



Antibiotic Resistance Patterns of Gram-Negative Bacilli Isolated from Inpatients Admitted to Various Wards of a Tertiary Hospital in Tehran, Iran

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Abstract

Background: Hospital-acquired infections (HAIs), specifically those caused by gram-negative bacteria, are a significant public health concern with high mortality rates among patients worldwide, especially in developing countries.

Objectives: This survey aimed to determine the prevalence and antibiotic resistance patterns of gram-negative nosocomial infections among patients admitted to different wards of a tertiary hospital in Iran.

Methods: This cross-sectional study was conducted using samples collected from patients admitted to coronary care unit (CCU), intensive care unit (ICU), neonatal intensive care unit (NICU), general, and surgery wards of Yas Hospital, Tehran, Iran, from October 1st, 2022, to February 28th, 2023. After the detection of bacteria, antibiotic susceptibility tests were performed using the Kirby-Bauer disk diffusion method by the guidelines of the clinical and laboratory standards institute (CLSI) for gentamycin (GM), ciprofloxacin (CIP), cefepime (FEP), ceftazidime (CAZ), cefotaxime (CTX), imipenem (IPM), meropenem (MR), and piperacillin-tazobactam (TZP), and the results were analyzed using SPSS v22 software.

Results: Two hundred and sixty isolates were collected from clinical specimens, most of which were isolated from the ICU ward (53.8%). Among the collected specimens, 98 (37.7%) were isolated from urine, 92 (35.4%) from sputum, 45 (17.3%) from blood, 17 (6.5%) from surgical wounds, and 8 (3.1%) belonged to other specimen types. Among detected bacteria, *Klebsiella pneumoniae* and *Escherichia coli* were found to be responsible for 78% of total infections. Based on the Kirby-Bauer disk diffusion results, *Acinetobacter baumannii* demonstrated the highest resistance to the antibiotics tested, with a resistance rate ranging from 92% to 97%. This was followed by *K. pneumoniae*, which demonstrated resistance rates between 70% and 85% across all classes of tested antibiotics.

Conclusions: The study highlights a high prevalence of nosocomial infections in the ICU, with significant antibiotic resistance, particularly from *A. baumannii*. It emphasizes strict hospital monitoring, infection control, and responsible antibiotic use.

Keywords: Nosocomial Infections, Gram-Negative Bacteria, Intensive Care Units, Antibiotic Resistance

1. Background

Hospital-acquired infections (HAIs) manifest during receiving medical care, typically developing in a hospital or other healthcare setting, and are defined as those that emerge 48 hours or more after admission or

within 30 days following healthcare interventions (1). They are associated with high rates of morbidity and mortality in hospitals globally. These infections also contribute to higher hospital costs due to prolonged hospital stays and escalate antimicrobial resistance rates (2, 3). Hospital-acquired infections are mainly

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associated with urinary tract infections (UTIs), respiratory tract infections (RTIs), infections of the circulatory system, and surgical site infections (4). According to World Health Organization (WHO) data, nosocomial infections occur in 15% of patients in developing countries and 7% in developed countries (5). Bacteria are responsible for most nosocomial infections; however, other microbes, such as protozoans, fungi, and viruses, are less common (6). These infections are controlled by antibiotic treatment. However, injudicious antibiotic use has led to an increase in drug-resistant bacteria, resulting in the emergence of significant threats to patients' health (7). The WHO has released a list of antibiotic-resistant "priority pathogens" categorized into critical, high, and medium priority groups. The critical group comprises gram-negative multidrug-resistant (MDR) bacteria, such as *Enterobacterales* (*Klebsiella pneumoniae*, *Escherichia coli*), *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, which have resisted commonly used antibiotics (8). The studies conducted in Iran show that the prevalence and antibiotic resistance pattern of nosocomial infections varies in Iranian hospitals (9-12). Therefore, monitoring drug resistance patterns of nosocomial infections in hospitals is crucial in healthcare management (13). The primary goal of the infection prevention and control program is to eliminate nosocomial infections. However, it is still necessary to conduct epidemiological surveillance to demonstrate performance improvements and achieve this goal (14).

2. Objectives

This study aims to summarize the surveillance results of prevalence and antibiotic susceptibility of gram-negative HAIs isolated from patients referred to a tertiary hospital for 5 months.

