

Original Article

Six-Year Retrospective Analysis of Bacterial Isolates and Antibiotic Resistance in Endotracheal Tube Cultures at an ICU in Tabriz, Iran

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Received: 22 February, 2026; Accepted: 01 August, 2026

DOI: 10.22037/nbm.v14i4.51854

Abstract

Background: Infections in the intensive care unit (ICU) account for a significant portion of hospital infections and are thought to be frequent and important contributors to patient mortality and length of stay. Respiratory and blood infections account for the majority of infections in this section. In this study, endotracheal tube samples from intubated patients admitted to the intensive care unit (ICU) of Imam Sajjad Hospital in Tabriz over 6 years will be examined for the types of bacteria present and their antibiotic resistance.

Materials and Methods: The results of 309 endotracheal tube culture samples from the intensive care unit of Imam Sajjad Hospital in Tabriz were assessed in this descriptive-retrospective study between October 2019 and June 2025.

Results: Out of the 309 samples that were examined, 163 were male (52.7%), and 146 were female (47.3%). Ninety-four (52.2%) of the 180 positive tracheal culture samples were female. *Klebsiella* (45.5%) and *Acinetobacter* (18.9%) were the most frequently isolated bacteria from the tracheal tube. Cefixime was associated with the highest level of antibiotic resistance in *Klebsiella* species, whereas *Acinetobacter* species were resistant to all antibiotics.

Conclusion: Bacterial contamination of the endotracheal tube was found in approximately 58.2% of patients. Timely endotracheal tube replacement and adherence to sterile conditions during intubation are advised because bacteria isolated from these tubes can cause nosocomial pneumonia and antibiotic resistance in patients.

Keywords: Endotracheal tube culture, Antibiotic resistance pattern, Antibiotic sensitivity pattern, Intensive care unit

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Please cite this article as: Khalili Amoli P, Naeili H, Najafi Boket A, Baghban Vahid S, Hamidi F. Six-Year Retrospective Analysis of Bacterial Isolates and Antibiotic Resistance in Endotracheal Tube Cultures at an ICU in Tabriz, Iran. Novel Biomed. 2026;14(4):173-80.

Introduction

Hospital-acquired infections (HAIs) are linked to high rates of morbidity and mortality among hospitalized patients, making them a significant public health concern in hospitals across the globe¹. HAIs currently pose a serious burden in developed nations, where

they impact at least 50% of patients in intensive care units (ICUs) and 5% to 15% of patients admitted to general wards. Urinary tract infections, lower respiratory tract infections, and surgical wound infections are the most frequent infections acquired in hospitals². A WHO study found that acute surgical and orthopedic wards, as well as the intensive care unit,

have the highest rates of hospital-acquired infections³. Because they are more susceptible, patients who are elderly, have underlying medical conditions, or are undergoing chemotherapy have a higher infection rate. Patients receiving chemotherapy, those with underlying medical conditions, and older patients are more susceptible to infection⁴. It is crucial to remember that patients in intensive care units are more susceptible to nosocomial infections due to the fact that endotracheal intubation and mechanical ventilation can result in infection acquisition, and underlying medical conditions can weaken their immune systems⁵. Further predisposing factors that raise the risk of nosocomial infections are longer hospital stays for these patients. Pneumonia, also known as ventilator-associated pneumonia (VAP), is one of the most significant forms of these hospital-acquired infections⁶. It typically happens in conjunction with endotracheal intubation and mechanical ventilation. It should be noted that, among all complications, ventilator-associated pneumonia (VAP) is the most common hospital-acquired infection in the intensive care unit (ICU), affecting about 20% of ICU patients and 30% of those on mechanical ventilation⁷. In patients on mechanical ventilation, VAP appears 48 hours after tracheostomy or intubation. Early-onset and late-onset VAP are the two varieties. The latter occurs within 96 hours after intubation. Typically, endotracheal intubation is used to restore hypoxemic or hypercarbic respiratory failure, prevent aspiration, and maintain a secure airway in critically ill patients⁸. Emergency intubation is an independent risk factor for primary VAP and does not prevent colonization of the tracheal mucosa by endogenous flora. Because the indwelling tracheal tube decreases bacterial clearance, increases secretion leakage around the cuff, and damages the airway, patients on mechanical ventilation are more likely to develop respiratory tract infections⁹. *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Staphylococcus aureus* are the most common causative microorganisms of intubation-associated respiratory infections in children¹⁰. The prevalence of antibiotic-resistant bacteria raises concerns about hospital-acquired infections by increasing morbidity, mortality (13–25%), and related expenses¹¹. It's

