Prevalence of Common Nosocomial Infections and Evaluation of Antibiotic Resistance Patterns in Patients with Secondary Infections in Hamadan, Iran

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Introduction: The prevalence of nosocomial infections in patients hospitalized to three hospitals of Shahid Beheshti, Farshchian, and Be’ saat in Hamadan was investigated for 2 years (2018 to 2020).

Materials and Methods: The samples were cultured and characterized using morphological and diagnostic biochemical tests. The analysis of the frequency of the isolates and their antibiotic resistance were calculated using SPSS (version 22) at a significant level of P-value < 0.05.

Results: Bacterial isolates were collected from the 1194 clinical specimens, of which 1394 were isolated from urine, 16 from CSF, and 588 from tracheal aspiration. Also, 654 (54.8%) isolates were obtained from females and 540 (45.2%) from males with the age range 15–73 years (P> 0.05). The results showed that 22.1% were gram-positive and 77.9% were gram-negative. In our study, the frequency of Klebsiella pneumoniae bacteria was higher than in some studies, and this indicates the genetic changes and resistance of this bacterium to many antibiotics.

Conclusion: To prevent further spread of resistance, increase the effectiveness of antibiotics and prevent multidrug resistance, it is essential to establish a precise schedule for the use of antibiotics and assess the resistance pattern periodically in each region based on the antibiotic resistance pattern.

Keywords: nosocomial infections, UTI, trachea, CSF, antimicrobial resistance pattern

Introduction
Nosocomial infections (NI) include infections that are acquired from a hospital or other health care centers, which appear for the first time within 48 hours of hospital admission, 3 days of discharge or 30 days of operation. However, in some cases, the NIs occur within 30 days after discharge, such as surgical site infections. Over recent decades, NIs have become a major health concern worldwide. Due to the increasing number of health centers and hospitals, emerging new infectious diseases or the re-emergence of old infectious diseases, increasing antibiotic resistance and prolonging the length of stay for hospitalized patients receiving specialist care, NIs are rapidly increasing, especially in developing countries. There are no accurate statistics on the prevalence of NIs, their adverse health effects, and financial complications in Iran. Based on the previous studies on the prevalence and antimicrobial resistance, it can be concluded that there is a different prevalence of NIs...
Many factors can cause NIs, including patient age (children and elderly individuals are more susceptible to contracting NIs), underlying diseases (e.g., failure in various organs, impaired mucosal defense in burn wound, trauma, surgery and immunodeficiency disorder), receiving immunosuppressive drugs, malnutrition and catheter-related infections. The most common causes of NIs include *Escherichia coli* (the main cause of urinary tract infections), *Staphylococcus aureus* (the main cause of surgical site and respiratory tract infections), *Pseudomonas aeruginosa* (the cause of respiratory tract infections), and Gram-positive bacteria (most common microorganisms in the development of primary bacteremia). The three major sites for NIs include urinary tract system (31%), respiratory system (24%), and bloodstream (16%). The NIs can also occur in the skin and other organs. Pneumonia, urinary tract infection, and septicemia are the most commonly diagnosed NIs on the three major sites. It has been reported that Ventilator-associated Pneumonia (VAP) is the most common nosocomial infection in the intensive care unit (ICU) and responsible for approximately fifty percent of all hospital-acquired pneumonia cases and UTI is the most common NIs in the developed countries. Catheterization leads to about 80% of hospital-acquired UTIs due to the formation of microbial biofilms and especially biofilms composed of *Klebsiella* spp. and *Candida*. Since the formation of biofilm is an important factor in increasing antibiotic resistance and decreasing drug sensitivity, it is essential to pay particular attention to the catheters used in hospitalized patients and urinary catheter users.

Due to the importance of hospitals in providing health services, emerging new infectious diseases, increasing antibiotic resistance, prolonging the length of stay for hospitalized patients and lack of accurate information and statistics on NIs in Iran, this study aimed to provide the prevalence of NIs in Hamadan, west of Iran in comparison with the international statistics. Moreover, this article also aimed to determine the antimicrobial resistance patterns and prevalence of the resistant isolates in NIs in hospitalized patients.

