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Retrospective Cohort Study of Tracheal Etiological Agents and their Drug Resistance in Patients Referred to Imam Khomeini Hospital in Ardabil City from 2012 to 2022

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Abstract:

Background: Endotracheal tube-associated infections, an important cause of mortality in patients admitted to Intensive Care Units (ICU), are mainly caused by opportunistic, Multidrug-Resistant (MDR) bacteria. Accurate diagnosis of etiological agents and investigation of their antibiotic resistance patterns could improve the clinical management of these patients. This study, conducted over 11 years, aimed to examine the epidemiological changes of bacteria and the trends in drug resistance at a referral medical center in the northwest of Iran by analyzing endotracheal culture data.

Methods: In this retrospective cohort study, tracheal swab samples were collected from patients referred to Imam Khomeini Hospital in Ardabil, from 2012 to 2022. The samples were cultured on appropriate culture media, including blood agar, chocolate agar, and McConkie agar, to provide conditions for bacterial growth. After incubation for 24 to 48 hours, bacterial colonies were identified using microscopic and chemical methods. The antibiotic susceptibility patterns of the isolated bacteria were determined using the disk diffusion method. All data were analyzed with SPSS software version 25.

Results: In total, 598 samples examined were positive for endotracheal tube-related infections. Following *Acinetobacter* spp. (n = 204 [34.1%]), *Acinetobacter baumannii* (n = 148 [24.7%]) and *Klebsiella* spp. (n = 78 [13%]) were the most prevalent bacteria isolated. The majority (87.6%) of isolated bacteria were MDR, and most bacteria were isolated from the ICU. Ceftazidime was more common than gentamicin.

Discussion: *Acinetobacter* spp. and *Klebsiella* spp. are the most important infectious agents found in the tracheal cultures of hospitalized patients. The elevated levels of antibiotic resistance are a serious challenge in hospital settings. Gentamicin was identified as one of the most effective drugs against the aforesaid isolates.

Conclusion: The study's findings highlight the necessity of strengthening infection control programs, in addition to critically evaluating antibiotic use and establishing treatment protocols based on regional data.

Keywords: Trachea, Antibiotic resistance, Multidrug-Resistant (MDR), Intensive Care Unit (ICU).

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1. INTRODUCTION

Using invasive methods for airway management in critically ill patients has significantly risen in the Intensive Care Unit (ICU) in the last few decades [1, 2]. Endotracheal intubation is an important technique for respiratory support in patients, but it carries several risks, most notably respiratory infections such as tracheitis, pneumonia, and aspiration pneumonia. The introduction of a foreign body into the respiratory system disrupts the normal functioning of defense mechanisms, leading to the colonization of microorganisms and the formation of biofilms on the inner surface of the endotracheal tube [3, 4].

Biofilms are complex structures formed by varying bacteria and fungi, and elevate their resistance to antibiotic treatments and the body's immune defenses. These structures complicate treatment and could cause chronic infections, extended hospital stays, high medical costs, and even mortality. In addition, failure to accurately and promptly identify etiological factors in tracheostomized patients may lead to unnecessary or ineffective antibiotic use, ultimately contributing to increased drug resistance [5-7].

Given the high prevalence of ventilator-associated pneumonia, the most common acquired infection in the ICU, and the role of biofilm in the pathophysiology of these infections, it is essential to investigate the patterns of common microbial agents in tracheal cultures and assess their drug susceptibility [8].

A clear understanding of microbial patterns and antibiotic resistance can help improve treatment protocols, prevent drug resistance, and enhance patient care. Therefore, the present study was designed to investigate the etiological factors found in the tracheal cultures of patients hospitalized in the ICU of Imam Khomeini Hospital in Ardabil, from 2012 to 2022. Our goal was to provide valuable information that can optimize the diagnosis and treatment of these patients by analyzing microbial and drug data.

2. MATERIALS AND METHODS

2.1. Study Type and Population

The current retrospective cohort study was conducted in a clinical setting. The study population included all patients admitted to Imam Khomeini Hospital in Ardabil between 2012 and 2022 who were suspected of having endotracheal tube infections. The demographic information of the patients was collected confidentially and coded.

