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Prevalence of Gram-negative bacteria and antibiotic resistance in intensive care unit in King Fahad Specialist Hospital, Kingdom of Saudi Arabia

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ABSTRACT

Backgroundand aim:The prevalence of gram-negative bacteria and antibiotic resistance in intensive care units is a significant concern in healthcare settings worldwide. Gram-negative bacteria pose a substantial threat to ICU patients due to their ability to cause healthcare-associated infections.So, we aimed to investigate the prevalence and distribution of MDR gram-negative bacteria in the ICU of King Fahad Specialist Hospital, their risk, and prognostic factors.

Methods:1089 specimens were collected from the patients in the King Fahad Specialist Hospital in Buraydah (Qassim region) from 2020 to 2022.

Results: 66% of cases resulted in death, with the majority of patients being over 70 years old (48%). The trachealaspirate was the most common sample type, followed by blood culture and urine. The prevalence of multi-drug resistance (MDR) was 37.6%, with 32.7% showing no resistance, and 26.8% producing ESBL enzymes. Males had a higher prevalence of MDR, ESBL, and ESBL (MDR) compared to females. K. pneumoniae, A. baumannii, P. aeruginosa, and E.coli were frequently detected, while P. vulgarise was rare. K.pneumoniae and A. baumannii were more prevalent in older age groups. Additionally, K. pneumoniae exhibited higher resistance rates than E. Coli across various antibiotics, with Ampicillin showing the highest resistance rate at 95.1%.

Conclusion:K. pneumoniae, A. baumannii, P. aeruginous, and E.coli were the highly detected bacteria in ICU mainly in Tracheal aspirate. While P. valgaris was the least detected one.Ampicillin, followed by Piperacillin, Oxacillin, Cefuroxime, Cefotaxime, Ceftriaxone, Amoxycillin/ Clavulanic acid, Moxifloxacin, and Aztreonam had the highest resistance rates.

Keywords: Gram-negative, antibiotics resistant, intensive care unit, Saudi Arabia

INTRODUCTION

Gram-negative bacteria aredistinguished by their lipopolysaccharides cell wall with the lipid A moiety, Which is essential for host-pathogen interactions with the innate immune system.(Maldonado, Sá-Correia, & Valvano, 2016). In Saudi Arabia, the prevalence of gram-negative bacteria among patients diagnosed with infections varied from 1% to 11% over the period spanning from 1981 to 2015. Comparatively, Gulf Cooperation Council (GCC) hospitals exhibited a higher risk of central line-associated bloodstream infection (CLABSI) by 146% compared to hospitals affiliated with the US National Healthcare Safety Network. However, the risk of CLABSI in GCC hospitals was 33% lower than that observed in hospitals under the jurisdiction of the foreign National Health Commission of China (NHCC)(Azab et al., 2021).

Gram-negative bacteria, including organisms such as Escherichia coli, Klebsiella pneumonia, and Pseudomonas

aeruginosa, pose a significant threat to patients in this setting due to their ability to cause healthcare-associated infections. These infections are often associated with high mortality rates and increased healthcare costs.(Vading, Nauclér, Kalin, & Giske, 2018).Furthermore, despite advancements in healthcare, sepsis represents a substantial contributor to neonatal morbidity and mortality. In particular, the transplantation of hematopoietic stem cells is often accompanied by significant morbidity and mortality related to sepsis. In the intensive care unit (ICU) setting, end-stage liver disease is a prominent cause of acquired infections. Notably, bloodstream infections caused by antimicrobial-resistant microorganisms have been associated with the highest mortality rates among hospitalized individuals.

On a global scale, approximately 30 million individuals experience bloodstream infections, resulting in an estimated 6 million deaths annually. In this context, sepsis infections affect 3 million infants and 1 million patients annually.(Borgio et al., 2021).In recent years, the emergence and spread of antibiotic resistance among gram-negative bacteria have further complicated the management of infections in the ICU.(Cantón, Gijón, & Ruiz-Garbajosa, 2020). Antibiotic resistance occurs when bacteria develop mechanisms to withstand the effects of commonly used antibiotics, rendering them ineffective.(Darby et al., 2023). This phenomenon has become a global concern, increasing morbidity, mortality, and healthcare expenditures((Dhingra et al., 2020). As per the World Health Organization (WHO) findings, microorganisms, specifically bacteria, that exhibit antibiotic resistance possess the ability to withstand antimicrobial interventions. This resistance phenomenon compromises the effectiveness of treatment and facilitates the persistence and dissemination of pathogens.(Akbar et al., 2022). While multidrug resistance (MDR) is a prevalent concern, there has been a notable upsurge in the number of individuals with compromised immune systems, including organ transplant recipients, severely burned patients, end-of-life individuals, and those susceptible to infections. Consequently, these vulnerable populations become susceptible targets for acquiring hospital-associated infectious diseases, amplifying the proliferation of multidrug-resistant strains. Extensive research conducted by the WHO has demonstrated alarmingly high resistance rates(Vivas, Barbosa, Dolabela, & Jain, 2019).This study aims to investigate the prevalence and distribution of MDR gram-negative bacteria in the intensive care area of King Fahad Specialist Hospital, their risk, and prognostic factors. The study also detects and evaluates the resistance patterns of isolated gram-negative bacteria and their impacts on morbidity and mortality based on different methods used in determining the sensitivity with the detection of the appropriate treatment of each organism. Finally, the correlation of antibiotic resistance data with the patient population's demographic and clinical data will also be examined.

METHODOLOGY

The present study is a cross-sectional study. A randomsample of 1089 specimens was collected from the patientsin the Department of Microbiology, King Fahad Specialist Hospital in Buraydah (Qassim region) from 2020 to 2022.Aseptic techniques were rigorously adheredto during the collection of clinical samples, encompassing blood, urine, and wound swabs.In the laboratory, specialized tests were conducted to identify gram-negative bacteria and assess their susceptibility to antibiotics. Additionally, patient data including demographic details, medical histories, and data on antibiotic usagewere obtained through electronic medical records.

Isolation and identification of bacteria

Regarding blood specimens, bottleswere pretested to detect the growth of the microbes using the Automatic Blood Culture Device. Samples with signals for microbial growth were used to isolate bacterial pathogens. The samples werethen cultured on blood agar, chocolate agar, McConkey agar, and mannitol salt agar plates at 37°C under aerobic conditions for 18 h. After incubation, the bacterial cultures were purified on blood agar plates to obtain pure cultures.The classification of the isolated bacteria was tested by Gram staining, and the gram-negative and gram-positive isolates were identified using Microscan Walkaway 96 instrument incorporating version and Phoenix. For this, organisms were removed from cold storage and sub-cultured. The culturing of gram-negative organisms was performed over MacConkey agar,whichwas crystal blue, according to the instructions of the producer. The organism that was unable to grow over this agar was provided with another type of culture, such as trypticase soy agar, along with the addition of soy or blood agar from sheep. After 20 to 24 hours, the colonies produced were taken, and then inoculation and incubation of them were performed following the manufacturer's guidelines or instructions.

Antimicrobial susceptibility

All clinical isolates were also tested for antimicrobial susceptibility. A large number of antimicrobials were tested in this study. The disc diffusion test did the confirmation. Using a computer-assisted microbiology laboratory database, patients with MDR organisms-associated infections were included as the case group, while those infected with non-MDR organisms were selected as a control group. The clinicalcharacteristics, demographic data, type of organisms, and resistance patternwere collected and analyzed.

