Antibiotic Susceptibility of Aerobic Gram-Negative Bacilli Isolated From Patients Admitted in Intensive Care Units of Sina Hospital, Tabriz, Iran

Shahrokh Hagi Saghati[1], Haedeh Mobaiyen[2], Zhinous Bayatmakoo[3*]

Abstract

Objective: Due to critical clinical situation and use of mechanical devices, managements including antibiotics and contact with hospital environment and healthcare workers, patients in intensive care units (ICUs) are susceptible to various infections, particularly those arising from aerobic gram-negative bacilli with multi-drug resistance. Knowledge of the pattern of antibiotics susceptibility and their empirical therapy helps prevent and reduce morbidity and mortality of infectious disease in ICU.

Materials and Methods: In this retrospective study, the medical records of all patients admitted to ICUs of Sina hospital of Tabriz from 2011 to 2015 were evaluated, and the information was extracted from questionnaires and statistically analyzed with SPSS 16.

Results: Among a total of 332 patients, 101 (30.42%) had nosocomial infection with gram-negative bacilli and *Escherichia coli* (*E. coli*) had the highest prevalence (35.6%). Urinary tract infection (UTI) (76.23%), pneumonia (18.81%), and septicemia (4.9%) were the most frequent nosocomial infections. Forty-one patients (12.3%) had positive cultures for gram-positive and fungal infections. Additionally, 46.53% of patients were female, and no significant difference existed between the gender quality variable and the isolated aerobic gram-negative bacilli ($P > .005$). The highest antibiotic resistance was against ampicillin, amikacin and piperacillin and the highest sensitivity was to imipenem.

Conclusion: Urinary tract and respiratory tract infections were the first and second common source of infection in ICU and *E. coli* as an aerobic gram-negative bacillus was the most important etiologic agent. The highest antibiotic susceptibility was to imipenem.

Keywords: Gram-negative aerobic bacteria, Drug resistance, Intensive care units

Introduction

Patients admitted to the intensive care units (ICU) are susceptible to various infections including multidrug resistant bacteria because of intubation and connection to ventilator, NG tubes, urinary, vascular, and central catheters, use of antibiotics and other drugs, critical situation, contact with healthcare workers, and nosocomial infection conditions (1). Gram-negative bacilli are among the important etiologic factors and cause a wide range of problems in managing the disease due to their resistance patterns as well as emergence of broad spectrum b-lactamase-producing enzyme (ESBL) strains (1-8).

Hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP) are the main syndromes in the ICU and refer to the occurrence of respiratory tract infection in patients 48 hours after hospitalization and infection in the ICU 48 hours after intubation and mechanical ventilation, respectively. These syndromes are the major

cause of morbidity and mortality and high cost of therapy (1). Bacteria originating from the endogenous flora of patients, hospital staff, equipment, and environmental factors are often the cause of these syndromes (1).

Bacterial pathogens in the ICU have a dynamic range and vary according to time, hospital, type of ICU, and specific community of patients (1). HAP and VAP are caused by gram-negative bacilli in 60% of the cases and by *Staphylococcus aureus* in 20%-40%. The prevalence of *Acinetobacter baumannii* has increased as a source of infection in the ICU in the last decade and its control and eradication is difficult because of its resistance to most antibiotics (1). Urinary Tract Infection (UTI) rising from urinary catheterization is another common syndrome which occurs as a hospital-acquired infection in the ICU. Most gram-negative bacilli are multiresistant making it difficult to treat and manage (1). Infection of wound (bedsore, surgical wound) in the ICU can result from gram-negative, gram-positive

[1]Collage of Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran. [2]Department of Microbiology, College of Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran. [3]Infections and Tropical Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran, Department of Infectious Disease, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
***Corresponding author:** Zhinous Bayatmakoo, Tel: +98 4133375411, Email: zhinous_b@yahoo.com

and anaerobic bacteria and drug resistance may complicate the problem (1).

