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ORIGINAL ARTICLE

Nosocomial Infections: Multicenter surveillance of antimicrobial resistance profile of *Staphylococcus aureus* and Gram negative rods isolated from blood and other sterile body fluids in Iran

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ABSTRACT

Background and Objective: Antibiotic resistance is increasing, especially in healthcare-associated infections causing significant public health concerns worldwide. National information is required to make appropriate policies, update list of essential drugs for treatment, and evaluate the effects of intervention strategies. A nationwide surveillance of antimicrobial resistant bacteria in nosocomial infections was established in Iran in 2008, so that the data obtained through the surveillance would enable us to construct a database.

Materials and Methods: Seven major teaching hospitals in Shiraz, Tabriz, Sari, Mashhad, Sanandaj, Ahwaz and Isfahan participated in this study. A total of 858 strains isolated from blood and other sterile body fluids were tested. Identification at the species level was performed with conventional biochemical methods and the API system. Susceptibility tests were done using disk diffusion method. The methicillin-resistance in *S. aureus* (MRSA) was determined by the oxacillin agar screen plate and respective MIC values were assessed using the E-test strips. The confirmatory disk diffusion methods were applied for phenotypic identification of extended-spectrum β- lactamase (ESBL) production for *E. coli* and *K. pneumoniae*, according to CLSI guidelines.

Results: Cultivation and re-identification of the strains yielded 858 isolates, consisting of 224 *S. aureus*, 148 *Klebsiella* spp., 105 *Serratia* spp., 146 *E. coli*, 67 *Acinetobacter* spp., 38 *Enterobacter* spp., 95 *Pseudomonas* spp., 71 *P.aeruginosa*. 35 *Stenotrophomonas* sp., and 8 other organisms. MRSA was detected in 37.5% of the isolates. No vancomycin-resistant or vancomycin-intermediate resistant *S. aureus* was detected. With the exception of *Acinetobacter* and *Stenotrophomonas*, 85% of the Gram-negative isolates were found to be susceptible in vitro to imipenem. Overall, about 61% of *K. pneumoniae* and 35% of *E. coli* isolates were ESBL producing.

Conclusion: Multidrug resistant isolates of Gram-negative organisms and methicillin-resistant strains of *S. aureus* have been detected in many hospitals in this study.

Keywords: Blood, Sterile body fluids, Gram negative bacteria, *Staphylococcus aureus*, Antimicrobial resistance, Iran.

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INTRODUCTION

Nosocomial infections occur worldwide and affect both developed and developing countries. Bacterial infections acquired in health care settings are among the major causes of death and increased morbidity and mortality among hospitalized patients. Nosocomial infections can be defined as those occurring within 72 hours of hospital admission, 3 days of discharge or 30 days of an operation. Many different microorganisms including bacteria, viruses, fungi and parasites may cause nosocomial infections (1, 2). Emerging patterns of antibiotic resistance of bacteria have altered outcome for critically ill patients. Physicians increasingly are faced with challenges to provide their patients with effective regimens while using antibiotics so that it does not result in further drug resistance. Antibiotic resistance is increasing and as a result significant public health problems are emerging (1-3). Moreover, it is now important to define local and national resistance rates for a range of pathogens in the blood and other strile body fluids in order to provide baseline data capable of serving as important reference for monitoring changes in resistance and empirical therapy. Data on antimicrobial resistance amongst pathogens recovered from blood and sterile body fluid infections in a nation wide study are limited.

Antibiotic resistance in Gram-positive cocci is a persistent issue. Methicillin resistant *S. aureus* (MRSA) is currently recognized as a major problem in hospitals throughout the world (3-5). Non-fermenter bacteria with high multidrug resistance, pose a particular challenge for healthcare management (6). Resistance due to the production of extended-spectrum B-lactamases (ESBLs) is a difficulty in the handling of Enterobacteriaceae infections, but other mechanisms of resistance are also emerging, leading to multidrug resistance and threatening to create panresistant species (7-9).

