Jundishapur J Microbiol. 2023 May; 16(5):e133257. https://doi.org/10.5812/jjm-133257.

Published online 2023 July 29. **Research Article**

Comparison of Respiratory Microbiota in Patients with and Without Hospital-Acquired Infection

Farzad Mohammadi Ebli [1], Zoheir Heshmatipour [2,*], Khadijeh Daneshjou [3] and Seyed Davar Siadat [4]

[1]Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2]Department of Microbiology, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran
[3]Department of Pediatrics, Imam Khomeini Hospital, School of Medicine, Tehran University of Medical Science, Tehran, Iran
[4]Department of Mycobacteriology and Pulmonary Research, Microbiology Research Center, Pasteur Institute of Iran, Tehran, Iran

[*]*Corresponding author*: Department of Microbiology, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran. Email: z.heshmatipour2020@gmail.com

Received 2022 November 16; **Revised** 2023 July 08; **Accepted** 2023 July 11.

Abstract

Background: Nosocomial infections have increased among patients admitted to the intensive care unit (ICU).
Objectives: This study investigated the microbiota pattern of the respiratory system in hospitalized patients with treatment-resistant respiratory infections compared to those without treatment-resistant respiratory infections.
Methods: This case-control study utilized sputum samples from hospital-acquired infection (HAI) and non-HAI (NHAI) patients over 52 years old hospitalized in the ICU. Identification and determination of the drug sensitivity of the bacteria responsible for treatment-resistant respiratory infections were made by culture method in selective and differential media and VITEK 2 device. Finally, quantitative polymerase chain reaction (qPCR) was used to analyze the microbiota of the respiratory system.
Results: Excessive prescription of antibiotics, long hospitalization, and history of surgery were important risk factors for nosocomial infections. The study of antibiotic resistance of pathogens causing hospital infections indicated their high resistance to most common antibiotics. Also, nosocomial infections led to a change in lung microbiota in HAI patients. The frequencies of *Streptococcus pyogenes*, *S. pneumoniae*, and *Haemophilus influenzae* were higher in patients with treatment-resistant respiratory infection ($P < 0.05$), but the frequency of *Neisseria* spp. was higher in patients without treatment-resistant respiratory infection ($P < 0.05$).
Conclusions: The pathogens responsible for nosocomial infections had acquired resistance to a wide range of antibiotics, leading to changes in their respiratory microbiota.

Keywords: Respiratory Microbiota, *Streptococcus*, Pathogen, Intensive Care Unit

1. Background

Nosocomial infections or hospital-acquired infections (HAIs) are one of the major problems in hospital environments, especially intensive care units (ICU), and cause increased morbidity and mortality (1). There has been resistance to antimicrobial agents in many types of HAI, which causes problems in treatment, augmenting morbidity and mortality (2, 3). The widespread use of antibiotics and drugs that inhibit the immune system has increased the number of vulnerable people to these infections (4). On the other hand, the transmission of antibiotic resistance among pathogenic agents has worsened HAI (5). In the world, more than two million hospital infections are reported annually, and the high costs of treating these infections reach 17 to 29 billion dollars (6). The prevalence of HAI in Iran has also been reported at 10.85%, with the most common site of infection being the lung and the most common microbial agent being *Acinetobacter* (7). Resistant nosocomial infections are one of the most common problems in human societies that not only are a major health challenge but also lead to the waste of many economic resources. Thus, there is a need to provide effective therapeutic approaches to combat HAI (8).

The human microbiota is a tremendously complex community of microorganisms, including bacteria, fungi, and viruses, that reside in various body organs, especially in the mucosal and epidermal layers of the skin, mouth, lungs, vagina, and intestine (9). It has been proven that the normal profiling of this microbial population plays a critical role in maintaining human health, and

its imbalance is associated with various diseases such as diabetes, obesity, allergies, and cancers (10). Based on reports, the improper and excessive administration of broad-spectrum antibiotics to hospitalized patients can disrupt the balance of their organs' microbiota, resulting in various drug-resistant nosocomial infections, including refractory type (11). Lungs are one of the most important organs for the residence of microbiota. Due to their vital function of exchanging respiratory gases, they are frequently exposed to different pathogens (11).

Nowadays, developed countries are trying to identify better the change patterns of lung microbiota in patients with nosocomial infections that are resistant to treatment to find a suitable therapeutic strategy for promoting their healing process (11). Growing evidence from recent studies in countries such as Germany, China, and the United Kingdom suggests that altering lung microbiota profiling and its function increases the risk of respiratory bacterial infections (12, 13). However, it should be noted that the pattern of normal microbiota is unique in different populations and ages (14).

2. Objectives

The present study investigates the dominant bacterial species in the respiratory microbiota of patients with drug-resistant nosocomial respiratory infections to provide a beneficial treatment method and address the related medical gaps.

3. Methods

3.1. Study Design and Sampling

This case-control study was conducted on patients with respiratory diseases hospitalized in the ICU-general of Pars hospital, Tehran, Iran, from April 2018 to September 2019. Patients $\geq$ 52 years old were divided into HAI (n = 50) and non-HAI (NHAI) (n = 50) groups. The HAI patients were identified based on microbiological records and confirmation from doctors and nurses. The NHAI group was selected from patients hospitalized in the ICU who had no hospital infection, confirmed by doctors and nurses. The sputum samples were quickly transferred to the bacteriology laboratory, and Gram staining and culture in selective media, including blood agar, chocolate agar, and MacConkey agar, were performed.