3. Methods

This study was approved by the local Ethics Committee of Shahid Beheshti University of Medical Sciences ([IR.SBMU.MSP.REC.1402.376](#)). Between October 1, 2022, and February 28, 2023, the study was conducted at a tertiary hospital affiliated with Tehran University of Medical Sciences (Yas Hospital). The study included all confirmed patients with nosocomial infections, such as UTIs, central line-associated bloodstream infections, surgical site infections, ventilator-associated pneumonia, and hospital-acquired pneumonia.

Specimens from outpatients and the emergency ward were excluded from the study. All specimens, including midstream urine, blood, respiratory specimens (bronchial lavage fluid and tracheal secretions), surgical wound samples, and other specimens from patients admitted to the coronary care unit (CCU), intensive care unit (ICU), neonatal intensive care unit (NICU), as well as general and surgical wards, were collected according to standard guidelines and transported to the hospital laboratory (15). Afterward, bacterial identification was conducted through colony characteristics, gram staining, and various biochemical tests following established protocols (16). The antibiotic susceptibility test was measured by the Kirby-Bauer disk diffusion method according to the clinical and laboratory standards institute (CLSI) guidelines, and the resistance rate was reported according to the guideline criteria (17). The antimicrobial agents (Padtanteb, Tehran, Iran) included in this study were gentamicin (GM; 10 µg), ciprofloxacin (CIP; 5 µg), cefepime (FEP; 30 µg), ceftazidime (CAZ; 30 µg), cefotaxime (CTX; 30 µg), imipenem (IPM; 10 µg), meropenem (MR; 10 µg) and piperacillin/tazobactam (TZP; 100/10 µg). The *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as quality controls. The isolates that were resistant to at least one potentially effective antimicrobial drug in three or more classes (such as penicillin/cephalosporins, fluoroquinolones, carbapenems, and aminoglycosides) were considered MDR strains (18, 19).

3.1. Statistical Analysis

Data were analyzed using SPSS software version 22. Nominal variables were displayed as frequencies and percentages. Chi-square and Fisher's exact test assessed the significant difference between antibiotic resistance, wards, clinical samples, and gram-negative bacteria. P-values < 0.05 were considered statistically significant.

4. Results

A total of 260 gram-negative nosocomial isolates were collected over five months. Among these, 140 isolates (53.8%) were obtained from the ICU, followed by 45 isolates (17.3%) from the surgery ward, 35 isolates (13.5%) from the general ward, 26 isolates (10%) from the CCU, and 14 isolates (5.4%) from the NICU. Table 1 presents the distribution of gram-negative bacteria isolated from various samples. Most isolates, 98 (37.7%), were obtained from urine samples. Following this, 92

(35.4%) were isolated from respiratory infections, 47 (17.3%) from blood samples, and 17 (6.5%) from surgical wounds. The lowest number of isolates, 8 (3.1%), was found in other specimens. Table 1 indicates that this study's most commonly identified pathogens were *K. pneumoniae* (N = 132, 50.8%), followed by *E. coli* (N = 71, 27.3%) and *Acinetobacter* spp. (N = 36, 13.8%), and *P. aeruginosa* (N = 21, 8.1%). Most bacteria isolated from blood, respiratory, surgical, wound, and other specimens were identified as *K. pneumoniae*. However, *E. coli* was the most frequently isolated in UTIs (P-value = 0.005). Figure 1 illustrates the antimicrobial susceptibility patterns of the isolated bacterial pathogens. Among gram-negative bacteria, *Acinetobacter* spp. exhibited the highest resistance rates to all antibiotics tested (P-value = 0.001). Specifically, it demonstrated a prevalence of 97% resistance to carbapenems and cephalosporins, 95% to TZP, and 92% to both CIP and GM. Subsequently, *K. pneumoniae* exhibited resistance between 70% and 85% to antibiotics. In contrast, 96% and 98% of *E. coli* isolates displayed sensitivity to IPM and MR, respectively. Notably, Figure 1 shows that *E. coli* isolates had the lowest resistance rates to TZP and GM (21% - 31%) (P-value = 0.006). Depending on the tested antimicrobial, the resistance rates for *P. aeruginosa* isolates ranged from 33% to 81%. Moreover, the most frequent MDR strain was *Acinetobacter* spp. (35/36, 97%), followed by *K. pneumoniae* (98/132, 74%), *P. aeruginosa* (9/21, 43%) and *E. coli* (24/71, 34%) (P-value = 0.001). As shown in Table 2, the findings further revealed that strains isolated from the ICU exhibited the highest resistance to antibiotics (P-value = 0.0045). Notably, isolates from the surgery ward had the lowest resistance rates to carbapenems and TZP (8.9% - 17.8%). Moreover, all isolates from the NICU were sensitive to CIP. Multidrug-resistant strains were also most prevalent in the ICU, followed by those from the general ward and CCU (P-value = 0.003).