important to remember that 50% of antibiotic prescriptions in the intensive care unit are for VAP management. Without a worldwide strategy to address this issue, it is estimated that 10 million people will perish each year from antibiotic resistance by 2050¹². However, patients are at risk for superinfection with fever, reduced respiratory signs and symptoms, and a higher number and proportion of polymorphonuclear leukocytes in sputum when a large number of potentially harmful bacteria colonize the trachea¹³. Because of this, nosocomial pneumonia is a prevalent and potentially fatal issue for critically ill patients receiving mechanical ventilation. Pneumonia can range in incidence from 9 to 68%, with a high mortality rate of 50 to 80%, particularly when caused by bacteria resistant to antibiotics. A new problem for intensive care unit doctors is the appearance of bacteria that are resistant to antibiotics in critically ill patients¹⁴. Antibiotic resistance varies by geographic location, according to the findings of multiple studies, and resistance to new-generation antibiotics can be a warning sign in certain cases¹⁵.

For the results to be a useful guide to the appropriate administration of antibiotics in that region, particularly in the intensive care unit, each region must monitor its resistance and sensitivity patterns dynamically and sustainably rather than cross-sectionally. Thus, the current study's objectives were to assess bacteria contaminating the endotracheal tube and to examine antibiotic resistance among patients who were intubated and admitted to the intensive care unit of Imam Sajjad Hospital in Tabriz.

Methods

A retrospective cohort study was conducted at Imam Sajjad Hospital in Tabriz between October 2019 and June 2025. Following approval from the ethics committee (IR.IAU.TABRIZ.REC.1403.523), the study and sample collection were carried out. Samples were taken from the tracheal tubes of endotracheal aspiration patients who were admitted to the intensive care unit and exhibited clinical signs of pneumonia. Inclusion criteria were age >18 years, at least 2 days of intubation, and survival for 5 days after intubation. Patients who survived less than 5 days after intubation were excluded because they did not have sufficient endotracheal tube

exposure for reliable microbiological evaluation and culture collection.

Samples from endotracheal tubes were put in a sterile tube, brought to the lab, inoculated into thioglycolate broth, and then incubated for 24 hours at 37°C. The next day, the broth medium was smeared directly with Gram stain to check for the presence of bacteria. Positive samples were subcultured on blood, MacConkey, and chocolate agar and incubated for 24 to 48 hours at 37°C. Following that, both macroscopic and microscopic features (such as the analysis of colony color, shape, and Gram staining) were investigated. Following morphological analysis, standard biochemical tests were conducted to identify gram-negative bacteria. These tests included oxidase and catalase, reaction in trisaccharide iron agar (TSI) culture medium, Acetate utilization test in Simmons citrate medium, indole production and mobility, urea utilization, and tests for staphylococci using catalase, coagulase, and DNase. The bacteria were then isolated using disk diffusion and tested for antibiotic susceptibility. For both gram-positive and gram-negative cocci, antibiotic discs were used. Lastly, a checklist was created by extracting patient data from each patient's file, including gender, age, type of cultured microorganism, sensitivity and resistance to tested antibiotics, and the presence or absence of a positive culture of tracheal secretions. Finally, using SPSS 25, the research data were analyzed using descriptive statistics, including mean, standard deviation, and frequency. The relationship between gender and bacterial infection and between age and bacterial infection was analyzed using Chi-square. *P-value* <0.05 was considered significant.

Results

Of the 309 intratracheal samples taken from intubated patients in the intensive care unit, 163 patients (52.7%) were male, and 146 patients (47.3%) were female. The mean age of patients was 53.12±0.52.

The results of this study showed that 180 samples (58.2%) tested positive for tracheal culture, with 94 (52.2%) of those samples being female. *Klebsiella* (45.5%) and *Acinetobacter* (18.9%) species were the most frequent bacteria found in the tracheal tube. The most *Klebsiella*-infected patients were in the 55-64 years age group. There was no significant association

between age and bacterial infection (*P* = 0.4). Also, no significant association was observed between gender and bacterial infection (*P* = 0.09). Frequency and Distribution of Microorganisms Isolated from Endotracheal Tube Samples and the distribution of microorganisms by gender and age was shown in Tables 1-3, respectively.

The highest antibiotic resistance in *K. pneumoniae* was

Table 1. Frequency and distribution of microorganisms isolated from endotracheal tube samples.

