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Investigation of Metallobetalactamase (*blaIMP & blaVIM*) and Carbapenemase (*blaKPC & blaGES*) Genes in Gram Negative Rods Isolated from Cancer Patients

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ABSTRACT

Backgrounds: Bacterial infections are the most common complication in cancer patients. Infection with multi-drug resistant bacteria has recently become a worrying phenomenon in cancer patients. This study focused on Gram-negative bacteria isolated from clinical samples of cancer patients. The purpose of this study was to evaluate the presence and prevalence of drug resistance genes, including metallobetalactamase (*blaIMP* and *blaVIM*) and carbapenemase (*blaKPC* and *blaGES*) genes, in the main bacteria agents of nosocomial infections in cancer patients, such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli*.

Materials & Methods: Common biochemical methods were used to identify bacterial isolates. Antimicrobial susceptibility testing was performed according to the standard method recommended by the Clinical and Laboratory Standards Institute (2019). Polymerase chain reaction (PCR) method was also used to check the presence and prevalence of resistance genes.

Findings: During six months, from May to November 2020, 250 clinical samples were collected from cancer patients in Ayatollah Khansari hospital in Arak city, Iran. From which 80 Gram-negative bacilli were isolated, including 33 (41.2%) *E. coli*, 15 (18.7%) *A. baumannii* complex, 12 (15%) *P. aeruginosa*, eight (10%) *K. pneumoniae*, seven (8.7%) *Citrobacter freundii*, and five (6.2%) *Enterobacter aerogenes* isolates. The frequency of *blaKPC, blaGES, blaIMP*, and *blaVIM* genes was 39.95, 21.25, 16.25, and 17.45%, respectively.

Conclusion: The present study emphasizes the importance of identifying Gram negative rods and their resistance genes (metallobetalactamase and carbapenemase genes) in cancer patients, carrying out preventive instructions to prevent the transmission of resistance genes, and reducing mortality in these patients.

Keywords: Infections, Gram-negative bacteria, Carbapenemase, Drug resistance, Cancer patients.

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Introduction

In recent years, several studies on cancer patients have shown a changing trend in the epidemiology and prevalence of bacterial infections in these patients from Gram-positive cocci to Gram-negative rods and the widespread emergence of antibiotic-resistant strains among Gram-negative organisms [1, 2]. Nosocomial or hospital-acquired infections (HAI) are one of the most prevalent life-threatening complications among patients admitted to hospitals. Bacterial infections in cancer patients are considered as serious complications in cancer treatment. Bacterial infections in these patients originate from normal flora or during therapeutic procedures such as surgical operation through direct contact with hospital staff, contaminated inanimate environment and medical equipment, etc [3]. As a result of drug-induced immunosuppression, opportunistic infections including nosocomial infections often occur in cancer patients [5].

In general, these patients experience neutropenia after therapeutic procedures, chemotherapy, and radiotherapy. People with cancer generally experience neutropenia during chemotherapy. Infection is an unavoidable complication of neutropenia and immune system dysfunction after chemotherapy in cancer patients. Most of these infections are hospital-acquired because patients have long and frequent contact with the hospital environment and are exposed to many sources of infection [4, 5]. The pathogens responsible for primary infections during fever and neutropenia are mainly bacteria, followed by fungi and viruses [6].

The increase in drug resistance is mostly due to antimicrobial prophylaxis regimens and ineffective empirical therapies that are performed in oncology treatment centers [7]. In recent decades, chemotherapy in cancer patients has made significant progress, but bacterial infections are still one of the leading causes of mortality among these patients with or without neutropenia. One of the main concerns in healthcare centers for cancer patients is the increase in the prevalence of highly resistant bacteria. Recently, the prevalence of infections caused by Gram-negative rods has significantly increased. Studies in recent decades have shown that the frequency of carbapenem-resistant *Enterobacteriaceae* as well as bacteria with metallobetalactamase (MBL)- and carbapenemase-mediated resistance has increased [2].

According to numerous studies performed on cancer patients, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii, Klebsiella pneumoniae*, and *Stenotrophomonas maltophilia* are the most frequently isolated Gram-negative bacteria from these patients. Among which, *A. baumannii* is the most important because of its high resistance to different groups of antibiotics, including carbapenems [2, 8-12].