**Materials and Methods**

In this study, the prevalence of nosocomial infections in hospitalized patients to three hospitals of Shahid Beheshti, Farshchian, and Besaat in Hamadan was evaluated for 2 years (2018 to 2020).

The prevalence of pulmonary infection (VAP), UTI, cerebrospinal fluid (CSF) infection were investigated in terms of causing agent and its frequency as well as the pattern of antibiotic resistance to commonly used antibiotics. The samples were taken from patients at the time of 48 hours after hospital admission. The samples were cultured and characterized using morphological and biochemical tests according to microbiological guidelines. Briefly, for urine samples, midstream urine samples were collected and then cultured on blood agar and MacConkey agar media. Finally, the cultured samples were incubated at 37 °C for 24–48 h. Tracheal samples were taken from the lower respiratory tract under sterile conditions and cultured on blood or chocolate and MacConkey agar. The cultured samples were incubated at 37 °C for 24 h. Diagnostic biochemical tests were performed to identify the agents isolated from the infections depending on the isolate type, sampling site, and gram-positive or negative bacteria. Also, catalase, coagulase, optochin, and novobiocin disks, CAMP, and esculin agar tests were utilized for the identification of the isolated gram-positive bacteria. Moreover, the indole diagnostic, Triple Sugar Iron Agar (TSI), citrate, lysine decarboxylase, oxidase, and motility tests were used for the identification of the isolated gram-negative bacteria. Then, the antibiotic resistance of the isolates was analyzed using Modified Disk Diffusion Method (MDDM) by Mueller-Hinton agar medium according to the Clinical & Laboratory Standards Institute (CLSI) instructions. For this purpose, a suspension of pure bacteria with a concentration of 0.5 McFarland (1.5 × 10^8 CFU.mL^{-1}) was prepared in sterile saline and the antibiotic resistance of the isolates was evaluated against different antibiotic groups.

In the present study, *E. coli* strain (ATCC 25922) was used as the control strain for antibiotic susceptibility testing. Various groups of antibiotic including aminoglycosides (amikacin and gentamicin), carbapenem (imipenem), first-generation cephalosporins (cephalosoline), second-generation cephalosporins (cefotaxime), third-generation cephalosporins (ceftazidime, ceftriaxone), nitrofurantoin, nitrofurans, ciprofloxacin and nalidixic acid, sulfonamides (co-trimoxazole), glycopeptides (vancomycin), macrolides (azithromycin) were investigated. After 24 h of incubation at 37 °C, the diameter of the growth zone was measured and the sensitivity of the isolates was estimated according to the CLSI instructions. The frequency of the isolates and their antibiotic resistance were analyzed by using SPSS (version 22) with a 0.05 significant level.
Results
In this research, bacterial isolates were obtained from 1194 clinical specimens, of which 1394 were isolated from urine, 16 from CSF, and 588 from tracheal aspiration. Also, 654 (54.8%) isolates were collected from females and 540 (45.2%) from males with the age range 15–73 years (P> 0.05). The results showed that 22.1% were gram-positive and 77.9% were gram-negative.

The most frequent in gram-negative samples were in the order of *E. coli* with 832 (59.6%), *K. pneumoniae* with 139 (9.9%), *P. aeruginosa* 71 (5.09%), *Enterobacter* spp. 31 (2.2%), *Acinetobacter baumannii* 30 (2.15%), *Klebsiella oxytoca* 18 (1.2%) which isolated from UTI specimens; *K. pneumoniae* 3 (18.7%), *P. aeruginosa* 1 (6.25%) which isolated via CSF and *K. pneumoniae* 164 (27.8%). *A. baumannii* 118 (20.06%), *P. aeruginosa* 97 (16.4%), *E. coli* 57 (9.6%) which were isolated from tracheal aspiration.