2.2. Inclusion and Exclusion Criteria

Inclusion criteria comprised patients over 18 years of age presenting clinical symptoms related to respiratory infections and those who underwent tracheal culture for diagnostic purposes. Exclusion criteria included patients who were uncooperative in providing information, those for whom a pathogen could not be identified after culture, and individuals with underlying diseases that could affect the results.

2.3. Culture of Samples and Identification of Isolated Bacterial Colonies

Tracheal samples were cultivated on proper culture media, including blood agar, chocolate agar, and eosin methylene blue. The plates were subsequently incubated at 37°C for 24-48 hours. After we grew the bacterial colonies on the above media, Gram staining was conducted for initial identification. We then performed diagnostic tests for sensitivity to bacitracin, optochin, and trimethoprim-sulfamethoxazole. We also conducted enzyme tests, including catalase and oxidase [9].

2.4. Antibiotic Susceptibility and Resistance Patterns

To test the sensitivity and resistance of bacterial isolates, the Kirby-Bauer disk diffusion method was used on Mueller-Hinton Agar (Merck, Germany) according to CLSI guidelines [10]. A bacterial suspension (containing 1.5×10^8 CFU/mL) equivalent to the half McFarland standard was then used. Antibiotic disks (Padtan Teb Co., Iran) were also used for ceftazidime, cefotaxime, imipenem, amoxiclav, gentamicin, ceftriaxone, and cotrimoxazole. After incubation of the plates at 35 to 37°C for 18-24 hours, the diameters of the inhibition zones were measured. The results were finally reported as susceptible, resistant, or semi-susceptible as per the CLSI guidelines. Isolates resistant to at least three different classes of antibiotics were classified as MDR bacteria [9].

2.5. Statistical Analysis

Analyses of data were performed using SPSS version 25. Descriptive statistics were used to describe the data. The relationship between variables was examined by Chi-square and Fisher's exact tests. In all analyses, the significance level was considered to be less than 0.05.

3. RESULTS

3.1. Demographic Information

This study involved 598 participants over 11 years. Of the samples collected, 329 were from men and 269 from women. Most samples (n = 530; 88.60%) were obtained from ICU wards. The incidence of positive tracheal cultures was notably higher in 2022 than in other years. September 2019 (n = 69; 11.5%) had the highest tracheal infection, followed by December 2019 (n = 61; 10.2%), August 2019 (n = 60; 10%), November 2019 (n = 59; 9.9%), and July 2020 (n = 58; 9.7%) (Table **1**).

3.2. Prevalence and Diversity of Microbial Strains Isolated

The highest number of bacterial isolates was obtained from the ICU (n = 530; 88.60%), while only 2 (0.3%) isolates were collected from the infectious ward. In the ICU, Acinetobacter, *Acinetobacter baumannii*, *Klebsiella* spp., and *Pseudomonas aeruginosa*, with 139, 181, 72, and 49 isolates, were the most common bacteria isolated, respectively (Table **2**).

Table 1. Distribution of Tracheal etiologic agents based on demographic data extracted over 11 years at Imam Khomeini Hospital, Ardabil.

	Frequency	Percentage	Valid Percentage	Cumulative Percentage
Wards				
Cardiology	5	0.8	0.8	0.8
CCU	18	3.0	3.0	3.8
ICU	530	88.6	88.6	92.5
Infectious	2	.3	.3	92.8
Internal	39	6.5	6.5	99.3
Surgery	4	.7	.7	100.0
Gender				
Male	329	55.0	55.0	55.0
Female	269	45.0	45.0	100.0
Year				
2012	3	0.5	0.5	0.5
2013	6	1.0	1.0	1.5
2014	22	3.7	3.7	5.2
2015	34	5.7	5.7	10.9
2016	33	5.5	5.5	16.4
2017	44	7.4	7.4	23.7
2018	69	11.5	11.5	35.3
2019	66	11.0	11.0	46.3
2020	30	5.0	5.0	51.3
2021	40	6.7	6.7	58.0
2022	77	12.9	12.9	70.9
Months				
1	28	4.7	4.7	4.7
2	49	8.2	8.2	12.9
3	40	6.7	6.7	19.6
4	44	7.4	7.4	26.9
5	38	6.4	6.4	33.3
6	44	7.4	7.4	40.6
7	58	9.7	9.7	50.3
8	60	10.0	10.0	60.4
9	69	11.5	11.5	71.9
10	48	8.0	8.0	79.9
11	59	9.9	9.9	89.8
12	61	10.2	10.2	100.0

