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Original Article

Antimicrobial resistance pattern of Gram-positive bacteria during three consecutive years at the nephrology ward of a tertiary referral hospital in Shiraz, Southwest Iran

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ABSTRACT

Objective: The aim of the present study was to determine the pattern of antimicrobial resistance of Gram-positive bacteria during three consecutive years at the nephrology ward of Namazi Hospital in Shiraz, Southwest of Iran.

Methods: During a 3-year period from 2013 to 2015, data of all biological samples of hospitalized patients at the adult nephrology ward of Namazi Hospital were sent to the central laboratory for identification of Gram-positive microorganisms and subsequently, their antimicrobial susceptibility testing by Kirby–Bauer disc diffusion method were analyzed in a retrospective manner.

Findings: Coagulase-negative Staphylococci (CONS) (38.5%), *Staphylococcus aureus* (25.4%), and Enterococcus spp. (23.8%) were the most common isolated Gram-positive bacteria from all biological samples. All Enterococcus spp. isolates within the 3 years were resistant to oxacillin. The rate of vancomycin-resistant enterococci (VRE) increased from 40.63% in 2013 to 72.73% in 2015. Enterococcus spp. resistance rates to aminoglycosides during 3 years were above 85%. The frequencies of oxacillin-resistant *S. aureus* (ORSA) in 2013, 2014, and 2015 were 95.24%, 80.95%, and 36.36%, respectively. Two out of 11 (6.67%) *S. aureus* isolates were resistant to vancomycin. More than 90% of CONS were sensitive to vancomycin within the study period. The frequency of gentamicin-resistant CONS ranged from 40% to 57.14%.

Conclusion: The rates of ORSA, VRE, and aminoglycoside-resistant CONS as well as Enterococcus spp. in our clinical setting were considerably high and concerning. These may be due to the failure or lack of infection control activities and antimicrobial selection pressure.

Keywords: Antibiotic; Gram-positive microorganisms; nephrology; resistance pattern

INTRODUCTION

Infectious diseases are among the most important causes of morbidity and mortality worldwide.[1] Spread of resistant microorganisms is playing a significant role in this regard.[2] In the United States, it has been estimated that 50–60% of all nosocomial infections are caused by antibiotic-resistant bacteria.[3] Although global data are insufficient, the burden of antibacterial resistance in the United States has been reported to

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be 23,000 deaths per year. Beside mortality, the direct and indirect overall societal costs of antibacterial resistance in the US have been estimated to be $20 and $35 billion, respectively. In the European Union countries, an estimated 25,000 deaths are attributed to antibiotic-resistant infections, costing €1.5 billion annually in both direct and indirect costs. In these countries, antibacterial resistance led to 16 million extra days of hospitalization.[4] In several hospital settings in the US and Europe, about 40% of antibacterial prescriptions are reported to be inappropriate.[5] Findings from a study in six teaching hospitals at Shiraz from February to July 2004 demonstrated that antibiotics are inappropriately used in 98% of patients undergoing surgery.[6]

Among pathogens, Gram-positive bacteria, especially *Staphylococcus aureus* and *Streptococcus pneumoniae* are responsible for a large number of both community-acquired and health-care associated infections at different sites including bone and joint, upper and lower respiratory tract, bloodstream, central nervous system, and skin and soft tissue.[7] Among Gram-positive bacteria, methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant enterococci (VRE), and coagulase negative staphylococci (CONS) become major problems in clinical settings due to high morbidity as well as mortality and limited treatment options.[8-10] In contrast to Gram-negative infections, the prevalence and resistance pattern of Gram-positive pathogens have not been studied well worldwide. This issue has been largely overlooked in developing countries such as Iran. Knowledge about the most common causative microorganisms and their resistance pattern can be exploited to help clinicians in selecting an optimized antimicrobial agent for empirical therapy and developing rational prescription guidelines for definite therapy. The main purpose of the current study was to determine the pattern of antimicrobial sensitivity of Gram-positive bacteria during three consecutive years at the nephrology ward of a tertiary referral hospital in Shiraz.

