
18. Geneva: World Health Organization. Implementation manual to prevent and control the spread of carbapenem-resistant organisms at the national and health care facility level: interim practical manual supporting implementation of the guidelines for the prevention and control of carbapenem-resistant *Enterobacteriaceae, Acinetobacter baumannii* and *Pseudomonas aeruginosa* in health care facilities. 2019.
19. World Health Organization (WHO). Global action plan on antimicrobial resistance. 2015. http://apps.who.int/iris/handle/10665/193736
20. Kerneis S, Lucet JC. Controlling the diffusion of multidrug-resistant organisms in intensive careunits. *Semin Respir Crit Care Med.* 2019;40(4):558-568. doi: 10.1055/s-0039-1696980
21. Iskandar K, Molinier L, Hallit S, et al. Surveillance of antimicrobial resistance in low- and middle-income countries: a scattered picture. *Antimicrob Resist Infect Control.* 2021;10(1):63. doi: 10.1186/s13756-021-00931-w
22. Leekha S, Terrell CL, Edson RS. General principles of antimicrobial therapy. *Mayo Clin Proc.* 2011;86(2):156-167. doi: 10.4065/mcp.2010.0639
23. Washington CW Jr, Stephen DA, William MJ, et al. Koneman's color atlas and text book of diagnostic microbiology. 6th ed. Philadelphia:Lippincott Williams and Wilkins. 2006.
24. Wayne, PA. Performance standards for antimicrobial susceptibility testing: 31st informational supplement. CLSI. M100Ed31; 2021.
25. Abayneh M, Tesfaw G, Abdissa A. Isolation of Extended-Spectrum β-lactamase- (ESBL-) Producing Escherichia coli and *Klebsiella pneumoniae* from Patients with Community-Onset Urinary Tract Infections in Jimma University Specialized Hospital, Southwest Ethiopia. *Can J Infect Dis Med Microbiol J Can Mal Infect Microbiol Medicale.* 2018;4846159. doi: 10.1155/2018/4846159
26. Basak S, Singh P, Rajurkar M. Multidrug Resistant and Extensively Drug Resistant Bacteria: A Study. *J Pathog.* 2016;4065603. doi: 10.1155/2016/4065603
27. Al Bshabshe A, Al-Hakami A, Alshehri B, et al. Rising *Klebsiella pneumoniae* infections and its expanding drug resistance in the intensive care unit of a tertiary Healthcare Hospital, Saudi Arabia. *Cureus.* 2020;12(8):e10060. doi: 10.7759/cureus.10060
28. vom Steeg LG, Klein SL. SeXX Matters in Infectious Disease Pathogenesis. *PLoS Pathog.* 2016;12(2):e1005374. doi: 10.1371/journal.ppat.1005374
29. Mythri H, Kashinath K. Nosocomial infections in patients admitted in intensive care unit of a Tertiary Health Center, India. *Ann Med Health Sci Res.* 2014;4(5):738. doi: 10.4103/2141-9248.141540
30. Bianco A, Capano MS, Mascaro V, Pileggi C, Pavia M. Prospective surveillance of healthcare-associated infections and patterns of antimicrobial resistance of pathogens in an Italian intensive care unit. *Antimicrob Resist Infect Control.* 2018;7:48. doi: 10.1186/s13756-018-0337-x
31. Ibrahim ME. High antimicrobial resistant rates among Gram-negative pathogens in intensive care units: A retrospective study at a tertiary care hospital in Southwest Saudi Arabia. *Saudi Med J.* 2018;39(10):1035-1043. doi: 10.15537/smj.2018.10.22944