Table 2. Distribution of Trachea etiologic agents by wards over 11 years at Emam Khomeini Hospital, Ardabil.

Bacteria	Wards (n; % of total)						Total (n; %)
	Cardiology	CCU	ICU	Infectious	Internal	Surgery	
Acinetobacter baumannii	1 (0.2)	2 (0.3)	139 (23.2)	0 (0)	6 (1)	0 (0)	148 (24.7)
Acinetobacter spp.	2 (0.3)	5 (0.8)	181 (30.3)	0 (0)	15 (2.5)	1 (0.2)	204 (34.1)
Burkholderia cepacia	0 (0)	0 (0)	1 (0.2)	0 (0)	0 (0)	0 (0)	1 (0.2)
Citrobacter spp.	0 (0)	0 (0)	10 (1.7)	0 (0)	1 (0.2)	0 (0)	11 (1.8)
E. coli	0 (0)	1 (0.2)	5 (0.8)	0 (0)	1 (0.2)	0 (0)	7 (1.2)
Enterobacter spp.	0 (0)	0 (0)	16 (2.7)	0 (0)	1 (0.2)	0 (0)	17 (2.8)
Enterococcus	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.2)	0 (0)	1 (0.2)
Klebsiella aerogenes	0 (0)	0 (0)	2 (0.3)	0 (0)	0 (0)	0 (0)	2 (0.3)
Klebsiella spp.	0 (0)	1 (0.2)	72 (12)	0 (0)	5 (0.8)	0 (0)	78 (13)
Methicillin resistant staph-aureus(MRSA)	0 (0)	2 (0.3)	8 (1.3)	0 (0)	2 (0.3)	1 (0.2)	13 (2.2)
Methicillin-resistant staph-coagulase-negative	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.2)	0 (0)	1 (0.2)
Proteus spp.	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.2)	0 (0)	1 (0.2)
Pseudomonas aeruginosa	0 (0)	0 (0)	49 (8.2)	2 (0.3)	1 (0.2)	0 (0)	52 (8.7)
Pseudomonas spp.	0 (0)	0 (0)	22 (3.7)	0 (0)	0 (0)	0 (0)	22 (3.7)

Bacteria	Wards (n; % of total)						Total (n; %)
	Cardiology	CCU	ICU	Infectious	Internal	Surgery	
S. aureus	1 (0.2)	3 (0.5)	9 (1.5)	0 (0)	0 (0)	1 (0.2)	14 (2.3)
Staphylococcus epidermidis	1 (0.2)	4 (0.7)	14 (2.3)	0 (0)	4 (0.7)	1 (0.2)	24 (4.0)
Stenotrophomonas maltophilia	0 (0)	0 (0)	2 (0.3)	0 (0)	0 (0)	0 (0)	2 (0.3)
Total	5 (0.8)	18 (3)	530 (88.6)	2 (0.3)	39 (6.5)	4 (0.7)	598 (100)

3.3. Distribution of MDR Microbial Agents Isolated from Tracheal Cultures

Of the bacteria isolated from the hospital, 524/598 (87.6%) were MDR. *Acinetobacter* spp. (n = 198; 33.1%) was the predominant MDR bacterium. This trend was followed by *Acinetobacter baumannii* and *Klebsiella* spp., with incidence rates of 138 (23.1%) and 70 (11.7%), respectively. However, *Enterococcus* was the only bacterial species with no MDR isolates (Table **3**).