The term multidrug-resistant (MDR) applies to a bacterium that is resistant to: (1) several antibiotics to which they would normally be susceptible, or (2) all but one or two antibiotic classes, regardless of the mechanism of resistance (and often susceptible to only one or two commercially available antibiotics) (8, 9).

National information is required to develop appropriate policy, update lists of essential drugs and national guidelines for treatment, and evaluate the effects of intervention strategies. The present study aims to investigate antibiotic resistance among *S. aureus* and Gram-negative rods isolated from bloodstream and sterile body fluids, and evalute reduced methicillin and vancomycin susceptibility in *S. aureus* and extended-spectrum beta lactams in *K. pneumoniae* and *E. coli*.

MATERIALS AND METHODS

Seven major teaching hospitals located in different geographic areas of Iran (Shiraz, Tabriz, Sari, Mashhad, Sababdah, Ahwaz, Isfahan) in collaboration with the professor Alborzi clinical Microbiology Center (PACMRC), participated in this multicentre collaborative study over the period 2008-2009. The study focused on the most important pathogens responsible for nosocomial infections. These centers sent all the bacteria isolated from blood and sterile body fluids every two weeks to PACMRC. Isolated bacteria were stored at -80°C until reidentification and the antimicrobial susceptibility testing was conducted in PACMRC. Cultivation and re-identification of the strains yielded 858 isolates, consisting of *S. aureus*, *Klebsiella* spp., *E.coli*., *Serratia* spp., *Acinetobacter* spp., *Enterobacter* spp., *Pseudomonas* spp., *Stenotrophomonas* sp. Data regarding the antimicrobial susceptibility results were analysed by SPSS version 13.

Identification and confirmation of the isoltated bacteria. Gram positive cocci isolates were identified as *S. aureus* by traditional biochemical tests, including catalase, coagulase, and acid production from D-mannitol (10). Identification of Gram negative bacteria at the species level was performed using conventional biochemical methods and tests incorporated in the API system (bio Merieux SA, Marcy-1, Etoile, France) (10).

Antimicrobial susceptibility testing. Susceptibility tests were performed by the disk diffusion method, according to CLSI recommendations (11). Results were evaluated based on the respective standards for antimicrobial susceptibility testing.

Susceptibility of *S. aureus* isolates were tested for: clindamycin (CD, 2 µg), erythromycin (E, 15µg), linezolid (LZD, 30 µg), penicillin G (PG,

10µg), co-trimoxazole (SXT, 1.25/23.75µg), rifampin (RP, 5µg), oxacillin (OX, 1µg), ciprofloxacin (CIP, 5µg), chloramphenicol (C, 30µg), cephalothin (KF, 30µg), amikacin (AK, 30µg), tetracycline (T, 30µg), vancomycin (VA, 30µg), quinupristin-dalfopristin (SYN, 15µg), gentamicin (GM, 10µg) and fusidic acid (FC, 10µg).

Susceptibility of Gram negative rods were tested for the following 16 antimicrobial agents: Imipenem (IMP, 10µg), meropenem (MEM, 10µg), piperacillin-tazobactam (PTZ, 100/10µg), ciprofloxacin (CIP, 5µg), levofloxacin (LEV, 5µg), co-trimoxazole (SXT, 1.25/23.75µg), amoxicillin (A, 25 µg), nitrofurantion (NI, 200µg), cephalotin (KF, 30µg), amikacin (AK, 30µg), gentamicin (GM, 10µg), tobramycin (TB, 10µg), ceftriaxone (CRO, 30µg), cefixime (CFM, 5 µg), cefotaxime (CTX, 30µg), cefepime (CPM, 30µg), ceftazidime (CAZ, 30µg), aztreonam (ATM, 30µg) and ticarcillin (TC, 75µg).

Detection of methicillin resistant *S. aureus* (MRSA): Oxacillin agar screen plate: The oxacillin agar screen plate, prepared in-house, performed well in the detection of methicillin resistance in *S. aureus.* Ten microliters of the 10^6 CFU/ml bacterial inocula (final concentration=10^4 CFU/ml) was dropped onto MHA plates containing 4% NaCl and 6 µg/ml of oxacillin (11). If any growth occurred within 48h incubation at 33-35°C, the isolate was considered to be oxacillin resistant.