Name of microorganism	Number	Frequency (%)
<i>Klebsiella pneumoniae</i>	82	45.5
<i>Acinetobacter spp.</i>	34	18.8
<i>Staphylococcus aureus</i>	25	13.8
<i>Escherichia coli</i>	16	8.8
<i>Streptococcus group D</i>	9	5.1
<i>Pseudomonas aeruginosa</i>	6	3.34
<i>Candida albicans</i>	6	3.34
<i>Citrobacter freundii</i>	2	1.03

Table 2. Distribution of microorganisms by gender.

Name of microorganism	Female	Male
<i>Klebsiella pneumoniae</i>	47	35
<i>Acinetobacter spp.</i>	20	14
<i>Staphylococcus aureus</i>	7	18
<i>Escherichia coli</i>	8	8
<i>Streptococcus group D</i>	4	5
<i>Pseudomonas aeruginosa</i>	5	1
<i>Candida albicans</i>	3	3
<i>Citrobacter freundii</i>	0	2

observed against cefixime (97.5%); cefotaxime and ciprofloxacin (94.9% each); ceftriaxone, ceftazidime, and ceftazidime (92.23% each).

Acinetobacter isolates showed 100% resistance to gentamicin, amikacin, imipenem, ciprofloxacin, ceftriaxone, ceftazidime, ceftizoxime, cefotaxime, and cefixime.

S. aureus exhibited 100% resistance to imipenem, ceftazidime, and cefotaxime, as well as 91.7% resistance to cefixime and 83.4% resistance to ceftriaxone and ceftizoxime. *E. coli* showed the highest levels of resistance to ceftazidime and cefixime (100% each), followed by ceftizoxime (85.8%).

Table 3. Distribution of microorganisms by age groups (year).

Bacteria types	<i>K. pneumoniae</i>	<i>Acinetobacter spp.</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>Streptococcus group D</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>C. freundii</i>
Age Order (year)								
25-34	0	0	0	1	0	0	0	0
35-44	10	6	8	7	2	1	1	1
45-54	18	14	10	3	3	2	4	0
55-64	38	12	7	4	3	3	1	1
65-74	14	2	0	1	1	0	0	0
75-84	1	0	0	0	0	0	0	0
85-94	1	0	0	0	0	0	0	0
Total	82	34	25	16	9	6	2	2

Table 4. Antibiotic resistance patterns of the isolated bacteria.

Antibiotic*	<i>K. pneumoniae</i>		<i>Acinetobacter spp.</i>		<i>S. aureus</i>		<i>E. coli</i>		<i>Streptococcus group D</i>		<i>P. aeruginosa</i>		<i>C. freundii</i>	
%	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Gentamicin	23	77	0	100	41.6	58.4	71.4	28.6	0	100	50	50	0	100
Amikacin	23	77	0	100	41.6	58.4	71.4	28.6	0	100	0	100	8.47	91.53
Imipenem	12.8	87.2	0	100	0	100	28.5	71.5	0	100	0	100	0	100
Ceftriaxone	7.7	92.23	0	100	16.6	83.4	28.5	71.5	33.33	66.67	0	100	0	100
Ceftizoxime	7.7	92.23	0	100	0	100	0	100	33.33	66.67	50	50	0	100
Cefotaxime	7.7	92.23	0	100	16.6	83.4	14.2	85.8	33.33	66.67	0	100	0	100
Ceftazidime	5.1	94.9	0	100	0	100	28.5	71.5	33.33	66.67	0	100	0	100
Cefixime	2.5	97.5	0	100	8.3	91.7	0	100	66.66	33.34	0	100	0	100
Ciprofloxacin	5.1	94.9	0	100	16.6	83.4	28.5	71.5	33.33	66.67	50	50	0	100

* The antimicrobial susceptibility panel represents the routine testing panel used by the hospital microbiology laboratory during the study period and does not necessarily correspond to current organism-specific CLSI recommendations.

Interestingly, *Citrobacter* isolates were resistant to nearly all tested antibiotics. The detailed antibiotic resistance patterns of the other isolated bacteria are presented in Table 4.

Discussion

Treatment and early diagnosis of nosocomial infections depend on several key factors, including the types of microorganisms, trends in antibiotic resistance, and the appropriate use of antibiotics to reduce resistance¹⁶. VAP is still the most common cause of death for patients with nosocomial infections, despite the implementation of comprehensive strategies to lower its incidence¹⁷. VAP can develop due to several factors, and its overall prevalence in the intensive care unit ranges from 10 to 70%. One of the primary factors that raises the risk of VAP, which can result in nosocomial pneumonia and micro aspiration of oropharyngeal bacterial colonization, is

intubation¹⁸. However, among patients admitted to the intensive care unit, the length of time spent on mechanical ventilation is linked to a higher risk of ventilator-associated pneumonia¹⁹.