Gram-negative rods encode several resistance genes belonging to the metallobetalactamase and carbapenemase families, such as *blaIMP, blaVIM, blaNDM, blaOXA48, blaGIM, blaGES,* and *blaKPC* [13, 14].

Also, nosocomial infections have been shown to significantly increase treatment costs for cancer patients [15, 16]. By identifying the causative agents of these infections, treatment costs could be significantly reduced. In addition, controlling the spread of these strains by the hospital infection control team (adherence to and implementation of instructions), eliminating biofilms, using preventive methods to prevent transmission of strains, and predicting resistance could be helpful.

Studies have shown that the mean prevalence of metallobetalactamase- and carbapenemase-producing strains in Iran is about 17% [17-21] .

Objectives: Since very few studies have been conducted on the resistance of these

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Table 1) PCR primers used to detect *KPC*, *GES*, *IMP*, and *VIM* genes based on PCR method in the present study

Genes	Sequence 3 -´5	Product Size(bp)	References
blaKPC	F: GTATCGCCGTCTAGTTCTGC R: GGTCGTGTTTCCCTTTAGCC	646	27
blaGES	F: GGCTACTAACCTCTTACTGA R: TACCAGTTTTCTCTCCAACA	311	Current study
blaIMP	F: GAAGGCGTTTATGTTCATAC R:TGTAAGTTTCAAGAGTGATGCGTC	586	24 ,25
blaVIM	F: GATGGTGTTTGGTCGCATA R: CGAATGCGCAGCACCAG	389	26 ,27

strains in cancer patients, this research was conducted to evaluate the frequency of antibiotic resistance genes among Gram-negative bacilli recovered from cancer patients in Arak city to help control their spread among these patients.

Materials and Methods

Bacterial isolates: In the present cross-sectional research, 80 Gram-negative bacilli were isolated from 250 clinical samples of cancer inpatients admitted to Ayatollah Khansari hospital in Arak from May to November 2020. The isolated bacteria were identified by conventional biochemical tests, such as oxidase, triple sugar iron agar (TSI agar), OF basal medium (OF glucose), methyl red, Voges-Proskauer broth (MR-VP), citrate, urea, lysine decarboxylase broth (LDC), and phenylalanine deaminase (PAD).

Antimicrobial susceptibility testing was performed for all isolated strains using disk diffusion technique according to Clinical and Laboratory Standards Institute (CLSI) guidelines (edition 29, 2019).

Carbapenem susceptibility testing: The MIC (minimum inhibitory concentration) value of meropenem was determined using E test based on the guidelines recommended by CLSI (2019).

Carbapenem inactivation method (CIM): CIM test was conducted according to the method previously described by Van der Zwaluw et al. (2015) [22]. For this purpose, a disk containing 10 µg of meropenem was submerged in a bacterial suspension containing a loop (10 µL) of the desired bacterium dissolved in 400 µL of physiological saline and incubated in an incubator at 37 °C for 2 hour. After incubation, the disc was taken out from the bacterial suspension and placed on Mueller-Hinton agar medium containing *E. coli* (ATCC 25922) suspension. The desired bacterium was again incubated at 37 °C overnight, and the results were read overnight. The suspension of carbapenemase-producing bacteria leads to the inactivation of antibiotics (meropenem). The zone of inhibition is ≤19 and ≤14 in the presence of carbapenemase-producing *Enterobacteriaceae* and *A. baumannii* bacteria, while the zone of inhibition is ≥23 and ≥18 in the presence of bacteria lacking carbapenemase enzymes.

Molecular method: DNA extraction was performed using YTA genomic DNA extraction kit for blood/cultured cells (YT9040, Yekta Tajhiz Company, Tehran, Iran) according to

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the kit protocol, and polymerase chain reaction (PCR) amplification of the studied genes was performed using the prepared DNA template.

PCR conditions for *blaKPC* and *blaGES* genes were as follows: an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 53.8 °C for 1 min, and extension at 72 °C for min and a final extension step at 72 °C for 9 min. Also, PCR conditions for *blaIMP* and *blaVIM* were as follows: an initial denaturation step at 94 °C for 4 min, followed by 28 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, and extension at 72 °C for 30 s and a final extension step at 72 °C for 8 min.