Statistical analysis

Data were analyzed using version 28.0 of the IBM SPSS software package (Armonk, NY: IBM Corp). Qualitative data were described using numbers and percentages (IQR). The relationship between two qualitative variables was analyzed by Utilizing the Chi-square test. Values less than 0.05 were considered to be statistically significant.

RESULTS

Demographic and clinical characteristics of the patients

Table 1shows the sample type distribution of the patients in the study. The result reveals a diverse array of samples, with tracheal aspirate cultures constituting the majority at 56.5%. Furthermore, the inclusion of various sample types, such as urine, blood cultures, wound swabs, and catheter tips, demonstrates the thoroughness of the research approach.

Table 1. Sample type distribution of the patients in the study

Sample types	Frequency	Per cent
Urine C/S	107	9.8
Body Fluid - Peritoneal - C/S	1	.1
CSF C/S	1	.1
Body Fluid-Pleural C/S	1	.1
Catheter Tip Culture &Sensitivity	14	1.3
Sputum C/S	44	4.0
Ear Swab C/S	2	.2
Tracheal Aspirate C/S	615	56.5
Body Fluid-Ascitic C/S	1	.1
Body Fluid-Drain Fluid C/S	3	.3
Body Fluid-Pericardial C/S	1	.1
Nasal C/S	1	.1
Wound C/S	103	9.5
Blood Culture - First Bottle - Aerobic	129	11.8
Blood Culture - Second Bottle - Anaerobic	23	2.1
Blood Culture -Third Bottle - Aerobic	26	2.4
Blood Culture - 4 Th Bottle - Anaerobic	9	.8
Tissue C/S	8	.7

Microorganisms' distribution in the study

Table 2 shows the distributions of bacteria isolated among the different study samples. Notably, the results established variations in bacterial prevalence across sample types. For instance, urinary samples demonstrate a higher prevalence of E. coli and K. pneumoniae, underlining their significance in urinary tract infections. In contrast, samples from catheter tips revealed a diverse range of bacteria, including E. coli, K. pneumoniae, E. aerogenes, and P. mirabilis, suggesting the complexity of infections related to catheter-associated sources.Moreover, the findings from blood cultures, both aerobic and anaerobic, illustrate the presence of various pathogens, with E. coli, K. pneumoniae, and A. baumannii being notable examples. The presence of E.coli in peritoneal fluid and E. cloacaein pleural fluid highlights the importance of identifying bacteria in these compartments, guiding clinicians in targeted therapeutic interventions.

Table 2. The percentage of prevalent bacteria isolated from the different samples.

	A. baumannii	E. coli	E. aerogenes	E. cloacae	K. pneumoniae	P. mirabilis	P. valgaris	P. stuartii	P. aeroginusa	S. marcescens	S. maltophilia
Urine C/S	10 (9.3)	30 (28.0)	2 (1.9)	2 (1.9)	41 (38.3)	4 (3.7)	0 (0.0)	3 (2.8)	13 (12.1)	2 (1.9)	0 (0.0)

Body Fluid - Peritoneal - C/S	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
CSF C/S	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Body Fluid-Pleural C/S	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Catheter Tip Culture &Sensitivity	2 (14.3)	0 (0.0)	1 (7.1)	1 (7.1)	6 (42.9)	3 (21.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (7.1)
Sputum C/S	12 (27.3)	0 (0.0)	1 (2.3)	4 (9.1)	15 (34.1)	4 (9.1)	0 (0.0)	2 (4.5)	6 (13.6)	0 (0.0)	0 (0.0)
Ear Swab C/S	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)
Tracheal Aspirate C/S	192 (31.2)	32 (5.2)	6 (1.0)	11 (1.8)	206 (33.5)	38 (6.2)	0 (0.0)	5 (0.8)	99 (16.1)	9 (1.5)	17 (2.8)
Body Fluid-Ascitic C/S	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Body Fluid-Drain Fluid C/S	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Body Fluid-Pericardial C/S	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Nasal C/S	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Wound C/S	14 (13.6)	7 (6.8)	1 (1.0)	6 (5.8)	29 (28.2)	19 (18.4)	1 (1.0)	1 (1.0)	23 (22.3)	1 (1.0)	1 (1.0)
Blood Culture - First Bottle - Aerobic	42 (32.6)	21 (16.3)	0 (0.0)	3 (2.3)	46 (35.7)	1 (0.8)	0 (0.0)	0 (0.0)	11 (8.5)	3 (2.3)	2 (1.6)
Blood Culture - Second Bottle - Anaerobic	3 (13.0)	2 (8.7)	0 (0.0)	2 (8.7)	8 (34.8)	2 (8.7)	0 (0.0)	3 (13.0)	2 (8.7)	0 (0.0)	1 (4.3)
Blood Culture -Third Bottle - Aerobic	11 (42.3)	2 (7.7)	0 (0.0)	0 (0.0)	5 (19.2)	1 (3.8)	0 (0.0)	2 (7.7)	4 (15.4)	0 (0.0)	1 (3.8)
Blood Culture - 4 Th Bottle - Anaerobic	0 (0.0)	0 (0.0)	0 (0.0)	1 (11.1)	8 (88.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Tissue C/S	2 (25.0)	2 (25.0)	0 (0.0)	0 (0.0)	2 (25.0)	1 (12.5)	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)
Total	290 (26.6%)	98 (9.0%)	11 (1.0%)	31 (2.8%)	370 (34.0%)	74 (6.8%)	1 (0.1%)	16 (1.5%)	160 (14.7%)	15 (1.4%)	23 (2.1%)

Distribution of isolated clinical bacteria about patient's age

Table 3 shows the distributions of bacteria isolated in the study regarding the patient's age. The results revealed that certain bacteria, notably Klebsiella pneumoniae and Proteus mirabilis, exhibit a notable escalation in prevalence with increasing age, shedding light on potential susceptibilities among elderly patients. Conversely, some bacteria, like Providencia stuartii and Serratia marcescens, display fluctuating prevalence rates across age groups, underscoring the intricacy of bacterial infections in the ICU milieu.

Table 3. The percentage of prevalent bacteria isolated among different age groups of patients.

Organism isolated	**< 40**	**41-70**	**> 70**
Acinetobacter baumannii	49 (16.9%)	115 (39.7%)	126 (43.4%)
E. coli	7 (7.1%)	43 (43.9%)	48 (49.0%)
Enterobacter aerogenes	1 (9.1%)	6 (54.5%)	4 (36.4%)
Enterobacter cloacae	3 (9.7%)	12 (38.7%)	16 (51.6%)

Klebsiella pneumoniae	65 (17.6%)	120 (32.4%)	185 (50.0%)
Proteus mirabilis	12 (16.2%)	26 (35.1%)	36 (48.8%)
Proteus valgaris	0 (0.0%)	0 (0.0%)	1 (100.0%)
Providencia stuartii	1 (6.3%)	2 (12.5%)	13 (81.3%)
Pseudomonas aeroginusa	26 (16.3%)	56 (35.0%)	78 (48.8%)
Serratia marcescens	3 (20.0%)	8 (53.3%)	4 (26.7%)
Stenotrophomonas maltophilia	3 (13.0%)	8 (34.8%)	12 (52.2%)
Total	170 (15.6%)	396 (36.4%)	523 (48.0%)

Distribution of isolated clinical bacteria regarding the multidrug-resistant

Table 4 shows the frequencies and percentages of the bacteria found in the study and their distribution among the multidrug-resistant. Particularly noteworthy is the substantial prevalence of MDR bacteria across multiple species, with Acinetobacter baumanniistanding out with a significant portion of MDR cases (87.2%)followed by Klebsiella pneumoniae (24.3%). This highlights the formidable challenge posed by the resistance of this bacterium, necessitating targeted interventions and focused antibiotic stewardship programs.The results further illuminate the existence of Extended-Spectrum Beta-Lactamase (ESBL) bacteria, indicating a troubling trend of resistance, especially in Escherichia coli and Klebsiella pneumoniae. Moreover, the presence of multidrug resistance in other bacteria, such as Acinetobacter baumannii, Enterobacter cloacae, and Proteus mirabilis underscores the pervasive nature of this challenge. Further, the high prevalence of MDR strains in Pseudomonas aeruginosa, frequently associated with healthcare-related infections, is disconcerting. Additionally, the emergence of MDR in Providencia stuartii and Serratia marcescens further accentuates the evolving challenges in managing these infections.

