Multidrug-Resistant Bacteria Isolated from Intensive-Care-Unit Patient Samples

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We examined epidemiological aspects and bacterial resistance patterns of bacteria isolated from intensive care unit (ICU) patient samples. During a 10 month period (from June 2006 to March 2007), 812 samples of blood, urine and cerebral spinal fluid (CSF) from 553 hospitalized patients, in ICU wards, including pediatric surgical, neonatal, adult surgical I, adult surgical II, general pediatrics, neurosurgical I, neurosurgical II, and internal medical, were collected. Minimum inhibitory concentration (MIC) of antibiotics for bacteria isolates was determined by the E-test method. The internal medicine ICU with 28.7% admissions gave the largest contribution. Coagulase negative staphylococci at frequencies of 66.7 % and 36.5 % and *E. coli* at 20.9% were the bacteria most frequently isolated from the blood, CSF and urine samples, respectively. Samples taken from patients 20-40 years old were the most frequent (32.2%), while the group of patients over sixty years contributed least (18.5%). Both Gram-positive and -negative isolates expressed resistance to most of the penicillins and cephalosporins tested. Combined therapy with vancomycin and meropenem or imipenem gave the most effective treatment against Gram-positive and Gram-negative isolates based on empirical therapy. High frequencies of multiresistant bacteria in ICUs warn us to administer a few effective antibiotics in our hospitals more wisely in order to reduce selective pressure on sensitive strains. This could help save the life of ICU patients and prevent of spread of resistant isolates in these critical wards. Due to continuous changes in antibacterial susceptibility patterns, periodical antibacterial sensitivity assessment in ICUs should be mandatory.

Key-Words: Intensive care unit, minimum inhibitory concentration, multidrug-resistant bacteria.

Patients with severe underlying disorders requiring intensive care are particularly prone to nosocomial infections caused by opportunistic pathogens or hospital strains of bacteria [1].These strains are often resistant to many antimicrobials, because of selective pressure due to extensive use of broad-spectrum antibiotics [2,3]. The application of hospital-wide antibiograms to guide clinicians in the initial choice of antibiotics is a rational and recommended approach, given the differences in susceptibility patterns among hospitals [1]. Susceptibility patterns may also vary among individual hospital wards [4,5]. If organisms that are more resistant are isolated from patients in the intensive care unit (ICU) but not in other hospital wards, this important information could be masked by the use of a hospital-wide antibiogram [2]. This is particularly important for the effectiveness of empirical therapy in critically ill patients.

There are a few published reports available on microbial analysis of patients' samples, determination of antibacterial susceptibility patterns, and duration of stay and effects of previous antibiotic therapy on patterns of antibacterial susceptibility in the region. Such data could be beneficial for the indication of appropriate antibiotics, reducing the length of stay in the hospital, as well as for reducing the morbidity/mortality rate. Furthermore, findings of such regional studies could be projected to other parts of the world.

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Material and Methods

Hospital Setting

We analyzed 812 non-duplicate blood, CSF and urine samples from 553 patients hospitalized in eight ICUs of Nemazee hospital (1,000 beds), affiliated with the Shiraz University of Medical Sciences, from June 2006 to March 2007. One, two, or three different samples were taken from each patient, depending on the infection sites. The criteria for nosocomial infections, i.e., infection symptoms observed at least after 48 hours post admission, with no signs of infection when admitted to the ICU wards, were met for all the samples. Depending on the underlying disorders requiring intensive care, patients were admitted to eight specific ICUs as follows: pediatric surgery (four bed unit), adult surgical I (six bed unit), neurosurgical I (nine bed unit), general pediatrics (10 bed unit), neonatal (10 bed unit), internal medical (11 bed unit), adult surgical II (five bed unit) and neurosurgical II (four bed unit). All necessary information, including demographic data, history of antibiotic therapy and duration of stay was collected in questionnaires.

Ethics Consideration

Written informed consent was obtained from all patients enrolled in the study, which was approved by the ethics board of Shiraz University of Medical Sciences.