5. Discussion

Hospital-acquired infections are a significant healthcare issue that causes economic losses and impacts productivity. They result in prolonged hospital stays, long-term disabilities, financial burdens on healthcare systems, antimicrobial resistance, increased costs for patients and families, and higher patient mortality rates (20). According to the present study, the overall rate and prevalence of antibiotic resistance and

MDR isolates in HAIs were more prevalent in the ICU than in other wards, which aligns with previous findings (12, 21-24). This can be attributed to the increased susceptibility of patients admitted to ICU to nosocomial infections compared to individuals in general wards. This increased susceptibility may be due to the frequent utilization of invasive medical devices among ICU patients and their heightened exposure to a greater diversity of antibiotic-resistant pathogens (25). Conversely, the lowest prevalence of nosocomial infections, accounting for 5.4% of total infections, was observed in the NICU, where no resistance to CIP was noted. This finding may be related to the off-label use of CIP in this population, partly due to the limited pharmacokinetic studies available for neonates (26). Regarding HAIs in terms of the type of infection, UTI was the most common type, accounting for 37.7% of all healthcare-associated infections. This confirms reports by other authors that have mentioned UTI consistently emerging as the leading cause of nosocomial infection (11, 27-29). The high prevalence of this infection can be associated with the use of medical instruments such as nephrostomy tubes, ureteric stents, suprapubic tubes, or Foley catheters (30). In contrast, the prevalence of surgical wound infections was among the lowest observed in the study, and these infections also exhibited the lowest rate of carbapenem resistance. This finding suggests that patients likely did not acquire these infections within the hospital environment. Instead, it may indicate that the surgical procedures were effectively managed with appropriate infection control measures. Additionally, the lower incidence of resistance in these infections may reflect effective prophylactic antibiotics and adherence to best practices in surgical care, further supporting the notion that these infections were acquired outside the hospital setting (31). The analysis based on the type of microorganism indicated that *K. pneumoniae* was identified as the predominant bacterium overall, while *E. coli* emerged as the primary microorganism among patients with UTIs. These results are comparable with those of other studies (32, 33). A similar study in Iran indicated that *E. coli* and *K. pneumoniae* were the most common infectious agents in UTI and respiratory samples, respectively (29). Another study by Tolera et al. elucidated that *E. coli* exhibited the highest prevalence among isolated gram-negative bacteria. Surprisingly, *K. pneumoniae* was primarily identified in cases of RTIs,

Table 1. Comparison of the Frequency of Isolates Among Different Clinical Specimens

Specimens	Number of Isolates (%)				Total (%)
	<i>Acinetobacter baumannii</i> Spp.	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	
Blood	9 (25)	6 (8.5)	27 (20)	3 (14)	45 (17.3)
Respiratory ^a	20 (55)	3 (4)	63 (48)	6 (28)	92 (35.4)
Urine	6 (17)	54 (76)	28 (21)	10 (48)	98 (37.7)
Surgical wound	1 (3)	6 (8.5)	9 (7)	1 (5)	17 (6.5)
Other	0 (0)	2 (3)	5 (4)	1 (5)	8 (3.1)
In total	36 (100)	71 (100)	132 (100)	21 (100)	260 (100)

^a Respiratory = bronchial lavage fluid and tracheal secretion.

