

Original Article



16S Ribosomal Ribonucleic Acid Gene Sequencing for Identification of Multiple Drug-Resistant Bacteria

Fatemeh Najafi¹, Safar Ali Alizadeh Kushkahi^{2*}, Mohammad Rasooli¹

¹Department of Biological Sciences and Technologies, Takestan Branch, Islamic Azad University, Takestan, Iran

²Department of Microbiology, Faculty of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran

Article history:

Received: July 4, 2025

Revised: September 3, 2025

Accepted: October 29, 2025

ePublished: August 25, 2026

***Corresponding author:**

Safar Ali Alizadeh Kushkahi,
Email: fatemeh.najafi4028@gmail.com and alizadeh120@yahoo.com

Abstract

Introduction: Combining 16S ribosomal ribonucleic acid (16S rRNA) gene sequence analysis with the assessment of antibiotic resistance genes can play a key role in identifying drug-resistant bacteria and optimizing treatment management. This study aimed to identify 16S rRNA gene sequencing for the identification of multiple drug-resistant (MDR) bacteria among different samples.

Methods: This epidemiological-descriptive study was conducted from September to December 2023 with bacteria isolated from the personnel and environment in Velayat Hospital, Qazvin City, Iran. The antibiotic resistance pattern was performed according to the Clinical and Laboratory Standards Institute (CLSI). Polymerase chain reaction (PCR) was performed using specific primers for 16S rRNA. Statistical Package for the Social Sciences (SPSS) 22 software, descriptive statistics, and Chi-square test (χ^2) were used for data analysis.

Results: Of the 186 collected samples, the highest and lowest isolates were related to *P. aeruginosa* (39.78%) and *Enterobacter* (9.67%). 74.86% of the total isolates produced the Extended Spectrum Beta-Lactamase (ESBL), and 24.59% of the isolates were MDR. There was no significant relationship between ESBL-producing and MDR isolates and sample types ($P > 0.05$); both types of isolates (isolates from the environment and personnel) showed ESBL-producing and MDR. Deoxyribonucleic acid (DNA) sequences in isolates were similar to the template sequence of bacteria. There was 94.70% genetic similarity between the strains isolated from personnel and the environment.

Conclusion: Isolates producing ESBL and MDR had a high abundance among bacterial isolates. High genetic similarity among bacteria indicates the distribution of genetically similar strains between personnel and the hospital environment.

Keywords: Multiple drug resistance, *Pseudomonas aeruginosa*, *Acinetobacter*, *Enterobacteriaceae*, 16S ribosomal ribonucleic acid, Multiple drug-resistant

Please cite this article as follows: Najafi F, Alizadeh Kushkahi SA, Rasooli M. 16S ribosomal ribonucleic acid gene sequencing for identification of multiple drug-resistant bacteria. Chron Dis J 2026;14(3): 125-130. doi:10.34172/cdj.1005

Introduction

Clinical factors, selective pressures, and contaminated environments are factors that drive the evolution and spread of antibiotic resistance. Antibiotic resistance occurs when bacteria change in such a way that antibiotics cannot kill them or stop their growth. As a result, bacterial infections become very difficult to treat. Antibiotic resistance is transmitted through the transfer of bacteria and antibiotic resistance genes. Antibiotic resistance flows between humans, animals, and the environment. The flow of bacteria and genes frequently transfers new resistance factors from one species to another. The transfer of resistant strains that are already highly prevalent is common, measurable, and predictable.¹ The prevalence rate of infection acquired in the intensive care unit (ICU) has been estimated from 9%

to 37% in Europe and the United States of America (USA).² In a study conducted in the USA, a *P. aeruginosa* strain was found in several sink drains in the USA and in 3.7% of the blood samples analyzed, indicating the movement of this clone between the environment and patients.³ A study in Germany reported that, 25 of the 32 isolates of *Klebsiella pneumoniae* (*K. pneumoniae*) (New Delhi metallo-beta-lactamase 1 (NDM)- and oxacillinase-48 (OXA-48)-encoding) and *P. aeruginosa* (Verona integron-encoded metallo- β -lactamase (VIM) -encoding) belonged to the two dominant clusters in the wastewater, while the other two isolates belonged to a sequence type that was less frequently detected in the wastewater from patient rooms.⁴ In India, surgical site infections (SSIs) account for 14-17% of nosocomial infections.⁵ In Sudan, of



