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Frequency and Antibiotic Resistance Patterns in Gram-negative Bacteria in a Hospital in Makkah, Saudi Arabia

Maher N. Alandiyjany

Department of Clinical Laboratory Sciences, Faculty of Applied Medical Sciences, Umm Al-Qura University, Makkah 21955, Saudi Arabia.

Abstract

Cities across Saudi Arabia are grappling with expanding rates of antimicrobial resistance, fuelled in part by overuse of antibiotics. Nevertheless, surveillance of antimicrobial resistance has been scanty, especially in regions such as Makkah, which renders this study to be very significant in shaping up the regional and national public health policies. The study has been conducted at Al-Noor Specialist Hospital in Makkah from November 2024 to April 2025. Both sexes were included (30-70 years of age). A total 960 specimens were obtained from patients at Al-Noor Hospital in Makkah. The positive culture rates were as follows: *Escherichia coli* 310, *Klebsiella* 233, *Pseudomonas aeruginosa* 157, *Acinetobacter* 114, *Enterobacter* 80 and *Proteus* 66. Highest number of1152 isolates in bacteriological examination was 148 from bronchial aspiration samples. Subsequent gastrointestinal symptoms (132) and urine C/S tests done (122). The findings indicated that 69.6% of the isolates were multidrug-resistant (MDR), 18.2% were extensively drug resistant (XDR), and 11.7% were pandrug resistant (PDR). We observed high susceptibility to amikacin, gentamicin, and cefotaxime. These results indicate a pan-drug resistance situation, which calls for continuous monitoring, research and development to prevent further spread of resistance, and to safeguard the efficacy of present regimens. This study infers that the resistance rates of antibiotics in pathogens are higher in the Kingdom and some of the world studies.

Keywords: Multidrug-resistant Bacteria, Extensively Drug-resistant Bacteria, Gram-negative, Antibiotic Resistant

***Correspondence:** mnandiyjany@uqu.edu.sa

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INTRODUCTION

The battle against Gram-negative bacteria (GNB) resistance, whether in the context of the term multidrug-resistant or not, has become one of the most significant worldwide health threat due to the limitation of therapeutic armamentarium against a broad spectrum of infections.[1] Common microbial agents implicated in healthcare-associated infections include *Escherichia coli, Klebsiella* species*, Pseudomonas* species*,* and *Acinetobacter* species. A growing concern is the escalating resistance demonstrated by these organisms to multiple antimicrobial classes, including carbapenems, which are typically considered a therapeutic option of last resort.[2] What makes Gram-negative bacteria particularly formidable is their inherent structural and biochemical defenses. Their outer membrane, composed of lipopolysaccharides, not only enhances virulence but also acts as a formidable barrier to many antibiotics.[3] Moreover, these bacteria can enact various resistance mechanisms, including efflux pumps, porin alterations, as well as beta-lactamase production, among others, to escape even strong antimicrobial agents.[4] The capacity of Gram-negative bacteria to acquire resistance genes via horizontal gene transfer is a significant concern, facilitating the swift propagation of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains.[5] Consequently, healthcare professionals frequently encounter resistant Gram-negative bacterial infections in clinical settings, particularly in intensive care units (ICUs), surgical wards, and among immunocompromised individuals.[6] These infections manifest as urinary tract infections (UTIs), ventilator-associated pneumonia (VAP), bloodstream infections (BSIs), and skin and soft tissue infections. Recognizing the severity of the threat, the World Health Organization (WHO) has designated certain Gram-negative bacteria as "critical priority" pathogens, emphasizing the urgent need for novel antimicrobial agents and improved strategies for infection control.[7] In Saudi Arabia, the growing burden of MDR Gram-negative infections mirrors global trends but is intensified by unique local dynamics. Rapid healthcare expansion, high volumes of international travelers particularly during Hajj and Umrah and inconsistent antibiotic prescription practices have all contributed to a complex and evolving resistance landscape.[8] Studies from Saudi hospitals have documented high resistance rates, especially in tertiary care centers and ICU settings. Regional disparities also exist, with central and western regions reporting notably higher levels of resistance.[9] The prevalence of ESBL (extended-spectrum beta-lactamase) producing *E. coli* and *K. pneumoniae,* Carbapenem-resistant *A. baumannii* and *P. aeruginosa* has complicated the empirical therapy.[10] Unfortunately, resistance to colistin, one of the last therapeutic options available, is increasingly being reported.[11] These patterns are correlated with prolonged hospital stay, increased mortality, and enhanced costs of medical care. Although attempts have been made to enforce antimicrobial stewardship programs (ASPs) on a national level, compliance is notably lacking. The spread of resistance is still being driven by factors like overuse of broad-spectrum antibiotics, poor access to rapid diagnostic services, and poor infection prevention control practices.[12] The present study was conducted to determine the prevalence and resistance rates of Gram-negative pathogens isolated at Al-Noor Hospital in Makkah, Kingdom of Saudi Arabia. This is at least in part highly relevant since resistance rates differ considerably between classes of antimicrobials.