Given the importance of this issue, the morbidity and mortality and high diagnostic and therapeutic costs imposed by aerobic gram-negative bacilli in ICUs, the present study aimed at evaluating the prevalence of infectious gram-negative bacilli as well as the susceptibility pattern of bacteria isolated in the ICUs of Sina hospital of Tabriz (surgical, burns, internal, and infectious) in order to reduce morbidity and morbidity and overall costs of diagnosis and treatment through using these information for selecting appropriate antibiotics.

Materials and Methods

In this retrospective study, medical records of all patients admitted to different ICUs of Sina hospital (burns, internal, infectious, and surgical) were randomly evaluated from 2011 to 2015. The internal ICU was newly established and included the least studied cases.

The questionnaire consisted of demographic information including age, gender, occupation, type of ICU, socioeconomic situation, primary diagnosis of disease, severity (based on vital and clinical signs), chronic underlying diseases, organ infection, prescribed antibiotics, previous use of antibiotics, recent hospitalization, intubation, NG tube, urinary and central catheterization, laboratory test results (WBC count, ESR, CRP, U/A, U/C, B/C), respiratory secretions culture, wound and sterile areas culture, stool exam), antibiotic resistance and sensitivity pattern based on antibiogram, changing of antibiotics based on culture and antibiogram, radiological findings, results of arterial blood (ABG), duration of ICU stay, and patient outcome. The results are expressed as mean ± standard deviation and frequency percent using SPSS version 16. The quantitative variables were analyzed with t test and Pearson correlation coefficient, and qualitative variables with chi-square and if necessary, Fisher exact test. In all cases, $P < .05$ was considered statistically significant.

Results

In this retrospective study, among 332 patients admitted to the ICUs of Sina hospital including surgical, internal medicine, infectious diseases, and burns, 101 patients (30.42%) had positive cultures for gram-negative bacteria and 41 patients (12.34%) had positive cultures for gram-positive bacteria and fungi.

Minimum and maximum age was 12 and 96 years, respectively, with a mean of 61.98 ± 21.85 years. The age range of patients with gram-negative bacilli was 16 to 95 years with a mean of 59.11 ± 24.20 years. Among 101 patients with gram-negative infections, 46.53% were female. No significant difference existed between the gender qualitative variable and aerobic gram-negative bacilli isolated from patients hospitalized in ICUs of Sina hospital ($P > .005$). The primary cause of hospitalization in ICUs included malignancies, infections, fractures, surgery, renal failure, poisoning, and seizures, which were the primary causes of hospitalization of patients in the ward and patients with

hospital-acquired infections with a frequency of 28.82% and 29.2%, respectively; and a significant relationship existed between the primary cause of hospitalization and nosocomial infection ($P < .05$).

There was a history of surgery in 101 patients admitted to the ICU and 23 patients (22.77%) admitted to the general ward. A history of antibiotics consumption existed in 234 hospitalized patients (45.9%) and in 53 patients (47.52%) with nosocomial infections. No statistically significant correlation was found in this study between the underlying risk factors of surgery and antibiotic use with nosocomial infection ($P > .05$). The mean length of stay (LOS) of 332 patients admitted to ICUs was 8.05 ± 7.3 days and the mean LOS of patients with positive cultures for gram-negative bacteria was 13.04 ± 9.82 days.

Age distribution, LOS, and days of implant use according to Kolmogorov-Smirnov test was not normal, and the difference of means using Mann-Whitney test between the two groups of patients with and without nosocomial infections (231 patients without nosocomial infection symptoms) was assessed and statistical analysis showed significant differences in the mentioned three variables in the 2 groups.

In this study, 312 patients hospitalized in ICUs (61.2%) and 63 patients with nosocomial infections (62.72%) hospitalized in ICUs had an underlying disease such as diabetes, renal failure, and alcoholism. There was no significant difference between the underlying disease and affection with nosocomial infection ($P > .05$).

