Iranian Journal of Pharmaceutical Research (2008), 7 (4): 305-309
Received: March 2008
Accepted: July 2008

Original Article

Prevalence of Microorganisms Causing Septicemia and Determination of Antimicrobial Resistance in Intensive Care Unit

Yunes Panahi [a*], Mojtaba Mojtahedzadeh [b], Fatemeh Beiraghdar [c], Marzeyeh Pazooki [d] and Yashar Moharamzad [a]

[a]Research Center of Chemical Injuries, Baqiyatallah Medical Sciences University, Tehran, Iran. [b]Pharmaceutical Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. [c]Nephrology and Urology Research Center, Baqiyatallah Medical Sciences University, Tehran, Iran. [d]ICU of Sina Hospital, Tehran University of Medical Sciences, Tehran, Iran.

Abstract

The objective of this study was to determine the frequency of microorganisms isolated from blood samples of patients admitted to Intensive Care Unit (ICU), and the rate of their resistance to common administered antibiotics in an Iranian ICU. One-hundred patients with Systemic Inflammatory Response Syndromes (SIRS), hospitalized in ICU of Sina Hospital, Tehran, Iran, were entered to this cross-sectional study from March 2005 to March 2006. Blood samples were taken from these patients and transferred to culture medium. After detecting the type of microorganism, minimum density for inhibition of growth of microorganisms by antibiotics, using minimum inhibitors concentration (MIC) method, was determined for ceftazidime, ceftriaxone, amikacin, ciprofloxacin, meropenem and cefepime. Culture results of 38 patients were positive (38%). *Klebsiella* (14 patients, 37%), coagulase negative *Staphylococcus* (9 patients, 23.6%), *Pseudomonas* and *Acinetobacter* (each of them recovered in 6 patients, 15.8%), *Staphylococcus aureus* (one patient, 2.6%) and gram positive bacilli (2 patients, 5.2%) were detected microorganism. Only *Staphylococcus aureus* was resistant to all studied antibiotics. Gram-negative bacilli showed resistance to all antibiotics except for amikacin and meropenem.

The rate of septicemia detected was higher in comparison to developed countries. Clinical suspicion with appropriate microbiological tests is essential for early diagnosis to prevent adverse outcomes in patients admitted to ICU.

Keywords: Microbial resistance; Antibiotic; Septicemia; ICU.

Introduction

Systemic Inflammatory Response Syndrome (SIRS) includes infectious (local and general) and non-infectious causes (trauma, thermal injuries, etc) which would raise high inflammatory response of the host with systemic manifestations (1). Presence of evidences of infection together with systemic responses to this infection like fever, hypothermia, tachycardia, etc is described as sepsis (2). Sepsis is one of the prevalent infections among patients admitted to Intensive Care Units (ICUs), with a significant morbidity,

* Corresponding author:
E-mail: yunespanahi@yahoo.com

and the rate of its mortality is estimated to be from 25% to 80% (3, 4). Almost 751,000 persons with sepsis have been detected in US in 1995, resulting in 215,000 deaths. The average cost per case of sepsis was $22,100 with total costs of $16.7 billion nationally (5).

ICU-associated infections are mostly due to *Staphylococcus aureus* and coagulase negative *Staphylococcus* (like *Staphylococcus epidermidis*) and usually because of contaminated intra vascular devices like artificial heart valves, intravenous and intra-arterial catheters (6).

Administration of antibiotics to control infection, prescription of proper amounts of liquid, oxygen and vasoconstrictor medications are the basic treatments of sepsis syndrome (7).

Before accurate diagnosis by specific culture media is made, different antibiotics are prescribed for septic patients in ICUs. Selection of appropriate antibiotics that can cover both positive and gram negative microorganisms, at this phase is usually empiric. This selection is depending on factors like epidemiological evidences, antibiotic resistance patterns and probable infective causes (8). Prevalence of microorganisms causing septicemia in ICUs depends on environmental and geographical locations. In developing countries, many hospitals are not equipped with isolation facilities (9), and it is of high probability that prevalent microorganisms in hospitals of these countries may be different from those in developed countries. Apart from different prevalence, these microorganisms show various degrees of resistance to antibiotics. The aim of this study was to determine the prevalence of microorganisms extracted from blood samples of patients admitted to ICU of a university general hospital in Tehran, the capital of Iran and to recognize degree resistance of the micro organisms to commonly used antibiotics.