In the present study, 309 endotracheal tube cultures were collected from intubated patients admitted to the ICU, of which 52.7% were male (163 patients) and 47.3% were female (146 patients). Differences in hormones, the distribution of infectious pathogens, the impact of gender-related gene polymorphisms on drug immune responses, and variations in complications between men and women could all be contributing factors to gender differences as a risk factor for VAP²⁰. In this study, out of a total of 309 cultured samples, 180 samples (58.2%) were positive for tracheal culture. In the study by Cairns et al. (2020) from New Zealand, 20 out of 24 endotracheal tube samples collected were reported to be culture positive in ICU patients²¹.

In the present study, the most common bacteria

contaminating the endotracheal tube were *Klebsiella pneumoniae* 82 (45.5%), followed by *Acinetobacter* spp. 34 (18.8%). According to Khatri et al. *Acinetobacter* species (n=25) and *Klebsiella* species (n=23) were the most commonly isolated organisms from 93 endotracheal tube samples in 2023, which is in line with the results of the current investigation²². In a similar study conducted by Rahimi et al. (2022, Iran), *Acinetobacter* was the most frequently isolated bacteria from day 1 and day 5 cultures²³.

Another study conducted in Iran by Ghafouri et al. indicated that the most frequent bacteria found in patients with hospital-acquired infections was *Escherichia coli*, which contradicts the current study's findings²⁴. One reason for the difference in the types of bacteria isolated may be variation in the type of sample.

In Khoshfetrat et al.'s study, *Acinetobacter* was the most frequently isolated Gram-negative bacteria from the endotracheal tube, which is in line with our study²⁵.

In the studies of Momen-Heravi et al.²⁶ and Andayani et al.²⁷, the most prevalent isolates were *Acinetobacter* spp. and *Klebsiella* spp. Those were isolated from the endotracheal tubes of patients hospitalized in the intensive care unit. Also, in a study by Ahmadi et al., *Acinetobacter* had the highest prevalence among Intensive Care Unit isolates. These study results are in line with our study²⁸.

In this study, there was no significant association between age ($P = 0.4$) and gender ($P = 0.09$) and bacterial infection, which is similar to Amini et al.'s study²⁹.

Due to gut colonization and exposure to antibiotics, critically ill patients in the intensive care unit who are intubated may have a higher risk of nosocomial infections. The oral flora of these patients eventually shifts to the hospital flora that predominates after exposure to hospital isolates in the intensive care unit. This oropharyngeal flora can enter the lower respiratory tract through pulmonary aspiration^{30,31}.

The pathogens linked to VAP are often described as potentially drug-resistant or multidrug-resistant bacteria because they are isolates that are extremely resistant to antibiotics. The majority of the isolated bacteria in this study had very high rates of antibiotic resistance (33.3-100%) and were resistant to most

antibiotics. Overall, *Citrobacter*, *Acinetobacter* spp., and *K. pneumoniae* exhibited the highest levels of antimicrobial resistance among the isolated pathogens. However, among the antibiotics tested, Gentamicin and Amikacin showed the lowest resistance rates in *K. pneumoniae* isolates.

Acinetobacter isolates demonstrated extensive multidrug resistance, consistent with reports from previous ICU-based studies. *Citrobacter* isolates also exhibited extensive antimicrobial resistance, while *K. pneumoniae* demonstrated high resistance rates to several antimicrobial agents, indicating the widespread presence of multidrug-resistant Gram-negative pathogens in the ICU.

The incidence of pneumonia or other infections caused by *Acinetobacter* is high. These observations are comparable to previous studies in the ICU³². According to one study, *Citrobacter* and *Klebsiella* species were the most frequently isolated, and VAP incidence was high. Although these two bacteria exhibited high susceptibility to colistin (94%), they were highly resistant to carbapenems, an antibiotic frequently used in the ICU³³. Salehifar et al. (2012) revealed that 30% of *Acinetobacter* spp. were resistant to all antimicrobial agents. Antibiotic resistance was highest in isolates to ceftazidime (22.2%) and imipenem and tobramycin (70% each). All isolated *Acinetobacters* were resistant to ciprofloxacin and ceftriaxone, which is in line with our study³⁴.