In this study, aerobic gram-negative bacteria which are the most important cause of nosocomial infection were iso-

Table 1. Prevalence of Gram Negative Bacteria in Nosocomial Infection in ICU Patients

Gram Negative Bacteria	Number	Percent
Klebsiella pneumoniae	25	24.75
Escherichia coli	36	35.64
Pseudomonas aeruginosa	12	11.88
Acinetobacter baumannii	10	9.9
Enterobacter aerogenes	7	6.9
Proteus mirabilis	6	5.94
Serratia marcescens	2	1.98
Citrobacter freundii	2	1.98
Alcaligenes	1	0.99
Total	101	100

Table 2. Type of ICUs and Etiologic Agents

Type of ICU	Gram Negative Bacteria
Surgical	*E. coli, P. aeruginosa, K. pneumoniae*
Internal	*K. pneumoniae, E. coli, P. aeruginosa*
Infection	*P. aeruginosa, E. coli, K. pneumoniae*
Burns	*E. coli, P. aeruginosa, K. pneumonia*

Table 3. Prevalence of Gram Negative Bacterial Infection in ICU Patients

Bacteria	Site of Infection		
	Urinary tract	Pneumonia	Hematogen
Klebsiella pneumoniae	12.98	73.68	20
Escherichia coli	45.45	5.26	0
Pseudomonas aeruginosa	12.98	10.52	0
Acinetobacter baumannii	10.38	0	40
Enterobacter aerogenes	6.49	0	40
Proteus mirabilis	9.09	0	0
Serratia marcescens	1.29	0	0
Citrobacter freundii	1.29	5.26	0
Alcaligenes	0	5.26	0
Total	100	100	100

Table 4. Antibiotic; Resistance Pattern of Gram Negative Bacteria in ICU Patients

Antibiotic	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*A. baumannii*	*E. aerogenes*	*P. mirabilis*
Gentamicin	10 (27.77%)	12 (48%)	3 (25%)	10 (100%)	4 (57.1%)	3 (50%)
Pipracillin-Tazobactam	13 (36.11%)	16 (64%)	4 (33.33%)	4 (40%)	2 (28.6%)	1 (16.7%)
Ampicillin	29 (80.55%)	24 (96%)	11 (91.66%)	10 (100%)	7 (100%)	6 (100%)
Trimethoprim-sulfamethoxazole (TMP/SMX)	17 (47.22%)	18 (72%)	10 (83.33%)	9 (90%)	3 (57.1%)	4 (83.3%)
Nitrofurantoin	5 (13.88%)	13 (52%)	12 (100%)	9 (90%)	3 (42.9%)	4 (66.7%)
Ciprofloxacin	21 (58.33%)	17 (68%)	4 (33.33%)	6 (60%)	3 (42.9%)	1 (16.7%)
Cefotaxime	22 (61.11%)	22 (88%)	11 (91.66%)	22 (88%)	5 (71.4%)	4 (66.7%)
Ceftazidime	23 (63.89%)	23 (92%)	6 (50%)	9 (90%)	5 (71.4%)	1 (16.7%)
Cefepime	24 (66.66%)	22 (88%)	7 (58.33%)	10 (100%)	4 (57.1%)	4 (66.7%)
Amikacin	29 (80.55%)	24 (96%)	5 (41.66%)	10 (100%)	7 (100%)	6 (100%)
Imipenem	3 (8.33%)	2 (88%)	3 (25%)	5 (50%)	0 (0%)	1 (16.7%)
Piperacillin	24 (66.66%)	22 (88%)	6 (50%)	8 (80%)	5 (71.4%)	4 (66.7%)
Ceftriaxone	23 (63.88%)	23 (92%)	8 (66.66%)	10 (100%)	4 (57.1%)	2 (33.3%)

lated from 101 patients (30.42%; Table 1). The most common etiologic agents of gram-negative bacilli were studied according to the types of ICUs (Table 2). Various hospital-acquired infections caused by gram-negative bacteria were UTI (76.23%), pneumonia (18.81%), and sepsis (4.9%) (Table 3). The results of antibiogram were assessed based on sensitivity and resistance (Tables 4 and 5).

