Disease and Diagnosis

Dis Diagn. 2022; 11(2):58-62

10.34172/ddj.2022.12

Original Article

Determination of Antibiotic Susceptibility of Bacteria Isolated From Endotracheal Tube in Pulmonary Intensive Care Unit of a Hospital in Bandar Abbas

Arash Rahimi[1], Maede Ansari[2], Parivash Davoodian[1], Saeed Shoja[1], Saeed Hosseini Teshnizi[1], Hesam Alizade[1]*

[1]Infectious and Tropical Diseases Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.
[2]Student Research Committee, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.

Abstract

Background: The present study attempted to determine the antibiotic susceptibility of bacteria isolated from endotracheal tube culture on patients admitted to the intensive care units (ICUs) of Shahid Mohammadi hospital in Bandar Abbas.

Materials and Methods: This cross-sectional study was conducted on 114 patients hospitalized in the pulmonary ICU of Shahid Mohammadi hospital in Bandar Abbas from March 2018 to March 2019. The samples of the patients' endotracheal tube secretions were collected for the culture purpose on the first and fifth days of hospitalization in the ICU where the disk diffusion method was used to determine antibiotic susceptibility.

Results: The most common isolated microorganisms from the first day of culture were 26 *Acinetobacter* spp. isolates (22.8%), 10 *Candida* isolates (8.8%), and 9 *Pseudomonas aeruginosa* isolates (7.9%). The most common microorganisms which were isolated on the fifth day comprised *Acinetobacter* spp. with 57 isolates (51.8%), *Candida* with 6 isolates (5.5%), and *P. aeruginosa* with 6 isolates (5.5%). The highest resistant bacteria isolated from *Acinetobacter* spp. samples on the first-day were amikacin (92.3%) and meropenem (84.6%). The highest resistance of *P. aeruginosa* was against meropenem (77.8%), while the highest resistance of *Acinetobacter* spp. isolates was found to be amikacin (94.7%) and meropenem (86%) from the fifth-day samples. Further, *P. aeruginosa* isolates had the highest resistance in meropenem (83.3%), co-trimoxazole, and cefepime (66.7% for each of them).

Conclusion: Ventilator-associated pneumonia (VAP) is considered a warning condition due to multi-drug-resistant bacteria. According to the present study, the most common VAP-causing bacteria are gram-negative, especially *Acinetobacter*, with high antibiotic resistance.

Keywords: Ventilator-associated pneumonia, Endotracheal intubation, Antibiotic resistance, ICU

***Correspondence to**
Hesam Alizade, Infectious and Tropical Diseases Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.
Tel: +98-76-33710393,
Fax: +98-76-33710393,
Email: alizade.h2000@yahoo.com

Received: February 1, 2022, Accepted: February 13, 2022, ePublished: April 1, 2022

Introduction

Nosocomial infections are counted as one of the most challenging problems worldwide. This complication imposes high costs on society and patients and sometimes results in death or severe morbidities. Exposure to nosocomial infections is higher in intensive care unit (ICU) patients since accompanying underlying diseases could suppress their immune systems, and the use of endotracheal tubes and mechanical ventilation contributes to the contraction of infection. In addition, more prolonged hospital stay for those patients is regarded as another predisposing factor that increases the risk of nosocomial infections (1). Several types of bacteria cause nosocomial infections, including *Acinetobacter*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, and *Enterococcus* (2). Respiratory tract infection is the most common infection in ICUs. Intubated patients on mechanical ventilation are at a greater risk of infection (3). Ventilator-associated pneumonia (VAP) is considered a major health problem for patients who experience intubation in the ICU. VAP develops in mechanically ventilated patients after 48 hours of intubation or tracheostomy. There are two types of VAP: early-onset and late-onset.