In gram-positive isolates, the most abundance samples were *S. aureus* 70 (5.02%) and coagulase-negative staphylococci species including, *S. epidermidis* 52 (3.7%), *S. saprophyticus* 13 (0.9%), *Micrococcus* 35 (2.5%), *S. pyogenes* 9 (0.06%) and Enterococcus 9 (0.06%) in UTI samples. The most frequent gram-positive isolates in the tracheal sample including *S. aureus* 52 (8.8%), *S. epidermidis* 27 (4.5%); and *S. viridans* 11 (68.7%) and *S. aureus* 1 (6.25%) were isolated from CSF samples (Table 1).

Moreover, 123 antibiotic-resistant *S. aureus* isolates were detected. *S. aureus* 70 (5.02%), 52 (8.8%), and 1 (6.25%) were isolated from UTI, trachea, and CSF respectively. The rest of the resistant isolates were collected from other parts of the patient’s body. The maximum antibiotic resistance in *K. pneumoniae* was observed against cefotetan 81 (58.2%), in UTI (Table 2), and the tracheal (Table 3) and CSF samples (Table 4) ceftazidime 120 (73.1%) and 3 (100%) were observed respectively.

For *S. epidermidis* isolates, as observed, 80 isolates of *S. epidermidis* were resistant to the studied antibiotics including 52 (3.7%), 27 (4.5%), and 1 (6.25%) resistance isolates were obtained from the urine, trachea, and CSF samples, respectively. According to the results, there were 41 resistance samples among the *Enterobacter* isolates. Of them, 31 (2.2%) and 10 (1.7%) were isolated from the urine and trachea of the patients, and no resistance was observed in the *Enterobacter* samples isolated from CSF. Of all *Pseudomonas* isolates, 169 specimens showed antibiotic resistance. Among them, 71 (5.09%), 97 (16.4%), 1 (6.25%) specimens were isolated from urine, tracheal tube, and CSF respectively. Also, there were 892 resistance samples in *E. coli* isolates, of which 832 (59.6%), 57 (9.6%), and 3 (15.7%) specimens were isolated from urine, trachea, and CSF, respectively.

Based on the results, there were 148 antibiotic-resistant isolates were detected among the *Acinetobacter* isolates. Of them, 30 (2.1%), 118 (20.06%) resistant isolates were isolated from urine and trachea, respectively. There were 26 antibiotic-resistant isolates among Proteus spp. isolates. Of them, 15 (1.07%) *Proteus mirabilis* were isolated via UTI. *Proteus vulgaris* 7 (1.1%) and *Proteus mirabilis* 4 respectively. The rest of the resistant isolates were collected from other parts of the patient’s body. The maximum antibiotic resistance in *K. pneumoniae* was observed against cefotetan 81 (58.2%), in UTI (Table 2), and the tracheal (Table 3) and CSF samples (Table 4) ceftazidime 120 (73.1%) and 3 (100%) were observed respectively.

### Table 1 The Most Prevalent Bacterial Isolates According to Their Sampling Sites

| Isolates  | UTI (1394 Isolates) | Percentage (%) | Tracheal (588 Isolates) | Percentage (%) |
|-----------|---------------------|----------------|------------------------|----------------|
| E. coli   | 832                 | 59.6           | K. pneumoniae          | 164            | 27.8           |
| K. pneumoniae | 139               | 9.9            | A. baumannii           | 118            | 20.06          |
| P. aeruginosa | 71               | 5.09           | P. aeruginosa          | 97             | 16.4           |
| S. aureus | 70                  | 5.02           | E. coli                | 57             | 9.6            |
| S. epidermidis | 52                | 3.7            | S. aureus              | 52             | 8.8            |
| Micrococcus | 35                | 2.5            | S. epidermidis         | 27             | 4.5            |
| Enterobacter | 31                | 2.2            | Enterobacter           | 10             | 1.7            |
| A. baumannii | 30                | 2.15           | K. oxytoca             | 9              | 1.5            |
| K. oxytoca | 18                  | 1.2            | S. marcescense         | 9              | 1.5            |
| Alkaligenes | 18                 | 1.2            | S. viridans            | 11             | 68.7           |
| P. mirabilis | 15                | 1.07           | K. pneumoniae          | 3              | 18.7           |
| S. saprophyticus | 13               | 0.09           | P. aeruginosa          | 1              | 6.25           |
| S. pyogenes | 9                  | 0.06           | S. aureus              | 1              | 6.25           |
| Enterococcus | 9                 | 0.06           |                        |                |                |