Discussion
ICU includes all critical cares related to patient's outcome; to be more precise, it refers to care of patients with chronic and life threatening illnesses, with a wide range of clinical conditions and somehow dysfunction of a single or multiple organs, in particular respiratory and cardiovascular systems. These units are the reservoirs of especially virulent and resistant bacteria which can easily disseminate through personnel's hands and equipment to the patients. On appreciated antibacterial therapy can also lead to colonization of resistant organisms. Among the organisms, aerobic gram-negative species and Staphylococcus species

are the most prevalent agents for this type of infections (9). In this study, among 332 patients admitted to the ICUs of Sina hospital of Tabriz, 101 patients (30.42%) were infected with gram-negative bacilli. *E. coli* which was the most common species among of isolated gram-negative bacilli, and urinary tract was the most common source of infection. Antibiotic resistance was observed in the maximum isolates.

Most studies show that the high prevalence of gram-negative bacteria isolated from patients in ICUs is associated with a high rate of mortality (10).

The incidence of nosocomial infections with gram-negative bacteria was 30.42% which is almost consistent with the prevalence of nosocomial infections in the ICU mentioned in other references (11). Other studies reported a prevalence of 17.1% and 15.6% (12,13).

No significant relationship was found between age and gender and nosocomial infection; this is consistent with studies carried out in Brazil (3). In other studies (12,14), a significant relationship was reported between age and

Table 5. Antibiotic; Sensitivity Pattern of Gram Negative Bacteria in ICU Patients

Antibiotic	E. coli	K. pneumoniae	P. aeruginosa	A. beumannii	E. aerogenes	P. mirabilis
Gentamicin	24 (66.66%)	12 (48%)	8 (66.66%)	0 (0%)	4 (42.9%)	3 (50%)
Pipracillin-Tazobactam	17 (47.22%)	8 (32%)	6 (50%)	4 (40%)	3 (42.9%)	5 (83.3%)
Ampicillin	3 (8.66%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Trimethoprim-Sulfamethoxazole (TMP/SMX)	17 (41.22%)	7 (28%)	0 (0%)	0 (0%)	3 (42.9%)	1 (16.7%)
Nitrofurantoin	30 (83.3%)	7 (28%)	0 (0%)	1 (10%)	2 (28.6%)	2 (33.3%)
Ciprofloxacin	14 (38.88%)	5 (20%)	8 (66.66%)	3 (30%)	4 (57.1%)	4 (66.7%)
Cefotaxime	13 (36.11%)	3 (12%)	1 (8.33%)	3 (12%)	2 (28.6%)	1 (16.7%)
Ceftazidime	13 (36.11%)	2 (8%)	5 (41.66%)	0 (0%)	2 (28.6%)	4 (66.7%)
Cefepime	12 (33.33%)	3 (12%)	4 (33.33%)	0 (0%)	2 (28.6%)	1 (16.7%)
Amikacin	3 (8.66%)	0 (0%)	7 (58.33%)	0 (0%)	0 0%)	0 (0%)
Imipenem	33 (91.66%)	21 (84%)	9 (75%)	4 (40%)	7 (100%)	5 (83.3%)
Piperacillin	10 (27.77%)	2 (8%)	6 (50%)	2 (20%)	2 (28.6%)	1 (16.7%)
Ceftriaxone	11 (30.55%)	2 (8%)	1 (8.3%)	0 (0%)	2 (28.6%)	1 (16.7%)

nosocomial infections.

A significant relationship existed in this study between the primary cause of hospitalization, the number of days of implant use, the number of days of hospital stay, and nosocomial infection which was consistent with other studies (15,16).