MATERIALS AND METHODS

Ethical Aspects

This study was conducted to evaluate the level of medical care and its impact on patient satisfaction with medical and nutritional health services in the Makkah region of Saudi Arabia. The study was approved by the Medical Research Ethics Committee in Makkah, Saudi Arabia. Written informed consent was obtained from each participant before completing the study questionnaire. The confidentiality of all research data was guaranteed, and participation was voluntary. The following were explained:

- The nature of the study and its protocols.
- The potential risks and benefits associated with the study.
- Voluntary participation.

• The freedom to withdraw from the study at any time.
• The reliability of the results.

Study setting, period, and population

Random samples were collected from 960 patients in the Microbiology Department at Al-Noor Specialist Hospital (ASH) in Makkah between November 2024 to April 2025. Strict sterile testing was performed on patients during clinical sample collection, including blood, urine, and wound samples. In the laboratory, susceptibility to antibiotics was evaluated based on the trends emerging from all the reports containing resistance data for the six Gram-negative bacterial isolates (*Escherichia coli*, *Acinetobacter* species, *Klebsiella* species, *Pseudomonas* species, *Enterobacter* species and *Proteus* species) responsible for the infections.

During the study period, all patients visiting Al-Noor Hospital in Makkah to avail healthcare services were requested to provide the relevant information. At least gender, age, and ward type were included in the study. Data with missing information on age, gender, and ward type were excluded.

Sample collection, isolation, and identification

The entire collection of samples was done in sterile containers at different times during the research period, and undoubtedly treated to eliminate the possibility of contamination and quick transportation to the lab. The examination was conducted according to the standard procedures and was done within six hours after the collection. The samples were inoculated with a sterile loop on different types of culture media like blood agar, cysteine lactose electrolyte agar, xylose lysine deoxycholate agar, and MacConkey agar. The aforementioned media were selected because they can isolate Gram-negative bacteria from clinical specimens effectively, specifically and reliably. They also support the growth of bacteria and at the same time, the pathogens can be identified easily, which is vital for the studies of drug resistance and treatment strategy development. Plates were incubated for 24 hours at 33 °C, except for chocolate agar plates (incubated for 24 hours at 37 °C without air). Automated methods using a BD-Phoenix 100 analyzer were used to identify the organisms correctly.

Antimicrobial susceptibility

The determination of antibiotic susceptibility was done by applying the Kirby-Bauer disk diffusion method. The broth microdilution technique was employed to ascertain the MICs of important antibiotics. The interpretation of resistance was based on the guidelines of the Clinical and Laboratory Standards Institute (CLSI). The GNB isolates were tested for susceptibility to the following antibiotics: ceftriaxone, ampicillin, cefuroxime, ciprofloxacin, levofloxacin, amikacin, gentamicin, colistin, erythromycin, and mupirocin.

Statistical analysis

The source data pertaining to the isolates was compiled, refined, and coded using Microsoft Excel 2016. Descriptive statistical analysis, incorporating means and percentages, was conducted to determine: the proportion of patients affected by multidrug-resistant Gram-negative bacteria; a comparative evaluation of resistance profiles across various demographic and clinical categories; and the identification of specific types of multidrug-resistant bacteria.