METHODS

This retrospective study was performed on microbiological records of patients hospitalized at a 20-bed adult nephrology ward of Namazi Hospital, a multispecialty healthcare university setting affiliated to Shiraz University of Medical Sciences, between 2013 and 2015. The study was approved by the Institutional Review Board and the Medical Ethics Committee of the hospital.

Biological isolates of hospitalized patients such as blood, urine, sputum, wound drainage, abscess, synovial, pleural, ascitic, and cerebrospinal fluid were sent to the central laboratory of the hospital. They were taken either before (as empirical therapy) or during antibacterial treatment (as definite therapy) for assessing microbiological response of both community-acquired and nosocomial infections. Identification of bacteria was performed by the initial Gram-staining and additional biochemical tests such as catalase, coagulase, and DNase. Mueller-Hinton agar culture medium (Merck, Germany) was inoculated with a saline suspension of isolated aerobic Gram-positive bacteria equivalent to McFarland 0.5 standard. After that, antibiotic discs (Padtan Teb Co., Iran) were placed on the surface of the agar. The antimicrobial susceptibilities of Gram-positive bacteria were determined by the Kirby–Bauer disc diffusion method based on the Clinical and Laboratory Standard Institute guidelines.[11] After 16–18 h incubation at 37°C, the isolated aerobic Gram-positive bacteria were categorized to be resistant, intermediately resistant, or sensitive based on the size of inhibition zone. The antibiotic discs (per unit disc) used for antimicrobial susceptibility of Gram-positive bacteria were as follows: oxacillin (5 mcg), ampicillin (10 mcg), cephalexin (30 mcg), ceftriaxone (30 mcg), cefotaxime (30 mcg), ceftizoxime (30 mcg), erythromycin (5 mcg), azithromycin (15 mcg), clindamycin (2 mcg), trimethoprim/sulfamethoxazole (25 [1.25/23.75] mcg), gentamicin (10 mcg), amikacin (30 mcg), vancomycin (30 mcg), ciprofloxacin (5 mcg), ofloxacin (5 mcg), nitrofurantoin (300 mcg), and chloramphenicol (30 mcg).

Categorical variables were expressed as percentage. Descriptive analyses were carried out by the Statistical Package for the Social Sciences (SPSS) version 20 software (IBM company, New York, NY, United States).

RESULTS

During the 3-year period, 374 Gram-positive bacteria were isolated from biological samples. The three most common isolated Gram-positive bacteria from all samples were *S. aureus* (38.30%), CONS (25.26%), and Enterococcus spp. (23.67%). Most Gram-positive bacteria were isolated from urine (37.97%) followed by blood (36.10%) [Table 1].

The 3-year antimicrobial resistance pattern of Gram-positive bacteria to different antimicrobials is demonstrated in Tables 2 and 3. The highest (68.6–78.1%) and lowest (14.85–39.29%) antibacterial resistance rates were seen with Enterococcus spp. and *S. aureus*, respectively. Regarding the antibacterial agent, the most frequent resistance was associated, with ofloxacin (80–90.91%) and oxacillin (79.31–86.67%). Nitrofurantoin, vancomycin,

Table 1: Frequency of isolated Gram-positive bacteria from different biological specimens (*n*=374)

Microorganism/ sample	Blood (*n*)	Urine (*n*)	Sputum (*n*)	Other* (*n*)
Enterococcus spp.	10	68	1	10
Coagulase negative staphylococci	68	29	3	44
S. aureus	52	7	9	27
Streptococcus spp.	5	38	1	2
Total	135	142	14	83
Percentage	36.1	37.97	3.74	22.19

*Including abscess, synovial, pleural, ascitic, and cerebrospinal fluids.
S. aureus=Staphylococcus aureus

and chloramphenicol were identified as the most active antibacterial agents with the resistance rates of 3.7–10%, 9.52–23.88%, and 17.44–26.92%, respectively.