In our study, no significant association was found between the use of antibiotics, history of surgery, and underlying disease with hospital-acquired infections; this was different from other studies (17,18).

The most common isolated gram-negative bacteria was *E. coli* and the most common nosocomial infection was UTI (76.23%); these findings were consistent with the study by Sava et al (19) reporting *E. coli* as the most important pathogen of UTI. This study was also consistent with other studies (20-22), all of which had *E. coli* as the most common organism. UTI was also highly frequent in a study by Duque et al (3).

In the present study, *Acinetobacter baumannii* strains had high resistance to most antibiotic discs that was consistent with other studies (23-26). The highest resistance was seen in amikacin, ampicillin, piperacillin discs and imipenem was the most effective antibiotic against all isolated gram-negative strains responsible for nosocomial infections except *Acinetobacter baumannii* which is consistent with other studies (27-30).

Ethical issues
This prospective study is approved by ethical committee of Islamic Azad University Branch Tabriz.

Conflict of interests
The authors declare no conflict of interests.

Acknowledgments

References
1. Jarvis WR. Epidemiology of nosocomial infections in pediatric patients. Pediatr Infect Dis J 1987;6(4):344-351.
2. Haley RW. The scientific basis for using surveillance and risk factor data to reduce nosocomial infection rates. J Hosp Infect 1995;30(Suppl 3):3-14.
3. Duque AS, Ferreira AF, Cezario RC, Gontijo Filho PP. Nosocomial infections in two hospitals in Uberlandia, Brazil. Rev Panam Infcctol 2007;9(4):14-18.
4. Weinstein RA. Nosocomial infection update. Emerg Infect Dis 1998;4(3):416-419.
5. Fridkin SK, Weibel SF, Weinstein RA. Magnitude and prevention of nosocomial infections in the intensive care unit. Infect Dis Clin North Am I997;l l(2):479-496.
6. Gain A, Singh K. Recent advances in the management of nosocomial infections. JK Science 2007;9(1):3-8.
7. Rowland SS, Walsh SR, Till LD, Carnahan AC. Pathogenic and Clinical Microbiology: A Laboratory Manual. 1st ed. London: Little Brown and company; 1994:103-185.
8. National Committee for Clinical Laboratory Standards (NCCLS) for disk diffusion antimicrobial susceptibility test for bacteria grown aerobically. 4th ed. Wayne, PA, USA; 2000.
9. Sharifi M, Assefzadeh M, Javadi A, Kargar A. Prevalence of methicillin-resistant Staphylococcus aureus colonization in patients hospitalized in ICUs in Qazvin's university hospitals-2006 (In Persian). Iran J

Med Microbiol 2009;3(2 and 3):40-46.

10. Vincent JL, Bihari DJ, Suter PM, et al. The prevalence of nosocomial infection in intensive care units in Europe. European Prevalence of Infection in Intensive Care (EPIC) Study. EPIC International Advisory Committee. JAMA 1995;274(8):639-644.

11. Edmond MB, Wenzcl RP. Nosocomial infection. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and Practice of Infectious Diseases.. 5th ed. New York: Churchill Livingstone; 1999: 2988.

12. Minnahdavi F. Study of nosocomial infection in Imam Khomeini hospital, Tabriz. Iranian Conference on Infectious Diseases and Tropical Medicine. Abstract book 2002;46-48.

13. Hajibagheri K, Afrsiabian SH. An epidemiologic study of nosocomial infections and its related factors at the intensive care unit of Tohid Hospital, in Sanandaj during 2003-2004. SJKU 2006;10(4):44-50. [In Persian]

14. Izquierdo Cubas F, Zambrano A, Frometa I, et al. National prevalence of nosocomial infections. Cuba 2004. J Hospital Infect 2008;68(3):234-240.