RESULTS

Most common Gram-negative pathogens

Antimicrobial resistance is a dynamic and multifaceted phenomenon, influenced by complex interactions between various factors. These factors include direct elements, such as antimicrobial misuse, as well as indirect factors, such as environmental pollution. The inherent characteristics of bacteria also play a significant role. Researchers have identified prior antibiotic exposure, underlying health conditions, and medical procedures as key risk factors associated with antibiotic resistance. Therefore, Gram-negative bacteria have become the primary cause of healthcare-associated infections (HAIs) in all the hospitals in Saudi Arabia. Besides the fact that their prevalence is extremely high in the ICUs, surgical departments, and organ transplant units, nowadays, these pathogens are not just getting more common but also more resistant, and the presence of multidrug-resistant (MDR)

Table 1. Gram-negative bacteria are the principal cause of infections that are acquired in healthcare settings

Organism	Typical Infections	Notes
Escherichia coli	UTIs, BSIs, wound infections	Frequently ESBL-producing
Klebsiella species	Pneumonia, BSIs, UTIs	Rising carbapenem resistance
Pseudomonas species	Respiratory, burns, device-associated infections	High intrinsic resistance
Acinetobacter species	VAP, bloodstream, ICU infections	Often XDR; outbreaks reported
Enterobacter spp.	BSIs, surgical wound infections	Emerging resistance to cefepime
Proteus spp.	UTIs	Less prevalent but often MDR

Table 2. Characteristics of Patients Sample type distribution of the patients in the study

Sample types	No. of Isolates	Percent(%)	Female (%)	Male (%)
Fever	86	8.9	44 (51.1%)	42 (48.8%)
Tracheal Aspirate C/S	148	15.4	83 (56.1%)	65 (43.9%)
Catheter Tip Culture & Sensitivity	13	1.3	8 (61.5%)	5 (38.4%)
Body Fluid-Drain Fluid C/S	16	1.6	10 (62.5%)	6 (37.5%)
Blood Culture	207	21.5	105 (50.7%)	102 (49.2%)
Respiratory infections	75	7.8	32 (42.6%)	43 (57.3%)
Chest tightness and shortness of breath	84	8.7	45 (53.5%)	39 (46.4%)
Gastrointestinal symptoms (vomiting, diarrhea)	132	13.7	69 (52.3%)	63 (47.7%)
Urine C/S	122	12.7	64 (52.4%)	58 (47.5%)
Wound C/S	77	8.01	43 (55.8%)	34 (44.1%)
Total	960	100	514 (53.5%)	446 (46.5%)

and extensively drug-resistant (XDR) strains is increasing, as demonstrated in Table 1.

Patient demographic and clinical characteristics

Bacteriological examination revealed the isolation of 960 organisms from clinical samples, including female and male 86 Fever samples, 148 Tracheal Aspirate C/S, 11 Catheter Tip Culture & Sensitivity samples, 16 Body Fluid-Drain Fluid C/S, 207 blood samples, 75 Respiratory infections, 84 Chest tightness, 132 Gastrointestinal symptoms, 122 Urine C/S and 77 Wound C/S. The results showed a diversity of samples, with blood cultures constituting the largest proportion at 21.5%. Furthermore, the inclusion of different sample types, such as urine samples, wound examinations, catheter tips, fever, and respiratory infections, demonstrates the rigor of the research methodology. Females were slightly more represented than males in most isolated samples (53.57% male, 46.43% female) as shown in Table 2.

Distribution and characteristics Gram-negative bacteria of isolated strains

Of the 960 isolated strains, the Gram-negative bacteria included *Escherichia coli* 310 (32.3%), *Klebsiella* spp. 233 (24.3%), *Pseudomonas* spp. 157 (16.3%), *Acinetobacter* spp. 114 (11.8%), *Enterobacter* spp. 80 (8.3%) and *Proteus* spp. 66 (6.8%). Table 3 shows the distribution of isolated bacteria among the different study samples. The results showed differences in bacterial prevalence across sample types. For example, urine samples showed a higher prevalence of *Escherichia coli* and *Klebsiella* spp. In contrast, tracheal aspiration (C/S) samples revealed a variety of bacteria, including *Escherichia coli, Klebsiella* spp., and *Acinetobacter* spp. Furthermore, blood cultures revealed the presence of various pathogens, most notably *Escherichia coli* and *Klebsiella* spp. Analysis of the samples showed that most of the bacterial organisms were of the types *Escherichia coli Klebsiella* spp. and *Pseudomonas* spp. that cause most infections and diseases

Table 3. Distribution of isolated Gram-negative bacteria among the different study samples