Experimental

In this observational study which conducted from March 2005 to March 2006, 100 septic patients hospitalized in ICU of Sina Hospital were included. The inclusion criteria were age older than 18 years, and presence of SIRS. This syndrome was defined as presence of at least two of the following criteria (2): fever or hypothermia ($38\,^{\circ}C<T<36\,^{\circ}C$), leukocytosis or leucopenia ($12000/mm^3<$white blood cell count$<4000/mm^3$ or Band cell$>10\%$), tachypnea ($RR>20$/min or $PCO2<32$ mmHg), and tachycardia ($HR>90$ beat/min). The score on the Acute Physiology and Chronic Health Evaluation (APACHE) II (10) at admission was also calculated.

Blood samples were taken from patients' veins using sterile syringes. Five milliliters of the sample was transferred to aerobic and anaerobic culture media. This sample was then taken to the laboratory and cultured in nutrient agar environment using four-zone method. It was kept in incubator for 24 h and then taken to the microbiology laboratory. After appearance of bacteria colonies, each colony would be cultured separately in order to get pure bacterial colonies. Type of microorganism identification (bacillus, coccus or yeast) in singel colony was done by microscope after gram staining and for accurate recognition, it was cultured in selectiv medium as follow:

1. Cetrimide agar culture medium for *Pseudomonas*, that existence of *Pseudomonas* would cause green mucoid colony. Existence of cetrimide would prevent growth of confounding bacteria.
2. EMB (Eosin Methylene Blue) and TSI (Triple Sugar Iron Agar) culture media for Klebsiella, which would cause red colored colony in EMB medium and in TSI medium would change into Acid/Acid with yellow color.
3. In order to detect *Staphylococcus aureus*, manitol salt agar private culture medium was used on which turns color of the medium to yellow.
4. EMB culture medium for *Acinetobacter* that causes red colored colony.

Other materials used in this study were Mueller-Hinton agar & broth, nutrient agar, casein-peptone soymeal-peptone agar (CASO Agar), soybean casein digest agar (SCDA), *Salmonella, Shigella*, EMB and manitol salt culture medium, which were purchased from Merck Co., Germany.

Minimum inhibitory density of growth of microorganisms was determined by MIC (Minimum Inhibitory Concentration) method.

Table 1. Demographic, APACHE score, serum glucose level, and mean arterial pressure in two group of ICU admitted patients according to presence of septicemia.

	Non-septicemia (n = 62)	Septicemia (n = 38)	P Value
Age, year	55.7 (±20.2)	55.5 (±19.6)	0.9
Gender			
Male	41 (66.1%)	23 (60.5%)	
Female	21 (33.9%)	15 (39.5%)	0.5
Hospitalization duration, days	16.3 (±15.2)	23 (±16)	0.04
APACHE			
At admission	16.1 (±6.8)	16.1 (±6.2)	0.9
At day 7	17.6 (±6.7)	16.8 (±5.6)	0.6
Serum glucose level, mg/dl	174.3 (±85.5)	135 (±41.5)	0.01
Mean arterial pressure, mmHg	89.6 (±14.1)	97.5 (±15.4)	0.02

All data are expressed as means (± standard deviation) other than gender.

MIC is the minimum density of antibiotic (µg/ml) that can prevent visible growth of microorganisms. In this study, broth micro dilution method (a common method in microbiology laboratories) was used for MIC test. Antibiotics used in this study included cefepime, ceftazidime, amikacin, ciprofloxacin, meropenem, and ceftriaxone. Antibiotics dilution was done by two fold (1/2) method with maximum and minimum density of 64 and 0.5 (µg/ml), respectively. In order to evaluate MIC results, NCCLS (National Committee for Clinical Laboratory Standards) recommendations were used (11).

For statistical analyses, descriptive indices like frequency, percentage, mean, and standard deviation (±SD) were used to express data. Comparisons were done using the chi-square and student t-tests, and linear correlation coefficient and Pearson correlation tests. All statistical analyses were done by statistical software SPSS (Ver. 13.0, Chicago, IL).

Results

One hundred patients (36 females, and 64 males) were enrolled. The mean (±SD) age of patients were 55.6 (±19.9). Mean (±SD) duration of hospitalization was 18.8 (±15.9) days. Sixty four percent of patients showed SIRS, that 28 cases (43.7%) were progressed to sepsis. Prevalence of bacteremia was 38% that 28 patients of them (73.7%) had sepsis. Table 1 shows demographics, APACHE II scores, serum glucose level, and mean arterial pressure of patients with septicemia and those without septicemia.

Among the positive culture results (38 patients), *Klebsiella* was the most common pathogen recovered (14 patients, 36.8%). Table 2 presents the frequency of detected microorganisms in 38 patients with septicemia.

Antimicrobial resistance status of microorganisms isolated is shown in Table 3.

Table 2. Frequency of microorganisms isolated from 38 patients with septicemia admitted to ICU.