Table 4. The percentage of prevalent bacteria isolated among different multidrug-resistant groups in the study.

Organisms isolated	**ESBL**	**ESBL(MDR)**	**MDR**	**Null**	**Total**
Acinetobacter baumannii	4 (1.4)	4 (1.4)	253 (87.2)	29 (10.0)	290 (100.0)
E. coli	44 (44.9)	2 (2.0)	4 (4.1)	48 (49.0)	98 (100.0)
Enterobacter aerogenes	3 (27.3)	0 (0.0)	1 (9.1)	7 (63.6)	11 (100.0)
Enterobacter cloacae	12 (38.7)	1 (3.2)	3 (9.7)	15 (48.4)	31 (100.0)
Klebsiella pneumoniae	183 (49.5)	18 (4.9)	90 (24.3)	79 (21.4)	370 (100.0)
Proteus mirabilis	37 (50.0)	5 (6.8)	5 (6.3)	27 (33.8)	74 (100.0)
Proteus valgaris	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)
Providencia stuartii	7 (43.8)	0 (0.0)	4 (25.0)	5 (31.3)	16 (100.0)
Pseudomonas aeroginusa	1 (0.6)	2 (1.3)	44 (27.5)	113 (70.6)	160 (100.0)
Serratia marcescens	1 (6.7)	0 (0.0)	1 (6.7)	13 (86.7)	15 (100.0)
Stenotrophomonas maltophilia	0 (0.0)	0 (0.0)	4 (17.4)	19 (82.6)	23 (100.0)
Total	292 (26.8)	32 (2.9)	409 (37.6)	356 (32.7)	1089 (100.0)

Values represent numbers (percentage)

Moreover, Table5focuses on the distributions of the multidrug resistance in E. coli and K. pneumoniae isolated samples.

Table 5. The distributions of antibiotics in E. Coli and K. Pneumoniae isolated samples

	E. coli	**K. pneumoniae**	**total**
NULL	48 (37.8)	79 (62.2)	127 (100.0)
ESBL	44 (19.4)	183 (80.6)	227 (100.0)
ESBL(MDR)	2 (10.0)	18 (90.0)	20 (100.0)
MDR	4 (4.3)	90 (95.7)	94 (100.0)

Multidrug resistance distribution in the study

Table 6 shows the Multidrug resistance percentage and frequencies observed in the study. The colony isolatedwere ESBL, ESBL(MDR), and MDR. The result points to a significant challenge posed by antibiotic resistance, with 37.6% of the studied bacteria demonstrating multidrug resistance (MDR), underscoring the rapid evolution of these pathogens, rendering them resistant to multiple drugs and complicating effective treatment strategies.Furthermore, the identification of Extended-Spectrum Beta-Lactamase (ESBL) in 26.8% of cases amplifies the gravity of the situation. ESBL-producing bacteria pose a significant problem due to their resistance to a broad spectrum of antibiotics, severely limiting available treatment options. The subset of ESBL

(MDR) bacteria at 2.9% represents an even more dangerous category, combining extended resistance with multidrug resistance.

Table 6. Multidrug resistance observed in the study

Colony count	Frequency	Per cent
NULL	356	32.7
ESBL	292	26.8
ESBL (MDR)	32	2.9
MDR	409	37.6
Total	1089	100.0

The association between MDR and mortality

The association between MDR frequencies and patients' mortality is shown in Table 7. The data reveals a concerning pattern: patients infected with MDR bacteria exhibited a mortality rate of 70.9%, significantly higher than the 29.1% mortality rate observed in patients without MDR strains. Patients infected with ESBL strains faced a mortality rate of 66.8%, compared to 33.2% among patients without ESBL infections. The subgroup of ESBL (MDR) strains painted an even grimmer picture, boasting a mortality rate of 62.5%, underscoring the lethal potential of these highly resistant pathogens. However, no statistically significant association was found between the different NDR types and the mortality of the patients in the study, P-value= 0.370.

Table 7. The association between MDR and mortality of the patients

	Alive	Died	P-value
MDR	119 (29.1)	290 (70.9)	0.370
ESBL	97 (33.2)	195 (66.8)	
ESBL(MDR)	12 (37.5)	20 (62.5)	
Total	228 (31.1)	505 (68.9)	

Values represent numbers (percentage). The P-value is calculated by a Pearson Chi-Square Test.

The association between MDR and antibiotics used in the study

The association between MDR frequencies and antibiotics is shown in Table 8. Notably, amikacin, a crucial antibiotic, encountered resistance in 55.5% of cases, signifying a high level of resistance against this medication. Likewise, cephalosporins such as cefepime, ceftazidime, and ceftriaxone also displayed significant resistance among MDR strains. This resistance pattern extends to other essential antibiotics like fluoroquinolones (ciprofloxacin and levofloxacin), carbapenems (imipenem and meropenem), and aminoglycosides (gentamycin and tobramycin), further narrowing treatment options for patients afflicted with MDR gram-negative bacteria. Furthermore, the elevated resistance rates observed in other antibiotics such as amoxicillin/clavulanic acid, ampicillin, and sulfamethoxazole/trimethoprim underscore the widespread prevalence of MDR, complicating the selection of effective antibiotics for treatment.

Table 8. The association between MDR and antibiotic

Antibiotics	MDR	ESBL	ESBL(MDR)	Total
Amikacin	406 (55.5)	292 (39.9)	34 (4.6)	732 (100.0)
Amoxycillin / Clavulanic(augmentin)	140 (31.3)	282 (62.9)	26 (5.8)	448 (100.0)
Ampicillin	32 (11.0)	256 (87.7)	4 (1.4)	292 (100.0)
Ampicillin / Cloxacillin	1 (50.0)	1 (50.0)	0 (0.0)	2 (100.0)
Ampicillin + Sulbactam	27 (67.5)	8 (20.0)	5 (12.5)	40 (100.0)
Azithromycin	3 (100.0)	0 (0.0)	0 (0.0)	3 (100.0)
Aztreonam	179 (64.2)	62 (22.2)	38 (13.6)	279 (100.0)
Cefepime	406 (55.9)	289 (39.8)	31 (4.3)	726 (100.0)
Cefotaxime	181 (36.4)	284 (57.1)	32 (6.4)	497 (100.0)
Cefoxitin	2 (50.0)	2 (50.0)	0 (0.0)	4 (100.0)
Ceftazidime	403 (55.7)	290 (40.1)	31 (4.3)	724 (100.0)
Ceftriaxone	9 (64.3)	4 (28.6)	1 (7.1)	14 (100.0)
Cefuroxime	159 (33.5)	286 (60.2)	30 (6.3)	475 (100.0)