Sample types	microorganism												Frequency
	Escherichia coli		*Klebsiella* spp.		*Pseudomonas* spp.		*Acinetobacter* spp.		*Enterobacter* spp.		*Proteus* spp.		
	n	%	n	%	n	%	n	%	n	%	n	%	
Fever	22	25.5	15	17.4	31	36.1	7	8.1	8	9.3	3	3.4	86
Tracheal Aspirate C/S	38	25.6	37	25	20	13.5	32	21.6	12	8.1	9	6.1	148
Catheter Tip & Sensitivity	2	18.2	4	36.3	3	27.2	0	0	1	9.1	1	9.1	11
Body Fluid-Drain	3	18.7	9	56.2	4	25	0	0	0	0	0	0	16
Blood Culture	65	31.4	49	23.6	37	17.8	16	7.7	25	12.1	15	7.2	207
Respiratory infections.	10	13.3	20	26.6	21	28	7	9.3	11	14.6	6	8	75
Chest tightness and breath	29	34.5	17	20.2	6	7.1	11	13.1	7	8.3	14	16.6	84
Gastrointestinal symptoms	55	41.6	19	14.3	22	16.6	18	13.6	7	5.3	11	8.3	132
Urine C/S	49	40.1	42	34.4	9	7.4	17	13.9	3	2.4	2	1.6	122
Wound C/S	35	45.4	21	27.2	4	5.2	6	7.8	6	7.8	5	6.4	77
Total	310	32.3	233	24.3	157	16.3	114	11.8	80	8.3	66	6.8	960

Distribution of isolated clinical bacteria by patient's age

Table 4 shows the distribution of bacteria isolated in the study according to patient age. The results showed that some bacteria, particularly *Klebsiella* spp. 144 (50.5%) and *Acinetobacter* spp. 133 (46.1%), exhibited a significant increase in prevalence with advancing age, highlighting the likelihood of infection in elderly patients. Increases were also observed in middle-aged patients, but to a lesser extent in the elderly. Other bacterial isolates such as *Enterobacter* spp. 8 (13.7%) *Proteus* spp. 11 (14.6%) showed slight increases in younger age groups.

Patient Demographics and bacterial composition

Table 5 shows the results of different age groups in relation to infection with different types of bacteria. Patients aged between 30 and 70 years were the most susceptible to infection with *Klebsiella* bacteria, followed by *Escherichia coli,* then *Acinetobacter* spp., and then *Klebsiella* spp. Females were more susceptible than males in most infections, particularly *Klebsiella* spp. female 157 (55.1%) and male 128 (44.9%) and *Pseudomonas* spp. accounting female 81 (53.2%) and male 71 (46.7%), Conversely, *Enterobacter* spp. In the age group of males over 30 years old Lowest value (25.0%) and *Proteus* spp. In the age group of males 30-70 years old (27.7) was less common among males than females.

Prevalent bacteria isolated among different multidrug-resistant groups in the study

In the event that a group of 960 Gram-negative bacilli (GNBs) were isolated, with *E. coli* being the predominant group, comprising 310 isolates (32.3%). Among these, 106 (34.1%) exhibited resistance. including 85 (80.1%) (MDR). isolates, 12 (11.3%) (XDR) isolates and 9 (8.4%) (PDR) isolates. *Klebsiella* spp. represented 233 (24.3%) of the isolates, with 108 (46.3%) exhibiting resistance, comprising 73 (67.5%) (MDR) isolates, 19(17.5%) (XDR) isolates and 16 (14.8%) (PDR) isolates. *Pseudomonas* spp. exhibited 157 isolates (16.3%), of which 124 (78.9%) were resistant, comprising 84 (67.7%) (MDR) isolates, 24 (19.3%) (XDR) isolates and 16 (12.9%) (PDR) isolates. *Acinetobacter* spp. accounted for 114(11.9%) isolates, of which 96 (84.2%) were resistant,

Table 4. The percentage of prevalent bacteria isolated among different age groups of patients

Organism isolated	<30		31-50		51-70		Total
	n	%	n	%	n	%	
Escherichia coli	12	11.7	41	40.1	49	48	102
Klebsiella spp.	57	20	84	29.4	144	50.5	285
Pseudomonas spp.	22	14.4	56	36.8	74	48.7	152
Acinetobacter spp.	45	15.6	110	38.2	133	46.1	288
Enterobacter spp.	8	13.7	18	31.1	32	55.1	58
Proteus spp.	11	14.6	27	36.0	37	49.3	75
Total	155	16.1	336	35.0	469	48.8	960

comprising 64 (66.6%) (MDR) isolates, 21 (21.8%) (XDR) isolates and 11 (11.4%) (PDR) isolates. *Enterobacter* and *Proteus* spp. made up 80 (8.3%) and 66 (6.8%) of the isolates, and were more (MDR) (80.9) and (50.0) than both (XDR) and (PDR) as shown in Table 6.