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Table 2 Antibiotic Resistance Profile of Organisms Isolated from UTI Infections

| Bacterial Isolates | Antibiotics Disk (No./Percent%) | FEP (15.1%) | GM (17.2%) | AN (30.9%) | SAM (21.5%) | SXT (0.7%) | FM (43.1%) | CN (58.2%) | CRO (1.4%) | LEV (38.8%) | CZ (2.8%) | IMI (30.2%) | MEN (1.4%) | PTZ (21.5%) | OFX (18.7%) | V |
|-------------------|--------------------------------|-------------|------------|------------|-------------|------------|-------------|-------------|------------|-------------|--------|-------------|--------|---------------|-------------|---|
| Klebsiella pneumoniae | 21 (15.1%) | 24 (17.2%) | 43 (30.9%) | 30 (21.5%) | 60 (43.1%) | 81 (58.2%) | 2 (1.4%) | 54 (38.8%) | 4 (2.8%) | 42 (30.2%) | 2 (1.4%) | 30 (21.5%) | 16 (11.5%) | 26 (18.7%) |
| Staphylococcus aureus | 3 (4.2%) | 2 (2.8%) | 0 (1.4%) | 19 (27.1%) | 5 (7.1%) | 0 (0.7%) | 6 (8.5%) | 2 (2.8%) | 14 (20%) | 0 (0.7%) | 2 (2.8%) | 9 (12.8%) |
| Staphylococcus epidermidis | 3 (5.7%) | 4 (7.6%) | 2 (3.8%) | 1 (1.9%) | 16 (30.7%) | 3 (5.7%) | 0 (0.7%) | 8 (15.3%) | 3 (5.7%) | 2 (3.8%) | 1 (1.9%) | 0 (0.7%) | 4 (7.6%) | 2 (3.8%) | 4 (7.6%) |
| Enterobacter spp. | 3 (9.6%) | 8 (25.8%) | 4 (12.9%) | 1 (3.2%) | 6 (19.3%) | 1 (3.2%) | 10 (32.2%) | 0 (0.7%) | 5 (16.1%) | 0 (0.7%) | 3 (9.6%) | 0 (0.7%) | 1 (3.2%) | 0 (0.7%) |
| Pseudomonas aeruginosa | 19 (27.1%) | 18 (25.7%) | 24 (34.2%) | 12 (17.1%) | 34 (48.5%) | 34 (48.5%) | 2 (2.8%) | 24 (34.2%) | 4 (5.7%) | 12 (17.1%) | 5 (7.1%) | 15 (21.4%) | 12 (17.1%) | 14 (20%) |
| Escherichia coli | 83 (9.9%) | 127 (15.2%) | 54 (6.4%) | 8 (0.9%) | 444 (53.3%) | 98 (11.7%) | 11 (1.3%) | 302 (36.2%) | 14 (1.6%) | 209 (25.1%) | 4 (0.4%) | 20 (2.4%) | 37 (4.4%) | 145 (17.4%) | 0 (0.7%) |
| Acinetobacter baumannii | 10 (33.3%) | 16 (53.3%) | 20 (66.6%) | 2 (6.6%) | 21 (70%) | 20 (66.6%) | 3 (10%) | 16 (53.3%) | 2 (6.6%) | 16 (53.3%) | 1 (3.3%) | 16 (53.3%) | 3 (10%) | 8 (26.6%) | 1 (3.3%) |
| Staphylococcus saprophyticus | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Abbreviations: FEP, cefepime; GM, gentamicin; AN, amikacin; SAM, sulfactam; SXT, trimethoprim-sulfamethoxazole; FM, nitrofurantoin; CN, cefotetan; CRO, ceftriaxone; LEV, levofloxacin; CZ, cefazolin; IMI, imipenem; MEN, meropenem; PTZ, piperacillin/tazobactam; OFX, ofloxacin; V, vancomycin.
| Bacterial Isolates                     | CP     | CAZ   | FEP   | GM    | AN    | SAM   | SXT   | CN    | CRO   | LEV   | AK    | CZ    | IMI   | MEN   | PTZ   | V     |
|--------------------------------------|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| **Klebsiella pneumoniae**            | 95     | 120   | 114   | 58    | 73    | 30    | 8     | 23    | 56    | 42    | 9     | 1     | 72    | 114   | 96    | 10    |