E-test Method: Methicillin (oxacillin) MICs were determined using the E-test strips (AB Biodisk, Solna, Sweden), according to the manufacturer's instructions on 150-mm-diameter MHA plates inoculated with 0.5 MacFarland density by swabbing in three directions. In case of heterogeneous growth, the highest MIC (inner limit of the inhibition zone) was read. *S. aureus* ATCC 25923 was tested with each batch of medium, as the standard strain.

Detection of vancomycin resistant *S. aureus* (VRSA)

BHI Agar Screen Plate: All *S. aureus* isolates were examined for reduced vancomycin susceptibility by an agar incorporation. Ten µL of a 0.5 Macfarland bacterial suspension (final concentration=10^6 CFU/ml) was spotted on the brain heart infusion (BHI) agar (Merck, Germany) containing 6 µg/ml vancomycin, allowed to air dry for approximately 5 min, and incubated at 35°C (11). Plates were examined at 24 and 48 h for any growth.

E-test Method: Standard E-test procedure was performed using a 2.0 McFarland inoculum on Mueller-Hinton agar (MHA) plates (Merck, Germany), according to the manufacturer's manual, using vancomycin E-test strips. Plates were incubated at 35°C for full 24h period. MIC endpoints were read, according to the manufacturer's recommendations. If heterogeneous growth occurred, the highest MIC (inner limit of the inhibition zone) was read. For quality control, *Entercococcus faecalis* ATCC 29212 as the susceptible control and *E. faecalis* ATCC 51299 as the resistant control, were used.

Detection of extended-spectrum β- lactamase (ESBL) in *E.coli* and *K. pneumoniae*

Combination Disc Diffusion Method: All *E. coli* and *K. pneumoniae* isolates were screened for extended-spectrum β- lactamase (ESBL) production, according to CLSI guidelines using confirmatory disk diffusion methods (11). A cefotaxime (30µg) and a cefotaxime + clavulanic acid (30µg+10µg), ceftazidime (30 µg) and ceftazidime + clavulanic acid (30µg+10µg) discs (Mast, UK) were placed at a distance of 25 mm on a Mueller-Hinton Agar plate, inoculated with a bacterial suspension of 0.5 McFarland turbidity standards and incubated overnight at 37°C. A ≥ 5mm increase in the diameter of inhibition zone for the combination disc versus ceftazidime disc, confirmed ESBL production. ESBL producing strain *K. pneumoneae* ATCC 700603 and non-ESBL producing strain *E. coli* ATCC 25922 were used as positive and negative controls.

RESULTS

Cultivation and re-identification of the strains yielded 858 isolates, consisting of 224 *S. aureus*, 148 *Klebsiella* spp., 146 *E. coli*., 105 *Serratia* spp., 67 *Acinetobacter* spp., 38 *Enterobacter* spp., 95 *Pseudomonas* spp., 71 *Pseudomonas aeruginosa*, 35 *Stenotrophomonas* sp. and 8 other organisms (Table 1, 2).

Table 1. Frequencies of isolates obtained from positive sterile body fluid cultures in different cities (N=858).