Antibiotic resistance pattern among MDR, XDR and PDR Gram-negative isolates

Antibiotic resistance patterns showed higher resistance among both MDR, XDR and PDR groups. In the MDR group (n = 380), resistance was highest to Amikacin (61.2%), Colistin (55.2%), and Ciprofloxacin (51.5%), Resistance were lower in the XDR (n = 99) groups isolates resistance was highest to Amikacin (35.3%), Colistin (36.3 %), and Erythromycin (34.3%). However, the resistance in PDR was less than in MDR and higher than in XDR, reaching in each of the Amikacin (50.0%), Erythromycin (62.5%) and Ciprofloxacin (64.1%), However, the lowest values in the three groups were in each of MDR Ceftriaxone 32.8% and Gentamycin 27.1% XDR (Gentamycin 17.1% and Mupirocin 23.2%) and PDR(Moxifloxacin 23.4% and Mupirocin 43.5%) respectively as shown in Table 7.

Major Gram-negative bacteria: comparative results for antimicrobial susceptibility

Bacterial resistance rates to antimicrobial agents vary significantly between different bacterial species, which are summarized in Table 8 as the mean resistance rates of the associated Gram-negative bacteria. With the discovery of multiple resistant strains, antibiotic susceptibility testing (Table 8). Revealed different patterns of bacterial resistance. Antibiotic-resistant bacteria exhibited high resistance to amikacin, cefotaxime, and ampicillin, while levofloxacin, moxifloxacin, and mupirocin isolates showed lower resistance. Some bacteria exhibited the highest resistance to cefoxitin (79.6%) in *Escherichia coli,* followed by ciprofloxacin (77.1%) in *Pseudomonas aeruginosa.* The lowest resistance was observed to levofloxacin (17.1%) in *Escherichia coli,* erythromycin (21.2%) in the same group, and mupirocin (21.6%) in *Pseudomonas aeruginosa.*

DISCUSSION

The existence of Gram-negative multidrug-resistant (MDR) bacteria has evolved into a serious concern for the world health due to the fact that they are now frequently found in hospitals. An example of such bacteria is *Escherichia coli*, *Klebsiella* species, *Proteus* species, and *Citrobacter* species which have acquired resistance against the greater part of the antibiotics thus the only options left are the ones that are less effective making the situation worse in the aspect of treatment failure and death.[13] It was stated that the bacteria belong to the Gram-negative bacteria group, an order called *Enterobacteriaceae*.[14] Certain microorganisms, including yeasts, have evolved significant resistance to a wide range of antimicrobial agents employed in human medicine.[15] The increasing prevalence of these resistant bacteria represents a substantial and growing concern, with recent investigations indicating that approximately 30% of community-acquired infections now exhibit resistance to commonly prescribed antibiotics.[16] Addressing antimicrobial resistance in humans requires a comprehensive and multidisciplinary approach. Given the high potential for human-to-human transmission, antimicrobial resistance

Table 5. Demographic Characteristics of Patients with Bacterial Infections

Organism isolated	<30		31-50		51-70		Total	
	Female number and percentage	Male number and percentage	Female number and percentage	Male number and percentage	Female number and percentage	Male number and percentage	Female number and percentage	Male number and percentage
Escherichia coli	7 (58.3%)	5 (41.6%)	23 (56.1%)	18 (43.9%)	29 (59.1%)	20 (40.8%)	59 (57.8%)	43 (42.1%)
Klebsiella spp.	32 (56.1%)	25 (43.8%)	46 (54.7%)	38 (45.2%)	79 (54.8%)	65 (45.1%)	157 (55.1%)	128 (44.9%)
Pseudomonas spp.	12 (54.5%)	10 (45.4%)	30 (53.5%)	26 (46.4%)	39 (52.7%)	35 (47.2%)	81 (53.2%)	71 (46.7%)
Acinetobacter spp.	23 (51.1%)	22 (48.8%)	59 (53.6%)	51 (46.3%)	74 (55.6%)	50 (37.5%)	156 (54.1%)	132 (45.8%)
Enterobacter spp.	6 (75.0%)	2 (25.0%)	13 (72.2%)	5 (27.7%)	18 (56.2%)	14 (43.7%)	37 (63.7%)	21 (36.2%)
Proteus spp.	6 (54.5%)	5 (45.4%)	18 (66.6%)	9 (33.3%)	21 (56.7%)	16 (43.2%)	45 (60.0%)	30 (40.0%)

should be addressed across all populations.[17] Accordingly, a systems-oriented perspective within ecological contexts is essential and constitutes a fundamental component of antimicrobial stewardship (AMS) initiatives designed to promote judicious antimicrobial utilization in human populations.[18] Furthermore, some studies have been published indicating an increase in cases of antibiotic hypersensitivity syndrome in humans. However, information regarding the isolation, resistance, and susceptibility patterns of bacteria remains very limited.[19]