| (57.9%)                              | (73.1%)| (69.5%)| (35.3%)| (44.5%)| (18.2%)| (4.8%)| (14.0%)| (34.1%)| (25.60%)| (5.48%)| (0.6%)| (43.9%)| (69.5%)| (58.5%)| (6.09%)|
| **Staphylococcus aureus**            | 20     | 7     | 4     | 0     | 0     | 0     | 0     | 9     | 0     | 0     | 0     | 0     | 2     | 10    | 2     | 8     |
| (38.4%)                              | (13.4%)| (7.6%)| (0%)   | (0%)   | (0%)   | (0%)   | (0%)   | (17.3%)| (0%)   | (0%)   | (0%)   | (0%)   | (19.2%)| (3.8%)| (15.3%)| (13.4%)|
| **Staphylococcus epidermidis**       | 8      | 8     | 6     | 4     | 0     | 0     | 0     | 5     | 2     | 2     | 0     | 0     | 1     | 0     | 4     | 0     |
| (29.6%)                              | (29.6%)| (22.2%)| (14.8%)| (3.7%)| (18.5%)| (7.4%)| (7.4%)| (11.1%)| (3.7%)| (4.1%)| (19.2%)| (3.8%)| (15.3%)| (13.4%)| (1.9%)| (3.8%)| (11.1%)|
| **Enterobacter spp.**                | 3      | 5     | 3     | 2     | 2     | 1     | 1     | 0     | 1     | 1     | 1     | 1     | 5     | 5     | 0     |
| (27.2%)                              | (45.4%)| (27.2%)| (18.1%)| (9.0%)| (9.0%)| (9.0%)| (9.0%)| (9.0%)| (9.0%)| (9.0%)| (9.0%)| (9.0%)| (9.0%)| (45.4%)| (45.4%)| (45.4%)| 0     |
| **Pseudomonas aeruginosa**           | 10     | 40    | 50    | 19    | 43    | 15    | 8     | 26    | 13    | 8     | 4     | 1     | 43    | 46    | 41    | 0     |
| (10.3%)                              | (41.2%)| (51.5%)| (19.5%)| (44.3%)| (15.4%)| (8.2%)| (13.4%)| (26.8%)| (8.2%)| (4.1%)| (1.0%)| (44.3%)| (47.4%)| (42.2%)| 0     |
| **Escherichia coli**                 | 30     | 20    | 20    | 17    | 4     | 3     | 6     | 0     | 17    | 9     | 0     | 0     | 14    | 10    | 27    | 0     |
| (52.6%)                              | (35.0%)| (35.0%)| (29.8%)| (7.01%)| (5.2%)| (10.5%)| (0.5%)| (29.8%)| (15.7%)| (0.5%)| (0.5%)| (0.5%)| (24.5%)| (17.5%)| (47.3%)| 0     |
| **Acinetobacter baumannii**         | 81     | 101   | 93    | 45    | 91    | 14    | 6     | 9     | 53    | 38    | 11    | 0     | 72    | 96    | 94    | 1     |
| (68%)                                | (85.5%)| (78.8%)| (38.1%)| (77.1%)| (11.8%)| (5.0%)| (7.6%)| (44.9%)| (32.2%)| (9.3%)| (0.6%)| (12.5%)| (12.5%)| (81.3%)| (79.6%)| (0.84%)|
| **Staphylococcus saprophyticus**     | 3      | 0     | 0     | 0     | 0     | 0     | 0     | 2     | 2     | 2     | 1     | 0     | 1     | 2     | 0     |
| (37.5%)                              | (0%)   | (0%)  | (0%)  | (0%)  | (0%)  | (0%)  | (0%)  | (25%) | (25%) | (25%) | (12.5%)| (12.5%)| (25%) | (25%) | (0%)  |