Ciprofloxacin	403 (55.5)	291 (40.1)	32 (4.4)	726 (100.0)
Clindamycin	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)
Colistin	279 (50.6)	254 (46.1)	18 (3.3)	551 (100.0)
Daptomycin	0 (0.0)	2 (100.0)	0 (0.0)	2 (100.0)
Ertapenem	187 (40.4)	254 (57.9)	22 (4.8)	463 (100.0)
Erythromycin	5 (100.0)	0 (0.0)	0 (0.0)	5 (100.0)
Gentamycin	407 (55.2)	295 (40.0)	35 (4.7)	737 (100.0)
Imipenem (tienam)	393 (56.0)	276 (39.3)	33 (4.7)	702 (100.0)
Levofloxacin	374 (45.0)	287 (41.4)	32 (4.6)	693 (100.0)
Linezolid	5 (55.6)	4 (44.4)	0 (0.0)	9 (100.0)
Meropenem	406 (55.6)	292 (40.0)	32 (4.4)	730 (100.0)
Moxalactam	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)
Moxifloxacin	122 (68.2)	39 (21.8)	18 (10.1)	179 (100.0)
Mupirocin	1 (50.0)	1 (50.0)	0 (0.0)	2 (100.0)
Nitrofurantoin	14 (35.0)	22 (55.0)	4 (10.0)	40 (100.0)
Norfloxacin	12 (41.4)	15 (51.7)	2 (6.9)	29 (100.0)
Oxacillin	5 (83.3)	1 (16.7)	0 (0.0)	6 (100.0)
Penicillin	3 (60.0)	2 (40.0)	0 (0.0)	5 (100.0)
Pip/Tazo	214 (39.5)	298 (55.0)	30 (5.5)	542 (100.0)
Piperacillin	12 (63.2)	6 (31.6)	1 (5.3)	19 (100.0)
Sulfamethoxazole / Trimethoprim (cotrimox)	373 (53.5)	292 (41.9)	32 (4.6)	697 (100.0)
Tetracycline	16 (64.0)	9 (36.0)	0 (0.0)	25 (100.0)
Tigecycline	143 (35.4)	237 (58.7)	24 (5.9)	404 (100.0)
Tobramycin	153 (39.1)	236 (60.4)	2 (0.5)	391 (100.0)
Trimethoprim/Sulfamethoxazole	0 (0.0)	4 (100.0)	0 (0.0)	4 (100.0)
Vancomycin	3 (50.0)	3 (50.0)	0 (0.0)	6 (100.0)
Total	5480 (48.9)	5176 (46.2)	550 (4.9)	11206 (100.0)

The association between MDR and patients' gender in the study

Table 9 shows the association between MDR frequencies and patients' gender in the study. The results expose a notable trend, with a significantly higher incidence of MDR among male patients in comparison to their female counterparts (65.3% vs. 34.7%). Additionally, the Extended-Spectrum Beta-Lactamase (ESBL) and ESBL(MDR) bacteria also reveal variances rooted in gender, with higher proportions observed among male patients. This observed association between gender and antibiotic resistance was statistically significant, p-value= 0.019, underscoring a statistically meaningful difference in MDR occurrence based on gender.

Table 9. The association between MDR and patients' gender

	Female	**Male**	**Total**	**P-value**
MDR	142 (34.7)	267 (65.3)	409	0.019*
ESBL	129 (44.2)	163 (55.8)	292	
ESBL(MDR)	9 (28.1)	23 (71.9)	32	
Total	280 (38.2)	453 (61.8)	733	

Values represent numbers (percentage). The P-value is calculated by a Pearson Chi-Square test.

The association between MDR and patients' age in the study

Table 10 shows the distribution of MDR bacteria in relation to the patient's age. Among patients below 40 years old, 17.4% were afflicted by MDR bacteria, a figure that increased to 36.7% in the 41-70 age group and further spiked to 46.0% in patients aged over 70 years. A similar pattern emerges with Extended-Spectrum Beta-Lactamase (ESBL) and ESBL(MDR) bacteria, demonstrating a consistent uptick in prevalence as age advances. However, no statistically significant association was found, P-value= 0.267.

Table 10. The percentage of prevalent MDR bacteria isolated among different age groups of patients.

Colony count	< 40	41-70	> 70	P-value
ESBL	42 (14.4%)	96 (32.9%)	154 (52.7%)	0.276
ESBL(MDR)	4 (12.5%)	15 (46.9%)	13 (40.6%)	
MDR	71 (17.4%)	150 (36.7%)	188 (46.0%)	
Total	117 (16.0%)	261 (35.6%)	355 (48.4%)	

Values represent numbers (percentage). The P-value is calculated by a Pearson Chi-Square test.

Antibiotic results in the study

Table 11 shows the results of culture and sensitivity tests for bacteria in the study. Amikacin, while exhibiting a relatively balanced sensitivity and resistance rate, remains a viable option for treatment, given its 42.8% sensitivity rate. However, the alarming rates of resistance seen in several antibiotics, such as Ampicillin, Ciprofloxacin, and Levofloxacin, underscore the challenges faced by healthcare professionals in selecting effective treatments. Also, the high resistance rates in commonly used antibiotics like Ampicillin, Ciprofloxacin, and Gentamycin emphasize the need for stringent antibiotic stewardship programs. Moreover, the limited effectiveness of key antibiotics like Imipenem and Meropenem, with 36.7% and 38.9% sensitivity, respectively, highlights the urgent requirement for novel therapeutic approaches. Interestingly, the sensitivity of antibiotics like Colistin (59.9%) and Tigecycline (71.2%) signifies their potential as last-resort treatments. However, the emergence of resistance even in these options raises concerns and emphasizes the importance of judicious use to prevent further resistance development.The results also highlight the diversity in bacterial response to different antibiotics. For instance, while some bacteria are highly resistant to multiple antibiotics, others exhibit varying degrees of sensitivity.

Table 11. The antibiotic results in the study

Antibiotic	Intermediate	Resistant	Sensitive	Total
Amikacin	74 (6.9)	539 (50.3)	458 (42.8)	1071 (100.0)
Amoxycillin	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)
Amoxycillin / Clavulanic (Augmentin)	29 (4.4)	490 (75.0)	134 (20.5)	653 (100.0)
Ampicillin	3 (0.7)	388 (95.1)	17 (4.2)	408 (100.0)
Ampicillin / Cloxacillin	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)
Ampicillin + Sulbactam	16 (40.0)	5 (12.5)	19 (47.5)	40 (100.0)
Azithromycin	1 (14.3)	6 (85.7)	0 (61.8)	7 (100.0)
Aztreonam	16 (3.6)	324 (73.5)	101 (22.9)	441 (100.0)
Cefepime	45 (4.2)	765 (72.0)	253 (23.8)	1063 (100.0)
Cefotaxime	8 (1.1)	560 (77.7)	153 (21.2)	721 (100.0)
Cefoxitin	2 (25.0)	6 (75.0)	0 (0.0)	8 (100.0)
Ceftazidime	25 (2.3)	794 (73.7)	259 (24.0)	1078 (100.0)
Ceftriaxone	0 (0.0)	15 (75.0)	5 (25.0)	20 (100.0)
Cefuroxime	11 (1.6)	551 (79.4)	132 (19.0)	694 (100.0)
Ciprofloxacin	23 (2.2)	760 (71.2)	284 (26.6)	1067 (100.0)
Clindamycin	0 (0.8)	2 (16.7)	10 (83.3)	12 (100.0)
Colistin	175 (23.1)	128 (16.9)	453 (59.9)	756 (100.0)
Daptomycin	0 (0.0)	0 (0.0)	6 (100.0)	6 (100.0)
Ertapenem	15 (2.3)	398 (61.3)	236 (36.4)	649 (100.0)
Erythromycin	1 (7.1)	6 (42.9)	7 (50.0)	14 (100.0)
Gentamycin	45 (4.2)	566 (52.7)	464 (43.2)	1075 (100.0)
Imipenem (Tieman)	72 (7.0)	580 (56.3)	378 (36.7)	1030 (100.0)
Levofloxacin	87 (8.4)	638 (61.6)	311 (30.0)	1036 (100.0)
Linezolid	1 (5.0)	0 (3.5)	19 (95.0)	20 (100.0)
Meropenem	27 (2.5)	627 (58.5)	417 (38.9)	1071 (100.0)