the 120 bacterial samples, all isolates were resistant to kanamycin, cefuroxime, and amoxicillin. A nucleotide substitution mutation, T550C, was identified in the 16S rRNA gene of the most resistant isolates.⁶ According to the research conducted in Iran, the prevalence of nosocomial infections was reported as 4.6%.⁷ In another study in Iran, the prevalence of this type of infection in the ICU was estimated at 8.5%.⁸ In the ICU of a hospital in Iran, the prevalence of nosocomial infections was 10.85%.⁹ A study conducted in Iran showed that among 212 bacterial samples isolated from the environment, 94.8% were isolated from hospital surfaces and 36.8% from the ICU. *P. aeruginosa* was the most common pathogen in patients (34.9%).¹⁰ The significant increase in the prevalence of infections caused by multiple drug-resistant (MDR) bacteria and extended-spectrum beta-lactamases (ESBL)-producing bacteria has caused great concern. ESBLs are generally defined as beta-lactamases that render bacteria resistant to penicillins, cephalosporins, and aztreonam by hydrolyzing antibiotics.⁷⁻⁹ During the last few years, resistance to important groups of antibiotics, including quinolones and piperacillin, along with tazobactam and carbapenem, has increased among gram-negative bacteria. Their alternative agents are ceftazidime-avibactam, amikacin, meropenem, and tigecycline.^{11,12} Also, the role of the environment, working personnel, and the patient population of a department in preventing infection or causing hospital infection in an endemic or epidemic form has been proven.¹³ Genetic epidemiology discusses the interaction between genes and the environment. It investigates the cycle of disease transmission in communities and how they are distributed in society.¹⁴ In Qazvin province, there are not many studies available on the transfer of bacterial resistance genes between the environment and hospital personnel, so the results of this study can provide new results and help manage and control the transfer of bacterial resistance genes in the hospital environment. We investigated the prevalence of *Enterobacteriaceae*, *Acinetobacter*, and *Pseudomonas aeruginosa* (*P. aeruginosa*) and their resistance to several antibiotics using 16S rRNA.

Methods

This epidemiological-descriptive study was conducted from September to December 2023 on the samples received from different parts (sanitary facilities, dirty workroom sink, patient's hand pulse, personnel hands, ventilators, and beds) and personnel of the ICU and operating room of the Velayat Hospital, Qazvin province, Qazvin city, Iran. All the collected samples were transferred to the microbiology laboratory within 30 minutes. Samples from Stewart's transfer medium on blood agar, MacConkey agar, and eosin methylene blue agar (all media; Merck, Germany) were cultured and incubated for 24 hours at 37°C. Gram staining and standard microbiology laboratory tests were performed to identify Gram-negative bacteria.¹⁵ According to the 2018 Clinical and Laboratory Standards Institute (CLSI), the following antibiotics were selected to test the bacteria's antibiogram: cefotaxime (30 µg), gentamicin (10

µg), imipenem (10 µg), and ceftazidime (30 µg) were used in this study (all antibiotics; Patan Teb, Iran).^{15,16}

The antibiotic resistance pattern of bacteria isolated from clinical samples was determined by the disk diffusion test by the Kirby-Bauer method, based on the Clinical Standards Institute (CLSI). To determine ESBL production, one disc was ceftazidime (30 µg) alone, and one disc of ceftazidime (30 µg)-clavulanic acid (10 µg). Both discs were placed at least 25 mm apart, center to center, on the test Gram-negative culture isolate on a Mueller-Hinton agar plate and incubated overnight at 37°C. Then the difference in the diameter of the zone with and without clavulanic acid was measured. A ≥ 5 mm increase in the diameter of the zone of inhibition around the ceftazidime (30 µg)-clavulanic acid (10 µg) combination disc, versus the diameter of the zone of inhibition around the ceftazidime (30 µg) disc alone, confirmed ESBL production.¹⁵ After identifying the resistant strains from the phenotypic point of view, to detect the genetic relationship of the resistant strains, the polymerase chain reaction (PCR) technique was performed using specific primers (Universal 16S rRNA bacterial primers, 1500 base pairs (bp)) of 16S rRNA. A deoxyribonucleic acid (DNA) extraction kit (MBST, Iran) was used to extract the bacterial genome. A PCR reaction in a final volume of 27.5 µl was prepared (Table 1).^{16,17}