Multiple-drug-resistant bacterial strains have emerged throughout the previous years while Gram-negative pathogens now display universal drug resistance according to research.[20] The bacteria received their name because they demonstrate resistance to three or more antimicrobial drug classes according to laboratory results. The World Health Organization (WHO) has released antibiotic-resistant pathogen lists which include critical bacteria such as carbapenem-resistant *Acinetobacter* and *Pseudomonas aeruginosa*.[21] The drug resistance mechanisms can be classified into several main groups such as the production of ESBL enzymes, the modification of aminoglycosides, the action of chloramphenicol acetyltransferase, and changes in the cell permeability which ultimately results in reduced drug accumulation inside the bacteria. Recent studies on the distribution pattern of antimicrobial resistance have revealed a sharp increase in resistant infections particularly in intensive care units. Patients in these units are more susceptible to infection due to undergoing various surgical procedures. Furthermore, many of the medications used may suppress their immune response.[22]

In this study, which analyzed 960 Gram-negative bacterial samples, the most valuable isolates came from blood cultures, tracheal aspiration, gastrointestinal symptoms, and urinary tract examinations, respectively, while the least valuable came from catheter tip cultures and fluid drainage. Samples of concern regarding bacterial infection, such as urine samples, were the most common source, totaling 122 samples (12.7%), with females predominating (64 samples) (52.4%), consistent with the known epidemiology of urinary tract infections. Wound examinations accounted

Table 6. The Number and percentage of prevalent bacteria isolated among different multidrug-resistant groups strains of respective bacteria

Organisms isolated	No. of Isolates	MDR (%)	XDR (%)	PDR (%)	Total resistant isolates
Escherichia coli	310 (32.3)	85 (80.1)	12 (11.3)	9 (8.4)	106 (34.1)
Klebsiella spp.	233 (24.3)	73 (67.5)	19 (17.5)	16 (14.8)	108 (46.3)
Pseudomonas spp.	157 (16.3)	84 (67.7)	24 (19.3)	16 (12.9)	124 (78.9)
Acinetobacter spp.	114 (11.3)	64 (66.6)	21 (21.8)	11 (11.4)	96 (84.2)
Enterobacter spp.	80 (8.3)	51 (80.9)	8 (12.6)	4 (6.3)	63 (78.8)
Proteus spp.	66 (6.8)	23 (50.0)	15 (32.6)	8 (17.3)	46 (69.6)
Total	960 (100)	380 (69.9)	99 (18.2)	64 (11.7)	543 (56.5)

Table 7. Antibiotic Resistance Pattern among MDR, XDR and PDR Gram-negative Isolates

Antibiotic	MDR resistance (n = 380)		XDR resistance (n = 99)		PDR resistance (n = 64)	
	n	%	n	%	n	%
Amikacin	234	61.5	35	35.3	32	50.0
Ampicillin	178	46.8	29	29.2	25	39.1
Cefotaxime	191	50.2	26	26.6	27	42.1
Ceftriaxone	125	32.8	28	28.2	34	53.1
Ciprofloxacin	196	51.5	25	25.2	41	64.1
Colistin	210	55.2	36	36.3	28	43.7
Erythromycin	119	31.3	34	34.3	40	62.5
Gentamycin	103	27.1	17	17.1	33	51.2
Levofloxacin	174	45.7	18	18.1	49	76.5
Moxifloxacin	186	48.9	30	30.3	15	23.4
Mupirocin	159	41.8	23	23.2	28	43.5

for 77 samples (8.01%), with the majority of these being female 43 samples (55.8%). This is likely due to trauma and postoperative wound infections. Respiratory tract infection samples yielded 75 cases (7.8%), with a high proportion being male (43 samples, 57.3%), suggesting a higher incidence of respiratory tract infections among males, possibly due to occupational exposure. Blood contributed to 207 samples (21.5%), the highest proportion among isolated samples, with similar proportions between the sexes, indicating more severe infections, such as sepsis, in both sexes. These findings are consistent with observations confirming the presence of Gram-negative bacteria in critical care settings.[23,24]