32. Sahu M, Siddharth B, Choudhury A, et al. Incidence, microbiological profile of nosocomial infections, and their antibiotic resistance patterns in a high volume Cardiac Surgical Intensive Care Unit. *Ann Card Anaesth.* 2016;19(2):281. doi: 10.4103/0971-9784.179625
33. Rao CM, Rout P, Pattnaik AP, Singh N, Rajendran A, Patro S. The Microbial Profile and Resistance Pattern of Pathogens Isolated From Long COVID Pneumonia Patients and Their Correlation to Clinical Outcome: Our Experience From a Tertiary Care Hospital. *Cureus.* 2022;14(3):e23644. doi: 10.7759/cureus.23644
34. Ghenea AE, Cioboata R, Drocas AI, et al. Prevalence and Antimicrobial Resistance of Klebsiella Strains Isolated from a County Hospital in Romania. *Antibiot Basel Switz.* 2021;10(7):868. doi: 10.3390/antibiotics10070868
35. Despotovic A, Milosevic B, Milosevic I, et al. Hospital-acquired infections in the adult intensive care unit-Epidemiology, antimicrobial resistance patterns, and risk factors for acquisition and mortality. *Am J Infect Control.* 2020;48(10):1211-1215. doi: 10.1016/j.ajic.2020.01.009
36. Tajeddin E, Rashidan M, Razaghi M, et al. The role of the intensive care unit environment and health-care workers in the transmission of bacteria associated with hospital acquired infections. *J Infect Public Health.* 2016;9(1):13-23. doi: 10.1016/j.jiph.2015.05.010
37. Moremi N, Claus H, Mshana SE. Antimicrobial resistance pattern: a report of microbiological cultures at a tertiary hospital in Tanzania. *BMC Infect Dis.* 2016;16(1):756. doi: 10.1186/s12879-016-2082-1
38. Rodloff AC, Goldstein EJC, Torres A. Two decades of imipenem therapy. *J Antimicrob Chemother.* 2006;58(5):916-29. doi: 10.1093/jac/dkl354
39. Wani FA, Bandy A, Alenzi MJS, et al. Resistance Patterns of Gram-Negative Bacteria Recovered from Clinical Specimens of Intensive Care Patients. *Microorganisms.* 2021;9(11):2246. doi: 10.3390/microorganisms9112246
40. Sader HS, Mendes RE, Streit JM, Carvalhaes CG,

Castanheira M. Antimicrobial susceptibility of Gram-negative bacteria from intensive care unit and non-intensive care unit patients from United States hospitals (2018-2020). *Diagn Microbiol Infect Dis.* 2022;102(1):115557. doi: 10.1016/j.diagmicrobio.2021.115557

41. Xiao S, Chen T, Wang H, et al. Drug Susceptibility and Molecular Epidemiology of *Klebsiella pneumoniae* Bloodstream Infection in ICU Patients in Shanghai, China. *Front Med.* 2021;8:754944. doi: 10.3389/fmed.2021.754944
42. Kumari HBV, Nagarathna S, Chandramuki A. Antimicrobial resistance pattern among aerobic gram-negative bacilli of lower respiratory tract specimens of intensive care unit patients in a neurocentre. *Indian J Chest Dis Allied Sci.* 2007;49(1):19-22.
43. Leelarasamee A, Janyapoon K. Antimicrobial resistance of 100 serial gram-negative isolates in two intensive care units. *J Med Assoc Thail Chotmaihet Thangphaet.* 1992;75(12):680-687.