3.2. Identification and Susceptibility Testing of Nosocomial Infection by VITEK 2 Compact

The VITEK 2 compact device (Biomerieix, France) was used to identify nosocomial infection-causing isolates using special cards (GN, GP, AST-GP-75, and GN-76 cards) to detect different bacterial groups and determine their antibiogram patterns. The AST card and broth microdilution (MB) methods based on the CLSI protocols were used to identify the sensitivity of strains to antibiotics (15). Also, the latter approach (MB) was used to determine the MIC (15). For this purpose, bacteria (10^5 CFU/mL) were cultured in Muller-Hinton broth. It is worth mentioning that *S. pneumonia* ATCC 19615, *S. pyogenes* ATCC 49619, *H. influenza* ATCC 33930, *Neisseria* PTCC 1773, *Bacillus fragilis* ATCC 25285, and *Moraxella catarrhalis* ATCC 19976 were used as standard strains.

3.3. Molecular Analysis

3.3.1. DNA Extraction

The extraction of DNA was done using the DNA-TECHNOLOGY kit (Moscow, Russia) after sputum treatment with mycolysin buffer. All steps followed the manufacturer's instructions.

3.3.2. Quantitative Polymerase Chain Reaction

We used 50 μL DNA for quantitative polymerase chain reaction (qPCR) (Rotor-Gene 6000, QIAGEN Corbett, Hilden, Germany) using a QuantiTect SYBR® Green PCR kit. First, the primers related to the 16s rRNA gene of each bacterial group were synthesized (Table 1), and then the qPCR was performed using a kit (Yekta Tajhiz Azma, Tehran, Iran). We used Gene Runner software for designing the sequences of primers. Then, we blasted the sequences in the NCBI database, and the primers were synthesized after ensuring the sequences' accuracy. The thermo-time program of the qPCR consisted of one cycle of 95°C for 60 s, 40 cycles of 95°C for 5 s, 55°C and 72°C each for 30 s, and a final cycle of 95°C for 5 s, 60°C and 95°C for 60 s and 1 s, respectively. Then, a thermal melting analysis was conducted. Gene expression analysis was done using the threshold cycle values (Ct) method.

3.4. Statistical Analysis

Qualitative variables were analyzed using Pearson's chi-square test, and quantitative variables were analyzed using one-way analysis of variance (ANOVA) after determining their normal distribution. An independent *t*-test was used to compare the means between HAI and NHAI groups. Data analysis was done by SPSS v.22 software.

4. Results

4.1. Patients Characteristics

The mean age of patients was 69.74 in the HAI group and 71.12 in the NHAI group, with no significant difference.

Table 1. The Primers Used for Amplification of 16s rRNA Genes

Bacteria	Primers
Streptococcus pneumonia	
Forward	5'-GGCATTGTGAATGGATTGATTG-3'
Reverse	5'-TCATGTGCATCCCAAGCTACA-3'
S. pyogenes	
Forward	5'-AAAGACCGCCTTAACCACCT-3'
Reverse	5'-TGGCAAGGTAAACTTCTAAAGCA-3'
Moraxella catarrhalis	
Forward	5'-CGTGTTGACCGTTTTGACTTT-3'
Reverse	5'-CATAGATTAGGTTACCGCTGACG-3'
B. fragilis	
Forward	5'-TGATTCCGCATGGTTTCATT-3'
Reverse	5'-CGACCCATAGACCCTTCATC-3'
Haemophilus influenza	
Forward	5'-CCAGCTGCTAAAGTATTAGTAGAAG-3'
Reverse	5'-TTCACCGTAAGATACTGTGCC-3'
Neisseria	
Forward	5'-CAACTATTCCCGATTGCG-3'
Reverse	5'-GTTATACAGCTTCGCCTGAA-3'

Also, regarding gender, no significant difference was observed between the two groups ($P > 0.05$). Nevertheless, regarding laboratory data such as IQR, CRP, ESR, and platelet, significant differences were observed between the two groups ($P = 0.001$), and they all were higher in the HAI group than in the NHAI group. Comorbidities in HAI and NHAI patients are listed in Table 2. The average hospitalization days, residence in nursing homes, and duration of antibiotic prescription were higher in HAI patients than in NHAI ones (Table 2).

4.2. Identification of Nosocomial Infection Isolates

The genus and species of bacteria were identified in sputum samples by Gram staining (Figure 1), cultivation in differential medium, and VITEK 2 compact device. The results indicated 50 strains of *Acinetobacter baumannii*, *Staphylococcus aureus*, *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Escherichia coli*. The results showed that in patients with a respiratory infection resistant to treatment, *A. baumannii* (24%) and *E. faecium* (22%) played the largest role, and *K. pneumonia* played the least role (10%).