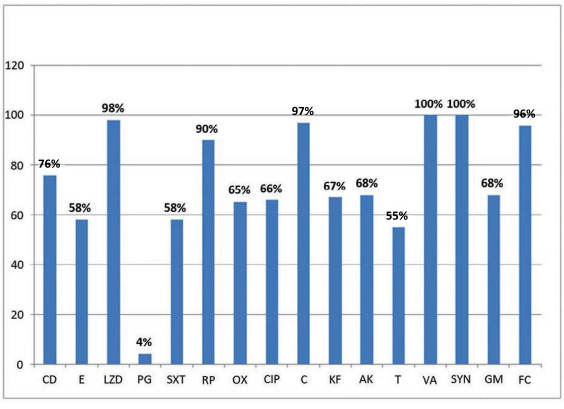
	City							
	Shiraz	**Sari**	**Tabriz**	**Mashhad**	**Sanandaj**	**Ahwaz**	**Esfahan**	**Total**
Gram positive cocci (N=224)								
S. aureus	54(24%)	11(5%)	35(15.5%)	1(0.5%)	36(16%)	85(38%)	2(1%)	224
Gram negative bacilli (N=634)								
***Klebsiella* spp.**	22(15%)	47(32%)	12(8%)	31(21%)	16(11%)	8(5%)	12(8%)	148
E. coli	36(24.5%)	39(27%)	13(9%)	25(17%)	21(14.5%)	2(1%)	10(7%)	146
***Serratia* spp.**	11(10.5%)	17(16%)	5(5%)	16(15%)	33(31.5%)	22(21%)	1(1%)	105
***Enterobacter* spp.**	8(21%)	14(37%)	2(5%)	2(5%)	5(13.5%)	4(10.5%)	3(8%)	38
***Pseudomonas* spp.**	19(20%)	45(47.5%)	5(5.3%)	16(16.8%)	4(4.1%)	3(3.2%)	3(3.2%)	95
***Acinetobacter* spp.**	20(30%)	4(6%)	1(1.5%)	24(35.5%)	5(7.5%)	3(4.5%)	10(15%)	67
***Stenotrophomonas* spp.**	5(14%)	10(28.5%)	1(3%)	14(40%)	1(3%)	3(8.5%)	1(3%)	35
Total	175	187	74	129	121	130	42	

Table 2. Distribution of isolates obtained from positive sterile body fluid cultures (N=858).

	Hospital Units						
	Blood	**CSF**	**Pleural Fluid**	**Ascitic Fluid**	**Joint Fluid**	**Peritunium**	Total
Gram positive cocci (N=224)							
S. aureus	210(94%)	5(2%)	5(2%)	3(1.5%)	1(0.5%)	-	224
Gram negative bacilli (N=634)							
***Klebsiella* spp.**	121(82%)	4(2.5%)	11(7.5%)	6(4%)	1(0.5%)	5(3.5%)	148
E. coli	112(77%)	11(7.5%)	6(4%)	13(9%)	1(0.5%)	3(2%)	146
***Serratia* spp.**	97(92%)	3(3%)	2(2%)	3(3%)	-	-	105
***Enterobacter* spp.**	24(63%)	2(5%)	6(16%)	4(11%)	-	2(5%)	38
***Pseudomonas* spp.**	65(68.5%)	6(6%)	4(4.5%)	7(7.5%)	7(7.5%)	6(6%)	95
***Acinetobacter* spp.**	60(89.5%)	2(3%)	4(6%)	1(1.5%)	-	-	67
***Stenotrophomonas* spp.**	26(74%)	-	1(3%)	1(3%)	7(20%)	-	35
Total	715	33	39	38	17	16	

Findings for *Staphylococcus* isolates. Of 224 *S. aureus* isolates, 210 were from blood, 5 from pleural fluid, 5 from CSF, 3 from ascitic fluid and 1 isolate from joint fluid. *S.aureus* was the species with the highest frequency (224/858; 26%) among the isolates in this study. The *in vitro* antimicrobial susceptibilities for *S. aureus* isolates to 16 antibacterial agents are shown in Table 2. Of the 224 *S. aureus* isolates, 84 (37.5%) were methicillin resistant (MRSA). No vancomycin resistance (VRSA), defined as MIC VA>8µg/ml, and vancomycin-intermediate resistance (VISA), defined as MIC VA 4-8µg/ml, were detected among *S. aureus* isolates. Also, no resistance to quinupristin-dalfopristin was observed. Susceptibility rates for linezolid, chloramphenicol and fusidic acid were 98%, 97% and 96%, respectively, and susceptibility rate for both erythromycin and co-trimoxazole was 58%. Among aminoglycosides the susceptibility rate for amikacin and gentamicin was found to be 68% (Fig. 1).

Fig. 1. Antimicrobial susceptibilities profile of S.aureus isolates.