The latter occurs more than 96 hours after intubation (4). Endotracheal intubation is usually performed on critically ill patients to prevent aspiration, keep a secure airway, and restore hypoxemic or hypercarbic respiratory failure. Emergency intubation does not prevent tracheal mucosal colonization by the normal endogenous flora and is an independent risk factor for early VAP (5, 6). Infection is caused by accumulation of pharyngeal secretions

behind the endotracheal tube cuff, loss of cough reflex, and the need to use suction to keep draining secretions. Furthermore, the transmission of infection through the personnel hand touches or medical equipment and the presence of antibiotic-resistant bacteria increase the risk of those types of infections (7). This study attempted to evaluate the bacteria isolated from endotracheal tube culture and their antibiotic susceptibility in patients admitted to the pulmonary ICU of a leading tertiary teaching hospital in Bandar Abbas.

Materials and Methods

Sampling

This cross-sectional study was conducted from March 2018 to March 2019. The participants comprised a total of 114 endotracheal intubated patients with respiratory failure (i.e., hypoxemia or hypercapnia) in the pulmonary ICU of the tertiary teaching hospital (Shahid Mohammadi) of Bandar Abbas, located in the south of Iran (Payambare-Azam-therapeutic complex). They were selected randomly among all admitted patients during this period. Eligible criteria were 15-year- old or above ICU patients who were intubated and underwent mechanical ventilation. Patients whose legal guardians did not sign informed consent were excluded from the study. Samples were collected non-invasively with endotracheal aspiration on the first and fifth day of intubation. The presence or absence of infection, strain change, and transformation of initial negative culture were evaluated during two samplings. The specimens were transferred to blood agar, chocolate agar as an enriched medium, and McConkey agar as a selective and differential medium, and then the plates were incubated at a temperature of 37°C for 48 hours. Different isolates were examined microscopically using the gram staining technique. The standard bacteriological and biochemical tests were performed to determine the genus and species (8).

Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing of bacteria was performed by disc diffusion method, following the Clinical Laboratory Standards Institute (2016) guidelines (9). The commercial antimicrobial disk (Padtan Teb. Co., IR) was used, containing antibiotics such as ciprofloxacin (5 µg), ampicillin (10 µg), gentamicin (10 µg), penicillin (10 µg), piperacillin (100 µg), ampicillin-sulbactam (10/10 ug), piperacillin-tazobactam (30/6 ug), imipenem (10 µg), meropenem (10 µg), and cefalexin (30 µg). The other included antibiotics were ceftazidime (30 µg), cefotaxime (30 µg), cefixime (5 µg), ceftriaxone (30 µg), cefepime (30 µg), amikacin (30 µg), tetracycline (30 µg), erythromycin (15 µg), clindamycin (2 µg), and trimethoprim/sulfamethoxazole (1.25/23.75 ug). Antibiotic susceptibility was determined after performing the intubation process from the isolated bacteria on the first and fifth days.

Results

Bacteria Isolated From Culture on the First Day

The mean age of the 114 endotracheal intubated patients with respiratory failure was 17 to 88 years, containing 67 males and 47 females. The duration of hospitalization was between 2 up to 26 days. Based on the present study's findings, the result of the first-day culture for 48 patients (42.1%) was negative. The isolation culture samples of the first day were 26 *Acinetobacter* spp. isolates, 10 *Candida* isolates (8.8%), 9 *P. aeruginosa* isolates (7.9%) , 7 *S. aureus* isolates (6.1%), 6 *K. pneumonia* isolates (5.3%), 2 *E. coli* isolates (1.8%), 1 *Stenotrophomonas maltophilia* isolate (0.9%), 1 *Citrobacter* isolate (0.9%), 1 *Streptococcus viridans* isolate (0.9%), 1 beta-hemolytic *streptococcus* isolate (0.9%), 1 *Enterococci* isolate (0.9%), and 1 *Enterobacter* isolate (0.9%) as represented in Table 1.

Antibiotic Resistance of the Isolated Bacteria From the First Day of Culture

The isolated bacteria of the first-day samples had the highest antibiotic resistance in *Acinetobacter* spp. against amikacin (92.3%), meropenem (84.6%), piperacillin-tazobactam (69.2%), cefepime (61.5%), ceftazidime (57.7%), co-trimoxazole (42.3%), ciprofloxacin, and imipenem (35.5% for each of them). The highest antibiotic resistance of *P. aeruginosa* was for antibiotics as meropenem (77.8%), co-trimoxazole, cefepime (44.4% for each of them), ciprofloxacin (33.3%), ceftazidime, and imipenem (22.2%). *S. aureus* was resistant to clindamycin and erythromycin (57.1% for each of them), ampicillin (42.9%), and co-trimoxazole (14.3%). The highest antibiotic resistance of *K. pneumonia* was against piperacillin-tazobactam and cefepime (50% for each of them), meropenem (33.3%), ampicillin-sulbactam, ceftriaxone, amikacin, gentamicin, and co-trimoxazole (16.7% for each of them). The details of the antibiotic resistance of other bacteria are presented in Table 1.