Microbiological Cultures

Clinical samples for microbiological culture comprised peripheral blood, urine and CSF. Cultures were processed using standard microbiological methods. Blood cultures were run using an automated Bactec 92490 (Becton Dickinson

Table 1. Duration of hospitalization in different intensive care unit wards.

Hospitalization (days)	Patients n (%)	Internal Medical n (%)	Neuro Surgical I n (%)	Adult Surgical I n (%)	Neuro Surgical II n(%)	General Pediatrics n (%)	Adult Surgical II n (%)	Neonatal n (%)	Pediatrics Surgical n (%)
<7	329 (59)	99 (62)	50(50)	53 (68)	45 (60)	35(52)	15 (54)	22 (78)	9 (53)
7-14	165(30)	44 (28)	41 (40)	15 (20)	21 (28)	22 (34)	11 (39)	6 (22)	5 (29)
>14	59(11)	16 (10)	10 (10)	10 (12)	9 (12)	9 (14)	2 (7)	-	3 (18)
Total	553 (100)	159 (100)	101 (100)	78 (100)	75 (100)	67(100)	28 (100)	28 (100)	17(100)

Table 2. Distribution of positive and negative cultures from intensive care unit patient isolates, ranked by patient age.

Age (year)	Patients n(%)	Negative culture n (%)	Positive culture n (%)	Total samples n (%)	p value
<20	155 (28)	183 (30)	53 (28)	236 (29.1)	0.91
20-40	189 (34)	197 (32)	65 (34)	262 (32.3)	0.91
40 -60	110 (20)	124 (20)	40 (21)	164 (20.1)	0.91
>60	99 (18)	116 (18)	34 (17)	150 (18.5)	0.91
Total	553 (100)	620 (100)	192 (100)	812 (100)	0.91

Table 3. Antibacterial susceptibility pattern of Gram-positive bacteria isolated from patients in intensive care unit wards.

Antibiotics	Coagulase-negative staphylococci				Enterococci				Streptococci			
	MIC		Susceptibility		MIC		Susceptibility		MIC		Susceptibility	
	50	90	Pattern (%)		50	90	Pattern (%)		50	90	Pattern (%)	
			S	R			S	R			S	R
Penicillin G	>32	>32	2.3	97.7	>32	>32	11.1	89.9	8	-	0	100
Vancomycin	2	4	100	0	1	2	89.5	10.5	1	2	100	0
Imipenem	4	>32	59.1	40.1	16	>32	26.3	73.7	1.5	3	100	0
Meropenem	>32	>32	40.1	50.9	>32	>32	5.3	94.7	>32	>32	0	100
Ceftriaxone	>32	>32	11.4	88.6	>32	>32	0	100	>32	>32	0	100
Ceftazidime	>256	>256	25	75	>256	>256	0	100	>256	>256	0	100
Cefotaxime	>32	>32	34.1	65.9	>32	>32	5.3	94.7	>32	>32	0	100
Cefazolin	64	>256	0	100	>256	>256	5.3	94.7	>256	>256	0	100
Ciprofloxacin	>32	>32	27.3	72.7	>32	>32	5.3	94.7	>32	>32	0	100
Co-trimoxazole	>32	>32	63.6	36.4	>32	>32	5.3	94.7	>32	>32	0	100
Ampicillin	16	64	5.6	94.4	4	>256	52.6	47.2	3	8	100	0
Piperacilline/ Tazobactam	8	>256	50	50	32	>256	36.8	63.2	4	6	100	0
Clindamycin	>256	>256	18.2	81.8	>256	>256	94.7	5.3	>256	>256	0	100

Figure 1. Percent of samples from the intensive care unit patients.

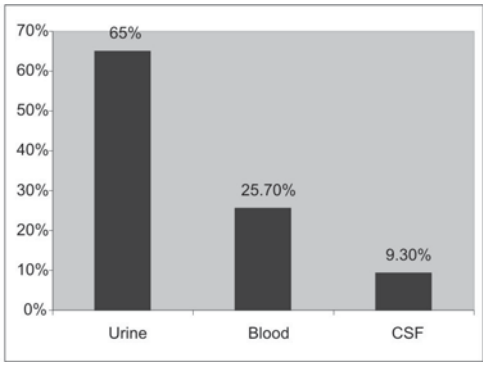

Figure 2. Percent of positive and negative culture results for urine, blood and cerebral spinal fluid (CSF) samples.