Moxalactam	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)
Moxifloxacin	5 (1.8)	206 (74.1)	67 (24.1)	278 (100.0)
Mupirocin	0 (0.0)	2 (40.0)	3 (60.0)	5 (100.0)
Nitrofurantoin	6 (9.1)	26 (39.4)	34 (51.5)	66 (100.0)
Norfloxacin	0 (0.0)	30 (58.8)	21 (41.2)	51 (100.0)
Oxacillin	1 (6.7)	12 (80.0)	2 (13.3)	15 (100.0)
Penicillin	0 (0.0)	12 (70.6)	5 (29.4)	17 (100.0)
Piperacillin	0 (0.0)	20 (83.3)	4 (16.7)	24 (100.0)
Piperacillin / Tazobactam (Tazocin)	41 (4.9)	513 (60.9)	278 (34.2)	841 (100.0)
Sulfamethoxazole / Trimethoprim (cotrimox)	0 (0.0)	614 (62.9)	362 (37.1)	976 (100.0)
Tetracycline	2 (5.3)	24 (63.2)	12 (31.6)	38 (100.0)
Tigecycline	82 (15.2)	73 (13.6)	383 (71.2)	538 (100.0)
Tobramycin	37 (7.2)	260 (50.6)	217 (42.2)	514 (100.0)
Trimethoprim/Sulfamethoxazole	0 (0.0)	2 (50.0)	2 (50.0)	4 (100.0)
Vancomycin	0 (0.0)	2 (11.8)	15 (88.2)	17 (100.0)
Total	852 (5.2)	9945 (60.9)	5531 (33.9)	16328 (100.0)

Values represent numbers (percentage)

DISCUSSION

According to the World Health Organization (WHO), antimicrobial resistance is a threat to the prevention of and therapy against infectious diseases. It is a global problem caused mainly by poor administration, inadequate therapy, and the use of antimicrobials in an abusive manner without medical supervision (10). Antimicrobial resistance leads to longer hospital stays, higher medical costs, and increased mortality. Antimicrobial resistance increases by 700,000 the number of deaths annually, and for 2050, the number is estimated to be 10,000,000, overtaking that of cancer. Antimicrobial resistance is increasing globally (11).

In recent years, a great number of resistant strains have emerged in different pathogens, and multidrug-resistant bacteria (MDR) gram-negative pathogens are also becoming increasingly prevalent in the community (12). The specific definition of MDR is labeled as such because of their in vitro resistance to three or more antimicrobial classes of drugs. In a previous report, the WHO published a list of antibiotic-resistant priority pathogens, in which bacteria with critical priority included Acinetobacter spp. and Pseudomonas spp. with carbapenem resistance, and Enterobacteriaceae family members that produced the extended-spectrum beta-lactamases (ESBL) and carbapenemases (13).The mechanisms of drug resistance fall into several broad categories, including drug degradation/alteration, such as ESBL, aminoglycoside-modifying enzymes, or Chloramphenicol acetyltransferases, the modification of drug binding sites/target, and the changes in cell permeability and efflux pump expression, resulting in reduced intracellular drug accumulation (14). Recent studies of the prevalence of antimicrobial resistance have shown a high increase in resistant infections, especially in intensive care unit (ICU) areas. The patients in ICU are vulnerable as they are exposed to different invasive procedures, such as intubation, mechanical ventilation, and vascular access. In addition, many of the drugs employed can give rise to inhibition of their immune response (14).

Therefore, the current study aimed to investigate the prevalence and distribution of MDR gram-negative bacteria in the intensive care area of King Fahad Specialist Hospital, their risk and prognostic factors. Also, this study aimed to detect and evaluate the resistance patterns of isolated gram-negative bacteria and their impacts on morbidity and mortality on the basis of different methods used in determining the sensitivity with the detection of the appropriate treatment of each organism. Lastly, the determination of the correlation of antibiotic resistance data with demographic and clinical data for the patient population was measured.

The present study included 1089 patients. 38.5% of them were females and 61.5 % were males. Among the cases, 66% and 34% were Dead and alive, respectively. Most of the patients were> 70 years old, 48%, while 36.4% were 41-70 years old, and 15.6% were < 40 years old.The study of Maina et al sampled 162 critically ill patients admitted to an intensive care unit at the Nairobi West Hospital in Kenya. The study's findings diverged from our expectations concerning the age and gender distribution of the patients. The participants, spanning ages 1 to 88 years (44.2 ± 17.42), presented a diverse demographic. Surprisingly, the majority were male (64.2%, 104 out of 162). Interestingly, Maina et al supported our results, noting a similar trend where most patients were successfully discharged from the ICU alive (15).

The sample types in the present study were Urine C/S, Peritoneal fluid, CSF, Body Fluid-Pleural C/S, Catheter Tip Culture and Sensitivity, Sputum C/S, Ear Swab C/S, Tracheal Aspirate C/S, Body Fluid-Ascitic C/S, Body

Fluid-Drain Fluid C/S, Body Fluid-Pericardial C/S, Nasal C/S, Wound C/S, Blood Culture - First Bottle – Aerobic, Blood Culture - Second Bottle – Anaerobic, Blood Culture -Third Bottle – Aerobic, Blood Culture - 4Th Bottle – Anaerobic, and Tissue C/S. Tracheal aspirate followed by blood culture (First Bottle-Aerobic), urine, wound, and sputum had the most frequencies of 56.5%, 11.8%, 9.8%, 9.5%, and 4%, respectively.Tracheal aspirate had the highest culture rate. This prevalence is probably due to ventilator-associated pneumonia, one of the principal infections in ICU and which occurs 48–72 h after the initiation of mechanical ventilation (16).

Among the colonies isolated from the present study, ESBL, ESBL (MDR), and MDR, the colony counts were 37.6% had MDR, 32.7% were NULL, 26.8% produced ESBL, and 2.9% produced ESBL (MDR). Uc-Cachón et al were similar to this clinical results; they evaluated a total of 2.711 clinical samples, the majority of isolates were recovered from the clinical samples of bronchial secretions (n = 245), urinary samples (n = 91), and blood samples (n = 76) (17).

In addition, the association between MDR frequencies and patients' mortality was assessed. It was found that there is no association between MDR frequencies and patients' mortality. At the same time, the occurrence of MDR (70.9% vs. 29.1), ESBL (66.8% vs. 33.2), and ESBL (MDR) (62.5 vs. 37.5) was insignificantly higher among the Died patients that the alive patients. The contrast between no connection between MDR frequencies and mortality and the higher occurrence of MDR and ESBL in deceased patients presents a fascinating paradox. This contradiction could be attributed to various factors, such as the seriousness of the underlying illness, other medical conditions, or the effectiveness of the treatment plan. This finding focuses on the intricacies of treating infections in the context of multidrug resistance, highlighting the need for more detailed studies to understand how MDR and patient outcomes interact. It emphasizes the significance of considering multiple factors when assessing the impact of MDR on patient mortality instead of relying solely on the frequency of drug resistance.