Bacteria Isolated From Culture on the Fifth Day

The fifth-day culture was negative for 31 patients (28.2%), and four patients (3.5%) did not fulfill the fifth-day culture due to death. Out of 110 samples cultured on the fifth day, Table 2 indicated the following results: 57 *Acinetobacter* spp. isolates (51.8%), 6 *Candida* isolates (5.5%), 6 *P. aeruginosa* isolates (5.5%), 5 *K. pneumonia* isolates (4.5%), 3 *S. aureus* isolates (2.7%), 1 *Enterobacter* isolate (0.9%), and 1 *S. maltophilia* isolate (0.9%).

Antibiotic Resistance of Bacteria Isolated From Culture on the Fifth Day

The highest antibiotic resistance of *Acinetobacter* spp. isolates from the fifth-day samples was against amikacin (94.7%), meropenem (86%), piperacillin-tazobactam

Table 1. Antibiotic Resistance of the Isolated Bacteria From the First Day

Antibiotic	A	P. a	S. a	K. p	E. c	S. m	C	S. v	b-h S	E¹	E²
Penicillin	-	-	-	-	-		-	-	1 (100)	-	-
Piperacillin	1 (3.8)	-	-	-	-	-	-	-	-	-	-
Ampicillin	1 (3.8)	-	3 (42.9)	-	-	-	-	-	1 (100)	1 (100)	
Ampicillin sulbactam	5 (19.2)	1 (11.1)	-	1 (16.7)	-	-	-	-	-	-	1 (100)
Piperacillin tazobactam	18 (69.2)	1 (11.1)	-	3 (50)	-	-	-	-	-	-	-
Meropenem	22 (84.6)	7 (77.8)	-	2 (33.3)	-	-	-	-	-	-	-
Imipenem	10 (38.5)	2 (22.2)	-	-	-	-	-	-	-	-	1 (100)
Cefalexin	-	-	-	-	-	-	-	-	-	1 (100)	-
Ceftazidime	15 (57.7)	2 (22.2)	-	-	-	-	-	-	-	-	1 (100)
Ceftriaxone	6 (23.1)	1 (11.1)	-	1 (16.7)	-	-	-	-	-	-	-
Cefepime	16 (61.5)	4 (44.4)	-	3 (50)	1 (50)	1 (100)	-	-	-	-	1 (100)
Amikacin	24 (92.3)	1 (11.1)	-	1 (16.7)	1 (50)	1 (100)	-	-	-	-	1 (100)
Gentamicin	9 (34.6)	-	-	1 (16.7)	1 (50)	-	-	-	-	-	-
Tetracycline	-	-	-	-	-	-	-	-	-	1 (100)	-
Erythromycin	-	-	4 (57.1)	-	-	-	-	-	-	1 (100)	-
Clindamycin	-	-	4 (57.1)	-	-	-	-	-	-	-	-
Cotrimoxazole	11 (42.3)	4 (44.4)	1 (14.3)	1 (16.7)	1 (50)	-	-	-	-	1 (100)	-
Ciprofloxacin	10 (38.5)	3 (33.3)	-	-	1 (50)	-	-	-	-	-	-

Note. A: Acinetobacter; P. a: Pseudomonas aeruginosa; S. a: Staphylococcus aureus; K. p: Klebsiella pneumoniae; E. c: Escherichia coli; S. m: Stenotrophomonas maltophilia; C: Citrobacter; S. v: Streptococcus viridans; b-h *S:* Beta-hemolytic *Streptococcus;* E¹: *Enterococci;* E²: *Enterobacter.*