Regarding Enterococcus spp., all (100%) isolates within the 3 years were resistant to oxacillin. The sensitivity rate of Enterococcus spp. to vancomycin decreased from 34.37% in 2013 to 13.64% in 2015. In other words, the rate of VRE increased from 40.63% in 2013 to 72.73% in 2015. Enterococcus spp. resistance rate to aminoglycosides in 2013, 2014, and 2015 were 92.86%, 88%, and 91.3%, respectively. Resistance rate of Enterococcus spp. to ampicillin was 100% in 2013, 46.4% in 2014, and 66.67% in 2015.

The frequency of oxacillin-resistant *S. aureus* (ORSA) from isolated samples in 2013, 2014, and 2015 was 95.24%, 80.95%, and 36.36%, respectively. During the study period, two out of 11 (6.67%) *S. aureus* isolates were resistant to vancomycin. The sensitivity rate of *S. aureus* to clindamycin was 63.64% in 2014 and 66.67% in 2015. *S. aureus* sensitivity rates to trimethoprim/sulfamethoxazole in 2013, 2014, and 2015 were 95.45%, 66.67%, and 90.91%, respectively.

The sensitivity rate of CONS to vancomycin was 97.87% in 2013, 98.08% in 2014, and 93.55% in 2015. CONS resistance rates to oxacillin in 2013 and 2015 were reported to be 90.32% and 73.91%, respectively. The frequency of gentamicin-resistant CONS was 54.05% in 2013, 40% in 2014, and 57.14% in 2015.

Regarding *Streptococcus* spp., the rate of resistance to ceftriaxone in 2013, 2014, and 2015 was 75%, 23.08%, and 14.29%, respectively. The frequency of vancomycin-resistant *Streptococcus* spp. in 2013 was 23.08% and in 2014 was 21.43%. All (100%) *Streptococcus* spp. isolates were sensitive to vancomycin in 2015. In contrast, all *Streptococcus* spp. isolates were resistant to azithromycin in 2013 and 2014. About 80%, 50%, and 17% of *Streptococcus* spp. samples were resistant to ciprofloxacin in 2013, 2014, and 2015, respectively.

DISCUSSION

In the present study, *S. aureus* was the most frequent Gram-positive pathogen isolated from all samples. This is consistent with Japoni *et al*. survey between January 2001 and December 2004 on 9407 referred blood samples from patients at three main wards, neonates, pediatrics, and adults of Namazi Hospital.[12] The same study group identified *S. aureus* as the second most prevalent pathogenic bacteria isolated from 58 patients with nosocomial pneumonia over 9 months in the aforementioned clinical setting.[13] Among 97 samples of 46 patients with Intensive Care Unit (ICU)-acquired infections, *S. aureus* was the most prevalent Gram-positive pathogen from eight ICUs of two major teaching hospitals in Shiraz.[14] Similar findings have been reported from some other hospitals in Iran such as Khalili *et al*.[15] and Soltani *et al*.[16] from Imam Khomeini Hospital at Tehran.

Although it varied in different years, the frequency of ORSA in the current study was considerably high. However, the rate of ORSA was descending during the study period (from 95.24% to 36.36%). The definite explanation for the latter finding in our center was unknown. Lack of adequate antibiotic discs for assessing antibacterial resistance and invalid techniques of isolating and culturing bacteria in years 2013 and 2014 may be account for these results. In this regards, the sum number of *S. aureus* in the years 2013 and 2014 was 12 isolates, much less than that in 2015 (41 isolates). In contrast, the resistance rate of *S. aureus* to oxacillin increased from 60.78% to 72% during 4 years at a referral infectious diseases ward in Tehran.[15] The rate of MRSA in a nephrology ward at Tehran was 58.3%.[17] Hassanzadeh *et al*. reported all 9 (100%) *S. aureus* isolates from Namazi and Faghihi ICUs in Shiraz were resistant to cefoxitin (as a suggested index of MRSA).[14] The rate of MRSA reported from other studies in Iran ranges from 40% to 100%.[15,18] This ranges between 30% and 40% in different regions of the neighboring country, Saudi Arabia.[19] MRSA frequency largely depends on several factors such as infection control activities and antimicrobial selection pressure. Regarding the first issue; for example, it has been demonstrated that rigorous infection control practices set by the government in the United Kingdom have resulted in the sharp fall in MRSA prevalence from 40-45% during 2001–2005 to 36% in 2007.[20] In relation to antimicrobial selection pressure, beta lactams including amoxicillin, cefixime, and penicillin 6.3.3 are among the 10 most commonly prescribed medications by physicians in Iran based on the last National Rational Drug Use Committee official report in 2010.[21] According to our data, trimethoprim/