To confirm the presence of the gene, the PCR product was sent for sequencing (Khatam Qazvin laboratory, Iran). 16S rRNA was used as a standard for bacterial taxonomy and identification because it is faster and easier to sequence and contains sufficient genetic information to identify prokaryotes. This gene (16S rRNA) contains conserved regions between bacteria of the same species. The degree of difference in this sequence indicates how distant or close the bacteria are taxonomically. The DNA to be sequenced was combined in a microtube with a primer, DNA polymerase, nucleotides, and dye-labeled dd-deoxynucleotides. The point was that the amount of

Table 1. PCR reaction in the final volume of 27.5 microlitres (µl) and thermocycling conditions

PCR reaction	
Materials	Volume (µl)
Master mix	12.5
Forward primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3')	1.5
Reverse primer 1392R (5'-GGTTACCTTGTACGACTT-3')	1.5
Template DNA	5
Distilled water	7
Final volume	27.5
Thermocycling conditions (38 cycles)	
DNA reaction	Tempratures/Time
Initial denaturation	94 °C for 5 minutes (min)
Denaturation	95°C for 1 min
Annealing	50°C for 40 seconds (s)
Extension	72°C for 1 min
Final extension	72°C for 1 min

dd-deoxynucleotides was much lower than that of regular nucleotides. The temperature was then raised to anneal the two strands of DNA, and then lowered so that the primer could bind to the single-stranded DNA. After the primer had bound to the single-stranded DNA, the temperature was raised again so that the polymerase could begin making DNA from where the primer bound. The DNA polymerase then started adding nucleotides to the DNA, continuing the chain until it added a dd-deoxynucleotide instead of a regular nucleotide. At this point, no further nucleotides can be added, so the dideoxynucleotide (ddNTP) (A, C, T, and G) will be the last point in the chain built. The smallest DNA fragment, which consists of only one dd-deoxynucleotide after the primer, crossed the finish line first. Then the fragment consisting of one nucleotide and one dd-deoxynucleotide crossed the finish line, and so on, in order of length, from shortest to longest. Thus, the DNA sequence can be determined by the colors read one after the other by a detector. The information read by the device consists of peaks. After the sequence, the sequence files were checked and extracted with CHROMAS software (viewing and editing chromatograms). Basic local alignment search tool (BLAST) and alignment with the genes of standard strains registered in the National Center for Biotechnology Information (NCBI) GenBank were done.^{18,19} The obtained data were analyzed by Statistical Package for the Social Sciences (SPSS)-22 software, and descriptive statistics indicators and frequency tables were presented to obtain the percentage of resistance in bacteria. The chi-square test (χ^2) was used for the relationship between frequencies of ESBL-producing bacteria and prevalence of MDR isolates and the type of samples ($P \leq 0.05$).

Results

A total of 186 bacterial isolates were obtained, of which 183 (98.38%) were included in this study; three isolates of *Bacillus* spp. were excluded. The isolates included *P. aeruginosa* (74 isolates; 39.78%), *Acinetobacter baumannii* (*A. baumannii*; 37 isolates; 18.89%), *Klebsiella pneumoniae* (*K. pneumoniae*; 27 isolates; 14.51%), *Escherichia coli* (*E. coli*; 27 isolates; 14.51%), and *Enterobacter* spp. (18 isolates;

9.67%). Overall, 74.86% (137/183) of the isolates produced ESBL, and 32.84% (45/137) of the ESBL-producing isolates were MDR, representing 24.59% (45/183) of all isolates.