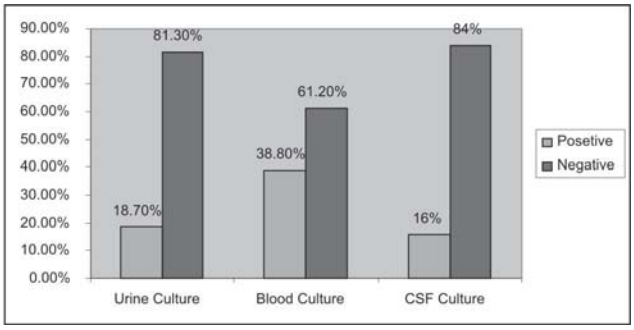

Table 4. Antibacterial susceptibility pattern of Gram-negative bacteria isolated from patients in intensive care unit wards.

Antibiotics	*Acinetobacter*				*Pseudomonas*				*Enterobacter*				*E.coli*				*Klebsiella*			
	MIC		Susceptibility Pattern (%)		MIC		Susceptibility Pattern (%)		MIC		Susceptibility Pattern (%)		MIC		Susceptibility Pattern (%)		MIC		Susceptibility Pattern (%)	
	50	90	S	R	50	90	S	R	50	90	S	R	50	90	S	R	50	90	S	R
Amikacin	96	>256	22.2	77.8	6	>256	62.5	37.5	4	24	82.4	17.6	3	128	62.5	37.5	2	4	71.4	28.6
Gentamicin	>32	>32	22.2	77.8	>32	>32	25	75	128	>256	17.6	82.4	4	>256	43.8	56.2	2	8	42.9	57.1
Imipenem	1.5	12	83.3	16.7	3	>32	75	25	1	6	82.4	17.6	0.250	1.5	87.5	12.5	1	3	71.4	28.6
Meropenem	1	4	88.9	11.1	0.5	4	50	50	0.094	0.250	94.1	5.9	0.032	0.125	87.5	12.5	0.125	1	71.4	28.6
Ceftriaxone	>32	>32	0	100	>32	>32	0	100	>32	>32	17.6	82.4	24	>32	25	75	0.094	>32	42.9	57.1
Ceftazidime	>256	>256	11.1	88.9	4	>256	50	50	>256	>256	29.4	70.6	48	>256	25	75	0250	>256	41.9	57.1
Cefotaxime	>32	>32	5.6	94.4	>32	>32	0	100	>32	>32	17.6	82.4	>32	>32	25	75	0250	>32	42.9	57.1
Cefazolin	>256	>256	0	100	>256	>256	0	100	>256	>256	0	100	>256	>256	0	100	>256	>256	0	100
Ciprofloxacin	>32	>32	11.1	88.9	0.25	>32	62.5	37.5	0.25	3	70.6	29.4	0.064	>32	50	50	0.125	0.5	71.4	28.6
Co-trimoxazole	8	>32	38.9	61.1	>32	>32	0	100	2	>32	41.2	58.8	24	>32	25	75	1	2	71.4	28.6
Ampicillin	>256	>256	0	100	>256	>256	12.5	87.5	>256	>256	5.9	94.1	>256	>256	12.5	87.5	>256	>256	14.3	85.7
Pipracilline/ tazobactam	>256	>256	22.2	77.8	6	>256	75	25	8	64	52.9	47.1	8	128	65	35	4	>256	42.9	57.1

Figure 3. Distribution of bacteria isolated from urine, blood and cerebral spinal fluid (CSF)samples.