3.4. Results of Antibiotic Resistance of Strains by the Disk Diffusion Method

Over the 11 years, the resistance rate to gentamicin was the lowest among the tested antibiotics, whereas resistance to ceftazidime was the highest (Table **4**).

Table 3. Distribution of MDR strains over 11 years at Emam Khomeini Hospital, Ardabil.

Bacteria	MDR (n; % of total)		Total (n; %)
	Non-MDR	MDR	
Acinetobacter baumannii	10 (1.7)	138 (23.1)	148 (24.7)
Acinetobacter spp.	6 (1)	198 (33.1)	204 (34.1)
Burkholderia cepacia	0 (0)	1 (0.2)	1 (0.2)
Citrobacter spp.	3 (0.5)	8 (1.3)	11 (1.8)
E. coli	2 (0.3)	5 (0.8)	7 (1.2)
Enterobacter spp.	3 (0.5)	14 (2.3)	17 (2.8)
Enterococcus	1 (0.2)	0 (0)	1 (0.2)
Klebsiella aerogenes	1 (0.2)	1 (0.2)	2 (0.3)
Klebsiella spp.	8 (1.3)	70 (11.7)	78 (13)
Methicillin resistant staph-aureus(MRSA)	2 (0.3)	11 (1.8)	13 (2.2)
Methicillin-resistant staph-coagulas-negative	0 (0)	1 (0.2)	1 (0.2)
Proteus spp.	0 (0)	1 (0.2)	1 (0.2)
Pseudomonas aeruginosa	9 (1.5)	43 (7.2)	52 (8.7)
Pseudomonas ssp.	2 (0.3)	20 (3.3)	22 (3.7)
S. aureus	8 (1.3)	6 (1)	14 (2.3)
Staphylococcus epidermidis	19 (3.2)	5 (0.8)	24 (4)
Stenotrophomonas maltophilia	0 (0)	2 (0.3)	2 (0.3)
Total	74 (12.4)	524 (87.6)	598 (100)

Table 4. Distribution of antibiotic resistance by years over 11 years at Emam Khomeini Hospital, Ardabil.

Year	Antibiotics (n; % of total)						
	Cefotaxime	Co-trimoxazole	Amoxiclav	Ceftazidime	Ceftriaxone	Gentamycin	Imipenem
2012	0 (0.0)	2 (0.5)	0(0.0)	5 (1.1)	1 (0.4)	1 (1.3)	6 (1.4)
2013	3 (0.7)	12 (2.8)	2 (1.2)	15 (3.2)	4 (1.6)	1 (1.3)	17 (4.1)
2014	5 (1.2)	15 (3.6)	4 (2.4)	21 (4.5)	5 (2)	0 (0.0)	22 (5.3)
2015	21 (5)	18 (4.3)	14 (8.3)	26 (5.6)	20 (8.1)	1 (1.3)	26 (6.3)
2016	29 (6.9)	22 (5.2)	12 (7.1)	34 (7.3)	18 (7.3)	1 (1.3)	30 (7.2)
2017	28 (6.7)	39 (9.2)	4 (2.4)	43 (9.2)	35 (14.2)	3 (1)	38 (9.1)
2018	52 (12.4)	40 (9.5)	27 (16.1)	24 (5.1)	28 (11.3)	5 (6.7)	43 (10.3)
2019	0(0.0)	26 (6.2)	19 (4.5)	20 (11.9)	26 (5.6)	5 (2)	1 (1.3)
2020	30 (7.2)	34 (8.1)	30 (17.9)	32 (6.9)	10 (4)	2 (2.7)	14 (3.4)
2021	56 (13.4)	42 (10)	48 (28.6)	55 (11.8)	1 (0.4)	17 (22.7)	16 (3.8)
2022	147 (35.1)	142 (33.6)	0(0.0)	149 (31.9)	103 (41.7)	3 (4)	136 (32.7)

4. DISCUSSION

Studies have identified infections as one of the main complications in tracheostomized patients, especially when the endotracheal tube is left in place for a long time. This prolonged presence can irritate the tracheal wall and provide conditions for bacterial colonization and ultimately, respiratory infection [9, 11].