The ESBL-producing isolates were obtained from sanitary facilities: 73 isolates (53.28%), dirty workroom sink; 36 isolates (26.27%), patient's hand pulse; 28 isolates (20.43%), personnel hands; 28 isolates (20.43%), ventilators; 9 isolates (6.56%), and bed; 9 isolates (6.56%). There was no significant relationship between ESBL-producing and MDR isolates with sample types ($P > 0.05$).

The numbers 110 to 170 indicate the nucleotide number; each nucleotide with the associated peak is indicated by a color. Longer peaks of equal size and color (peak heights may vary by up to 3-fold, which is normal) indicate accurate results. In our study, clean and distinct peaks indicated sample purity and accurate sequencing (Figure 1).

The BLAST results obtained from the sequence extracted from CHROMAS software showed that the DNA sequences were similar (97%) to the template sequence and sequences that produced significant alignments. It was based on statistical indices of their similarity and identity with the template sequence (Figure 2).

Max[imum] Score: the highest alignment score calculated from the sum of the rewards for matched nucleotides or amino acids and penalties for mismatches and gaps. Total Score: the sum of alignment scores of all segments from the same subject sequence. Query Cover[age]: the percent of the query length that is included in the aligned segments. E[xpect] Value: the number of alignments expected by chance with the calculated score or better. The expected value is the default sorting metric; for significant alignments, the E value should be very close to zero. Ident[ity]: the highest percent identity for a set of aligned segments to the same subject sequence (<https://usuhslibguides.com/c.php?g=468091&p=3260303>).

The alignment results of the PCR product, 16S rRNA (Length: 150, nucleotide number: 1025565 base pair (bp)), showed that there was 94.70% (Identities: 161/170) similarity between the isolated strains. There was no inter-genomic gap (Gap: 0/170, 0%). Both DNA strands (Query: Searched sequence and Subject: Database sequence) were

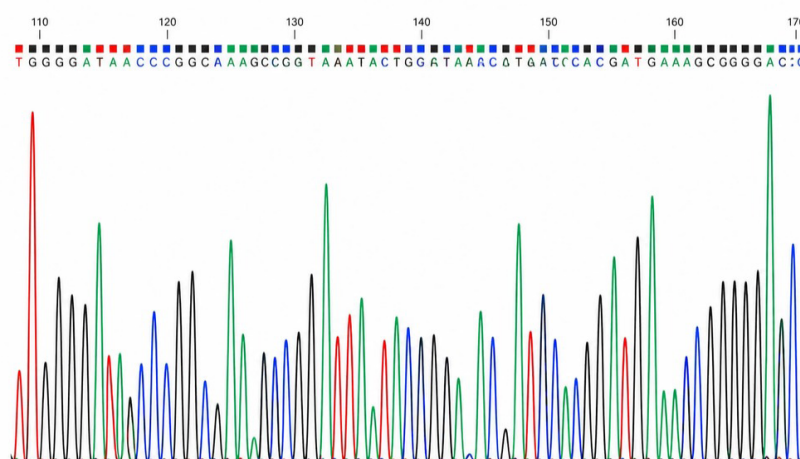


Figure 1. Sequencing results of strains isolated from the hands of personnel and the environment