Statistical analysis: Data were analyzed using Excel and SPSS (Version 21.0) software by employing Chi-square test, and data analysis results were presented descriptively in terms of percentage frequency.

Findings

During six months, 250 clinical samples were collected from cancer patients admitted to different wards of Ayatollah Khasnsari hospital in Arak, from which 80 Gram-negative bacilli were isolated. The clinical samples sent to the laboratory included urine, lung secretion, blood, wound, pus, and sputum specimens.

Among cancer patients included in this research, 67.5% were male, and 32.5% were female with a mean age of 54 years.

Most of the samples were related to patients with leukemia (n=19, 23.7%), gastric cancer (n=12, 15%), and breast cancer (n=12, 15%), and most of which were from ICU (intensive care unit) (n=37, 46.2%) and hematology (n=31, 38.7%) wards.

The frequency distribution of Gram-negative rods isolated was as follows: 33 (41.2%) *E. coli*, 15 (18.7%) *A. baumannii* complex, 12 (15%) *P. aeruginosa*, eight (10%) *K. pneumoniae*, seven (8.7%) *Citrobacter freundii*, and five (6.2%) *Enterobacter aerogenes* isolates.

The frequency of samples sent to the laboratory from different wards was as follows: 27 (33.7%) urine culture samples, 19 (23.7%) lung secretion culture samples, 18 (22.5%) blood culture samples, ten (12.5%) wound culture samples, five (6.2%) sputum culture samples, and one (1.25%) pus culture sample.

According to the results presented in Table 2, most of the lung secretion samples (15, 78.9%) were collected from ICU, most of the urine samples (11, 40.7%) were equally collected from the ICU and hematology wards, and most of the blood samples (9, 50%) were collected from the hematology department and sent to the laboratory. Also, most of the bacteria isolated from culture samples of lung secretions were *A, baumannii* complex (12, 63.1%), while most of the bacteria isolated from urine (20, 74.07%) and blood (7, 38.8%) culture samples were *E. coli*.

Most of the bacteria isolated from patients with cancers mentioned in Table 3 were *E. coli* (16, 48.4%) isolated from urine culture samples, *A. baumannii* complex (4, 26.6%) isolated from lung secretion culture samples, and *P. aeruginosa* (2, 16.6%) isolated equally from lung secretion, blood, and wound culture samples.

In addition, the frequency of the studied genes among the most prevalent bacteria in this study was examined.

As shown in Table 4, the frequency of *blaKPC* and *blaGES* genes was 39.95 and 21.25%, respectively. Also, 16.25 and 17.45% of the studied bacteria harbored *blaIMP* and *blaVIM* genes, respectively. The frequency of each gene among the studied bacteria is also listed separately in Table 4.

Discussion

Cancer patients develop opportunistic infections such as nosocomial infections due to immunodeficiency. These patients are

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Table 2) Frequency of culture samples sent to the laboratory from different departments of Ayatollah Khansari hospital, Arak, Iran