On the other hand, the antibiotics in this study, including Amikacin, Amoxycillin/Clavulanic, Ampicillin, Ampicillin / Cloxacillin, Ampicillin + Sulbactam, Azithromycin, Aztreonam, Cefepime, Cefotaxime, Cefoxitin, Ceftazidime, Ceftriaxone, Cefuroxime, Ciprofloxacin, Clindamycin, Colistin, Daptomycin, Ertapenem, Erythromycin, Gentamycin, Imipenem, Levofloxacin, Linezolid, Meropenem, Moxalactam, Moxifloxacin, Mupirocin, Nitrofurantoin, Norfloxacin, Oxacillin, Penicillin, Piperacillin, Sulfamethoxazole/Trimethoprim, Tetracycline, Tigecycline, Tobramycin, Trimethoprim/Sulfamethoxazole, Vancomycin.The patients were further categorized into ESBL, MDR, and ESBL (MDR) and determined their association with antibiotics. Gentamycin (n=7373), Amikacin (n=732), Meropenem (n=730), Cefepime (n=726), Ciprofloxacin (n=726), Ceftazidime (n=724), Imipenem (n=702), Sulfamethoxazole/Trimethoprim (n=697), Levofloxacin (n=693), Cefotaxime (n= 497), Cefuroxime (n=475), Ertapenem (n=463), Amoxycillin /Clavulanic (n=448), and Tigecycline (n=404) were the most tested antibiotics.In addition, the Prevalence of MDR, ESBL, and ESBL (MDR) was statistically significantly higher in males than females (p-value=0.019). MDR (n=409) was 65.3% in males and 34.7% in females, while ESBL (n=292) was 55.8% in males and 44.2% in females. Both ESBL and MDR (n=32) were 71.9% in males and 28.1% in females. Conversely, the Percentage of ESBL, ESBL (MDR) and MDR were insignificant among the age groups <40, 41-70, and > 70. ESBL was 52.7%, 32.9%, and 14.4% in patients aged >70, 41-70, and < 40 years old, respectively. ESBL (MDR) was higher in patients aged 41-70 years old (46.9%), followed by patients aged >70 years old (40.6%) and patients aged <40 (12.5%).This finding was consistent with several recent studies that demonstrated a higher rate of multidrug resistance in males than females. For example, Wang, Guo et al analyzed patients with suspected or confirmed Mycobacterium tuberculosis (MTB) infection and found that the rates of multidrug-resistant tuberculosis (MDR-TB) were higher in males compared to females. The study also revealed that rates of resistance to rifampicin (RFP) and isoniazid (INH) were higher in males than females (18).Wang, Zhang et alfocused on patients using levofloxacin eye drops preoperatively before cataract surgery. The authors found that male patients with hypertension and diabetes mellitus (DM) had a greater risk of having positive bacterial cultures, including multidrug-resistant strains (19).

The observed gender disparity in multidrug resistance can be attributed to various factors. Firstly, behavioral aspects play a significant role. Males may encounter greater exposure to environments or engage in activities that elevate the risk of contracting drug-resistant infections. This increased exposure could stem from occupational hazards or a lower propensity to adhere to prescribed medication regimens, thereby facilitating the development of resistance due to incomplete treatment.Additionally, inherent biological differences between males and females may influence their susceptibility to drug-resistant infections. These variances might encompass divergent immune system responses, distinct genetic makeup, or hormonal factors, all of which can affect how each gender responds to infections and their subsequent treatments.Another critical aspect is the differing patterns in healthcare utilization between males and females. It is conceivable that males, who may be less inclined to seek medical assistance or who might delay seeking help until the infection has progressed, face a heightened risk of severe illness. This delay can increase the probability of encountering drug-resistant strains when treatment is eventually sought.

Finally, the prevalence of comorbid conditions predisposing individuals to multidrug-resistant (MDR) infections may vary based on gender. For instance, a higher propensity among males for certain chronic diseases

associated with an increased risk of MDR infections could partially account for the gender-based discrepancies observed in the prevalence of these infections. Thus, the interaction of these diverse factors provides a comprehensive explanation for the gender disparity noted in multidrug resistance.MDR highly occurred in patients aged >70 years old with 46%, followed by patients aged 41-70 years old (36.7%) and then patients aged <40 (17.4%). From the previous finding, ESBL, ESBL (MDR) and MDR are more likely to occur in older patients but without significance.This finding was aligned with numerous previous studies. For example, Older patients (≥ 65 years) are more likely to harbor multidrug-resistant organisms (MDROs) at hospital admission compared to younger patients (20).

Multidrug-resistant bacterial infections in ventilator-associated pneumonia (VAP) were more common in older age, and age ≥ 65 years was associated with an increased risk of methicillin-resistant Staphylococcus aureus (MRSA) infections (21).

Increased multidrug resistance (MDR) among older adults is a multifaceted issue closely linked to the aging process and associated healthcare challenges. A critical factor in this trend is immunosenescence, the natural weakening of the immune system that occurs with age, which renders older adults more susceptible to infections, including those caused by drug-resistant bacteria (22). Furthermore, the propensity for increased antibiotic exposure in this demographic, often due to chronic illnesses or frequent hospitalizations, contributes significantly to the development of antibiotic resistance in bacteria (23).

In addition to these factors, the prevalence of chronic health conditions such as diabetes, heart disease, and lung diseases in the elderly compromises their overall health and increases vulnerability to drug-resistant infections. The environment of long-term care facilities, such as nursing homes, also plays a crucial role. These settings, characterized by close living quarters and shared healthcare facilities, are conducive to the spread of drug-resistant organisms (24).

Moreover, Polypharmacy, the practice of using multiple medications concurrently, is a common issue in older adults. This practice can result in drug interactions that diminish the efficacy of antibiotics or unintentionally foster the development of drug-resistant bacteria. Additionally, the frequent requirement for medical interventions in older adults, such as surgeries or medical devices like catheters, escalates the risk of acquiring hospital-acquired infections, which are often resistant to multiple drugs (25).

On the other hand, A. Baumannii, E.coli, E. Aerogenes, E. cloacae, K. pneumoniae, P. mirabilis, P. valgaris,P.stuartii, P. aeroginusa, S. marcescens, and S. maltophiliawere the most bacterial isolated in this study. In the present study, the percentage of prevalent bacteria isolated regarding the sample type was evaluated. In tracheal aspirate samples, there were 206 K. Pneumoniae, followed by 192 A. baumannii, then 99 P. aeroginusa, and 38 P. mirabilis, 32 E.coli, 17 S. maltophilia, 11 E. cloacae, 9 S. marcescens, 6 E. aerogenes, 5 P.stuartii, and 0 P. valgaris.