Table 2: Sensitivity, intermediate resistance, and resistance frequencies of isolated Gram-positive bacteria to different antimicrobials during three sequential years

Antimicrobial agent/microorganism	Sensitive (*n*)			Intermediate (*n*)			Resistant (*n*)		
	2013	2014	2015	2013	2014	2015	2013	2014	2015
Trimethoprim/sulfamethoxazole									
Streptococcus spp.	2	6	-	1	2	2	9	11	1
S. aureus	21	18	9	-	-	-	1	7	1
Coagulase negative staphylococci	3	21	11	-	1	-	5	26	18
Enterococcus spp.	-	5	3	-	-	3	22	25	18
Total	26	50	23	1	3	5	37	69	38
Percentage	40.63	40.98	34.85	1.56	2.46	7.58	57.81	56.56	57.58
Amikacin									
Streptococcus spp.	-	2	1	-	-	-	10	13	5
S. aureus	1	-	-	-	-	-	1	3	1
Coagulase negative staphylococci	7	10	1	-	1	1	5	1	1
Enterococcus spp.	1	-	-	-	-	-	8	20	9
Total	9	12	2	-	1	1	24	37	16
Percentage	27.27	24	10.53	-	2	5.26	72.73	74	84.21
Gentamicin									
Streptococcus spp.	1	4	1	-	1	-	10	13	4
S. aureus	18	12	10	3	2	-	14	11	-
Coagulase negative staphylococci	17	28	8	-	2	3	20	20	16
Enterococcus spp.	2	1	1	-	4	1	18	24	12
Total	38	45	20	3	9	4	62	69	32
Percentage	36.89	36.59	35.71	2.91	7.32	7.14	60.19	56.1	57.14
Ciprofloxacin									
Streptococcus spp.	1	5	3	-	5	2	12	8	1
S. aureus	-	10	10	3	2	1	3	14	1
Coagulase negative staphylococci	10	21	11	-	1	-	3	26	18
Enterococcus spp.	1	-	1	2	-	1	17	30	18
Total	12	36	25	5	8	4	35	78	38
Percentage	23.08	29.51	37.31	9.62	6.56	5.97	67.31	63.93	56.72
Ofloxacin									
Streptococcus spp.	-	1	-	-	-	-	4	3	-
Coagulase negative staphylococci	-	-	-	-	-	-	1	-	-
Enterococcus	1	-	-	-	1	-	5	5	5
Total	1	1	1	-	1	-	10	8	5
Percentage	9.09	10	16.67	-	10	-	90.91	80	83.33
Nitrofurantoin									
Streptococcus spp.	-	10	6	1	2	-	1	2	-
S. aureus	2	3	1	-	-	-	-	-	-
Coagulase negative staphylococci	13	11	2	-	1	2	-	-	-
Enterococcus spp.	-	20	8	2	5	4	1	-	2
Total	15	44	17	3	8	6	2	2	2
Percentage	75	81.48	68	15	14.81	24	10	3.7	8
Ceftriaxone									
Streptococcus spp.	2	10	5	-	-	-	6	4	1
S. aureus	1	-	1	-		-	-	3	-
Coagulase negative staphylococci	4	6	-	-	-	1	7	6	3
Total	7	16	6	1	1	1	13	13	4
Percentage	33.33	53.33	54.55	4.76	3.33	9.09	61.9	43.33	36.36
Cefotaxime									
Streptococcus spp.	-	2	3	-	-	-	-	3	1
S. aureus	-	-	1	-	-	-	-	2	1
Coagulase negative staphylococci	-	1	-	-	-	-	-	3	1
Total	-	3	4	-	-	1	-	8	3
Percentage	-	27.27	50	-	-	12.5	-	72.73	37.5

Contd...