Alignments Download ▾ GenBank Graphics Distance tree of results							
	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☐	Burkholderia cenocepacia strain FDAARGOS_734 chromosome 3	1058	4223	97%	0.0	100.00%	CP054820.1
☐	Burkholderia sp. ASPD2_16S ribosomal RNA gene, partial sequence	1058	1058	97%	0.0	100.00%	KJ375115.1
☐	Burkholderia cenocepacia strain 842 chromosome 1, complete sequence	1058	4209	97%	0.0	100.00%	CP015033.1
☐	Burkholderia cenocepacia strain NML110041 chromosome 3, complete sequence	1058	1058	97%	0.0	100.00%	CP102477.1
☐	Burkholderia cenocepacia strain NML110041 chromosome 2, complete sequence	1058	1058	97%	0.0	100.00%	CP102476.1
☐	Burkholderia cenocepacia strain NML110041 chromosome 1, complete sequence	1058	4232	97%	0.0	100.00%	CP102475.1
☐	Burkholderia cenocepacia strain toggle3 chromosome 3, complete sequence	1058	1058	97%	0.0	100.00%	CP073703.1
☐	Burkholderia cenocepacia strain toggle3 chromosome 1, complete sequence	1058	4218	97%	0.0	100.00%	CP073702.1
☐	Burkholderia cenocepacia strain toggle4 chromosome 3, complete sequence	1058	1058	97%	0.0	100.00%	CP073676.1
☐	Burkholderia cenocepacia strain toggle4 chromosome 1, complete sequence	1058	4214	97%	0.0	100.00%	CP073674.1
☐	Burkholderia cenocepacia strain toggle2 chromosome 1, complete sequence	1058	4218	97%	0.0	100.00%	CP073673.1
☐	Burkholderia cenocepacia strain toggle2 chromosome 3, complete sequence	1058	1058	97%	0.0	100.00%	CP073670.1
☐	Burkholderia cepacia isolate MINF_4A-sc-2280433 genome assembly, chromosome: 3	1058	1058	97%	0.0	100.00%	LR890525.1
☐	Burkholderia cepacia isolate MINF_4A-sc-2280433 genome assembly, chromosome: 2	1058	1058	97%	0.0	100.00%	LR890524.1

Figure 2. BLAST results of the strains isolated from the hands of personnel and the environment

(+) or Plus. Alignment results showed that there was also a 95% genetic similarity between the strains isolated from personnel and the environment (Figure 3).

Discussion

In this study, 16S rRNA gene sequencing for the identification of MDR bacteria isolated from the environment and personnel was applied. In our study, different bacteria were isolated from personnel and the environment of the hospital. Some of these isolates produced ESBL and were also MDR.

In recent years, dealing with and treating infections caused by bacteria with multiple drug resistance has increased, so it is important to investigate them. Due to the high ability of these bacteria to acquire antibiotic resistance genes and create MDR, the treatment of these infections has become difficult, expensive, and sometimes impossible. For this reason, the position of these bacteria has changed from a suspected pathogenic organism to an important infectious agent in hospitals around the world. In our study, 74.86 % of isolates were ESBL producers, and MDR bacteria were also detected in 24.59% of the isolates. The highest and lowest prevalence were related to *P. aeruginosa* (39.78%) and *Enterobacter* (9.67%), respectively. In a study conducted by Mosaffa et al, out of 70 blood samples taken from the ICU (8 cases from the nurse group and eight cases from the patient group), 16 of them had antibodies against *A.baumannii*. 10% of all bacterial infections were related to *Acinetobacter*.²⁰ In the study by Rasti et al, 133 (17.73%) cases of *Acinetobacter* were isolated from the 750 blood culture samples for 9 months.²¹ The comparison of the present study with other studies showed that the number of our findings was almost similar to other studies, and the slight difference in the percentage of some isolates is because the prevalence of hospital infections in different regions is due to risk factors such as how treatment of patients, infection control systems, geographical area, and health guidelines in hospitals are different.^{20,21} Antibiotic

resistance is caused by the use of various antibiotics in healthcare, environmental, and agricultural/livestock systems, and their transfer from one environment to another. Other important factors that are strong drivers of antibiotic resistance include sanitary environments, water and sanitation systems, drug quality, diagnostic and treatment methods, and travel or immigration quarantine. Depending on the mutations in the various genes residing in the bacterial chromosomes, the exchange of genetic material between organisms plays a role in antibiotic resistance.²² Also, in an international study conducted by Wang et al. in 76 countries, 51% of patients had nosocomial infections. In a retrospective observational cohort study in the ICU, out of 1298 patients who stayed at least 48 hours in the ICU, 9.53% of 231 patients 9.53% had nosocomial infections after admission to the ICU. According to these studies, it seems necessary to review infection control protocols in the ICU.²³ Similar to the studies conducted, the results obtained from the present study also showed that the most common microorganism isolated is *P. aeruginosa*. Considering the nature of the MDR of this bacterium and its rapid spread, it is very necessary to complete and implement infection control processes in intensive care units. Also, medical personnel should be informed about the methods of spreading infection from an infected patient to other patients. So a hospital can prevent the transmission of hospital infections and reduce the need for long-term antibiotic treatment.²¹⁻²³