	Frequency	Percentage
Klebsiella	14	36.8
Coagulase-negative *Staphylococci*	9	23.7
Pseudomonas	6	15.8
Acinetobacter	6	15.8
Staphylococcus aureus	1	2.6
Gram-positive bacilli	2	5.3
Total	38	100

Table 3. Antimicrobial resistance of microorganisms according to MIC[a].

	Klebsiella	Coagulase negative *Staphylococci*	*Pseudomonas*	*Acinetobacter*	*Staphylococcus aureus*	Gram-positive bacilli
Ceftriaxone	12 (85.7%)	8 (88.9%)	5 (83.3%)	6 (100%)	1 (100%)	2 (100%)
Ciprofloxacin	12 (85.7%)	9 (100%)	5 (83.3%)	5 (83.3%)	1 (100%)	2 (100%)
Cefepime	11 (78.6%)	9 (100%)	6 (100%)	6 (100%)	1 (100%)	2 (100%)
Amikacin	6 (42.9%)	8 (88.9%)	1 (16.7%)	1 (16.7%)	1 (100%)	2 (100%)
Cefixime	13 (92.8%)	9 (100%)	5 (83.3%)	6 (100%)	1 (100%)	2 (100%)
Meropenem	14 (100%)	9 (100%)	5 (83.3%)	0%	1 (100%)	0%

[a]MIC (Minimum Inhibitory Concentration) for tested antibiotics was as follow (in μg/ml): ceftriaxone: 32; ciprofloxacin: 4; cefepime: 32; amikacin: 64; cefixime: 64; meropenem: 16.

Various densities of these microorganisms were exposed to antibiotics in order to detect the resistance limit of them to common antibiotics. As shown, only *Staphylococcus aureus* was resistant to all studied antibiotics. Gram-negative bacilli showed resistance to all antibiotics except for amikacin and meropenem.

Discussion

According to obtained results, prevalence of septicemia was 38%, which is a higher percentage comparing to previous studies. For example, prevalence of bloodstream infections has been reported 18.2% in a Turkish university hospital (12). In another report from 17 ICUs of European countries, septicemia has been reported to be 12% (13). Central venous catheter, hyperglycemia, urinary tract infections (UTI), pneumonia, use of sedative drugs and surgery are among risk factors of bacteremia (12).

Another study which was performed by Brun-Buisson et al. showed that prevalence of SIRS in ICU patients was more than 80% while one-third of them (26.7%) had sepsis (1).

As body systems do not function individually, infection spreads to cause SIRS or sepsis when it defeats defending mechanisms of body. In advanced cases, while SIRS or sepsis occurs, we can detect the origin of infection, consider proper therapy and control infection in ICU based on type of bacteria detected in blood samples.

From the results obtained,it can be deduced that the highest rate of microbial resistance was to ceftriaxone, and the lowest rate to amikacin. *Klebsiella*, the most prevalent microorganism detected in this study, showed the lowest resistance to amikacin. The selected antibiotic for *Acinetobacter* microorganism is meropenem as no microbial resistance to meropenem was observed. Studies performed in European and American countries, revealed a resistance rate of 13.6% of *Acinetobacter* infection to meropenem (14), although one study reported the resistance percentage as 3.5% (15).

In the current study, septic group had higher levels of mean arterial pressure and lower blood glucose in comparison with non septic group. It should be mentioned that sepsis has the criteria to be considered as SIRS, for which a microbial etiology has been proven or suspected. However, severe sepsis is the situation accompanied by one or more signs of organ dysfunction and mean arterial pressure≤70 mmHg that respond to administration to of intravenous fluid (16, 17). Regarding this definition, the blood pressure is not a diagnostic criteria of sepsis but, in fact is one of the criteria of severe sepsis, responded to fluid resuscitation which in turn may lead to blood pressure normalization. This may explain the normal mean arterial pressure of this group, it has been shown that blood glucose decreases with insulin therapy in critically ill patients.It also results in less mortality (18) and morbidity (19). Blood glucose levels less than 8-9 mmol/lit (144-162 mg/dl) (20) or less than 145-150 mg/dl (21, 22) are ideal in these patients. It was shown that blood glucose decreasing with insulin therapy in critically ill patients, results in less mortality (18) and morbidity (19), and blood glucose levels less than 8-9 mmol/l (144-162 mg/dl) (20) or less than 145-150 mg/dl (21, 22) were more favorable.

Vancomycin is the drug of choice for infection

caused by methicillin resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* (22, 23). In conclusion, the hospitalized patients in ICU of a general university hospital in Iran are at high risk of developing septicemia. High clinical suspicion with appropriate microbiological tests is essential for early diagnosis and proper administration of antimicrobial treatments to reduce mortality rate.