Table 2: Contd...

Antimicrobial agent/microorganism	Sensitive (*n*)			Intermediate (*n*)			Resistant (*n*)		
	2013	2014	2015	2013	2014	2015	2013	2014	2015
Ceftizoxime									
Streptococcus spp.	3	8	1	-	-	-	-	-	1
S. aureus	2	-	-	-	-	-	-	-	-
Coagulase negative staphylococci	4	3	2	-	-	-	10	-	1
Total	9	11	3	-	2	-	10	14	2
Percentage	47.37	4.07	60	-	7.41	-	52.63	51.85	40
Cephalexin									
Streptococcus spp.	3	12	4	-	-	1	-	7	1
S. aureus	-	10	8	2	2	1	-	11	1
Coagulase negative staphylococci	-	33	18	4	-	1	-	16	10
Enterococcus spp.	1	4	1	-	1	2	5	26	18
Total	4	59	31	6	3	5	5	60	30
Percentage	26.67	48.36	46.97	40	2.46	7.58	33.33	49.18	45.45
Ampicillin									
Streptococcus spp.	-	8	4	-	1	-	4	8	2
Enterococcus spp.	-	9	7	-	6	-	16	13	14
Total	1	17	11	-	7	-	20	21	16
Percentage	4.76	37.78	40.74	-	15.56	-	95.24	46.67	59.26
Oxacillin									
Streptococcus spp.	-	-	-	-	-	-	4	5	-
S. aureus	-	4	2	1	-	2	20	12	-
Coagulase negative staphylococci	3	6	1	-	-	1	-	29	19
Enterococcus spp.	-	-	-	-	-		2	8	4
Total	3	10	3	1	-	3	26	54	23
Percentage	10	15.63	10.34	3.33	-	10.34	86.67	84.38	79.31
Vancomycin									
Streptococcus spp.	8	13	5	-	1	-	3	3	-
S. aureus	-	28	11	-		-	-	2	-
Coagulase negative staphylococci	46	51	29	-	1	-	1	-	-
Enterococcus spp.	11	13	3	8	7	3	13	7	16
Total	65	105	48	8	9	3	17	12	16
Percentage	72.22	83.33	71.64	8.89	7.14	4.48	18.89	9.52	23.88
Erythromycin									
Streptococcus spp.	-	-	-	-	-	-	4	4	-
S. aureus	-	10	7	-	-	-	-	11	3
Coagulase negative staphylococci	1	12	3	-	-	-	-	23	19
Enterococcus spp.	-	-	1	-	-	-	2	8	7
Total	1	22	11	-	-	-	6	48	29
Percentage	14.29	31.43	27.5	-	-	-	85.71	68.57	72.5
Azithromycin									
Streptococcus spp.	-	-	-	-	-	-	4	4	-
Total	-	-	1	-	-	-	4	4	1
Percentage	-	-	50	-	-	-	100	100	50
Clindamycin									
S. aureus	-	7	6	-	-	-	-	4	3
Coagulase negative staphylococci	-	14	5	-	-	1	-	9	17
Enterococcus spp.	-	-	1	-	-	-	-	3	5
Total	-	21	12	-	-	1	-	16	25
Percentage	-	56.76	31.58	-	-	2.63	-	43.24	65.79
Chloramphenicol									
Streptococcus spp.	-	2	-	-	-	-	-	3	-
S. aureus	34	26	10	-	-	1	2	3	-
Coagulase negative staphylococci	2	37	20	1	-	-	10	6	5
Enterococcus spp.	1	6	5	-	-	-	2	3	3
Total	37	71	35	1	-	1	14	15	8
Percentage	71.15	82.56	79.55	1.92	-	2.27	26.92	17.44	18.18