Diagnostic Instrument System Sparks, Mds). The Bactec bottles were incubated in the Bactec system as recommended by the manufacturer for seven consecutive days. During the seven day incubation, when the system indicated positive results, three to five drops of blood culture samples taken up with 1 mL sterile syringes were inoculated onto blood agar or chocolate agar containing 5% whole sheep blood and incubated aerobically overnight. The pure cultures were then stained with Gram's stain. The bacteria were identified based on morphological characters, Gram's stain and biochemical tests. Urine samples were cultured on eosine methylene blue (EMB) agar and on blood agar containing 5% whole sheep blood, and CSF samples were cultured on blood agar or chocolate agar containing 5% whole sheep blood. All the cultures were incubated aerobically, except the urine samples, which were incubated

on chocolate agar in 10% CO_2. Further identification of the bacteria was carried out based on Gram staining and standard biochemical tests.

Antibacterial Susceptibility Testing

Susceptibility of the isolated strains to 14 antibacterial agents was checked with the E test (AB Biodisk, Solna, Sweden). MIC breakpoints for each antibiotic were determined according to the manufacturer's recommendations. American Typing Culture Collection isolates of *E. coli* (ATCC 25923) and *Staphylococcus aureus* (ATCC 25922) were used as controls for MIC determination. Sets of antibiotics, including amikacin, gentamicin, imipenem, meropenem, ceftriaxone, ceftazidime, cefotaxime, cefazolin, ciprofloxacin, co-trimoxazole, vancomycin, penicillin G, ampicillin and piperacilline/tazobactam were used to evaluate the *in vitro* susceptibility of bacterial isolates to these antibiotics.

Statistical Analysis

Data collected from patients were compared using Chi-square, comparing the effects of length of stay in the hospital or previous antibiotic therapy on positive and negative culture results. The significance level was defined as $p<0.05$

Results

Samples consisting of urine (65%), blood (25.7 %) and CSF (9.2 %) were collected from patients (Figure 1). The rate of positive cultures for blood samples was higher than that for urine and CSF samples (Figure 2). Coagulase-negative Staphylococci (CNS) and *E. coli* were most frequently isolated from patient samples (Figure 3).

The number of hospitalized patients in the different wards was 17, 28, 28, 67, 75, 78, 101 and 159, corresponding to pediatric surgery, neonatal, adult surgical II, general pediatrics, neurosurgical II, adult surgical I, neurosurgical I and internal medical ICUs, respectively. The internal medicine ICU accounted for 28.7% of the admissions. Frequencies of admitted patients in each ward and length of hospitalization are shown in Table 1. Patients from 20 to 40 years old were the most prevalent in our ICUs (Table 2). The patients previously treated with antibiotics tended to have positive cultures (87.5%) more frequently than the patients who were not treated with antibiotics (81%, $p=0.022$). The *in vitro* evaluation of effectiveness of antibiotics against Gram positive and negative bacteria revealed that most pencillins, cephalosprins and clindamycin would not be effective in controlling infections in these ICU patients. Among the carbapenems, imipenem was most effective against Gram-positive bacteria (26% to 100%), while meropenem had the highest activity against Gram-negative bacteria (50% to 94%). Patterns of antibiotic sensitivity for Gram-positive and Gram-negative bacteria isolated from ICU patients are shown in Tables 3, and 4, respectively. *Staphylococcus aureus* was isolated from just one blood culture.

Discussion

Surveys of the prevalence and antibacterial susceptibility patterns of bacterial isolates are important for determining appropriate empirical therapy for infections in critically-ill patients [4,5]. Also, epidemiological analysis of patient data can be informative for appropriate management of patients in ICUs.