Table 2. Comparison of the Frequency of Antibiotic Resistance and Multidrug-Resistant of Gram-Negative Bacilli According to Various Hospital Wards

Antibiotics	Number of Isolates (%)					Total (%)
	CCU	ICU	General	NICU	Surgery	
GM	13 (50)	111 (79)	18 (51.4)	11 (78)	11 (24)	164 (63)
CIP	16 (61)	125 (89)	23 (66)	0 (0)	24 (53)	188 (72)
FEP	17 (65)	121 (86)	22 (63)	8 (57)	19 (42)	187 (72)
CAZ	19 (73)	130 (93)	28 (80)	9 (64)	25 (55)	211 (81)
CTX	19 (73)	129 (92)	31 (88)	11 (79)	24 (53)	214 (82)
IPM	6 (23)	117 (83)	14 (40)	4 (28)	5 (11)	146 (56)
MR	6 (23)	116 (83)	12 (34)	4 (28)	4 (9)	142 (54)
TZP	11 (42)	118 (84)	20 (57)	9 (64)	8 (18)	166 (64)
MDR	13 (50)	120 (86)	18 (51)	4 (28)	11 (24)	166 (64)

Abbreviation: MDR, multidrug-resistant; CCU, coronary care unit ; ICU, intensive care unit; NICU, neonatal intensive care unit; GM, gentamicin; CIP, ciprofloxacin; FEP, cefepime; CAZ, ceftazidime; CTX, cefotaxime; IPM, imipenem; MR, meropenem; TZP, piperacillin-tazobactam.

whereas *E. coli* prevailed as the most common microorganism in UTIs (34). The high propensity of *E. coli* to trigger UTIs can be attributed to its virulence factors. This microorganism produces numerous adhesins that facilitate bacterial adherence to host cells. Moreover, the production of various toxins by *E. coli* influences the immune response, and the presence of siderophores aids in iron uptake (35, 36). Although *K. pneumoniae* was the most prevalent bacteria, accounting for 50.8% of total isolated strains, *A. baumannii* showed a significantly higher level of antibiotic resistance, ranging from 92% to 97%. These findings are consistent with the results of a study by Zhang et al. in 2011, which indicated that although *K. pneumoniae* was more common, *A. baumannii* showed more excellent resistance to several antibiotics, including TZP, IPM, MR, CAZ, CTX, and FEP (37). The prevalent multidrug resistance pattern observed in *A. baumannii* clinical isolates has established carbapenems as frequently recommended treatment choices for

Acinetobacter infections in healthcare settings (38). However, this study revealed a high prevalence of carbapenem resistance among *A. baumannii* isolates, with rates just below 90% for IPM and MR, corroborating the findings of other studies (38-41). The rise in carbapenem utilization has led to the widespread emergence of carbapenem-resistant *A. baumannii* strains, presenting a substantial risk to global health and patient safety due to the ongoing reduction in available treatment modalities (38, 42). Given the alarming prevalence of antibiotic resistance within the healthcare sector, the implementation of stringent disinfection protocols for patient environments, meticulous screening of high-risk individuals, effective management of infection reservoirs, enhancement of routine hygiene practices, and the judicious prescription of narrow-spectrum antibiotics stand as imperative strategies to combat the dissemination of MDR strains and proactively avert potential outbreaks (43, 44). In this study, *E. coli* was identified as the second