S. aureus=Staphylococcus aureus

Table 3: Sensitivity, intermediate resistance, and resistance frequencies of isolated Gram-positive bacteria to different antimicrobials during three sequential years

Antimicrobial agent/microorganism	Susceptibility								
	Sensitive (*n*)			Intermediate (*n*)			Resistant (*n*)		
	2013	2014	2015	2013	2014	2015	2013	2014	2015
Streptococcus spp.									
Vancomycin	8	11	5	2	-	-	3	3	-
Gentamicin	1	2	1	2	11	1	10	12	4
Amikacin		1	1	1	1	-	10	13	5
Ciprofloxacin	1	3	3	2	5	2	12	8	1
Ofloxacin	-	-	-	1	-	-	4	3	-
Trimethoprim/sulfamethoxazole	1	4	2	2	2	2	2	10	1
Ampicillin	-	6	4	2	-	-	4	8	2
Oxacillin	-	-	-	-	-	2	4	3	-
Cephalexin	3	101	4	2	-	2	-	6	1
Ceftriaxone	2	10	5	-	-	1	6	3	1
Ceftizoxime	3	8	2	-	-	1	-	-	2
Cefotaxime	-	2	-	-	-	-	-	3	-
Nitrofurantoin	-	10	6	1	2	1	1	1	-
Erythromycin	-	-	-	-	-	2	4	4	-
Azithromycin	-	-	-	-	-	1	5	3	-
Chloramphenicol	-	1	-	1	-	1	-	2	-
Total	19	68	33	16	21	16	65	82	17
Percentage	19	39.77	50	16	12.28	24.24	65	67.77	25.76
S. aureus									
Vancomycin	-	28	11	-	-	-	-	2	-
Gentamicin	18	12	10	3	2	-	14	11	1
Amikacin	1	-	1	-	-	-	1	3	-
Nitrofurantoin	2	3	1	-	-	-	-	-	-
Trimethoprim/sulfamethoxazole	21	18	10	-	2	-	1	7	1
Ciprofloxacin	-	10	10	3	2	1	3	14	1
Oxacillin	-	4	5	1	-	2	20	17	4
Cephalexin	-	10	8	2	2	1	-	11	1
Ceftriaxone	1	-	1	-		-	-	3	-
Cefotaxime	-	-	1	-	-	-	-	2	1
Ceftizoxime	2			-	-	-	-	-	-
Erythromycin	-	10	7	-	-	-	-	11	3
Chloramphenicol	34	26	10	-	-	1	2	3	-
Clindamycin	-	7	6	-	-	-	-	4	3
Total	79	128	81	9	8	5	41	88	15
Percentage	61.24	57.14	80.19	6.98	3.57	4.95	31.78	39.29	14.85
Coagulase negative staphylococci									
Vancomycin	46	51	29	-	1	-	1	-	-
Gentamicin	17	28	9	-	2	3	20	20	16
Amikacin	7	10	2	-	1	1	5	1	1
Ciprofloxacin	10	21	11	-	1	-	3	26	18
Ofloxacin	-	-	-	-	-	-	1	-	-
Oxacillin	3	6	4	-	-	2	28	-	17
Ampicillin	1	-	-	-	-	-	--	-	-
Cephalexin		33	18	4	-	1	-	16	10
Ceftriaxone	4	6	-	-	-	1	7	6	3
Ceftizoxime	4	3	2	-	-	-	10	2	1
Cefotaxime		1	-	-	-	-	-	3	-
Nitrofurantoin	13	11	2	-	1	2	-	-	-
Trimethoprim/sulfamethoxazole	2	20	10	-	1	-	1	26	19
Chloramphenicol	2	37	20	2	-	-	10	6	5
Clindamycin	-	14	5	-	-	1	-	9	17

Contd...