Coagulase-negative Staphylococci (CNS) were isolated from 66.7% of the blood and 36.5% of the CSF samples. These values are high compared with data from German ICUs [5], but are similar to those of some other reports [6]. The high prevalence of CNS isolates warns us that special attention should be given to controlling the dissemination of these opportunistic bacteria in ICU patients. Appropriate antibiotic therapy and control measures could be adopted to prevent cross contamination of multidrug-resistant CNS bacteria from previous ICU patients to new patients and hospital staff [6,7]. Furthermore, CNS bacterial isolates are normal flora of skin and common contaminants of patient samples. Therefore, contamination of patients' samples with this potential contaminant should be taken into account when handling the patients. Nevertheless, evaluation of patients' symptoms, including fever and determination of time to positivity of CNS isolates in suspicious Bactec bottles can help to differentiate between potential contaminants and true pathogens [8,9]. Enterococci are also important pathogens for patients hospitalized in the ICU [10], particularly in view of the increasing frequency of resistance to vancomycin. Among our isolates, we detected only 10.5% resistant enterococci to vancomycin. Nevertheless, vancomycin can serve as an antibiotic to control our CNS and streptococci infections and is relatively appropriate for enterococci. Other investigators have also reported that vancomycin is still effective in controlling Gram-positive bacterial infections [11]. The streptococci were all susceptible to vancomaycin, ampicillin and piperacilline/tazobactam. It seems safe therefore to administer ampicillin to control streptococcal infections. Only one vancomycin-sensitive *S. aureus* strain was isolated from samples. Other reports have indicated that *S. arueus* are the most commonly isolated bacteria from ICU patients [12,13]. Distribution patterns of nososomial infections could be adapted to antibiotic consumption and the types of antiseptics and disinfectants used in hospitals [14-16]. Overall, vancomycin can effectively control Gram-positive cocci, while the high rate of bacterial resistance to cephalosporins and clindamycin implies that these drugs should not be used in our ICU wards. The MIC_{50} and MIC_{90} for vancomycin varied from 1 to 4μg/mL (Table 3), which indicates that this antibiotic should be effective at tolerable doses in patients [11]. In addition, imipenem was superior to meropenem for treatment grampositive bacteria.

The species of Gram-negative bacteria isolated from urine, blood, and CSF samples varied. In urine samples, *E. coli* was more prevalent, while in the blood and CSF, *Acinetobacter* and *Enterobacter* predominated, respectively (Figure 3). It is to be expected that *E. coli* is the common colonizing or infecting agent of the urinary tract system. Furthermore, *Enterobacter* spp. are also important pathogens that are frequently isolated from patients hospitalized in ICUs. *Acinetobacter* was isolated from CSF

samples at a relatively high frequency (18.2%), when compared to urine (13.4%) and blood (10.6%) samples. Of particular concern is an increase in multidrug resistance of *Acinetobacter* isolated from both ICU and non-ICU hospitalized patients [17,18]. In our study, we also recorded imipenem-resistant *Acinetobacter* strains. Imipenem-resistant *Acinetobacter* with a 16.7% resistance rate were found in our ICUs, which is close to the rate (17.1%) recently reported in a European study [19]. We also found that patients who had previously been treated with antibiotics had more positive cultures than those who had not. This result is consistent with some other reports that previous antibiotic therapy can favor predominance of antibiotic-resistant isolates [20]. Meropenem proved more effective against Gram-negative bacteria than imipenem. Previously, we reported superiority of *in vitro* activity of meropenem over imipenem for burn patients infected with *P. aeruginosa* [21].

We recorded more patients 20-40 years old in our ICUs, while other investigators found patients over 60 years to be more frequently admitted to ICUs [22]. One explanation for this discrepancy could be population distribution, that is in Iran has a younger population than many countries where such studies have been undertaken. Differences in social activities, nutrition, smoking and high rates of car accidents may also contribute to this variation. Sixty-five percent of the patients were admitted to the internal, adult surgery and neurosurgery ICUs. As also reported in other studies, most of the patients were suffering from malignancies [23,24] or were admitted to ICUs as a result of car accidents [25]. The high prevalence of patients in these wards means that measures should be taken to reduce such problems.

In conclusion, the high frequency of multidrug resistant bacteria in ICUs suggests that we need to prescribe broad-spectrum antibiotics more wisely in order to reduce pressure on sensitive strains. This could be beneficial for saving ICU patients and preventing the spread of resistant isolates in these critical wards. It appears that a combination of vacomycin with meropenem or imipenem can effectively treat most of our ICU patients who have bacterial infections when empirical therapy needs to be considered. However, clinical efficacy of monotherapy or combined administration of these antibiotics remains to be assessed. To overcome inappropriate treatment of patients, periodical antibacterial susceptibility surveys for nosocomial infections in ICU wards are warranted.

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