**Abbreviations:** CP, ciprofloxacin; CAZ, ceftazidime; FEP, cefepime; GM, gentamicin; AN, amikacin; SAM, sulbactam; SXT, trimethoprim-sulfamethoxazole; PM, nitrofurantoin; CN, cefotetan; CRO, ceftriaxone; LEV, levofloxacin; CZ, cefazolin; IMI, imipenem; MEN, meropenem; PTZ, piperacillin/tazobactam; OFX, ofloxacin; V, vancomycin.
| Bacterial Isolates           | FEP   | MEN   | IMI   | CRO   | AN    | PTZ   | SXT   | SAM   | LEV   | GM    | CAZ   | CP    |
|-----------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Klebsiella pneumonia        | 0     | 2 (66.6%) | 0 | 0 | 0 | 0 | 2 (66.6%) | 0 | 0 | 3 (100%) | 3 (100%) | 3 (100%) |
| Staphylococcus aureus       | 0     | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 1 (25%) | 0 | 4 (100%) |
| Pseudomonas aeruginosa      | 0     | 2 (9.5%) | 0 | 2 (9.5%) | 0 | 0 | 3 (14.2%) | 0 | 0 | 1 (4.7%) | 2 (9.5%) | 1 (4.7%) |
| Escherichia coli            | 0     | 0 | 1 (33.3%) | 0 | 0 | 2 (66.6%) | 1 (33.3%) | 0 | 1 (33.3%) | 2 (66.6%) | 3 (100%) |
| Staphylococcus epidermidis  | 0     | 0 | 0 | 0 | 0 | 0 | 1 (100%) | 0 | 0 | 0 | 0 | 0 |

**Abbreviations:** CP, ciprofloxacin; CAZ, ceftazidime; FEP, cefepime; GM, gentamicin; AN, amikacin; SAM, sulbactam; SXT, trimethoprim-sulfamethoxazole; CRO, ceftriaxone; LEV, levofloxacin; IMI, imipenem; MEN, meropenem; PTZ, piperacillin/tazobactam.
(0.6%) were isolated from tracheal samples. In total, 27 antibiotic-resistant isolates were identified in *K. oxycoza* bacteria, of which 18 (1.2%) and 9 (1.5%) were isolated from urine and tracheal samples, respectively. In *S. saprophyticus* isolates, 125 resistant isolates were detected, which 13 (0.09%) isolates obtained from UTI samples.

20 antibiotic-resistant isolates were detected in Group A 9 (0.6%) and B 9 (0.6%) streptococci, of which 18 (1.2%) were isolated from the UTI samples, and 2 (0.2%) others isolated from the trachea samples (Table 1). In the Enterococci (Group D streptococci) bacteria isolated from patients, 12 antibiotic-resistant isolates were identified, of which 3 (0.5%) and 9 (0.6%) samples were isolated from tracheal and urine samples, respectively. *Listeria* isolates were not isolated from the studied sites, including trachea, urine, CSF. *Morganella Morgani* 2 (0.1%) and *Moraxella catarrhalis* 2 (0.1%) showed antibiotic resistance and all of them (100%) were isolated in urine samples. Besides, in the tracheal samples 1 (0.1%) *Moraxella catarrhalis* was isolated.

In *Hafnia alvei* isolates, 3 resistant were identified, which 1 (0.07%) and 2 (0.3%) from the urine and tracheal samples were isolated. Also, 1 resistant isolate was detected among *Edwardsia* isolates, which all of them were isolated from urine samples (100%).