Table 3: Contd...

Antimicrobial agent/microorganism	Susceptibility								
	Sensitive (*n*)			Intermediate (*n*)			Resistant (*n*)		
	2013	2014	2015	2013	2014	2015	2013	2014	2015
Erythromycin	1	12	3	-	-	-	-	23	19
Total	110	253	115	6	7	11	86	138	126
Percentage	54.46	63.57	45.63	2.97	1.76	4.37	42.57	34.67	50
Enterococcus spp.									
Vancomycin	11	13	3	8	7	3	13	12	16
Gentamicin	2	1	1	-	4	1	18	24	12
Amikacin	1	1	-	-	-	-	21	20	9
Ciprofloxacin	1	-	1	2	-	1	17	30	18
Ofloxacin	1	-	-	-	1	-	5	5	-
Nitrofurantoin	-	20	8	2	5	4	1	-	2
Chloramphenicol	1	6	5	-	-	-	2	3	3
Erythromycin	-	-	1	-	-	-	-	9	8
Ampicillin	-	9	7	-	6	-	16	13	14
Oxacillin	-	-	-	-	-	-	-	9	5
Cephalexin	1	4	1	-	1	2	5	26	18
Trimethoprimw/sulfametoxazole	-	4	3	-	-	3	9	23	6
Clindamycin	-	-	1	-	-	-	-	3	5
Total	18	57	31	12	24	14	107	177	116
Percentage	13.14	22.1	19.25	8.76	9.3	8.69	78.1	68.6	72.05

S. aureus=Staphylococcus aureus

sulfamethoxazole and clindamycin can be considered as potentially favorable options for the treatment of *S. aureus* or even community acquired MRSA infections.

Two out of 11 (6.67%) *S. aureus* isolates were resistant to vancomycin during the study period. The first clinical vancomycin-resistant *S. aureus* (VRSA) in the world was isolated from a diabetic foot ulcer at the Michigan, US in 2002.[22] After that, several VRSA isolates confirmed by genetic analysis have been reported from different countries such as US, India, Pakistan, and Iran.[23] In Imam Khomeini Hospital in Tehran, Aligholi *et al*. identified 2 (0.56%) strains of VRSA confirmed by minimum inhibitory concentration of 64 and 512 mcg/ml for vancomycin as well as detection of vanA gene in 1 of the isolates through polymerase chain reaction (PCR).[24] At the same hospital in infectious diseases ward, Khalili *et al*. reported that 1.82% and 3.33% of detected *S. aureus* in 2009 and 2010 were resistant to vancomycin determined by the disc diffusion method.[15] A cross-sectional, observational study during 2 years at the nephrology and kidney transplant wards of Imam Khomeini Hospital demonstrated that all (100%) *S. aureus* isolates were sensitive to vancomycin.[17] Agar screening and PCR tests on 220 staphylococcal samples from three laboratories of Shiraz hospitals (Shahid-Faghihi, Namazi, and MRI) between March and December 2012 demonstrated that vanA and vanB resistant genes were positive in 34% and 37% of clinical isolates, respectively.[23] The development of VRSA could be attributed to overuse and selective pressure of vancomycin. In this regards, results of a cross-sectional study in a referral university-affiliated hospital in Shiraz at 2003 revealed that the prescription of vancomycin was only appropriate in 12 out of 200 (6%) patients according to the Hospital Infection Control Practice Advisory Committee guideline.[25] Although not studied yet, overuse of vancomycin for the empirical treatment of catheter-associated infections, as a common admission diagnosis in nephrology settings, can partially justify the current frequency of VRSA in our ward.