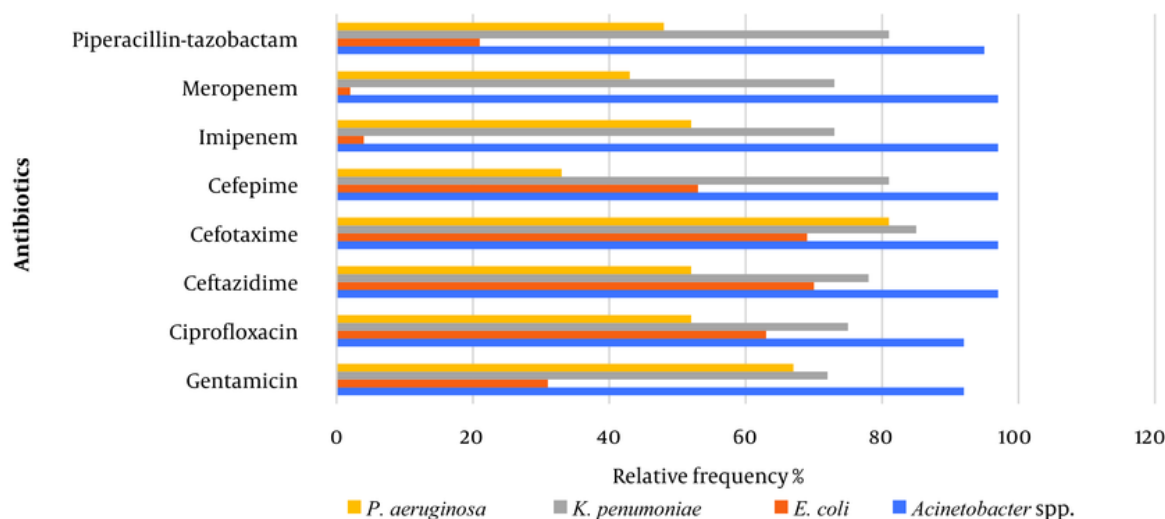


Figure 1. Relative frequency comparison of antimicrobial resistance rates among gram-negative bacilli isolated from patients with nosocomial infections

most prevalent strain, mostly isolated from UTI patients. However, the incidence of carbapenem resistance was not as high as that of *A. baumannii* and *K. pneumoniae* isolates. According to the results, supported by Chen et al.'s findings, *E. coli* isolates exhibited the most resistance to CIP and third-generation cephalosporins (45). The resistance observed can be attributed to the frequent use of fluoroquinolones or third-generation cephalosporins in hospital settings to provide empiric coverage for this pathogen in UTI patients. Consequently, the high prevalence of antibiotic resistance can compromise treatment efficiency by limiting available treatment options (46-48). As part of the study, *P. aeruginosa* was identified as the least prevalent bacterium isolated from patients. However, the fact that over 40% of these isolates exhibited carbapenem resistance raises significant concerns within the healthcare sector. Notably, the rate of carbapenem resistance in *P. aeruginosa* varies by geographical region, ranging from 10% to 50%. This variability is particularly alarming, as carbapenems are among the most effective antimicrobial agents for treating severe infections. Their role as one of the last-resort treatment options for MDR bacterial infections further underscores the urgency of addressing this growing resistance issue (49, 50). The study had several limitations as it was limited to a single territory hospital

within the province, potentially impacting the generalizability of the results. Therefore, it is advisable to replicate the study in other cities or hospitals within the province for a more comprehensive understanding. Additionally, the study focused solely on four types of nosocomial infections. Therefore, it is suggested that future studies explore additional types of nosocomial infections to provide a more comprehensive analysis. Another limitation of the study is the lack of patient follow-up post-discharge. Hospitals need to implement structured and systematic monitoring of nosocomial infections, particularly in high-risk wards, focusing on older patients and those with multiple risk factors for nosocomial infections.

5.1. Conclusions

This study found a high prevalence of nosocomial infections among patients admitted to hospital wards, particularly in ICU settings. There was a significant degree of resistance to the treating antibiotics in the bacteria that caused the infections in the ICU. Urinary tract infection was the most common hospital infection in the study. Additionally, it is noteworthy that *A. baumannii* displayed a higher level of phenotypic resistance than other bacteria. It suggests that to address HAIs and antibiotic resistance effectively; hospitals should continuously monitor MDR isolates,

especially within the ICU, so that the infection does not spread to other wards of the hospitals, particularly those that are antibiotic-resistant, enforce strict infection control measures, manage resistant pathogens like *A. baumannii*, and promote responsible antibiotic use through education and stewardship.

Footnotes

Authors' Contribution: P. N. conducted the tests. M. S. wrote the manuscript. S. D. and S. H. supervised the project, worked on the concept and design of the study, and critically revised the paper. S. H. and M. S. analyzed and interpreted data. S. H. critically revised the paper. All authors read and approved the final version of the manuscript and authorship list.

Conflict of Interests Statement: The authors declared that they have no conflict of interest.

Data Availability: The data presented in this study are uploaded during submission and are openly available for readers upon request.

Ethical Approval: This study was approved by the local Ethics Committee of Shahid Beheshti University of Medical Science (IR.SBMU.MSP.REC.1402.376).

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