Interestingly, the most prevalent GNB isolate for bronchial secretions was the NF-GNB was P. aeruginosa (42.30%) in the study by Uc-Cachón et al., 2019. Also, Moolchandani et al., 2017 agree with this finding in the ICU of a hospital in Southern India, NF-GNB Acinetobacter spp. (36.00%) and Pseudomonas spp. (27.6%) were those most prevalent in bronchial secretions. The previous finding suggests that this is due to an increase in the colonization of gram-negative bacteria in the respiratory tract of patients on prolonged mechanical ventilation (26).In blood culture (First Bottle-Aerobic), K. pneumoniaehas the highest prevalence at 35.7%, followed by A. baumanniiat 32.6% then E.coli at 16.3, and P. aeroginusa8.5%, while it was not detected in E. aerogenes, P. valgaris, and P.stuartii. In urine samples, the most detected isolate was K. pneumoniae(38.3%), followed by E.coli (28%), then P. aeroginusa(12.1%) and A. baumannii(9.3%). P. valgariswas not detected in urine samples.In the wound sample, the most detected isolate was K. pneumoniae(28.2%), followed by P. aeroginusa(22.3%), then P. mirabilis(18.4), A. baumannii (13.6%) and E.coli (6.8%). P. valgariswas detected only in one sample from a patient with > 70 years old. In the sputum sample, the most detected isolate was K. pneumoniae(34.1%), followed by A. baumannii(27.3%), then P. aeroginusa(13.6%) and E. cloacaeand P. mirabiliswith 9.1%. P. valgariswas not detected in any sputum.

From the previous results, K. pneumoniae, A. baumannii, P. aeroginusa, and E.coli were the highly detected bacteria in our samples. In contrast, P. valgariswas not detected in almost all of the samples. In line with this finding, Uc-Cachón et al., 2019 noted that P. aeruginosa was the predominant isolate (n = 156, 30.17%), followed by K. pneumoniae (n = 104, 20.12%), and E. coli (n = 83, 16.05%). Similar result was reported in studies conducted in three hospitals in Mexico. The most prevalent species found was P. aeruginosa (24%), followed by A. baumannii (12.5%) (27).

Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Escherichia coli have emerged as highly multidrug-resistant Gram-negative bacteria due to several factors. These bacteria possess an extraordinary capacity for genetic adaptability, allowing them to acquire and exchange resistance-conferring genes through mechanisms like plasmid exchange, transposons, and integrons. Their ability to develop efflux pumps is another critical factor, as these protein complexes actively expel antibiotics from the cell, thereby diminishing the drug's intracellular efficacy (28).

Furthermore, forming biofilms by these bacteria provides them with a protective layer, enhancing their

antibiotic resistance and shielding them from host immune responses, contributing to their survival in hostile environments (29). The production of beta-lactamases, particularly by Klebsiella pneumoniae and Escherichia coli, is a significant concern. These enzymes, especially extended-spectrum beta-lactamases (ESBLs) and carbapenemases, effectively inactivate a broad spectrum of beta-lactam antibiotics (30).The prevalence of these bacteria in healthcare settings, where antibiotic use is intensive, creates a selective pressure favoring the survival and proliferation of resistant strains. This environment also presents a high concentration of vulnerable patients, thereby increasing the risk of infection and transmission of multidrug-resistant bacteria. The global spread of these bacteria is exacerbated by travel and medical tourism, combined with inconsistent infection control practices worldwide. This variability in infection control can lead to outbreaks and further dissemination of resistant strains (31).Additionally, the lag in developing new antibiotics, particularly those effective against Gram-negative bacteria, has not matched the pace of emerging resistance. This gap leaves healthcare providers with limited therapeutic options to manage infections caused by these multidrug-resistant organisms (32). Therefore, addressing these challenges through enhanced antibiotic stewardship, robust infection control practices, and ongoing research into novel therapeutic approaches remains imperative in the fight against the rising tide of multidrug-resistant Gram-negative bacteria.

In our analysis of different age groups, it was observed that K.pneumoniae was present in 185 patients aged over 70 years, 120 patients between 41 and 70 years, and 6 patients below 40 years. A.baumannii was identified in 126 patients over 70 years old, 115 patients aged 41-70 years, and 49 patients under 40 years old. E.coli was detected in 48 patients aged over 70 years, 43 patients between 41 and 70 years, and 7 patients below 40 years.Additionally, P. aeruginosa was found in 78 patients over 70 years old, 56 patients aged 41-70 years, and 26 patients below 40 years. P. mirabilis was prevalent in 36 patients over 70 years old, 26 patients aged 41-70 years, and 12 patients below 40 years. E. cloacae was identified in 16 patients over 70 years old, 12 patients aged 41-70 years, and 3 patients below 40 years. E. aerogenes was present in 4 patients over 70 years old, 6 patients aged 41-70 years, and 419 patients below 40 years.

Furthermore, P.stuartii was found in 13 patients over 70 years old, 2 patients aged 41-70 years, and one patient below 40 years. S. marcescenswas detected in 4 patients over 70 years old, 8 patients aged 41-70 years, and 3 patients below 40 years. S.maltophilia was identified in 12 patients over 70 years old, 8 patients aged 41-70 years, and 3 patients below 40 years.Among these isolates, K.pneumoniae, A.baumannii, P. aeruginosa, E.coli, and P. mirabilis were most prevalent in patients over 70 years old, while patients below 40 years exhibited the lowest incidence of these bacterial strains.

Regarding multidrug-resistant groups, K.pneumoniae was prevalent in 183 cases in the ESBL group, 90 cases in the MDR group, 18 cases in the ESBL (MDR) group, and 79 cases in the NULL group. A.baumannii was detected in 4 cases in the ESBL group, 253 cases in the MDR group, 4 cases in the ESBL (MDR) group, and 98 cases in the NULL group. E.coli was identified in 44 cases in the ESBL group, 4 cases in the MDR group, 2 cases in the ESBL (MDR) group, and 48 cases in the NULL group. P.aeruginosa was present in one case in the ESBL group, 44 cases in the MDR group, 2 cases in the ESBL (MDR) group, and 113 cases in the NULL group. P. mirabilis was found in 37 cases in the ESBL group, 5 cases in the MDR group, 5 cases in the ESBL (MDR) group, and 27 cases in the NULL group. On the other hand, E. cloacae was present in 12 cases in the ESBL group, 3 cases in the MDR group, 1 case in the ESBL (MDR) group, and 15 cases in the NULL group. E. aerogenes was detected in 3 cases in the ESBL group, 1 case in the MDR group, 0 cases in the ESBL (MDR) group, and 7 cases in the NULL group. P.stuartii was found in 1 case in the ESBL group, 1 case in the MDR group, 0 cases in the ESBL (MDR) group, and 13 cases in the NULL group. S. marcescenswas identified in 1 case in the ESBL group, 1 case in the MDR group, 0 cases in the ESBL (MDR) group, and 13 cases in the NULL group. S.maltophilia was detected in 0 cases in the ESBL group, 4 cases in the MDR group, 0 cases in the ESBL (MDR) group, and 19 cases in the NULL group. As a result, it might be concluded that most of the bacterial isolates were in the NULL group, except for K.Pneumoniae and P. Mirabilis, in which most of the isolates were ESBL.