Youssef et al. showed that, of the 260 bacterial species tested by 16S rRNA, 45.9% were related to *Staphylococcus*, *Streptococcus*, and *Enterobacter* species. High-throughput 16S rRNA offers a promising tool for accurate bacterial identification. Increases and decreases in antibiotic resistance were observed in 31.3% and 41% of cases, respectively.²⁴ Plasmid transfer is the most important phenomenon that may transfer antibiotic resistance genes to the host cell, and antibiotics may affect this process by inducing the transfer of resistance elements. These

Alignments					
Sequence ID: Query_1025665 Length: 150 Number of Matches: 1					
Range 1: 11 to 180 Graphics Next Match Previous Match					
Score:	Expect:	Identities:	Gaps:	Strand:	
267 bits(295)	2e-76	161/170(95%)	0/170(0%)	Plus/Plus	
Query 3	GATTAGCTAGTTGGTGAGGTAAAGGCTACCAAGGCGACGATCAGTAGCTGGTCTGAGAG	62			
Sbjct 11	GATTAGCTAGTTGGTGGGTAAAGGCCCTACCAAGGCGACGATCAGTAGCTGGTCTGAGAG	70			
Query 63	GATGATCAGCCACTGGAAGTACGACGCTCCAGACTCTACGGGAGGACAGTGGG	122			
Sbjct 71	GACGACAGCCACTGGGACTGAGACAGGCCCCAGACTCTACGGGAGGACAGTGGG	130			
Query 123	GAATATTGCACAATGGGCGAAAGCCTGATGCAGCCATGCCGCGTTGTGA	172			
Sbjct 131	GAATTTTGCACAATGGGCGAAAGCCTGATCCAGCAATGCCGCGTTGTGA	180			

Figure 3. Alignment results of the strains isolated from personnel and the environment; *Sbjct; Subject, Query; searched sequence, Length; 150 base pairs, number of matches; one strand, The numbers such as 3, 62, 11, and ... show protein start and end locations, Bit Score; BLAST scoring metric; higher is better, the expected E-value, the number of perfect matching identities, the number of matches, and the number of gaps in the whole alignment. Expect: Expected number of random alignments with a particular score or better that could be found by chance in a given database search. E-values close to zero correspond to more significant alignments

antimicrobial drugs may also exert selective pressure for the emergence of resistance. The fecal-oral route is the most important route of transmission, especially for resistant pathogens of the Enterobacteriaceae family, which usually occurs due to poor hygiene.²⁵ Sequencing and detection of genetic similarity of bacteria have become an important tool for understanding the transmission and epidemiology of infectious diseases. With this method, sources of infection and routes of bacterial transmission are identified, thus having positive and important effects on management and healthcare in the desired settings. Several bacterial species persist better and longer in the hospital environment than in other environments. Therefore identifying species by genetic similarity helps determine the route of transmission of infection from the environment to staff and patients. Nosocomial infections, in addition to increasing mortality and longer hospitalization periods compared to patients with acquired infections, also increase the workload of the medical staff, increase the use of drugs, and ultimately significantly increase treatment costs. Therefore, the control of hospital infections should be done more carefully.¹

In this study, the number of samples used was small because we had time constraints. Also, due to financial constraints, other molecular techniques were not used. It is better to use other molecular techniques, such as repetitive sequence-based (REP) and enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR), to obtain more accurate results. Genetic similarity between the strains was observed. Although no significant relationship existed between resistant isolates and sample types in this study, considering the nature of MDR in bacteria, it is very necessary to review the infection control processes and prescribe standard antibiotics in the ICU.

Conclusion

In our study, *P. aeruginosa* and *Enterobacter* accounted for the most and the fewest bacterial strains, respectively. Isolates producing ESBL had a high frequency. The highest

frequency of resistant isolates was associated with sanitary facilities, and the lowest was associated with ventilators and patient beds.

Acknowledgments

The present research is taken from the MSc thesis of the first author (Fateme Najafi). Hereby, the researcher would like to express her gratitude to the Takestan Branch, Islamic Azad University, Takestan, Iran.