44. Wu C, Lu J, Ruan L, Yao J. Tracking Epidemiological Characteristics and Risk Factors of Multi-Drug Resistant Bacteria in Intensive Care Units. *Infect Drug Resist.* 2023:1499-509. doi: 10.2147/IDR.S386311
45. Moolchandani K, Sastry AS, Deepashree R, Sistla S,Harish BN, Mandal J. Antimicrobial Resistance Surveillance among Intensive Care Units of a Tertiary Care Hospital in Southern India. *J Clin Diagn Res.* 2017;11(2):DC01-DC07. doi: 10.7860/JCDR/2017/23717.9247
46. Sader HS, Castanheira M, Flamm RK, Mendes RE, Farrell DJ, Jones RN. Ceftazidime/avibactam tested against Gram-negative bacteria from intensive care unit (ICU) and non-ICU patients, including those with ventilator-associated pneumonia. *Int J Antimicrob Agents.* 2015;46(1):53-59. doi: 10.1016/j.ijantimicag.2015.02.022
47. Bandy A, Tantry B. ESBL Activity, MDR, and Carbapenem Resistance among Predominant Enterobacterales Isolated in 2019. *Antibiotics.* 2021;10(6):744. doi: 10.3390/antibiotics10060744
48. Ayukekbong JA, Ntemgwa M, Atabe AN. The threat of antimicrobial resistance in developing countries: causes and control strategies. *Antimicrob Resist Infect Control.* 2017;6(1):47. doi: 10.1186/s13756-017-0208-x
49. Hawkey PM, Warren RE, Livermore DM, et al. Treatment of infections caused by multidrug-resistant Gram-negative bacteria: report of the British Society for Antimicrobial Chemotherapy/Healthcare Infection Society/British Infection Association Joint Working Party. *J Antimicrob Chemother*. 2018;73(suppl_3):iii2-iii78. doi: 10.1093/jac/dky027
50. Indrajith S, Mukhopadhyay AK, Chowdhury G, et al. Molecular insights of Carbapenem resistance *Klebsiella pneumoniae* isolates with focus on multidrug resistance from clinical samples. *J Infect Public Health.* 2021;14(1):131-138. doi: 10.1016/j.jiph.2020.09.018
51. Neuner EA, Yeh JY, Hall GS, et al. Treatment and outcomes in carbapenem-resistant *Klebsiella pneumoniae* bloodstream infections. *Diagn Microbiol Infect Dis.* 2011;69(4):357-362. doi: 10.1016/j.diagmicrobio.2010.10.013
52. Bassetti M, Vena A, Sepulcri C, Giacobbe DR, Peghin M. Treatment of Bloodstream Infections Due to Gram-Negative Bacteria with Difficult-to-Treat Resistance. *Antibiotics.* 2020;9(9):632. doi: 10.3390/antibiotics9090632
53. Wilson GM, Fitzpatrick M, Walding K, et al. Meta-analysis of Clinical Outcomes Using Ceftazidime/Avibactam, Ceftolozane/Tazobactam, and Meropenem/Vaborbactam for the Treatment of Multidrug-Resistant Gram-Negative Infections. *Open Forum Infect Dis.* 2021;8(2):ofaa651. doi: 10.1093/ofid/ofaa651
54. Colkesen F, Tarakci A, Eroglu E, et al. Carbapenem-resistant Klebsiella pneumoniae infection and its risk factors in older adult patients. *Clin Interv Aging.* 2023;18:1037-1045. doi: 10.2147/CIA.S406214
55. Siwakoti S, Subedi A, Sharma A, Baral R, Bhattarai NR, Khanal B. Incidence and outcomes of multidrug-resistant gram-negative bacteria infections in intensive care unit from Nepal- a prospective cohort study. *Antimicrob Resist Infect Control.* 2018;7(1):114. doi: 10.1186/s13756-018-0404-3
56. Ben-David D, Kordevani R, Keller N, et al. Outcome of carbapenem resistant *Klebsiella pneumoniae* bloodstream infections. *Clin Microbiol Infect*. 2012;18(1):54-60. doi: 10.1111/j.1469-0691.2011.03478.x
57. Cosgrove SE. The Relationship between Antimicrobial Resistance and Patient Outcomes: Mortality, Length of Hospital Stay, and Health Care Costs. *Clin Infect Dis.* 2006;42(Suppl 2):S82-9. doi: 10.1086/499406
58. Blot S, Vandewoude K, De Bacquer D, Colardyn F. Nosocomial Bacteremia Caused by Antibiotic Resistant Gram Negative Bacteria in Critically Ill Patients: Clinical Outcome and Length of Hospitalization. *Clin Infect Dis.* 2002;34(12):1600-1606. doi: 10.1086/340616
59. Menashe G, Borer A, Yagupsky P, et al. Clinical significance and impact on mortality of extended-spectrum beta lactamase-producing *Enterobacteriaceae* isolates in nosocomial bacteremia. *Scand J Infect Dis.* 2001;33(3):188-193. doi: 10.1080/00365540151060806