When comparing the distributions of antibiotics in E. coliand K. pneumoniaeisolated samples, K. pneumoniaehas a higher prevalence than E. Coli in all resistance groups: NULL (n=127), ESBL(n=227), ESBL (MDR) (n=20), MDR (n=94).In alignment, Maina et al included 92% of the GNB isolates in their study that were MDR, with E. coli (27/30,90%), K. pneumoniae (25/28, 89.3%), and P. aeruginosa (13/13, 100%) as the most frequent isolates. In the NULL group, K. pneumoniaeand E. coliwere 62.2% and 37.8%, respectively. In the ESBL group, K. pneumoniaeand E. coliwere 80.6% and 19.4%, respectively. In the ESBL (MDR) group, K. pneumoniaeand E. coliwere 90% and 10%, respectively. In the MDR group, K. pneumoniaeand E. coliwere 95.7% and 4.3%, respectively (15). Furthermore, Uc-Cachón et al concluded that E.coli (MDR = 91.57%), A. baumannii (MDR = 86.79%), and K. pneumoniae (MDR = 83.65%) exhibited the highest percentage of MDR profile in clinical isolates and, overall, 71.65% of total GNB exhibited MDR profiles, respectively; which strongly support our findings (17).Similarly, in the study of Uc-Cachón et al, the enterobacterial isolates of K. pneumoniae, E. coli, and E. cloacae revealed high resistance rates, which was reversed by the addition of an ESBL inhibitor, such as Sulbactam and Tazobactam, cephalosporins (100–59.37%) except for Cefotetan,

Aztreonam (88.89–87.50%), and Tobramycin (79.01–48.39%). Additionally, clinical isolates of E. coli displayed high resistance rates (17).

Also, the clinical isolates of A. baumannii and P. aeruginosa in Uc-Cachón et al study exhibited high resistance rates (17). Other gram-negative bacteria that, including P.mirabilis, S.marcescens, Burkholderia spp., A. lwoffi, and K. oxytoca revealed high resistance rates to a variety of antibiotics. Besides, Moolchandani et al found that A.baumannii was 82.1%. However, these were higher for E. coli (26.3%) and Klebsiella spp. (52.4%) (26). Uc-Cachón et al have a higher percentage of bacteria producing ESBL, 83.13% (69/83) of the clinical isolates of E. coli, 78.84% (82/104), of K. pneumoniae, and 66.67% (6/9) of P. mirabilis were ESBL-producing.

On the other hand, from 2009–2011, 13.7% and 16.6% of clinical isolates of E. coli were ESBL-producing in the U.S. and European countries, respectively. The ESBL-producing isolates of Klebsiella spp. were reported to have undergone an increase in the prevalence of 27.5–42.8% in Europe during the same years in the study of Sader et al (33).

In the analysis of antimicrobial susceptibility among 1071 bacterial isolates, 50.3% exhibited resistance, 42.8% were sensitive, and 6.9% showed intermediate response to Amikacin. Similarly, among 653 bacterial isolates, 75% were resistant, 20.5% were sensitive, and 4.4% were intermediate to Amoxicillin/Clavulanic acid. Notably, Ampicillin demonstrated a high resistance rate at 95.1% in 408 bacterial isolates, with 4.2% sensitivity and 0.7% intermediate response.The analysis further revealed that 73.5% of 441 bacterial isolates were resistant to Aztreonam, while 22.9% were sensitive, and 3.6% exhibited intermediate response. Additionally, 77.7% of 721 bacterial isolates displayed resistance to Cefotaxime, whereas 21.2% were sensitive, and 1.1% showed intermediate susceptibility. Moreover, 73.7% of 1078 bacterial isolates were resistant to Ceftazidime, with 24% sensitivity and 2.3% intermediate response.Concerning Colistin, 16.9% of 756 bacterial isolates were resistant, 59.9% were sensitive, and 23.1% exhibited intermediate response. In contrast, Gentamycin showed resistance in 52.7% of 1075 bacterial isolates, with 43.2% sensitivity and 4.2% intermediate response. Imipenem displayed resistance in 56.3% of 1030 bacterial isolates, while 36.7% were sensitive, and 7% showed intermediate response. A similar pattern was observed for Levofloxacin, with 61.6% resistance, 30% sensitivity, and 8.4% intermediate response among 1036 bacterial isolates.

Moxifloxacin demonstrated high resistance at 74.1% in 278 bacterial isolates, whereas 24.1% were sensitive, and 1.8% exhibited intermediate response. Previous studies suggested that the higher resistance of Moxifloxacin among gram-negative bacteria may be due to its specific properties and its selective influence on antibiotic sensitivities. A recent study analyzed the antibiotic susceptibility patterns between 2005 and 2020 in ocular infections found that the resistance of Moxifloxacin among gram-negative bacteria was higher compared to other fluoroquinolones. The resistance rate of Moxifloxacin was as high as 74.3%, while other fluoroquinolones showed lower resistance rates (34).Piperacillin/Tazobactam also exhibited substantial resistance at 60.9% in 841 bacterial isolates, with 34.2% sensitivity and 4.9% intermediate response. This high resistance percentage suggested that the majority of the bacteria were resistant to this combination (35). Additionally, 62.9% of 976 bacterial isolates were resistant to Sulfamethoxazole/Trimethoprim, while 37.1% were sensitive, and none showed intermediate response. Tetracycline displayed resistance in 63.2% of 38 bacterial isolates, with 31.6% sensitivity and 2% intermediate response. Tigecycline demonstrated 13.6% resistance, 71.2% sensitivity, and 15.2% intermediate response among 538 bacterial isolates. Tobramycin exhibited resistance in 50.6% of 514 bacterial isolates, with 42.2% sensitivity and 7.2% intermediate response. Vancomycin displayed low resistance at 11.8% in 17 bacterial isolates, while 88.2% were sensitive, and none exhibited an intermediate response. Nitrofurantoin showed resistance in 39.4% of 66 bacterial isolates, with 51.5% sensitivity and 9.1% intermediate response. Mupirocin, tested in five bacterial isolates, exhibited 40% resistance and 60% sensitivity, with no intermediate response.Interestingly, Colistin, Tigecycline, Vancomycin, Nitrofurantoin, and Mupirocin showed higher sensitivity rates compared to their resistance patterns. These antibiotics are thus recommended for use in the intensive care unit based on our study. Noteworthy is the high resistance observed in Ampicillin (95.1%), Piperacillin (83.3%), Oxacillin (80%), Cefuroxime (79.4%), Cefotaxime (77.7%), Ceftriaxone (75%), Amoxicillin/Clavulanic acid (75%), Moxifloxacin (74.1%), and Aztreonam (73.5%). This high resistance could be attributed to the prevalence of ESBL-producing bacteria, particularly Escherichia coli (44.9%) and Klebsiella pneumoniae (49.5%).These findings align with the Uc-Cachón et al study, which reported a similarly high resistance rate for Ampicillin (95.85%), which was followed by Cefuroxime (84.17%), Piperacillin (82.93%), Cefotaxime (78.07%), Ceftriaxone (77.41%), Aztreonam (75.23%), Cefazolin (75.00%), and Ceftazidime (73.19%) (17).

CONCLUSION

Based on the study results, several key recommendations regarding the prevalence of Gram-negative bacteria and antibiotic resistance in the ICU at King Fahad Specialist Hospital were suggestedImplement robust antibiotic stewardship programs, Enhance infection control practices, Develop clinical pathways for MDR infections, Utilize alternative antibiotics wisely, Enhance microbiology capabilities, Implement active surveillance,In addition, several potential avenues of future research and investigation can be recommended

based on the study findings,including:Further characterize resistance genes and mechanisms, Investigate risk factors for acquisition, Assess infection control measures, Examine alternative and combination therapies, Identify pharmacodynamic targets and PK/PD breakpoints,

Ethical considerations

This study was approved by the National Committee of Bioethics (NCBE) before data collection. Informed consent was obtained from patients or their legal guardians after the purpose and procedures of the study were explained to them. The confidentiality and anonymity of participants were assured.

Conflict of Interest

authors declare no conflict of interest.

Authors Contribution

All authors contributed to the sudmittrd manuscript**.**

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