A demographic analysis of patients showed that the majority of positive tracheal cultures were obtained from male patients, mainly those hospitalized in the ICU. The high prevalence in men may be associated with the high prevalence of underlying diseases, smoking, or occupational exposure to various risk factors. Cases were also more often in the ICU (88.6%), which shows the important contribution of mechanical ventilation and severity in the risk of nosocomial respiratory infections. Interestingly, 2022 had the highest number of culture-positive cases, possibly due to the pandemic of COVID-19 worldwide and a rise in pulmonary hospitalizations. Additionally, the highest numbers of tracheal infections were observed in the warm months and early fall, which could relate to increased hospitalizations from respiratory infections and/or inadequate ventilation in these seasons. These results underscore the need for greater attention to infection prevention, protection, and compliance with mechanical ventilation practices in ICUs, especially during periods of higher seasonal or epidemic prevalence. In a previous study performed in 2019-2021 at the 5th Military Hospital with a Polyclinic in Krakow, 60 patients (20% female and 20% male) were studied [12]. The highest rate of microbial agents isolated from tracheal culture samples was obtained from the patients in the ICU, with a significant frequency of 88.6%. This observation illustrates the impact of mechanical ventilation, prolonged hospital stay, disease severity, and excessive antibiotic use on the risk of bacterial colonization and infection in this ward.

In this study, *Acinetobacter* spp. (including *Acinetobacter baumannii*), *Klebsiella* spp., and *Pseudomonas aeruginosa* were the most common bacteria isolated. Many of these bacteria are MDR and opportunistic pathogens. They are frequently seen in patients with endotracheal tubes and in ICUs. Their presence is generally associated with poor clinical outcomes, such as mortality and prolonged hospitalization. These pathogens are also less likely to be present in other hospital wards (such as infectious, surgical, or internal), indicating that they may be transmitted *via* secondary transmission from ICU patients or that mechanical ventilation is used less frequently in those wards.

The results of our study underscore the need to regularly monitor for nosocomial infections, the necessity of strict implementation of infection control measures in the ICU, and the need to continually conduct microbial screening to enable the proper and specific treatment of lower respiratory tract infections. Although the most dominant pathogens isolated included most of the bacterial isolates, including species of *Acinetobacter* and *Klebsiella*, as well as *Pseudomonas* aeruginosa, the detection of bacteria with low abundance is of paramount importance. *Staphylococcus epidermidis* (4%), *Staphylococcus aureus* (3.2%), species of *Enterobacter* and *Citrobacter*, and *Escherichia coli* were the bacteria that were isolated in smaller numbers. This result indicates other sources of the infection, *i.e.*, normal flora of the patient, invasive catheters, and horizontal transmission within the hospital. We also explored a small proportion of resistant bacteria, such as *Stenotrophomonas maltophilia* and methicillin-resistant *Staphylococcus aureus* (MRSA), with a prevalence of less than 3%. The results of this finding indicate that the above-mentioned bacteria are not abundant. These bacteria, however, if not detected rapidly, may cause severe respiratory infections and complicate treatment, particularly in patients who are immunocompromised or have not been intubated for long time periods. The detection of bacteria (*e.g.*, *Burkholderia cepacia* and *Proteus spp*.) is rare and indicates that the hospital environment or equipment is contaminated and requires further epidemiological investigation. Therefore, screening these low-abundant bacteria in hospitals and developing treatment protocols are suggested.

In studies investigating bacterial identification in endotracheal tubes, *Acinetobacter* spp., *Pseudomonas aeruginosa*, and generally Gram-negative bacilli have been identified as the most common agents [2, 11]. In the study by Sanders *et al*., it has been noted that children with long-term tracheostomy tubes are often colonized with *Pseudomonas aeruginosa*. They also indicated an increase in the isolation of this bacterium following tracheostomy [13]. The endotracheal tube makes a connection to the outside environment, which can compromise the upper respiratory tract's mechanism of defence. In addition, hospital areas are typically contaminated with Gram-negative bacilli, such as *Acinetobacter* spp. and *Pseudomonas aeruginosa*. These are bacteria that can readily form biofilms on plastic devices (endotracheal tubes), and their control is very important. As a result, to prevent further complications, it is vital to control their growth using various methods [14].