Table 2. Antibiotic Resistance of Isolated Bacteria From the Fifth Day

Antibiotic	*A*	*P. a*	*S. a*	*K. p*	*S. m*	*E. b*
Penicillin	1 (1.8)	-	-	-		-
Piperacillin	3 (5.3)	-	-	-	-	-
Ampicillin	-	-	2 (66.7)	-	-	-
Ampicillin sulbactam	6 (10.5)	-	-	-	-	-
Piperacillin tazobactam	43 (75.4)	1 (16.7)	-	2 (40)	-	1 (100)
Meropenem	49 (86)	5 (83.3)	-	-	-	-
Imipenem	22 (38.6)	3 (50)	-	-	-	-
Cefalexin	1 (1.8)	-	-	-	-	-
Ceftazidime	33 (57.9)	1 (16.7)	-	-	-	-
Cefotaxime	3 (5.3)	-	-	-	-	-
Cefixime	3 (5.3)	-	-	-	-	-
Ceftriaxone	13 (22.8)	2 (33.3)	-	1 (20)	-	-
Cefepime	39 (68.4)	4 (66.7)	-	2 (40)	1 (100)	-
Amikacin	54 (97.4)	1 (16.7)	-	1 (20)	1 (100)	-
Gentamicin	17 (29.8)	-	-	-	-	-
Tetracycline	1 (1.8)	-	-	-	-	-
Erythromycin	1 (1.8)	-	1 (33.3)	-	-	-
Clindamycin	-	-	1 (33.3)	-	-	-
Cotrimoxazole	20 (35.1)	4 (66.7)	1 (33.3)	1 (20)	-	1 (100)
Ciprofloxacin	35 (61.4)	2 (33.3)	-	-	-	-

Note. A: Acinetobacter; P. a: Pseudomonas aeruginosa; S. a: Staphylococcus aureus; K. p: Klebsiella pneumoniae; S. m: Stenotrophomonas maltophilia; E. b: *Enterobacter.*

(75.4%), cefepime (68.4%), ciprofloxacin (61.4%), ceftazidime (57.9%), and imipenem (38.6%). Further, *P. aeruginosa* isolates revealed the highest antibiotic resistance to meropenem (83.3%), cotrimoxazole and cefepime (66.7% for each of them), as well as imipenem (50%). In addition, *K. pneumonia* isolates showed the highest resistance to piperacillin-tazobactam and cefepime (40% for each), and *S. aureus* isolates had the highest resistance to ampicillin (66.7%), clindamycin, erythromycin, and co-trimoxazole (33.3% for each of them). The details of the antibiotic resistance of other bacteria on the fifth day are illustrated in Table 2.

Discussion

The most important principles of treatment were documented to be the early detection of nosocomial infections, type of microorganisms, trends of antibiotic resistance, and proper use of antibiotics for diminishing drug resistance (10). Despite comprehensive strategies to reduce VAP incidence, it remains the leading cause of death in patients facing nosocomial infections (11). Krishnamurthy et al suggested that the overall prevalence of VAP in the ICU was between 10 and 70% (12). They revealed that several factors might contribute to the spread of VAP. Intubation was counted as one of the leading factors that increase VAP risk, causing nosocomial pneumonia and microaspiration of bacterial colonization from the oropharynx (11). The present study investigated 114 patients hospitalized in ICUs. The most common bacteria isolated from the first and fifth-day cultures were *Acinetobacter*. In Iran, a study by Ghafouri et al identified *E. coli* as the most common bacterium isolated from ICU patients with acquired pneumonia (13). The observed difference in results of isolated bacteria could be due to the differences in the type of samples.