CD, clindamycin; E, erythromycin; LZD, linezolid; PG, penicillin G; SXT, co-trimoxazole; RP, rifampin; OX, oxacillin; CIP, ciprofloxacin; C, chloramphenicol; KF, cephalothin; AK, amikacin; T, tetracycline; VA, vancomycin; SYN, quinupristin-dalfopristin; GM, gentamicin; FC, fusidic acid.

Findings for Gram-negative isolates. Of the 634 Gram-negative isolates, 511 were from the blood, 48 from acitic fluid, 32 from pleural fluid, 27 from CSF and 16 isolates from synovial fluid. The distribution of bacterial species and the *in vitro* antimicrobial susceptibilities for Gram-negative isolates against different antimicrobial agents are shown in Table 3. The most frequent Gram-negative isolates were *Klebsiella* spp. (17.2%), *E.coli* (17%), *Serratia* spp. (12.2%) and *Pseudomonas* spp. (11%).

With the exception of *Acinetobacter* and *Stenotrophomonas*, imipenem retained acceptable *in vitro* activites against the other Gram-negative isolates (>85%, susceptibilities). The susceptibility rate for *Serratia* and *Enterobacter* to imipenem was 100%. About 71% of *Stenotrophomonas* and 36% of *Acinetobacter* strains were resistant or intermediate-resistant to imipenem. The sensitivity of *Acientobacter* to imipenem was 64%.

In addition to imipenem, only piperacillin-tazobactam, nitrofurantion and amikacin had acceptable *in vitro* activities (≥80%) against *E. coli* isolates. Imipenem and levofloxacin were the most effective agents against (≥80%), followed by ciprofloxacin, pieracillin-tazobactam, nitrofurantion and amikacin which exhibited (60-80%) efficacy against *Klebsiella* isolates. As revealed, third generation ceplalosporines had poor activity against *Klebsiella*.

Overall, about 61% of *K. pneumoniae* isolates (90 out of the 148 isolates tested) and 35% of *E. coli* isolates (51/146) were ESBL producing. Imipenem (100%), piperacillin-tazobactam (84%), levofloxacin (96%) and ciprofloxacin (93%) functioned actively against *Serratia* isolates. Most strains of *Serratia* were resistant to amoxicillin, ampicillin, cephalothin and nitrofurantoin and most third generation cephalosporins except ceftazidime showed poor activity against *Serratia* isolates.

Most strains of *Enterobacter* were found susceptible to imipenem, levofloxacin, ciprofloxacin and piperacillin-tazobactam (100%, 92%, 87%, and 79%, respectively). Third generation cephalosporins were not active enough against the *Enterobacter* isolates tested (60%). Piperacillin-tazobactam exhibitied excellent (95%) and meropenem, imipenem and ticarcillin good activity (>80%) against *Pseudomonas* isolates. Only piperacillin-tazobactam, ceftazidime and co-trimoxazole had acceptable *in vitro* activities against *Stenotrophomonas* isolates (>75% susceptibilities) that were highly resistant to carbapenems (imipenem an meropenem). Levofloxacin, ciprofloxacin, amikacin, cefepime, piperacillin, carbencillin and tricarcillin were fairly effective (60-80%) against *Stenotrophomonas* (Table 3).

DISCUSSION

To arrive at a more accurate view about the most common bacteria isolated from patients with nosocomial infections and their antimicrobial susceptibility, we established a multicenter surveillance program in 2008 in Iran. Despite a large number of sporadic reports on antimicrobial susceptibity of bacteria from single hospitals in Iran, there is no accessible and comprehensive database about the susceptibility of bacterial pathogens to the currently used antibacterial agents. This study was the first nationwide one to address the problem of drug resistance and susceptibility among nosocomial bacterial pathogens in Iran. The antimicrobial susceptibility rates of *S. aureus* and Gram-negative rods strains isolated from the blood and other sterile body fluids, were determined in the present study.