Acinetobacter species is naturally resistant to Chloramphenicol, Aminopenicillins, and first- and second-generation Cephalosporins; it can also become resistant to Tetracyclines, Aminoglycosides, broad-spectrum beta-lactams, and Fluoroquinolones³⁵.

Another study by Thakuria et al. reported a high prevalence of VAP caused by *Citrobacter* and *Klebsiella pneumoniae*, with high resistance to carbapenems and high susceptibility to polymyxin B and tigecycline³³. Direct comparison between their findings and ours should be interpreted with caution because antimicrobial susceptibility testing panels differed between the two studies, and our retrospective analysis was based on the routine antibiotic panel used in the hospital microbiology laboratory rather than a standardized organism-specific research panel.

The antimicrobial susceptibility results for *P. aeruginosa* reflected the routine testing panel used in

our microbiology laboratory during the study period and should be interpreted within this context. According to Ahsan et al. *Acinetobacter*, *Klebsiella*, and *Pseudomonas* also exhibited high levels of resistance (>80%) to GNB in contrast to Fluoroquinolones and third-generation cephalosporins. The resistance rates of *Pseudomonas* to Piperacillin-Tazobactam were lower than those of *Klebsiella* and *Acinetobacter*, but they were higher for aminoglycosides (>68%) and imipenem (>60%)³⁶. Although the common bacterial types and antibiotic resistance vary greatly across studies, all demonstrate that Gram-negative bacteria are the most troublesome cause of VAP³⁷. These findings emphasize the importance of continuous local surveillance of pathogen distribution and antimicrobial resistance to support infection control measures and antimicrobial stewardship programs.

The resistance patterns observed in the present study are consistent with the increasing global burden of multidrug-resistant Gram-negative pathogens in intensive care units. Recent surveillance data and reviews have highlighted the growing prevalence of carbapenem-resistant *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, particularly among mechanically ventilated patients and those with ventilator-associated pneumonia. These pathogens are increasingly associated with limited therapeutic options and poor clinical outcomes^{38, 39}.

The resistance patterns observed in this study provide useful information regarding the local epidemiology of bacterial isolates recovered from endotracheal tubes in our ICU. However, because antimicrobial susceptibility testing was based on the routine laboratory panel used during the study period, these findings should be interpreted with caution and should not be considered direct evidence for empirical therapeutic recommendations. Future prospective studies using organism-specific antimicrobial panels in accordance with current CLSI are warranted to better inform antimicrobial treatment strategies.

The limitations of the present study include the lack of use of molecular methods to detect genes responsible for antibiotic resistance and the lack of investigation of the relationship between pathogens isolated inside ET tubes and microbiological culture tests from lower

respiratory tract samples, such as endotracheal tube aspirates or bronchoalveolar lavage of individual patients, which could have clarified the pathogenic role of the isolated microorganisms. The absence of evaluation of fungal infections and the medications used to treat them, as well as the bacterial sensitivity to other antibiotics suggested in recent publications and guidelines, were further limitations of the current study. An additional limitation of this study is that antimicrobial susceptibility testing was performed using the routine antibiotic panel adopted by the hospital microbiology laboratory during the study period. Consequently, some antimicrobial agents were not included in accordance with current organism-specific CLSI recommendations. Therefore, susceptibility results should be interpreted cautiously and primarily reflect local laboratory surveillance rather than serving as recommendations for antimicrobial therapy.

Conclusion

The present study demonstrated that *Klebsiella pneumoniae* and *Acinetobacter* spp. were the predominant bacterial isolates recovered from endotracheal tubes of ICU patients, with bacterial colonization identified in 58.2% of the collected samples. The high prevalence of multidrug-resistant Gram-negative pathogens highlights the importance of continuous local microbiological surveillance and regular monitoring of antimicrobial resistance patterns in intensive care units. These findings provide valuable epidemiological information to support infection prevention strategies and antimicrobial stewardship programs. Because antimicrobial susceptibility testing was based on the routine laboratory panel used during the study period, the susceptibility results should be interpreted with caution and not considered direct evidence for therapeutic recommendations. Future prospective studies using standardized organism-specific antimicrobial susceptibility testing panels are warranted to further optimize antimicrobial management in critically ill patients.

Acknowledgment

We would like to express our sincere appreciation to the esteemed laboratory staff, ICU staff, and physicians of Imam Sajjad Hospital of Tabriz, and to the Research

Deputy of Tabriz Islamic Azad University of Medical Sciences, for their valuable assistance and support throughout this study.

Conflict of Interest

The authors declare no conflicts of interest related to this study.

Funding

None.

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