Colonization significantly increased in ICU patients who underwent intubation due to the inability to oral feed, reduced salivation, and decreased oral clearance and hygiene. For this reason, ICU patients are at a high risk of infection. Colonization varies in different geographical areas and even in each hospital unit (14, 15). The American Thoracic Society identifies gram-negative intestinal bacilli (i.e., *Enterobacter*, *E. coli*, *Klebsiella*, *Proteus*, and *Serratia marcescens*), *Haemophilus influenzae*, *Streptococcus pneumonia*, and *S. aureus* sensitive to methicillin as the major bacteria that cause nosocomial pneumonia, and assigns nosocomial pneumonia caused by them to the ATSI group; however, if the above microorganisms cause nosocomial pneumonia along with one of the pathogens of *Pseudomonas aeruginosa*, *Acinetobacter* spp. and methicillin-resistant *S. aureus* rank as ATSIII group (16). The first-day positive samples for *Acinetobacter* spp. showed the highest antibiotic resistance to amikacin.

On the other hand, in this study out of 57 cases of *Acinetobacter* spp. isolated from the fifth-day culture, they were most resistant to amikacin. However, Salehifar et al revealed that susceptibility of *Acinetobacter* spp. isolates had the highest antibiotic resistance to imipenem and tobramycin (70% for each of them) and ceftazidime (22.2%) (10). Although *Acinetobacter* spp. is inherently resistant to aminopenicillins, first and second-generation cephalosporins, and chloramphenicol, it can develop resistance to broad-spectrum beta-lactams, aminoglycosides, fluoroquinolones, and tetracyclines (17). Another study reported a high prevalence of VAP via *Citrobacter* and *K. pneumonia*, indicating high levels of resistance to carbapenems as common antibiotics used in the ICU while being highly sensitive to polymyxin B (94%) and tigecycline (96%) (18). Malik et al also reported *K. pneumonia* as the most common bacterium causing VAP with over 60% sensitivity to the combined drugs such as cefoperazone-sulbactam and piperacillin-tazobactam (19). This study revealed that *K. pneumonia* was about 50% sensitive to piperacillin-tazobactam. Although there are significant differences in the type of common bacteria and their antibiotic resistance in different studies, all studies agreed that the most problematic bacteria contributing to VAP are gram-negative bacteria with little antibiotic resistance among gram-positive bacteria. This difference in prevalence and antibiotic resistance suggests that hospitals should continuously monitor the prevalence of VAP-causing agents and identify their antibiotic resistance to be used in experimental therapies or after determining culture results. One of the limitations of the present study was the lack of evaluation of bacterial susceptibility compared to other antibiotics recommended in new articles and guidelines and the lack of evaluation of fungal infections and their control drugs. Results of the present study suggested that the most common bacteria that cause VAP were gram-negative bacteria, especially *Acinetobacter,* which have high antibiotic resistance, are resistant to most antibiotics commonly used in ICUs, and should be targeted with appropriate antibiotics.

Acknowledgments

The authors acknowledge Hormozgan University of Medical Sciences for the financial support.

Authors' Contribution

AR: Conceptualization and study design; SHT and MA: Experimental and laboratory bench work and data collection; PD and SS: Coordination and data collection; HA: Manuscript drafting. All authors reviewed and approved the final manuscript.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

Ethical Statement

This study was ethically endorsed by the Ethics Committee of Hormozgan University of Medical Sciences with number IR.HUMS.REC.1397.012.

Funding/Support

This work was supported by Hormozgan University of Medical

Sciences through the allocated grant. We are grateful to them for their support and financial provision.

Informed Consent

The present study was conducted after obtaining informed consent from all participants.