Department	Bacteria	Lung Secretions N=19 (23.7%)	Blood N=18 (22.5%)	Urine N=27 (33.7%)	Wound N=10 (12.5%)	Sputum N=5 (6.2%)	Pus N=1 (1.25%)	Total N=80 (100%)
1: ICU 2: Hematology 3: Other wards of hospital	*Escherichia coli*	1: 0 2: 0 3: 0	1: 0 2: 6 (33.3%) 3: 1 (5.5%)	1: 8 (29.6%) 2: 8 (29.6%) 3: 4 (14.8%)	1: 1 (10%) 2: 2 (20%) 3: 0	1: 0 2: 2 (40%) 3: 0	1: 0 2: 0 3: 1 (100%)	1: 9 2: 18 3: 6
	Acinetobacter baumannii complex	1: 10 (52.6%) 2: 2 (10.5%) 3: 0	1: 1 (5.5%) 2: 0 3: 0	1: 0 2: 0 3: 0	1: 1 (10%) 2: 0 3: 0	1: 1 (20%) 2: 0 3: 0	1: 0 2: 0 3: 0	1: 13 2: 2 3: 0
	Pseudomonas aeruginosa	1: 3 (15.7%) 2: 2 (10.5%) 3: 0	1: 1 (5.5%) 2: 3 (16.6%) 3: 0	1: 0 2: 0 3: 1 (3.7%)	1: 0 2: 2 (20%) 3: 0	1: 0 2: 0 3: 0	1: 0 2: 0 3: 0	1: 4 2: 7 3: 1
	Klebsiella pneumoniae	1: 0 2: 0 3: 0	1: 1 (5.5%) 2: 0 3: 0	1: 2 (7.4%) 2: 3 (11.1%) 3: 0	1: 1 (10%) 2: 0 3: 0	1: 1 (20%) 2: 0 3: 0	1: 0 2: 0 3: 0	1: 5 2: 3 3: 0
	*Others	1: 2 (10.4%) 2: 0 3: 0	1: 0 2: 0 3: 5 (27.7%)	1: 1 (3.7%)	1: 3 (30%)	1: 0 2: 1 (20%) 3: 0	1: 0 2: 0 3: 0	1: 6 2: 1 3: 5
Total		99.7%	99.6%	99.9%	100%	100%	100%	99.8%

*others: *Citrobacter freundii, Enterobacter aerogenes*

Table 3) Frequency of bacteria isolated from clinical specimens of patients with various cancers in Ayatollah Khansari hospital, Arak, Iran

Type of Cancer	Type of Bacteria			
	***Escherichia coli* (%)**	***Acinetobacter baumannii* (%)**	***Pseudomonas aeruginosa* (%)**	***Klebsiella pneumoniae* (%)**
Leukemia	63.1	10.5	15.7	0
Gastric cancer	33.3	33.3	16.6	0
Breast cancer	66.6	8.3	8.3	8.3

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Table 4) Frequency of metallobetalactamase and carbapenemase resistance genes among bacterial isolates from cancer patients in Ayatollah Khansari hospital, Arak, Iran

Type of Bacteria	*blaKPC*	*blaGES*	*blaIMP*	*blaVIM*
Klebsiella pneumoniae	2 (25%)	1 (12.5%)	1 (12.5%)	1 (12.5%)
Acinetobacter baumannii	11 (13.7%)	6 (7.5%)	1 (1.25%)	3 (3.7%)
Pseudomonas aeruginosa	1 (1.25%)	1 (1.25%)	2 (2.5%)	1 (1.25%)
Escherichia coli	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total	39.95%	21.25%	16.25%	17.45%

exposed to many of these infections due to immune system dysfunction after chemotherapy or radiotherapy as well as long-term hospitalization [4, 5]. Bacteria are mainly pathogens that cause infections [6], the prevalence of these infections is associated with antibiotic resistance, which is considered as one of the most important causes of death among these patients [11, 23].

This study was conducted on Gram-negative bacterial strains isolated from cancer patients in Arak city to investigate the prevalence of antibiotic resistance genes in these isolates.

E. coli (n=33, 41.2%), *A. baumannii* (n=15, 18.7%), *P. aeruginosa* (n=12, 15%), and *K. pneumoniae* (n=8, 10%) were the most prevalent bacterial isolates among cancer patients, respectively.

A. baumannii is a very successful pathogen [11] in causing infection because of its high resistance to many therapeutic measures. Most isolates of this bacterium are extensively drug resistant (XDR). Therefore, the problem of resistance of these isolates recovered from both patients and equipment to antimicrobial agents has become a serious challenge.

In some studies, the most commonly isolated bacteria have been reported to be *E. coli, P. aeruginosa, Klebsiella* spp., *Acinetobacter* spp., and *Proteus mirabilis* strains, which are consistent with the present study results [3, 16, 28].

In this research, most cancer patients with nosocomial infections were middle-aged (41-60 years), and more precisely, the mean age of the study participants was 54 years. The average age of men and women was 55 and 54 years, respectively. This study findings show that the prevalence of nosocomial infections is higher among middle-aged people. In a study by Zhao et al. (2016), age, surgery, and prolonged hospital stay were considered as risk factors for hospital-acquired infections (HAI). Their study results showed a clear age-related trend in the prevalence of nosocomial infections [16], which is consistent with the present study findings.