Following Gram-negative bacilli, *Staphylococcus aureus* was reported in the tracheal tube. While this bacterium is naturally present on both the skin and respiratory tract, it can become pathogenic and cause severe infections under specific conditions, for instance, long-term use of a tracheostomy [12]. In a recent study, Batool and associates examined microorganisms isolated from endotracheal tubes and tracheal aspirate specimens at a hospital in Lahore, Pakistan. The majority of the organisms (98 strains; 77.78%) were Gram-negative. However, 17 (13.49%) were Gram-positive, and 11 (8.73%) were yeasts. *Acinetobacter*, *Klebsiella*, and *Pseudomonas* species were identified as the most prevalent Gram-negative bacteria, and *Streptococcus* spp., MRSA, and *Staphylococcus epidermidis* as the most prevalent Gram-positive bacteria [15]. These findings are in line with the results obtained in the present study.

The current study found that 87.6% of bacteria isolated from tracheal cultures exhibited MDR patterns. This high level of resistance represents a serious challenge in the management of patients with nosocomial respiratory infections, especially those admitted to ICUs. *Acinetobacter* spp., especially *Acinetobacter baumannii*, with MDR frequencies of 198 (33.1%) and 138 (23.1%), respectively, were considered to be the most important resistant agents. Given the high innate and acquired resistance of this bacterium to antibiotics, it requires more stringent control strategies and careful monitoring in hospitals. Important resistance was also observed among *Klebsiella* spp. and *Pseudomonas aeruginosa* (70 and 43 MDR isolates, respectively), indicating the significant role of Gram-negative bacilli in the spread of MDR infections in the lower respiratory tract. Some bacteria, like *Enterococcus* spp., were found to be free of MDR isolates. In contrast, other bacteria, including the species of *Enterobacter* and *Citrobacter*, *Escherichia coli*, Gram-positive cocci (*Staphylococcus aureus*), and MRSA, contributed to MDR. Notwithstanding the low frequency of some of these agents, their presence could be an alarm signal for the potential spread of resistance in hospitals. Therefore, antibiotic resistance levels among hospital pathogens is not only widespread but also multifaceted and complex, and addressing this issue needs developing precise antibiotic programs, strengthening control systems of infections, and continuous screening of resistant bacteria. In Batool *et al.*'s study, *Acinetobacter* showed high levels of resistance to all antibiotics, except for colistin, doxycycline, and polymyxin B. Moreover, *Pseudomonas* was susceptible to all antibiotics except for ceftazidime (100% resistant). *Klebsiella* and MRSA showed high resistance to all antibiotics tested [15]. In our study, *Acinetobacter* spp. also showed high resistance and were mostly MDR. The species of *Acinetobacter* and Klebsiella, in our study, were the most common Gram-negative bacteria. We also identified coagulase-negative *staphylococci* and *Staphylococcus aureus*, with 87.6% of the bacteria exhibiting MDR. This figure was much higher than that reported in Dargahi's study.