4.3. Antimicrobial Susceptibility Testing

Acinetobacter baumannii showed resistance to the antibiotics such as piperacillin/tazobactam, ceftazidime, ceftriaxone, cefepime, imipenem, amikacin, gentamicin, ciprofloxacin, levofloxacin, trimethoprim/sulfamethoxazole, and meropenem. Therefore, it could be said that *A. baumannii* was pan-drug-resistant (PDR). However, colistin led to an 83% inhibition of this bacterium (Table 3). The drug sensitivity pattern of methicillin-resistant *S. aureus* (MRSA) was studied in the current research, and the results indicated the complete sensitivity of this bacterium to gentamicin, linezolid, and vancomycin antibiotics. However, it showed complete resistance to other antibiotics, such as cefoxitin and oxacillin. Also, MRSA showed 83% sensitivity to doxycycline and tetracycline antibiotics (Table 3).

Enterococcus faecium showed a multidrug resistance pattern to all antibiotics used in the current study, including ampicillin, ciprofloxacin, levofloxacin, erythromycin, linezolid, vancomycin, doxycycline, tetracycline, and nitrofurantoin (Table 3). *Pseudomonas aeruginosa* showed 100% and 57% sensitivity to colistin and amikacin, respectively. However, *P. aeruginosa* had resistance to other antibiotics such as piperacillin/tazobactam, ceftazidime, cefepime, imipenem, gentamicin, ciprofloxacin, levofloxacin, meropenem, and tobramycin. *Klebsiella pneumonia* was identified as a PDR species, as it showed resistance to all antibiotics, including ampicillin, cefazolin, piperacillin/tazobactam, ceftazidime, ceftriaxone, cefepime, imipenem, amikacin, gentamicin, ciprofloxacin, and levofloxacin. Also, *E. coli* was completely resistant to ampicillin, cefazolin, piperacillin/tazobactam, ceftazidime, ceftriaxone, and cefepime. However, it showed 100% sensitivity to imipenem, amikacin, trimethoprim/sulfamethoxazole, and cefixime (Table 3).

4.4. Quantitative Polymerase Chain Reaction

The respiratory microbiota in the HAI and NHAI groups was evaluated by the qPCR technique, and the results were expressed as copies per microliter of total microbial DNA. As can be seen, the frequency of S. pneumoniae, S. pyogenes, and H. influenzae was higher in HAI patients than in NHAI ones ($P = 0.001$). However, Neisseria spp. frequency was higher in NHAI than in HAI patients ($P = 0.001$, Figure 2). There was no significant difference between the two groups regarding Moraxella and Bacteroides.

5. Discussion

Establishing ICUs in hospitals has significantly reduced patient mortality. However, prolonged

Table 2. Clinical Characteristics of Hospitalized Patients with Hospital-Acquired Infection and Non-Hospital-Acquired Infection

Variables	HAI (n = 50)	NHAI (n = 50)	P-Value
Demographic data			
Age	69.74 (52 - 91)	71.12 (52 - 95)	0.553
Male	34 (68)	30 (60)	0.653
Female	16 (32)	20 (40)	0.427
Laboratory data			
IQR	55.35 (30.1 - 90.2)	5.06 (3 - 9.2)	0.001
CRP	33.72 (24.2 - 46.9)	3.74 (1 - 7.5)	0.001
ESR	32.24 (25 - 40)	14.02 (10 - 20)	0.001
Platelets	269.28 (189 - 321)	225.04 (126 - 389)	0.001
CBC	11106	9475	0.018
U/C (%)			
Negative	65	70	
Escherichia coli	15	5	
CoNS		25	
Acinetobacter	5		
Pseudomonas	5		
Klebsiella	5		
Diseases			
ARDS	2 (4)	0 (-)	-
Asthma	4 (8)	0 (-)	-
Bronchitis	4 (8)	2 (4)	0.812
Cerebrovascular	0 (-)	8 (16)	-
CF	6 (12)	3 (6)	0.037
COPD	6 (12)	2 (4)	0.001
Dementia	0 (-)	4 (8)	-
Embolism	2 (4)	7 (14)	0.001
Femoral fracture	2 (4)	6 (12)	0.001
Gastric cancer	3 (6)	0 (-)	-
Heart failure	0 (-)	6 (12)	-
Hypertension	2 (4)	6 (12)	0.003
Influenzae	5 (10)	0 (-)	-
Pleurisy	1 (2)	0 (-)	-
Pneumoniae	5 (10)	2 (4)	0.021
Rectal cancer	3 (6)	2 (4)	0.754
Sepsis	1 (2)	0 (-)	-
Tuberculosis	4 (8)	2 (4)	0.135
Risk factors			
Days of hospitalization, median	29 (16 - 41)	12 (7 - 23)	< 0.001
Previous surgery within 3 months, No. (%)	10 (35.71)	10 (17.85)	< 0.001
Residence in the nursing home, No. (%)	11 (21.15)	4 (8.16)	0.005
Duration of antibiotic exposure	11 (6 - 18)	5 (4 - 9)	0.000

Abbreviations: HAI, hospital-acquired infection, NHAI, non-HAI, CoNS, coagulase-negative *staphylococci*.