References

(1) Brun-Boissun C. The epidemiology of the systemic inflammatory response. *Intensive Care Med.* (2000) 26: s64-s74

(2) Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, Schein RM, Sibald WJ. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* (1992) 101: 1644-1655

(3) Angus DC and Wax R. The epidemiology of sepsis: an update. *Crit. Care Med.* (2001) 29: s109-s116

(4) Remick DG. Pathophysiology of sepsis. *Am. J. Pathol.* (2007) 170: 1435-44

(5) Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J and Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Crit. Care Med.* (2001) 29: 1303-1310

(6) Rangel-Frausto MS. Sepsis: still going strong. *Arch. Med. Res.* (2005) 36: 672-81

(7) Asfar P, De Backer D, Meier-Hellmann A, Radermacher P and Sakka SG. Clinical review: influence of vasoactive and other therapies on intestinal and hepatic circulations in patients with septic shock. *Crit. Care* (2004) 8: 170-9

(8) Leone M, Bourgoin A, Cambon S, Dubuc M, Albanese J and Martin C. Empirical antimicrobial therapy of septic shock patients: adequacy and impact on the outcome. *Crit. Care Med.* (2003) 31: 462-7

(9) Reza MW, Kazi BM, Mustafa M and Gould FK. Developing countries have their own characteristic problems with infection control. *J. Hosp. Infect.* (2004) 57: 294-9

(10) Knaus WA, Draper EA, Wagner DP and Zimmerman JE. APACHE II: a severity of disease classification system. *Crit. Care Med.* (1985) 13: 818-29

(11) National Committee for Clinical Laboratory Standards. Approved standard M7-A4. Method for dilution antimicrobial susceptibility test for bacteria that grow aerobically. 4th ed. NCCLS, Wayne (1997)

(12) Meric M, Willke A, Caglayan C and Toker K. Intensive care unit-acquired infections: incidence, risk factors and associated mortality in a Turkish university hospital. *Jpn. J. Infect Dis.* (2005) 58: 297-302

(13) Vincent JL, Bihari DJ, Suter PM, Bruining HA, White J, Nicolas-Chanoin MH, Wolff M, Spencer RC, Hemmer M. The prevalence of nosocomial infection in intensive care units in Europe. Results of the European revalence of infection in intensive care (EPIC) study. EPIC International Advisory Committee. *JAMA* (1995) 274: 639-44

(14) Jones ME, Draghi DC, Thornsberry C, Karlowsky JA, Sahm DF and Wenzel RP. Emerging resistance among bacterial pathogens in the intensive care unit-a European and North American surveillance study (2000-2002). *Ann. Clin. Microbiol. Antimicrob.* (2004) 3: 14

(15) Kucukates E and Kocazeybek B. High resistance rate against 15 different antibiotics in aerobic gram-positive bacteria isolates of cardiology intensive care unit patients. *Indian J. Med. Microbiol.* (2002) 20: 208-10

(16) Bernard GR, Vincent JL, Laterre PF, LaRosa SP, Dhainaut JF, Lopez-Rodriguez A, Steingrub JS, Garber GE, Helterbrand JD, Ely EW and Fisher CJ Jr. Recombinant human protein C worldwide evaluation in severe sepsis (PROWESS) study group. Efficacy and safety of recombinant human activated protein C for severe sepsis. *N. Engl. J. Med.* (2001) 344: 699-709

(17) American College of Chest Physician Society of Critical Care Medicine Consensus Conference. Definition of sepsis and organ failure and guidelines for the use of innovation therapies sepsis. *Chest* (1992) 101: 1644-1655

(18) van den Berghe G, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, Vlasselaers D, Ferdinande P, Lauwers P, Bouillon R. Intensive insulin therapy in the critically ill patients. *N. Engl. J. Med*. (2001) 345: 1359-67

(19) Van den Berghe G, Wilmer A, Hermans G, Meersseman W, Wouters PJ, Milants I, Van Wijngaerden E, Bobbaers H, Bouillon R. Intensive insulin therapy in the medical ICU. *N. Engl. J. Med.* (2006) 354: 449-61(2006) 354: 449-61

(20) Henderson WR, Chittock DR, Dhingra VK and Ronco JJ. Hyperglycemia in acutely ill emergency patients-cause or effect? *CJEM* (2006) 8: 339-43

(21) Finney SJ, Zekveld C, Elia A and Evans TW.Glucose control and mortality in critically ill patients. *JAMA* (2003) 290: 2041-7

(22) Ena J, Dick RW, Jones RN and Wenzel RP. The epidemiology of intravenous vancomycin usage in a university hospital. A 10-year study. *JAMA* (1993) 269: 598-602

(23) Roghmann MC. Predicting methicillin resistance and the effect of inadequate empiric therapy on survival in patients with Staphylococcus aureus bacteremia. *Arch. Intern. Med.* (2000) 160: 1001-4

This article is available online at http://www.ijpr-online.com