Authors' Contribution

Conceptualization: Safar Ali Alizadeh Kushkahi
Data Curation: Fateme Najafi, Safar Ali Alizadeh Kushkahi, Mohammad Rasooli
Formal Analysis: Fateme Najafi, Safar Ali Alizadeh Kushkahi, Najafi, Safar Ali Alizadeh Kushkahi, Mohammad Rasooli
Investigation: Fateme Najafi, Safar Ali Alizadeh Kushkahi, Mohammad Rasooli
Methodology: Safar Ali Alizadeh Kushkahi, Mohammad Rasooli
Project Administration: Safar Ali Alizadeh Kushkahi
Resources: Fateme Najafi, Safar Ali Alizadeh Kushkahi, Mohammad Rasooli
Software: Mohammad Rasooli
Supervision: Safar Ali Alizadeh Kushkahi
Validation: Safar Ali Alizadeh Kushkahi
Visualization: Safar Ali Alizadeh Kushkahi
Writing–Original Draft: Fateme Najafi, Safar Ali Alizadeh Kushkahi
Writing–Review & Editing: Fateme Najafi, Safar Ali Alizadeh Kushkahi

Competing Interests

The authors have no conflict of interest.

Ethical Approval

Takestan Branch, Islamic Azad University, Takestan, Iran, approved this study. Ethics code: IR.IAU.TON.REC.1402.147.

Funding

This research was not funded by any association.

References

1. Eyre DW. Infection prevention and control insights from a decade of pathogen whole-genome sequencing. *J Hosp Infect* 2022;122:180-6. doi:10.1016/j.jhin.2022.01.024
2. Izadi N, Eshrati B, Mehrabi Y, Etemad K, Hashemi-Nazari SS.