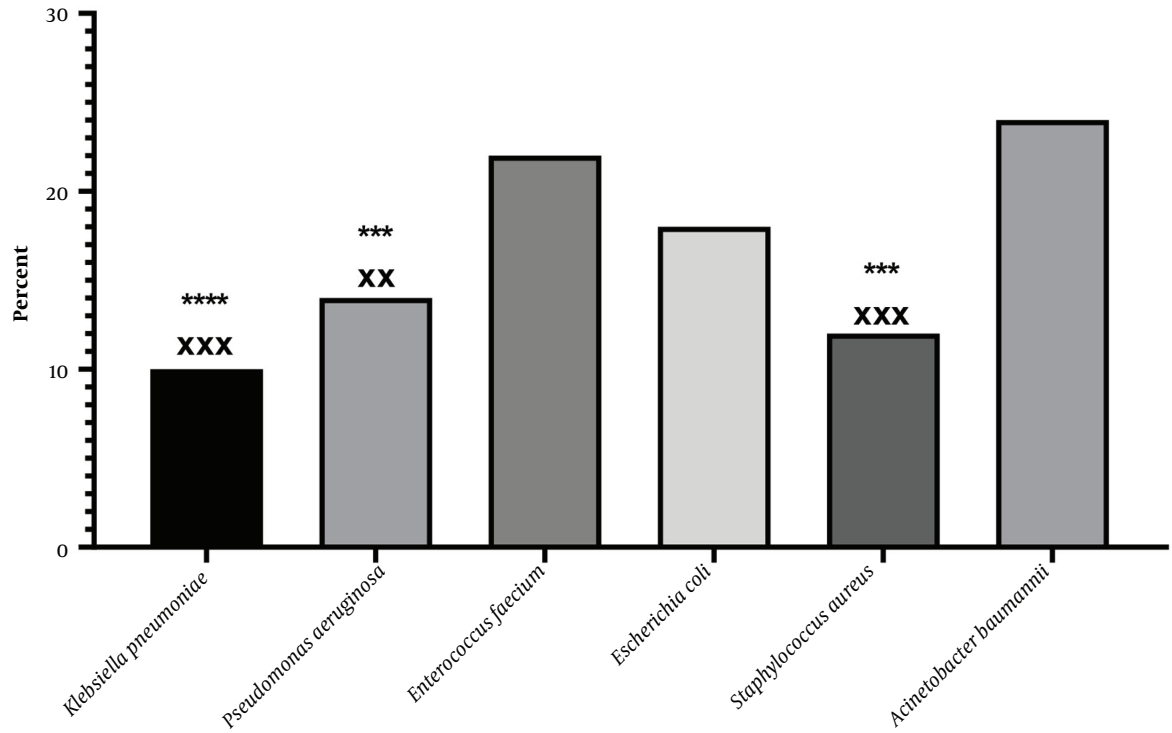

Figure 1. The frequency of nosocomial infectious bacteria from clinical samples. Compared to *Acinetobacter baumannii*: ***: P < 0.001, ****: P < 0.0001; compared to *Enterococcus faecium*: xx: P < 0.01, xxx: P < 0.001.

hospitalization and the use of various invasive monitoring devices and vascular catheters have caused the emergence of nosocomial infections in these departments, which can ultimately lead to the failure of other organs (16). These infections appear after 72 hours of patient hospitalization, increasing mortality and treatment costs (17). *Pseudomonas*, *Staphylococcus*, *Candida*, enterococci, and *Enterobacter* species are among the most important causes of nosocomial infections (18). Factors predisposing to nosocomial infections include the severity of the disease, age, immune system defects, excessive prescription of antibiotics, and the development of antibiotic-resistant microorganisms (16).

The patient's age is an important factor in getting nosocomial infections, so these infections are 10 times more prevalent in elderly patients (19). Nowadays, developed countries are trying to distingue changes in the pattern of lung microbiota in patients with nosocomial infections that are resistant to treatment so that they can help the healing process by recognizing changes in the microbial flora of the lungs (20). Therefore, the current study investigated the microbial flora in ICU patients with nosocomial infections and compared it with non-HAI patients. While identifying bacteria, the resistance pattern of bacteria to antibiotics was studied, and their number was compared between the HAI and non-HAI populations.

In this study, 50 strains of the pathogens *A. baumannii*, *S. aureus*, *E. faecium*, *P. aeruginosa*, and *K. pneumonia* were identified, and the results of the antibiogram study indicated that most strains were resistant to common antibiotics. Also, the results showed that long hospitalization, history of surgery, residence in nursing homes, and exposure to various antibiotics were the risk factors for nosocomial infections. One of the interesting findings of the current research was the lack of resistance of the identified strains to the antibiotics colistin, aminoglycoside, linezolid, and vancomycin. The highest rate of antibiotic resistance was observed in cephalosporin antibiotics. The low sensitivity of strains to this class of antibiotics can be attributed to their high prescription in the treatment of various infections (21), which has led to the emergence of resistance in this class of pathogens over time. The causative pathogens of nosocomial infections have the potential to transfer