Table 3. Antimicrobial susceptibilities profile of Gram negative isolates

	Klebsiella spp. N (%)	*E.coli* N (%)	*Serratia* spp. N (%)	*Pseudomonas* spp. N (%)	*Acinetobacter* spp. N (%)	*Enterobacter* spp. N (%)	*Stenotrophomonas* spp. N (%)
Imipenem	145(98)	145(99.3)	105(100)	81(85)	43(64)	38(100)	10(29)
Ciprofloxacin	109(74)	84(57)	98(93)	72(76)	18(27)	33(87)	23(66)
Levofloxacin	118(80)	92(63)	101(96)	73(77)	16(24)	35(92)	22(63)
Co-trimoxazole	48(32)	41(28)	70(67)	17(18)	20(30)	22(58)	29(83)
Amoxicillin	2(1)	16(11)	2(2)	-	2(3)	4(11)	-
Ampicillin	5(3)	15(10)	2(2)	-	4(6)	4(11)	-
Nitrofurantion	103(70)	141(96)	23(22)	-	1(2)	22(58)	-
Cephalotin	39 (26)	50(34)	3(3)	-	4(6)	5(14)	-
Amikacin	94(64)	133(91)	68(65)	76(80)	24(36)	26(69)	21(60)
Gentamicin	69(46)	105(72)	55(52)	64(67)	13(21)	24(63)	19(54)
Tobramycin	-	-	-	67(71)	-	-	17(49)
Ceftriaxone	48(32)	73(50)	50(48)	-	1(2)	18(47)	-
Cefixime	50(34)	73(49)	50(48)	-	1(2)	13(34)	-
Cefotaxime	44(30)	73(50)	45(43)	-	2(3)	19(50)	-
Cefepime	69(47)	101(69)	67(64)	38(40)	17(25)	24(63)	22(63)
Ceftazidime	67(45)	91(62)	69(66)	70(74)	20(30)	20 (52)	28(80)
Piperacillin-Tazobactam	104(70)	116(80)	88(84)	90(95)	27(40)	30(79)	29(83)

As observed, *E. coli* was the most common isolated Gram negative bacterium in some of the centers including Shiraz, and Tabriz, *Klebsiella* spp. was most frequent bacterium causing nosocomial infections in Esfahan and Mashhad, and finally, *Serratia* spp. was the most common in Sanandaj and Ahvaz. Ampicillin and co-trimoxazole were the least effective antibiotics against all the isolates, except Stenotrophomonas. More than 90% of *E. coli* isolates were sensitive to amikacin, and carbapenems (imipenem and meropenem). In aminoglycoside class of antibiotics, amikacin was the most effective agent against different isolated Gram negative bacteria. Among the isolates, *E. coli* was the most sensitive one to amikacin (91%). Imipenem was the most effective agent against *Klebsiella* spp. and only 20% of *Klebsiella* spp. strains showed susceptibility to all the tested antibiotics. Twenty-nine (83%) of *Stenotrophomonas* spp. isolates were susceptible to co-trimoxazole, which can act as the drug of choice against this bacterium.

The data analysis indicates that the antibiotics such as Beta-lactam agents, used extensively in the treatment of different infections, were active only against about 50% of total *Enterobacter* species tested. In some studies (12), cefepime showed a good activity against *Enterobacter* species, but we didn't find such a result. Approximately, 48% of *Enterobacter* species were resistant to ceftazidime, according to the studies of ICU isolates in the United States between 1987 and 1991 (13) and between 1994 and 1995 (14) and in 5 European countries (15), in agreement with the present study findings. Previous use of third generation cephalosporins is more likely to cause resistance to β-lactams in certain isolates of *Enterobacter* species.

Concerning the alarming types of resistance (i.e., resistance to third generation cephalosporins in *Klebsiella pneumoniae*, to quinolones in *E. coli*, and to methicillin in *S.aureus*) (16-18), our data showed that resistance to third generation cepholosporins tested was common (55% on average) among *Klebsiella* spp. As revealed, significant resistance to ciprofloxacin in *E. coli* (43%) and the percentage of MRSA strains (37.5%) were alarming. In international studies on antimicrobial resistance, the susceptibility of *Klebsiella pneumoniae* has been quite variable. In contrast to some reports that indicated low incidence of ESBL producing *K. pneumoniae* (19), about 61% of *K. pneumoniae* isolates in this study were ESBL producing, consistent with some other studies (20), which could be an alarming situation,too.