In terms of the type of clinical specimens, most of the clinical specimens sent to the laboratory were urine, pulmonary, and blood samples, which is in line with other studies in which most of the collected samples were respiratory aspiration, blood, and urine specimens [28, 29].

Nearly half of the patients were hospitalized in the ICU (47.5%), while the ICU itself is one of the most important hospital wards for multidrug-resistant (MDR) strains. In another study, more than half of the patients were hospitalized in the ICU (63.2%) [9, 28]. As the findings of various studies show,

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about half of the strains isolated are related to ICU patients, which could be due to the long-term hospitalization of patients in this department, the excessive use of antibiotics for these patients, and the presence of antibiotic-resistant strains in this department [9]. Therefore, considering the special conditions of patients hospitalized in this department and the weakness of the immune system of cancer patients and, as a result, more deaths among these patients, special importance should be given to infection control, identification of antibiotic-resistant strains, and evaluation of antibiotic resistance patterns of pathogens in this department.

Phenotypic detection of carbapenemases is often difficult. In this study, CIM test was used to confirm the activity of carbapenemases. Resistance to imipenem and meropenem was detected in the antibiogram, but *blaKPC* and *blaGES* carbapenemase genes were not completely detectable.

The first study conducted on *blaKPC* production in *A. baumannii* isolates showed that 4.3% of *A. baumannii* isolates carried *blaKPC* genes, which was further confirmed by sequencing of *blaKPC2*, *blaKPC3*, *blaKPC4*, and *blaKPC10* [30, 31].

Various studies have shown that the prevalence of *A. baumannii* strains carrying *blaKPC* and *blaGES* genes could vary from zero to 9.23% [28, 31-34] and 7.2 to 34.5% [35-37], respectively.

Some studies on *Enterobacteriaceae* (such as *Klebsiella* species especially *K. pneumoniae*) and *Pseudomonas* species have shown that the prevalence of *blaKPC* genes in these species varies from 1.48 to 19% [38, 39]; in another study conducted on *Pseudomonas*, the prevalence of this gene was reported to be 25.8% in *Pseudomonas* strains [28, 38, 39]. In addition, the prevalence of *blaGES* gene is estimated to be 55.2% in these species, while the prevalence of *blaVIM* and *blaIMP* genes has been shown to vary from zero to 18.4% in these species [39, 40].

In a study conducted by Zare and Fazeli (2019) in Iran, 27.7% of *E. coli*, 28.6% of *K. pneumoniae,* and 66.7% of *Proteus* species were MBL positive [18]. Amini and Namvar (2019) showed that from a total of 42 *P. aeruginosa* isolates recovered from ICU patients within 10 months, 23.8% harbored the *blaVIM-2* gene, 4.7% harbored the *blaIMP* gene, and 4.7% contained *blaIMP*-1, but none of them harbored the *blaKPC* gene [17]. In a study by Ghasemian et al. (2018), 55% of *P. aeruginosa* isolates were resistant to imipenem and meropenem, and 37.7% of them were MBL producers. Among MBL-producing isolates, *blaVIM* (12.91%±11.01%) and *blaIMP* (12.50%±23.56%) genes were dominant [20]. In another study in Tehran, the frequency of *blaIMP* and *blaVIM* genes in *P. aeruginosa* isolates was 9 and 33%, respectively [19].

In the present study, 21.2% of *A. baumannii*. 2.5% of *P. aeruginosa*, and 37.5% of *K. pneumoniae* strains were carbapenemase positive. Among *A. baumannii* isolates, 13.7% had the *blaKPC* gene, and 7.5% had the *blaGES* gene, and among *P. aeruginosa* isolates, 1.25% had the *blaKPC* gene, and 1.25% had the *blaGES* gene: also, 25 and 12.5% of *K. pneumoniae* isolates harbored *blaKPC* and *blaGES* genes, respectively. Overall, 39.95% of the strains isolated from cancer patients in this study harbored the *blaKPC* gene, and 21.25% harbored the *blaGES* gene.