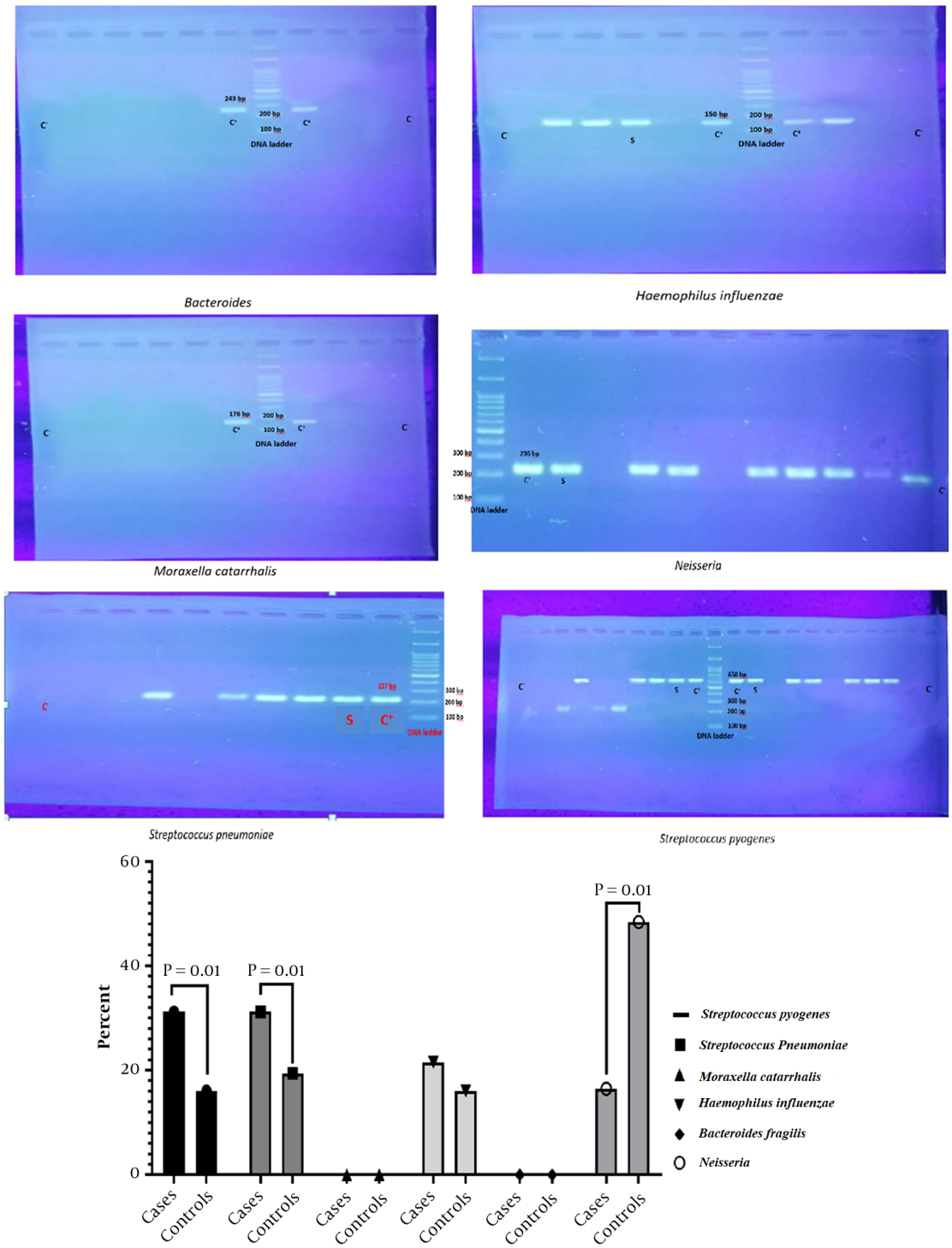

Figure 2. The quantitative polymerase chain reaction (qPCR) bands obtained from lung microbiota (above) and the frequency of lung microbiota from PCR samples (below)

from one region to another and spread epidemically or pandemically. Therefore, genotyping, identifying, and evaluating their drug resistance patterns are very important (22).

In our study, all nosocomial infection species showed a PDR pattern, indicating the necessity of new treatments to deal with this category of infections. Also, *S. pneumoniae*, *S. pyogenes*, and *H. influenzae* were higher in HAI patients than in non-HAI ones. However, *Neisseria* spp. frequency was higher in non-HAI than in HAI patients. The risk factors for HAI were prolonged hospital stay, previous surgery, residence in a nursing home, and exposure to a wide range of antibiotics. Lung microbiota is affected by antibiotics, and the effect remains 6 to 24 months after treatment (23).

In our study, the reduction in the population of *Neisseria* spp. and an increase in the populations of *S. pneumonia*, *S. pyogenes*, and *H. influenzae* were seen in the HAI group compared to the non-HAI group, indicating a change in the lung microbiota. This is in line with other studies that reported that the repeated administration of antibiotics led to changes in the lung microbiota of mice (24, 25). Other bacterial species in the lung microbiota may also change because competition for the ecological niche is created in this situation, and other bacterial species may decrease in population. The limitations of the current study include the small sample size and the clinical complexity of the patients, which could affect the results. In this field, large-scale clinical studies are needed.

5.1. Conclusions

In general, it is concluded that antibiotic-resistant strains of *A. baumannii* and *E. faecium* played an important role in respiratory infections in the patients. Nevertheless, all 6 species identified in the sputum samples of patients with respiratory infections showed the MDR pattern of antibiotic resistance, which necessitates new therapeutic approaches to deal with them. Genetic studies indicated the difference between the microbiota of the HAI group and NHAI, so that *S. pneumoniae*, *S. pyogenes*, and *H. influenzae* were higher in HAI patients than in NHAI ones.