In *Alcaligenes* spp isolates, there were 19 resistant specimens, 18 (1.2%) and 1 (0.1%) samples were isolated from urine and trachea, respectively. In *S. marcescens* isolates, 11 antibiotic resistance specimens were detected. Of the 9 (1.5%) and 2 (0.1%), isolates were obtained from the trachea and urine samples, respectively. Also, 5 resistant isolates were identified in diphtheroid isolates, 2 (0.1%) and 3 (0.5%) of which were isolated from urine and trachea samples.

**Discussion**

The spread of antibiotic-resistant NIs or healthcare-associated infections has become a public health concern worldwide. The rate of NI in developed and developing countries is 7% and 10% respectively. This difference in antimicrobial susceptibility depends on several factors including endemic resistant pathogens, misuse or overuse of antibiotics in the treatment of patients, the severity of the disease, longer hospitalization are the most important factors. Because these infections occur during hospitalization along with disability, prolong hospitalization, and economic burden. Increasing antibiotic resistance among the microorganisms causing NIs is associated with a high mortality rate in hospitalized patients. Investigating the prevalence of these resistant bacteria can be useful to control NIs.

In this study, the prevalence of gram-negative bacteria (77.9%) in NIs was much higher than that of gram-positive bacteria (22.1%). Hence, the main cause of NIs is gram-negative bacteria, which is consistent with the findings of the Sikka et al. Moreover, our findings implied that UTI is the main nosocomial infection caused by bacteria. According to the results, the most prevalent gram-negative bacteria causing NIs were in the order of *E. coli* > *Klebsiella* > *P. aeruginosa*, which is consistent with the findings of Tolera et al, and Sikka et al. Also, the most common bacterium causing NIs among gram-positive bacteria was *S. aureus*, which is in agreement with Wang et al.

The prevalence of antibiotic resistance in the studied bacteria was in the order of *E. coli* > *Klebsiella* > *Acinetobacter* > *S. aureus* that is in contrast with Amini et al in 2017 study in that reported Klebsiella with the low prevalence and our study antibiotic resistance to Klebsiella was increased and placed in the second prevalent bacteria. Generally, the most prevalent and antibiotic resistance in the studied bacteria were observed in the samples isolated from tracheal aspiration and UTIs. Agaba et al, also reported that *Klebsiella, Acinetobacter*, and *S. aureus* were the most cause of NIs. In the mentioned study, the most resistant isolates were isolated from tracheal aspiration and UTIs, which is in agreement with our findings. The resistance to *A. baumannii* as one of the most important pathogens acquired from hospitals is increasing and the evaluation of its antimicrobial patterns is so necessary.

In the present study, antibiotic resistance was observed to different antibiotics. The most resistant to cefotetan, nitrofurantoin, levofloxacin, amikacin, imipenem, piperacillin-tazobactam, co-trimoxazole, were found in *E. coli* isolates from urine samples. In Maechler study in 2015, Klebsiella isolates 13% was reported compared with the present research with 27.8% in the trachea. This difference may be attributed to the sampling site of the specimens because, in the mentioned study, all samples were only isolated from the ICU ward, where patients had a weakened immune system. In the current study, the highest prevalence of antibiotic resistance in Klebsiella strains was observed in trachea and urine specimens. Also, the antibiotic resistance to ciprofloxacin, ceftazidime, cefepime, amikacin, imipenem, meropenem, and piperacillin discs was higher than other studied antibiotics (Table 2).