The distribution of pathogens revealed that *Escherichia coli* was the most frequently isolated organism, with 310 isolates (32.3%), followed by *Klebsiella* spp. (233 isolates) (24.3%), *Pseudomonas* spp. (157 isolates) (16.3%), and *Acinetobacter* spp. (114 isolates) (11.3%). These species are common pathogens of community- and hospital-acquired infections, particularly urinary tract and lower respiratory tract infections. They were primarily isolated from respiratory tract and wound specimens. *Proteus* spp. (66 isolates) (6.8%) and *Enterobacter* spp. (80 isolates) (8.3%) were less frequently isolated. This distribution reflects a pattern of Gram-negative bacteria in healthcare facilities, where antagonistic bacteria predominate.[25]

Research conducted earlier by Samonis et al.[26] showed that *Pseudomonas* spp. and *Escherichia coli* and *Klebsiella* spp. were the dominant bacterial species found in the adult intensive care unit of a tertiary care hospital based in Riyadh, Saudi Arabia.[27] The bacteria identified in this study match the typical bacterial infections doctors encounter during routine medical practice. The research results match those from a Riyadh-based study conducted by Alkofide et al.[28] The study identified urine as the primary source of

Table 8. Number and rates of resistance of clinical Gram-negative bacteria to common antibiotics

Antibiotic	Microorganism											
	Escherichia coli 310		*Klebsiella* spp. (233)		*Pseudomonas* spp. (157)		*Acinetobacter* spp. (114)		*Enterobacter* spp. (80)		*Proteus* spp. (66)	
	n	%	n	%	n	%	n	%	n	%	n	%
Amikacin	173	55.8	171	73.3	123	78.3	63	55.2	66	82.5	33	50.0
Ampicillin	145	46.7	129	55.3	117	74.5	54	47.3	33	41.2	42	63.6
Cefotaxime	247	79.6	188	80.6	108	68.7	61	53.5	32	40.0	41	62.1
Ceftriaxone	98	31.6	120	51.5	72	45.8	44	38.5	27	33.7	24	36.3
Ciprofloxacin	97	31.2	83	35.6	121	77.1	39	34.2	18	22.5	29	43.9
Colistin	33	10.6	91	39.0	47	29.9	47	41.2	16	20.0	36	54.5
Erythromycin	66	21.2	87	37.3	32	20.3	38	33.3	16	20.0	35	53.0
Gentamycin	79	25.4	77	33.0	109	69.4	59	51.7	23	28.7	39	59.1
Levofloxacin	55	17.7	95	40.7	97	61.7	28	24.5	26	32.5	28	42.4
Moxifloxacin	131	42.2	62	26.6	43	27.3	42	36.8	25	31.2	31	46.9
Mupirocin	88	28.3	51	21.8	34	21.6	29	25.4	23	28.7	27	40.9

infection. The combination of medical factors including urinary catheter placement and pre-existing conditions and anatomical differences in patients makes them more susceptible to urinary tract infections which leads to increased positive test results from urine samples.

The results of this study showed that some bacteria become more prevalent with age, highlighting the potential for infection in elderly patients. Increases were also observed in middle-aged patients, but to a lesser extent in the elderly. Other bacterial isolates showed slight increases in younger age groups. Patients aged 30-70 years were most susceptible to *Klebsiella*, followed by *Escherichia coli*, and then *Acinetobacter*. Females were more susceptible than males in most cases. A study conducted in Saudi Arabia.[29] On the other hand, another study revealed that the female infection rate was higher than that of males.[30] Such variability is not simple to explain and should thus be seen in light of differences in sample collection procedures, study design, sample characteristics, inclusion of patients in the study, the settings and the hygiene procedures used.

One of the most important discoveries from the research was that of the great antimicrobial resistance tendency. Among the 960 Gram-negative isolates, 380 (69.9%) were identified as multidrug-resistant (MDR), while 99 (18.2%) were classified as extensively drug-resistant organisms (XDR). Only 64 (11.7%) were susceptible to severe pandrug resistant (PDR). This resistance has been internationally defined by the classification proposed by Magiorakos et al.[31] and reflects a worrying trend in resistance.