Footnotes

Authors' Contribution: Farzad Mohammadi Ebli and Zoheir Heshmatipour: Analysis and interpretation of data; Farzad Mohammadi Ebli: Drafting of the manuscript; Khadijeh Daneshjou: Critical revision of the manuscript for important intellectual content; Seyed Davar Siadat: Statistical analysis.

Conflict of Interests: This study was derived from the doctoral thesis by Farzad Mohammadi Ebli, who provided all costs. There is no conflict of interest among the authors.

Ethical Approval: The Helsinki Declaration principles were fully observed during the execution of the experiments. The ethics committee of Islamic Azad University, Science and Research Branch, approved the research.

Funding/Support: The authors received no funds from any organization.

Informed Consent: Informed consent was obtained from human subjects.

References

1. Trubiano JA, Padiglione AA. Nosocomial infections in the intensive care unit. *Anaesth Intensive Care Med*. 2015;**16**(12):598–602. https://doi.org/10.1016/j.mpaic.2015.09.010.
2. Remschmidt C, Schroder C, Behnke M, Gastmeier P, Geffers C, Kramer TS. Continuous increase of vancomycin resistance in enterococci causing nosocomial infections in Germany - 10 years of surveillance. *Antimicrob Resist Infect Control*. 2018;**7**:54. [PubMed ID: 29760912]. [PubMed Central ID: PMC5937822]. https://doi.org/10.1186/s13756-018-0353-x.
3. Ayoub Moubareck C, Hammoudi Halat D. Insights into Acinetobacter baumannii: A review of microbiological, virulence, and resistance traits in a threatening nosocomial pathogen. *Antibiotics (Basel)*. 2020;**9**(3):119. [PubMed ID: 32178356]. [PubMed Central ID: PMC7148516]. https://doi.org/10.3390/antibiotics9030119.
4. Furnkranz U, Walochnik J. Nosocomial infections: Do not forget the parasites!. *Pathogens*. 2021;**10**(2):238. [PubMed ID: 33669761]. [PubMed Central ID: PMC7923136]. https://doi.org/10.3390/pathogens10020238.
5. Suleyman G, Alangaden G, Bardossy AC. The role of environmental contamination in the transmission of nosocomial pathogens and healthcare-associated infections. *Curr Infect Dis Rep*. 2018;**20**(6):12. [PubMed ID: 29704133]. https://doi.org/10.1007/s11908-018-0620-2.
6. Brunner LS, Smeltzer SCO, Suddarth DS. *Brunner & Suddarth's textbook of medical-surgical nursing*. Philadelphia, PA: Lippincott Williams & Wilkins; 2010.
7. Ghazvini K, Rashed T, Boskabadi H, Yazdan Panah M, Khakzadan F, Safaee H, et al. [Neonatal intensive care unit nosocomial bacterial infections]. *Tehran Univ Med J*. 2008;**66**(5):349–54. Persian.
8. Pettigrew MM, Tanner W, Harris AD. The lung microbiome and pneumonia. *J Infect Dis*. 2021;**223**(12 Suppl 2):S241–5. [PubMed ID: 33330898]. https://doi.org/10.1093/infdis/jiaa702.
9. Fromentin M, Ricard JD, Roux D. Respiratory microbiome in mechanically ventilated patients: A narrative review. *Intensive Care Med*. 2021;**47**(3):292–306. [PubMed ID: 33559707]. [PubMed Central ID: PMC7871139]. https://doi.org/10.1007/s00134-020-06338-2.
10. Pathak JL, Yan Y, Zhang Q, Wang L, Ge L. The role of oral microbiome in respiratory health and diseases. *Respir Med*. 2021;**185**:106475. [PubMed ID: 34049183]. https://doi.org/10.1016/j.rmed.2021.106475.
11. Thibeault C, Suttorp N, Opitz B. The microbiota in pneumonia: From protection to predisposition. *Sci Transl Med*. 2021;**13**(576):eaba0501. [PubMed ID: 33441423]. https://doi.org/10.1126/scitranslmed.aba0501.
12. Kim S, Rigatto K, Gazzana MB, Knorst MM, Richards EM, Pepine CJ, et al. Altered gut microbiome profile in patients with pulmonary arterial hypertension. *Hypertension*. 2020;**75**(4):1063–71. [PubMed ID: 32088998]. [PubMed Central ID: PMC7067661]. https://doi.org/10.1161/HYPERTENSIONAHA.119.14294.
13. Zhang C, Zhang T, Lu W, Duan X, Luo X, Liu S, et al. Altered airway microbiota composition in patients with pulmonary hypertension. *Hypertension*. 2020;**76**(5):1589–99. [PubMed ID: 32921193]. https://doi.org/10.1161/HYPERTENSIONAHA.120.15025.