References

1. Maoulainine FM, Elidrissi NS, Chkil G, Abba F, Soraa N, Chabaa L, et al. [Epidemiology of nosocomial bacterial infection in a neonatal intensive care unit in Morocco]. Arch Pediatr. 2014;21(9):938-43. doi: 10.1016/j.arcped.2014.04.033.
2. Abedini P, Soleimani N. A review of designing new vaccines to prevent hospital-acquired antibiotic-resistant infections. Int Electron J Med. 2018;7(2):21-9. doi: 10.31661/iejm861.
3. Ebrahimi Fakhar HR, Rezaie K, kohestani HR. Effect of closed endotracheal suction on incidence of ventilator-associated pneumonia. Sci J Kurd Univ Med Sci. 2010;15(2):79-87. [Persian].
4. Cook DJ, Walter SD, Cook RJ, Griffith LE, Guyatt GH, Leasa D, et al. Incidence of and risk factors for ventilator-associated pneumonia in critically ill patients. Ann Intern Med. 1998;129(6):433-40. doi: 10.7326/0003-4819-129-6-199809150-00002.
5. Sirvent JM, Torres A, Vidaur L, Armengol J, de Batlle J, Bonet A. Tracheal colonisation within 24 h of intubation in patients with head trauma: risk factor for developing early-onset ventilator-associated pneumonia. Intensive Care Med. 2000;26(9):1369-72. doi: 10.1007/s001340000611.
6. Campbell W, Hendrix E, Schwalbe R, Fattom A, Edelman R. Head-injured patients who are nasal carriers of *Staphylococcus aureus* are at high risk for *Staphylococcus aureus* pneumonia. Crit Care Med. 1999;27(4):798-801. doi: 10.1097/00003246-199904000-00039.
7. Pneumatikos IA, Dragoumanis CK, Bouros DE. Ventilator-associated pneumonia or endotracheal tube-associated pneumonia? An approach to the pathogenesis and preventive strategies emphasizing the importance of endotracheal tube. Anesthesiology. 2009;110(3):673-680. doi:10.1097/ALN.0b013e31819868e0.
8. Church DL. Aerobic bacteriology: 3.3 Processing, Isolation, Detection, and Interpretation of Aerobic Bacteriology Cultures. In: Leber Al, ed. Clinical Microbiology Procedures Handbook. 4th ed. Washington, DC, USA: ASM Press; 2016.
9. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 26th ed. Wayne, PA: CLSI; 2016.
10. Salehifar E, Abedi S, Mirzaei E, Kalhor S, Eslami G, Ala S, et al. Profile of microorganisms involved in nosocomial pneumonia and their antimicrobial resistance pattern in intensive care units of Imam Khomeini hospital, Sari, 2011-2012. J Mazandaran Univ Med Sci. 2013;23(Suppl 1):151-62. [Persian].
11. Maebed AZM, Gaber Y, Bakeer W, Dishisha T. Microbial etiologies of ventilator-associated pneumonia (VAP) in intensive care unit of Beni-Suef University's Hospital. Beni Suef Univ J Basic Appl Sci. 2021;10(1):41. doi: 10.1186/s43088-021-00130-x.
12. Krishnamurthy V, Vijay Kumar GS, Prashanth HV, Prakash R, Kumar MS. Ventilator associated pneumonia: bacterial isolates and its antibiotic resistance pattern. Int J Biol Med Res. 2013;4(2):3135-8.
13. Ghafouri M, Garevani T, Seyed Sharifi SH, Moshirfar M. Bacterial isolation and its related factors in hospitalized patients with hospital acquired pneumonia in intensive care unit patients of Imam Reza hospital in Bojnurd, Iran. J North Khorasan Univ Med Sci. 2014;6(1):125-30. doi: 10.29252/jnkums.6.1.125. [Persian].
14. Sole ML, Poalillo FE, Byers JF, Ludy JE. Bacterial growth in secretions and on suctioning equipment of orally intubated patients: a pilot study. Am J Crit Care. 2002;11(2):141-9.
15. Salimi S, Akdemir N, Anami I, Nourouzinia S, Rastad MA. Effect of standardization of nursing cares on incidence of nosocomial infection in MICU. J Urmia Univ Med Sci. 2009;19(4):310-5. [Persian].
16. Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis. 2007;44 Suppl 2:S27-72. doi: 10.1086/511159.
17. Bergogne-Bérézin E, Towner KJ. Acinetobacter spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. Clin Microbiol Rev. 1996;9(2):148-65. doi: 10.1128/cmr.9.2.148.
18. Thakuria B, Singh P, Agrawal S, Asthana V. Profile of infective microorganisms causing ventilator-associated pneumonia: a clinical study from resource limited intensive care unit. J Anaesthesiol Clin Pharmacol. 2013;29(3):361-6. doi: 10.4103/0970-9185.117111.
19. Malik MI, Malik M, Chaudhary A. Antimicrobial susceptibility pattern of bacteria isolated from tracheal secretions in intensive care units admitted patients of Lahore General Hospital. Pak J Chest Med. 2018;24(2):72-7.