The situation in Makkah has been highlighted by these findings, which are a clear indicator of the increasing problem of microbial resistance and the necessity of prompt public health interventions of a specific nature. Our figure of multidrug-resistance was greater than that found in other parts of Saudi Arabia, but the figure for severe and complete drug resistance was comparably lower. As an instance, a research by Alnour et al.[32] in northwestern Saudi Arabia gave prevalence rates of 33.4%, 29.3%, and 12.4%, respectively. The large presence of the multidrug resistance phenomenon can be linked to antibiotic usage on a large scale, since antibiotics are the main factor that creates a more favorable environment for resistant strains to appear and spread among the bacterial populations.[33] On the other hand, though, *Acinetobacter* spp. isolates showed the highest prevalence of XDR and PDR phenotypes among all the bacteria tested, where 54% and 46% of the isolates were found to be XDR and PDR, respectively. These numbers are much greater than those of Said et al.[34] who reported 35% and 3% for XDR and PDR, respectively. Moreover, *Acinetobacter spp* is capable of acquiring multidrug-resistance due to its intrinsic mechanisms of resistance and diverse genetic makeup.[35]

In the same way, *Escherichia coli* and *Klebsiella* spp. got samples which showed a high level of multipledrug-resistance (MDR) and contained in addition the XDR and PDR phenotypes. The level of multipledrug-resistance in *Klebsiella* spp. was even higher than what was documented by Hafiz et al.[36] *Klebsiella* spp. ability to acquire and spread resistance genes is what accounts for the development of the strains which are multidrug-resistant. *Pseudomonas* spp. isolates also exhibited a high level of the multiple drug resistant strains as documented by Ibrahim.[37] *Pseudomonas* spp. is known to have a high level of resistance to a number of antibiotics and this resistance can increase by various ways including the development of efflux pumps and biofilms.[38,39]

Within the scope of our investigation into the sensitivity of laboratory-derived microbial strains to a range of antimicrobial compounds, we have characterized an organism as exhibiting multidrug resistance when demonstrating insensitivity to a minimum of three distinct antimicrobial agents. Antibiotic susceptibility testing revealed different patterns of bacterial resistance. Antibiotic-resistant bacteria showed high resistance to amikacin, cefotaxime, and ampicillin, while levofloxacin, moxifloxacin, and mupirocin isolates showed lower resistance. The same result was recorded in a study which was published by Silva et al.[40] Resistance in *Proteus* and *Escherichia coli* strains was very low in comparison to the first group.

Some bacteria showed the highest resistance to cefoxitin (79.6%) in *Escherichia coli*, followed by ciprofloxacin (77.1%) in *Pseudomonas aeruginosa*. The lowest resistance to levofloxacin (17.1%) was observed in *Escherichia coli,* erythromycin (21.2%) in the same group, and mupirocin (21.6%) in *Pseudomonas aeruginosa.* Studies by Narten et al.[41] reported that cefotaxime resistance to *Escherichia coli* (24%-54%) was high in *Staphylococcus aureus*, while high resistance to aminoglycosides was observed in 53.3% of *Enterococcus* species in the same study. It has been suggested that the higher amounts of resistant bacteria in Saudi Arabia are the result of the country's escalating antibiotic consumption.[42] A research study was carried out in Mecca, where it was found that the rate of resistance among Gram-negative bacteria was much higher than in other countries around the world, thus making a surveillance program to monitor the situation necessary. Therefore, implementing a national antibiotic policy and guidelines is a critical step in curbing the occurrence of multidrug-resistance as well as keeping the resistance to modern antibiotics in Saudi Arabia at a low level.[43]

CONCLUSION

The current research has drawn attention to the existence of multidrug-resistant and extensively drug resistant Gram-negative bacilli in clinical samples, posing a major challenge for the antibiotic treatment to be effective. It points out the necessity of strict sanitary measures and efficient antimicrobial management programs. Timely detection and careful antibiotic use are still vital in containing the resistant pathogens' spread. It would be a good idea to carry out more studies including a national project to check the susceptibility of Gram-negative bacteria from hospitals and health centers in Makkah. Additionally, knowing the resistance mechanisms of these bacteria will be helpful for marking their distribution within the study area.

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DATA AVAILABILITY

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

ETHICS STATEMENT

The study was approved by the Medical Research Ethical Committee, Umm Al-Qura University, Saudi Arabia (application number BIBB060326).

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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