According to our results, the resistance of bacteria isolated from tracheal cultures to antibiotics has been increasing, with significant differences among different antibiotics. The highest level of resistance was observed against ceftazidime, with resistance to this antibiotic reaching 31.9% in 2022. Also, the resistance level to ceftriaxone (41.7%) and cefotaxime (35.1%) has been rising in recent years. These results match investigations indicating that Gram-negative bacteria, particularly *Klebsiella* spp. and *Acinetobacter baumannii*, show high resistance to third-generation cephalosporins in ICUs [16-18]. This elevation is likely because of the frequent and sometimes improper use of these antibiotics in the empirical treatment of nosocomial infections for mechanically ventilated/critically ill patients who are vulnerable to MDR pathogens. In contrast, resistance to gentamicin has been significantly lower, remaining below 5% in most years. This observation may be due to the limited use of this aminoglycoside in general practice and the constraints of its systemic administration due to nephrotoxicity. However, for some Gram-negative bacteria, gentamicin is effective [19, 20]. Imipenem is a highly active carbapenem that was relatively resistant in the early years of the study but has increased at a considerable rate in recent years (up to 32.7% in 2022). Increasing resistance to imipenem indicates the spread of carbapenem-resistant bacteria, specifically *Acinetobacter baumannii* and *Klebsiella pneumoniae*, which are a threat to successful treatment within the hospital setting [21, 22]. Meanwhile, the level of resistance to cotrimoxazole reached more than 33% in the last years of the study, especially in 2022, meaning that it is not as effective as a first-line antibiotic in some respiratory infections. Resistance to amoxiclav also increased from about 2% in the early years to over 28% in 2021, and then reportedly dropped to zero in 2022. This elevation in resistance may come from the discontinuation of these antibiotics or an alteration in treatment patterns. Gram-negative bacteria (76.13%), fungi (17.04%), and Gram-positive cocci (6.81%) were the most prevalent organisms isolated from an ICU in Bangladesh. The species of *Acinetobacter* and *Klebsiella* were also the most common pathogens. Besides, the species of *Acinetobacter*, *Klebsiella*, and *Pseudomonas* were the most resistant Gram-negative bacteria, which exhibited high resistance levels (<80%) to third-generation fluoroquinolones and cephalosporins. There was also high resistance to aminoglycosides (>68%) and imipenem (>60%) (9). The findings of that study are in alignment with our study regarding the predominant bacterial species and the levels of antibiotic resistance to cephalosporins.

This study was conducted at a single medical center in northwestern Iran; therefore, generalization of the results to other regions or medical centers may be limited. Due to the descriptive observational nature of the study, we were unable to examine causal relationships among variables. In addition, possible changes in antibiotic prescribing patterns, infection control policies, and patients' clinical conditions over the 11-year study period may have affected the observed trends. Overall, the unavailability of certain clinical data on patients, such as their history of antibiotic use or underlying diseases, may have limited the comprehensive analysis of risk factors associated with multidrug resistance.

CONCLUSION

Considering the increasing levels of antibiotic resistance and the prevalence of MDR strains reported in the present study, the emergence of drug resistance in bacteria isolated from tracheal cultures is a serious concern for physicians and health policymakers. In hospitals, excessive antibiotic consumption, non-prescription use, and inadequate infection control are the main contributory factors to this high level of resistance. Therefore, to prevent further resistance, it is necessary to prescribe antibiotics, such as gentamicin and imipenem, only in specific cases and based on antibiogram results.

AUTHORS' CONTRIBUTIONS

The authors confirm their contributions to the paper as follows: R.R.: Supervision; R.R.: Conceptualization; M.M.: Investigation; P.SH.: Writing - original draft preparation; M.A.: Methodology; R.R.: Review and editing; M.A and A.M.: Data analysis.

LIST OF ABBREVIATIONS

ICU	=	Intensive Care Unit
MDR	=	Multidrug-resistant

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The present study was submitted to the Ethics Committee of Ardabil University of Medical Sciences and approved with the ethics number IR.ARUMS.MEDICINE. REC.1404.038.

HUMAN AND ANIMAL RIGHTS

This study was conducted in accordance with the ethical principles set forth in the Declaration of Helsinki.

CONSENT FOR PUBLICATION

Given that the present study was based on retrospective laboratory data analysis and no direct intervention was performed on the patients, the privacy and confidentiality of the patients' information were fully respected, and all data were recorded and reviewed anonymously before analysis. The protocol of this study was reviewed and approved by the Ethics Committee of Ardabil University of Medical Sciences. Also, due to the use of secondary data, obtaining informed consent from the patients was not deemed necessary; however, all stages of the research were conducted in accordance with national and international ethical standards in the field of human-related research.

STANDARDS OF REPORTING

STROBE guidelines were followed.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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