14. Zhang W, Li J, Lu S, Han N, Miao J, Zhang T, et al. Gut microbiota community characteristics and disease-related microorganism pattern in a population of healthy Chinese people. *Sci Rep*. 2019;**9**(1):1594. [PubMed ID: 30733472]. [PubMed Central ID: PMC6367356]. https://doi.org/10.1038/s41598-018-36318-y.
15. Kassim A, Omuse G, Premji Z, Revathi G. Comparison of Clinical Laboratory Standards Institute and European Committee on antimicrobial susceptibility testing guidelines for the interpretation of antibiotic susceptibility at a university teaching hospital in Nairobi, Kenya: A cross-sectional study. *Ann Clin Microbiol Antimicrob*. 2016;**15**:21. [PubMed ID: 27068515]. [PubMed Central ID: PMC4827198]. https://doi.org/10.1186/s12941-016-0135-3.
16. Despotovic A, Milosevic B, Milosevic I, Mitrovic N, Cirkovic A, Jovanovic S, et al. Hospital-acquired infections in the adult intensive care unit-Epidemiology, antimicrobial resistance patterns, and risk factors for acquisition and mortality. *Am J Infect Control*. 2020;**48**(10):1211–5. [PubMed ID: 32093978]. https://doi.org/10.1016/j.ajic.2020.01.009.
17. Ture Z, Ustuner T, Santini A, Aydogan S, Celik I. A comparison of nosocomial infection density in intensive care units on relocating to a new hospital. *J Crit Care Med (Targu Mures)*. 2020;**6**(3):175–80. [PubMed ID: 32864463]. [PubMed Central ID: PMC7430358]. https://doi.org/10.2478/jccm-2020-0028.
18. Kollef MH, Torres A, Shorr AF, Martin-Loeches I, Micek ST. Nosocomial infection. *Crit Care Med*. 2021;**49**(2):169–87. [PubMed ID: 33438970]. https://doi.org/10.1097/CCM.0000000000004783.
19. Li Y, Ren L, Zou J. Risk factors and prevention strategies of nosocomial infection in geriatric patients. *Can J Infect Dis Med Microbiol*. 2019;**2019**:6417959. [PubMed ID: 30931076]. [PubMed Central ID: PMC6410437]. https://doi.org/10.1155/2019/6417959.
20. Roquilly A, Torres A, Villadangos JA, Netea MG, Dickson R, Becher B, et al. Pathophysiological role of respiratory dysbiosis in hospital-acquired pneumonia. *Lancet Respir Med*. 2019;**7**(8):710–20. [PubMed ID: 31182406]. https://doi.org/10.1016/S2213-2600(19)30140-7.
21. Sibley CD, Parkins MD, Rabin HR, Duan K, Norgaard JC, Surette MG. A polymicrobial perspective of pulmonary infections exposes an enigmatic pathogen in cystic fibrosis patients. *Proc Natl Acad Sci U S A*. 2008;**105**(39):15070–5. [PubMed ID: 18812504]. [PubMed Central ID: PMC2567494]. https://doi.org/10.1073/pnas.0804326105.
22. Servick K. Of mice and microbes: The zoo of bacteria and viruses each lab animal harbors may confound experiments. *Science*. 2016;**353**(6301):741–3.
23. LeMessurier KS, Iverson AR, Chang TC, Palipane M, Vogel P, Rosch JW, et al. Allergic inflammation alters the lung microbiome and hinders synergistic co-infection with H1N1 influenza virus and Streptococcus pneumoniae in C57BL/6 mice. *Sci Rep*. 2019;**9**(1):19360. [PubMed ID: 31852944]. [PubMed Central ID: PMC6920369]. https://doi.org/10.1038/s41598-019-55712-8.
24. Dinwiddie R. Pathogenesis of lung disease in cystic fibrosis. *Respiration*. 2000;**67**(1):3–8. [PubMed ID: 10705255]. https://doi.org/10.1159/000029453.
25. Kennedy EA, King KY, Baldridge MT. Mouse microbiota models: Comparing Germ-free mice and antibiotics treatment as tools for modifying gut bacteria. *Front Physiol*. 2018;**9**:1534. [PubMed ID: 30429801]. [PubMed Central ID: PMC6220354]. https://doi.org/10.3389/fphys.2018.01534.