Our results for ceftazidime resistance among *E. coli* (38%), which is thought to be the result of ESBL production, are not in agreement with some reports from other countries like USA, which showed resistance to ceftazidime to be 4% (21). In the present study, 35% of *E. coli* isolates were ESBL positive.

In some studies, *Acinetobacter* and *Stenotrophomonas maltophilia* were the most resistant pathogens to many antibiotics (22,23). It was also the case with the present study. *Acinetobacter* is an increasingly infectious threat, especially in patients receiving broad spectrum antimicrobial therapy and requiring life support (24, 25). A Spanish study has shown that *Acinetobacter* isolates, usually acquired in the ICU, are multiresistant and may cause severe infections associated with a high mortality rate (26). Riely et al. recently described the failure to stop the spread of gentamicin resistant *A. baumannii* in an Australian ICU despite infection control measures (27).

P. aeruginosa species are naturally resistant to a number of antimicrobials and their resistance to the commonly used therapeutic agents has increased in recent years. Strains resistant to all available antimicrobial agents (pan-resistant strains) have emerged in hospitalized patients (28).

In our study, more than 95% of *Pseudomonas* isolates were sensitive to piperacillin-tazbactam and more than 80% of the isolates were sensitive to meropenem, imipenem and ticarcillin. As such a resistance can readily spread within a hospital setting and cause protracted outbreaks with high mortality rates, strict infection control procedures are highly recommended. Compared with other studies, imipenem exhibited excellent activity (100%) against *Serratia* isolates, in this study. piperacillin-tazobactam, levofloxacin and ciprofloxacin were highly active (84%, 96%, 93%, respectively) against *Serratia* isolates.

About 37.5% of *S. aureus* isolates in our study were MRSA, which is much higher , compared to the report from other countries (12.8%) between 2000 and 2001 as well as from studies performed in Tehran (Iran) (29, 30). The present study shows antibiotic resistance pattern within Iran, and has been conducted specifically on sterile body fluids, whereas others were limited to certain regions (30) and on a wide variety of sterile and non sterile clinical specimens (30, 31). Therefore, the present findings can serve as an index of actual antibiotic resistance across the nation. The prevalence of MRSA continues to increase worldwide, sometimes accounting for approximately 40-60% of all hospital acquired strains (32). No vancomycin resistant (VRSA) or vancomycin-intermdiate resistant *S.aureus* (VISA) isolates were detected. There could be many explanations for such differences, including: infection control measures, antibiotic prophylaxis and treatments used in each ward/hospital and last but not least, the clonal and epidemic nature of these microorganisms. While there are reports aroud the world indicating a tendency toward decreasing susceptibility to vancomycin in *S.aureus* (33), we had no VRSA of VISA isolates. This may be due to judicious and controlled use of vancomycin in our hospitals.

Multidrug resistant strains of Gram-negative organisms have emerged in many hospitals and has caused restrictions in the choice of antibiotics for empirical therapy and control of the increasing incidence of such organisms. There is a progressive increase in MRSA prevalence in Iran and an extremely low prevalence of VRSA or VISA was confirmed among *S.aureus*

clinical isolates. Vancomycin can serve as the drug of choice for treating multidrug resistant MRSA infections. It should be also noted that in many cases antibiotic resistance is transmited to humans and hospital environment through other sourses including food animals, plants, poultries, fish, and other industries, in which antibiotics are used for different purposes and may lead to emerging resistant strains (34-36). Isolation, identification and antimicrobial susceptibility of pathogens can be helpful in optimizing the antimicrobial use. It is, therefore, crucial to implement the rational use of available antimicrobials in everyday clinical practice to prevent selective pressure and the further development of resistance in these pathogens. Also, a regular surveillance of hospital associated infections, monitoring of antibiotic susceptibility pattern and formulation of practical antibiotic policy are suggested.

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