In the present study, 4.9% of *A. baumannii*, 3.7% of *P. aeruginosa,* and 25% of *K. pneumoniae* isolates were MBL positive. Among *A. baumannii* isolates, 1.25% had the *blaIMP* gene, and 2.5% had the *blaVIM* gene. Also, 2.5% of *P. aeruginosa* isolates had the *blaIMP* gene, and 1.25% had the *blaVIM* gene. In addition, the frequency of *blaIMP* and *blaVIM* genes in *K. pneumoniae* isolates was 12.5% each. In general, 16.25% of the strains isolated from cancer patients in this study harbored the *blaIMP* gene, and 17.45% har-

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bored the *blaVIM* gene.

The results show that the prevalence of nosocomial pathogens among cancer patients is increasing, and Gram-negative bacteria are one of the main causes of hospital-acquired infections in cancer patients.

In recent years, the prevalence rate of bacterial resistance genes among nosocomial pathogens has increased. The increase in antibiotic resistance is mostly due to antimicrobial prophylaxis regimens and ineffective empirical treatments that are performed in oncology treatment centers [7].

Today, by recording the results of drug sensitivity tests in the WHONET software in medical diagnostic laboratories, it is possible to predict the drug resistance tendency of a bacterium; in addition, this information could help the hospital's stewardship committee in choosing appropriate antibiotics, rationalizing the use of these drugs, and reducing the prevalence of resistant bacterial strains.

The hospital environment and personnel and patients' companions are a potential reservoir for pathogenic bacteria; thus, their transmission to patients should be prevented by following the instructions of the hospital infection control team.

Also, choosing suitable disinfectants for hand hygiene, contaminated surfaces, equipment, and operating room floors and using appropriate methods for sterilizing equipment could reduce the abundance of these bacteria and, as a result, the incidence of infectious diseases caused by them.

Studies have shown that hospital-acquired infections could significantly increase treatment costs and mortality in cancer patients [15, 16]. By examining antibiotic resistance patterns of pathogenic strains, identifying antibiotic resistance genes, and preventing arbitrary antibiotic prescription [11], treatment costs and mortality in these patients could be significantly reduced.

This study results showed that some target bacterial strains (such as *P. aeruginosa*) were phenotypically antibiotic sensitive, but carbapenemase resistance genes were shown in genotypic studies; thus, the spread of these strains should be controlled by the hospital infection control team by implementing and following instructions. It is necessary to reduce growth and proliferation and prevent exposure to strains that are the source of resistance gene transfer. Identification of bacterial clones and subclones should be done to establish a balance between drug sensitivity and resistance. The first principle is to reduce microorganisms and the growth of antibiotic-resistant strains by using appropriate disinfectants and implementing infection control protocols, and the second principle is to identify microorganisms and their resistance genes for the rational administration of antibiotics. When an antibiotic is used repeatedly, sensitive colonies are removed, and antibiotic-resistant colonies remain, which should be replaced again by sensitive strains through changing antibiotics to restore balance. In hospital environments, by implementing preventive measures such as the optimal use of disinfectants, the number and prevalence of bacteria causing hospital infections is reduced. Appropriate treatment is possible by predicting antibiotic resistance pattern and dealing with it. For this purpose, the stewardship based on data stewardship guidelines and WHONET application could be used.

This information helps doctors prevent the transmission of resistant strains, reduce costs and mortality, prescribe antibiotics correctly, and reduce the length of hospital stays for patients.

This research, like other studies, had special limitations, such as short study time, small sample size, and lack of examination of other antibiotic resistance genes.

Conclusion

It is necessary to have proper and sufficient

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knowledge of the distribution of pathogens in each region, determine their susceptibility pattern, and rapidly initiate effective antimicrobial therapies for severe complications arouse by Gram-negative organisms in cancer patients.

Since the resistance pattern of strains is constantly changing due to the transfer of resistance genes; therefore, taking appropriate measures on a daily basis to manage and prevent infections could be helpful, such as observing hand hygiene, disinfecting objects in the hospital environment, using personal protective equipment, using appropriate antiseptics, reducing the use of invasive methods for treating patients, not using broad-spectrum antibiotics, reducing the duration of hospitalization, and providing proper training to patients at the beginning of hospitalization and after discharge from the hospital.

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