- The national rate of intensive care units-acquired infections, one-year retrospective study in Iran. BMC Public Health 2021;21(1):609. doi:10.1186/s12889-021-10639-6
3. Sukhum KV, Newcomer EP, Cass C, Wallace MA, Johnson C, Fine J, et al. Antibiotic-resistant organisms establish reservoirs in new hospital built environments and are related to patient blood infection isolates. Commun Med (Lond) 2022;2:62. doi:10.1038/s43856-022-00124-5
 4. Neidhöfer C, Sib E, Neuenhoff M, Schwengers O, Dummin T, Buechler C, et al. Hospital sanitary facilities on wards with high antibiotic exposure play an important role in maintaining a reservoir of resistant pathogens, even over many years. Antimicrob Resist Infect Control 2023;12(1):33. doi:10.1186/s13756-023-01236-w
 5. Nayan A, Sarang B, Khajanchi M, Roy N, Jesudian G, Menon N, et al. Exploring the perioperative infection control practices & incidence of surgical site infections in rural India. Antimicrob Resist Infect Control 2023;12(1):65. doi:10.1186/s13756-023-01258-4
 6. Mohamed MS, Abd Alfadil NA, Gibril SI, Elsaman T, Mohamed MA. Identification and 16S rRNA gene sequence analysis of multidrug-resistant *Pseudomonas aeruginosa* in paper currency notes. Pharmacologyonline 2020;3:142-50.
 7. Mohammadi M, Vaisi Raiegan AA, Jalali R, Ghobadi A, Salari N, Barati H. The prevalence of nosocomial infections in Iranian hospitals. J Babol Univ Med Sci 2019;21(1):39-45. doi:10.22088/jbums.21.1.39
 8. Baghaei R, Mikaili P, Nourani D, Khalkhali HR. An epidemiological study of nosocomial infections in the patients admitted in the intensive care unit of Urmia Imam Reza hospital: an etiological investigation. Ann Biol Res 2011;2(5):172-8.
 9. Ghafouri M, Shakeri A, Shahsavand S, Aghamohammadi M. Knowledge, attitude, and practice of medical team about nosocomial Infections control and ways to protect against them in 6-educational hospitals of North Khorasan University of Medical Sciences in Bojnurd, 2013. J North Khorasan Univ Med Sci 2014;5(5):953-8.
 10. Akbari R, Fattahi Bafghi M, Fazeli H. Nosocomial infections pathogens isolated from hospital personnel, hospital environment and devices. J Med Bacteriol 2018;7(1-2):22-30.
 11. Ebrahimzadeh Leylabadlo H, Poursak T, Zahedi Bialvaei A, Aghazadeh M, Asgharzadeh M, Samadi Kafil H. Extended-spectrum beta-lactamase producing gram negative bacteria In Iran: a review. Afr J Infect Dis 2017;11(2):39-53. doi:10.21010/ajid.v11i2.6
 12. Lo Giudice D, Trimarchi G, La Fauci V, Squeri R, Calimeri S. Hospital infection control and behaviour of operating room staff. Cent Eur J Public Health 2019;27(4):292-5. doi:10.21101/cejph.a4932
 13. Spagnolo AM, Ottria G, Amicizia D, Perdelli F, Cristina ML. Operating theatre quality and prevention of surgical site infections. J Prev Med Hyg 2013;54(3):131-7.
 14. Kumar A, Chaudhry D, Goel N, Tanwar S. Epidemiology of intensive care unit-acquired infections in a tertiary care hospital of North India. Indian J Crit Care Med 2021;25(12):1427-33. doi:10.5005/jp-journals-10071-24058
 15. Nemati S, Mojtahedi A, Soltanipour S, Sharifgar Mavari M, Rouhi S. Evaluation of bacterial species to determine antimicrobial resistance in patients with chronic rhinosinusitis after surgery of paranasal sinuses referring to Amiralmomenin hospital in Rasht, 2018. Sci J Kurdistan Univ Med Sci 2020;25(2):1-13.
 16. Mollasalehi H, Vahedipour N, Alimi M. Differentiation and specific detection of *Shigella* genus using 16S Single Specific Primer PCR (16S SSP-PCR). Iran J Biol 2022;34(4):565-74.
 17. Vahedi A, Baghani A, Baseri Z, Pourmand MR. Frequency and antibiotic resistance patterns of isolated bacteria from positive blood culture of hospitalized patients. Tehran Univ Med J 2018;75(12):902-12.
 18. Srinivasan R, Karaoz U, Volegova M, MacKichan J, Kato-Maeda M, Miller S, et al. Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. PLoS One 2015;10(2): e0117617. doi:10.1371/journal.pone.0117617
 19. BLAST® Help [Internet]. Bethesda, MD: National Center for Biotechnology Information (US); 2008. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1762/>.
 20. Mosaffa N, Nafarieh T, Bandehpour M, Gachkar L, Taheri S, Hashemi A, et al. The study of serum antibody level in patients with *Acinetobacter baumannii* infection and comparing with healthy people and intensive care unit personnel. Pajoohandeh 2015;20(3):171-80.
 21. Rasti A, Erfani Y, Yazdanbod H. Prevalence and antibiotic susceptibility of isolated *Acinetobacter* from blood culture in Shariati hospital. Payavard Salamat 2010;3(3-4):70-5.
 22. Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S, Rasool MH, et al. Antibiotic resistance: a rundown of a global crisis. Infect Drug Resist 2018;11:1645-58. doi:10.2147/idr.S173867
 23. Wang Y, Ren J, Yao Z, Wang W, Wang S, Duan J, et al. Clinical impact and risk factors of intensive care unit-acquired nosocomial infection: a propensity score-matching study from 2018 to 2020 in a teaching hospital in China. Infect Drug Resist 2023;16:569-79. doi:10.2147/idr.S394269
 24. Youssef N, Boutros CF, Dakroub F, Akl F, Reslan L, Finianos M, et al. The clinical impact of 16S ribosomal RNA PCR and sequencing in the identification of bacterial infections: a 7-year report from a Lebanese tertiary care center. Front Cell Infect Microbiol 2025;15:1619640. doi:10.3389/fcimb.2025.1619640
 25. Kurniawan FD, Alia D, Shiraishi M, Higo M, Inoue Y, Hagiwara K. A systematic algorithm using 16S ribosomal RNA for accurate diagnosis of pneumonia pathogens. Sci Rep 2025;15(1):29253. doi:10.1038/s41598-025-14841-z