The prevalence of antibiotic resistance in gram-positive *S. aureus* and *S. epidermidis* was lower than in gram-negative...
bacteria. The results of Carlsen et al.,\textsuperscript{31} study on the susceptibility of urinary pathogens causing NIs showed that the highest antibiotic susceptibility to ciprofloxacin and nitrofurantoin was observed in \textit{E. coli} and \textit{K. pneumoniae} isolates, respectively and these results are consistent with our findings. In the present study, the more number of antibiotics were investigated, and there was a higher antibiotic susceptibility in some studied antibiotics such as nitrofurantoin, ampicillin, clindamycin, linezolid, penicillin G, oxacillin, piperacillin, and tobramycin. In our research \textit{Klebsiella} and \textit{E. coli} isolates susceptibility to co-trimoxazole are in contrast in UTI samples so that the resistance rate for Klebsiella and \textit{E. coli} was 1 (0.7\%) and 444 (53\%) against co-trimoxazole are the minimum and maximum resistance to co-trimoxazole respectively.\textsuperscript{31} This finding of Klebsiella is in contrast with the Sakkas H study in 2019\textsuperscript{32} that reported the prevalence of co-trimoxazole resistant bacteria 95\%. This difference may be due to the different geographical region and so in our region, the pattern of antibacterial resistance of klebsiella to co-trimoxazole is different from other areas significantly. It can be interpreted that co-trimoxazole prescription for UTI infection is a low rate. In addition, the resistance rate of \textit{K. pneumoniae} to fluoroquinolones including levofloxacin and ciprofloxacin is approximately 50\% in the urine and trachea sources and is in agreement with our previous study.\textsuperscript{33}

Khanal et al.,\textsuperscript{34} is in agreement with this research that reported the most gram-negative bacteria were isolated from the aspirate samples of \textit{Acinetobacter} spp. Then, \textit{K. pneumoniae} and subsequently \textit{Pseudomonas} strains with multi-drug resistance to combined cefotaxime and cefotaxime-clavulanate were also identified. In the current work, the rate of resistant \textit{Acinetobacter baumannii} to cefazidime and meropenem was 85.1\% and 81.3\% in the urine and trachea samples, respectively.

Moreover, in Malik et al.,\textsuperscript{35} \textit{K. pneumoniae} was identified as the most common bacteria. The highest sensitivity was also observed among the combined drugs of cefoperazone-sulbactam and piperacillin-tazobactam, in which over 60\% sensitivity was observed among gram-negative bacteria and 100\% sensitivity to vancomycin and linezolid was observed among gram-positive bacteria. These results are consistent with our findings in terms of the sequence of the strains. One \textit{S. aureus} isolated from the trachea was resistant to vancomycin although the confidential test to confirm or reject this finding is necessary. Also, resistance to vancomycin in \textit{S. aureus} was reported previously.\textsuperscript{32} In terms of Acinetobacter, a study in Pakistan in 2016\textsuperscript{36} that reported the high prevalence of resistance to 3\textsuperscript{rd} generation of cephalosporins approximately 100\%, is in contrast to the present work in which Acinetobacter isolates resistance rate was lower than 50\% that may be due to the overuse of aforementioned antibiotics in that country that is in neighboring of Iran.

\section*{Conclusion}
In this study, the prevalence of antibiotic resistance was high in most common pathogenic strains. Therefore, the results of this study demonstrated that antibiotics with a high resistance level must be less used for the treatment of infections. Moreover, to prevent the spread of resistance among various strains and improve the effectiveness of antibiotics, it is suggested to establish a precise schedule for antibiotic use in each region based on their antibiotic resistance pattern. Due to the increase in the number of tracheal infections as well as the emergence of antibiotic-resistant strains, it is necessary to take the necessary guidelines to minimize these cases including directional airflow in the room and the use of positive pressure to prevent the placement of infectious particles in the isolated room.

\section*{Abbreviations}
NIs, nosocomial infections; UTI, urinary tract infection; ICU, intensive care unit; VAP, ventilator-associated pneumonia.

\section*{Data Sharing Statement}
All data used are available. Please contact the corresponding authors for data requests.

\section*{Ethics Approval}
This study was approved by the ethics committee of Hamadan University of Medical Sciences.

\section*{Consent to Participate}
In this study, we weren’t in touch with the children directly, although all sample urines were collected from the laboratory of the Be’ sat, Beheshti, and Sina hospitals.

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\section*{Author Contributions}
All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of
data; took part in drafting the article or revising it critically for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Disclosure
The authors declare that they have no conflicting interests related to this manuscript.

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