Table 3. Antimicrobial Susceptibility of 50 Hospital-Acquired Infection Isolates

Bacteria and Antibiotics	Disk Potency (μg)	Sensitivity No. (%)	Inhibition Zone	MIC	E-Test	R. Type
Acinetobacter baumannii						MDR
Piperacillin/tazobactam	10	0 (0)	≥ 21	16 ≥	-	
Ceftazidime	30	0 (0)	≥ 18	8 ≥	-	
Ceftriaxone	30	0 (0)	≥ 21	8 ≥	-	
Cefepime	30	0 (0)	≥ 18	8 ≥	-	
Imipenem	10	2 (16)	≥ 22	2 ≥	-	
Amikacin	30	2 (16)	≥ 17	16 ≥	-	
Gentamicin	10	0 (0)	≥ 15	4 ≥	-	
Ciprofloxacin	5	0 (0)	≥ 21	1 ≥	-	
Levofloxacin	5	0 (0)	≥ 17	2 ≥	-	
Trimethoprim/sulfamethoxazole	1.25	0 (0)	≥ 16	2 ≥	-	
Colistin	10	10 (83)	-	-	2 ≥	
Meropenem	10	2 (16)	≥ 18	-	2 ≥	
Staphylococcus aureus						MDR
Cefoxitin	30	0 (0)	≥ 22	4 ≥	-	
Oxacillin	30	0 (0)	-	2 ≥	-	
Gentamicin	10	6 (100)	≥ 15	4 ≥	-	
Ciprofloxacin	5	3 (50)	≥ 21	1 ≥	-	
Levofloxacin	5	3 (50)	≥ 19	1 ≥	-	
Moxifloxacin	5	2 (33)	≥ 24	0.5 ≥	-	
Erythromycin	15	3 (50)	≥ 23	2 ≥	-	
Clindamycin	2	3 (50)	≥ 21	0.5 ≥	-	
Linezolid	30	6 (100)	≥ 21	16 ≥	-	
Daptomycin	10	4 (66)	≥ 21	4 ≥	-	
Vancomycin	30	6 (100)	-	2 ≥	-	
Doxycycline	30	5 (83)	≥ 16	4 ≥	-	
Tetracycline	30	5 (83)	≥ 19	4 ≥	-	
Nitrofurantoin	300	3 (50)	≥ 17	32 ≥	-	
Rifampicin	5	4 (66)	≥ 20	1 ≥	-	
Trimethoprim/sulfamethoxazole	1.25	3 (50)	≥ 16	2.38 ≥	-	
Enterococcus faecium						MDR
Ampicillin	10	0 (0)	≥ 15	8 ≥	-	
Ciprofloxacin	5	0 (0)	≥ 21	1 ≥	-	
Levofloxacin	5	0 (0)	≥ 17	2 ≥	-	
Erythromycin	15	0 (0)	≥ 23	0.5 ≥	-	
Linezolid	30	11 (0)	≥ 23	2 ≥	-	
Vancomycin	30	0 (0)	≥ 17	4 ≥	-	
Doxycycline	30	0 (0)	≥ 16	4 ≥	-	
Tetracycline	30	0 (0)	≥ 19	4 ≥	-	
Nitrofurantoin	300	0 (0)	≥ 17	32 ≥	-	
Pseudomonas aeruginosa						MDR
Piperacillin/tazobactam	10	0 (0)	≥ 21	16 ≥	-	
Ceftazidime	30	0 (0)	≥ 18	8 ≥	-	
Cefepime	30	0 (0)	≥ 18	8 ≥	-	
Imipenem	10	2 (28)	≥ 19	2 ≥	-	
Amikacin	30	4 (57)	≥ 17	16 ≥	-	
Gentamicin	10	0 (0)	≥ 15	4 ≥	-	
Ciprofloxacin	5	0 (0)	≥ 25	0.5 ≥	-	

Levofloxacin	5	0 (0)	≥ 22	1 ≥	-	
Colistin	10	7 (100)	-	-	-	
Meropenem	10	2 (28)	≥ 19	-	-	
Tobramycin	10	0 (0)	≥ 15	4 ≥	-	
Klebsiella pneumoniae						MDR
Ampicillin	10	0 (0)	≥ 17	8 ≥	-	
Cefazolin	30	0 (0)	≥ 23	2 ≥	-	
Piperacillin/tazobactam	10	0 (0)	≥ 21	16 ≥	-	
Ceftazidime	30	0 (0)	≥ 21	4 ≥	-	
Ceftriaxone	30	0 (0)	≥ 23	1 ≥	-	
Cefepime	30	0 (0)	≥ 25	2 ≥	-	
Imipenem	10	0 (0)	≥ 23	1 ≥	-	
Amikacin	30	3 (60)	≥ 17	16 ≥	-	
Gentamicin	10	2 (40)	≥ 15	4 ≥	-	
Ciprofloxacin	5	0 (0)	≥ 26	0.25 ≥	-	
Levofloxacin	5	0 (0)	≥ 21	0.5 ≥	-	
Trimethoprim/sulfamethoxazole	1.25	0 (0)	≥ 16	2 ≥	-	
Meropenem	10	0 (0)	≥ 23	1 ≥	-	
Ertapenem	10	0 (0)	≥ 221	0.5 ≥	-	
Cefixime	5	0 (0)	≥ 19	1 ≥	-	
Escherichia coli						MDR
Ampicillin	10	0 (0)	≥ 17	8 ≥	-	
Cefazolin	30	0 (0)	≥ 23	2 ≥	-	
Piperacillin/tazobactam	10	0 (0)	≥ 21	16 ≥	-	
Ceftazidime	30	0 (0)	≥ 21	4 ≥	-	
Ceftriaxone	30	0 (0)	≥ 23	1 ≥	-	
Cefepime	30	0 (0)	≥ 25	2 ≥	-	
Imipenem	10	9 (100)	≥ 23	1 ≥	-	
Amikacin	30	9 (100)	≥ 17	16 ≥	-	
Gentamicin	10	7 (78)	≥ 15	4 ≥	-	
Ciprofloxacin	5	4 (45)	≥ 26	0.25 ≥	-	
Levofloxacin	5	4 (45)	≥ 21	0.5 ≥	-	
Trimethoprim/sulfamethoxazole	1.25	9 (100)	≥ 16	2 ≥	-	
Meropenem	10	8 (89)	≥ 23	1 ≥	-	
Ertapenem	10	7 (78)	≥ 221	0.5 ≥	-	
Cefixime	5	9 (100)	≥ 19	1 ≥	-	

Abbreviations: R. type, resistance type, MDR, multidrug resistance.