15. Girou E, Stephan F, Novara A, Safar M, Fagan JY. Risk factors and outcome of nosocomial infections: results of a matched case-control study of ICU patients. Am J Respir Crit Care Med 1998;157(4 Pt 1):1151-1158.

16. Kawagoe JY, Segre CA, Pereira CR, Cardoso MF, Silva CV, Fukushima JT. Risk factors for nosocomial infections in critically ill newborns: a 5-year prospective cohort study. Am J Infect Control 2001;29(2):109-114.

17. Torres A, Aznar R, Gatell JM, et al. Incidence risk, and prognosis factors of nosocomial pneumonia in mechanically ventilated patients. Am Rev Respir Dis 1990;142(3):523-528.

18. Marik P. The ICU Therapeutics Hand Book. St Louis: Mosby Co; 1996:240-253.

19. Savas L, Guvel S, Onlen Y, Savas N, Duran N. Nosocomial urinary tract infections: micro-organisms, antibiotic sensitivities and risk factors. West Indian Med J 2006;55(3):188-193.

20. Aminzade Z, Zare Dehabadi M, Gachkar L, Shah Hoseini R. Evaluation of gram negative infections and their antibiotic susceptibility in Loghman Hakim Hospital, 2005. Iranian J Infect Dis Tropical Med 2005;10(30): x-x. [In Persian].

21. Pfaller MA, Jones RN, Doern GV, Kugler K. Bacterial pathogens isolated from patients with blood stream infection: frequencies of occurrence and antimicrobial surveillance program (United States and Canada, 1997). Antimicrob Agents Chemother 1998;42(7):1762-170.

22. Aubry Damon H, Courvalin P. Bacterial resistance to antimicrobial agents: selected problem in France, 1996 t 1998. Emerg Infect Dis 2000;5(3):315-320.

23. Mohammadimehr M, Feizabadi MM, Bahadori O, Motashaker Arani M, Khosravi M. Study of prevalence of gram- negative bacteria caused nosocomial infections in ICU in Besat hospital in Tehran and detection of their antibiotic resistance pattern-year 2007. Iran J Med Microbiol 2009;3 (2 and 3):47-54. [In Persian].

24. Jain R, Danziger LH. Multidrug resistant Acinetobacter infections: an emerging challenge to clinicians. Ann Pharmacother 2004;38(9):1449-1459.

25. Singh AK, Sen MR, Anupurba S, Bhattacharya P. Antibiotic sensitivity pattern of the bacteria from nosocomial infection in ICU. J Commun Dis 2002;34(4):257-263.

26. Hsueh PR, Teng LJ, Chen CY, et al. Pandrug-resistant Acinetobacter baumannii causing nosocomial infections in a university hospital, Taiwan. Emerg Infect Dis 2002;8(8):827-832.

27. Mendes C, Oplustil C, Sakagami E, Turner P, Kiffer C. Antimicrobial susceptibility in intensive care units: MYSTIC Program Brazil 2002. Braz J Infect Dis 2005;9(1):44-51.

28. Chen MJ, Wang H. [Continuous surveillance of antimicrobial resistance among nosocomial gram-negative bacilli from intensive care units in China]. Zhonghua Yi Xue Za Zhi 2003;83(5):375-381. [In Chinese].

29. Jones RN, Pfaller MA, Doern GV, Erwin ME, Hollis RJ. Antimicrobial activity and spectrum investigation of eight broad- spectrum beta-lactam drugs: a 1997 surveillance trial in 102 medical centers in the United States. Cefepime Study Group. Diagn Microbial Infect Dis 1998;30(3):215-228.

30. Hadadi A, Rasoulinejad M, Maleki Z, Yonesian M, Shirani A, Kourorian Z. Antimicrobial resistance pattern of Gram-negative bacilli of nosocomial origin at 2 university hospitals in Iran. Diagn Microbiol Infect Dis 2008;60(3):301-305.