The sensitivity rate of CONS to vancomycin in our survey was high and did not change considerably during the 3 years period. This is in accordance with most of the other reports from Iran. Khoshbakht *et al*. reported that more than four-fifth (92.3%) of *Staphylococcus saprophyticus* isolates from urine of patients with urinary tract infection in Karaj was susceptible to vancomycin.[26] In addition, two investigations in Tehran hospitals demonstrated that all (100%) CONS from different biological samples were sensitive to vancomycin.[16,27] In contrast to vancomycin, most of CONS isolates (73.91–90.32%) in the present study were resistant to beta lactams such as oxacillin. Similarly, at three Makkah Hospitals in Saudi Arabia, Asghar reported that 82.4% of CONS were resistant to oxacillin.[19] Isolated CONS from

Northern population of Jordan revealed the resistance rate of 98.2% and 97.3% to ampicillin and penicillin, respectively.[28] The resistance rate of *Staphylococcus epidermidis* to oxacillin at Imam Khomeini hospital in Tehran and Namazi Hospital in Shiraz was 57.1%[16] and 100%,[13] respectively. This disparity in the rate of *S. epidermidis* resistant to oxacillin in these two investigations can partially due to different study settings (all clinical wards vs. only ICUs, respectively), and the method of determining antibacterial resistance (disk diffusion vs. E-test, respectively).

Beside beta lactams, CONS exhibited high resistance to aminoglycosides including gentamicin (40–57.14%) in our study. This is relatively within the range (60–70%) in the US.[29] Al Tayyar *et al*. reported that 49.3% of isolated CONS in different local hospitals of Jordan were resistant to gentamicin.[28] The resistant rate of this pathogen to gentamicin in two hospitals in Tehran and one hospital in Shiraz was 42.9%,[16] 87.5%,[27] and 50%,[14] respectively. Altogether, vancomycin appears to be remaining as the mainstay empirical and definite therapies of CONS infections in our center.

In our study, Enterococcus spp. exhibited high resistance rate to various antibacterials such as ampicillin, gentamicin, and especially, vancomycin. The frequency of VRE in developed countries such as US, most European countries (other than the UK and Ireland), UK, and Ireland is reported to be 30%,[30] <10%, and up to 32%,[20] respectively. The rate of VRE from different clinical settings in Iran varies considerably. For example, in an infectious diseases ward of Imam Khomeini Hospital in Tehran, between 6.7% and 18.75% of Enterococcus spp. isolates were resistant to vancomycin from 2007 to 2010.[15] A cross-sectional study at different wards of the same hospital over 1 year period reported the VRE rate of 54.5%.[16] Two studies from ICUs of two teaching clinical settings including Namazi Hospital in Shiraz demonstrated that all of Enterococcus spp. isolates were sensitive to vancomycin.[14] In contrast, Askarian *et al*., at the same hospital, reported that 99 out of 700 patients (14%) were rectally colonized with VRE.[31] Apart from type of study and method of detecting the pathogen, variation in possible risk factors of VRE colonization and infection can justify these different findings. In addition to vancomycin selection pressure and duration of treatment (>7 days), underlying disease, hemodialysis, and history of using a third generation cephalosporin have been also identified as associated factors of VRE in Namazi Hospital.[31] Although not studied in our population, linezolid can be considered as an effective treatment option of VRE because at least two studies in Tehran[15] and Shiraz[13] teaching hospitals demonstrated that all Enterococcus spp. isolates were sensitive to this agent. In addition to linezolid, according to the present as well as Soltani *et al*. results,[16] another potentially available, relatively low-cost antibacterial agent for the treatment of VRE in the studied centers is chloramphenicol.

The resistance rate of Enterococcus spp. to aminoglycosides (including gentamicin and amikacin) during our study period was high and ranged from 88% to 92.86%. Three studies on hospital-acquired urinary tract infections from Tehran[27,32] and Karaj[26] hospitals reported that between 40% and 100% of Enterococcus spp. isolates were resistant to either amikacin or gentamicin. Samanipour *et al*. reported that Enterococcus spp. isolates from a nephrology ward at Tehran were resistant to gentamicin in 75% of cases.[17] It has been showed that 66.7% of isolated Enterococcus spp. from two ICUs in Shiraz (including Namazi Hospital) was resistant to gentamicin.[14] Widespread use of aminoglycosides for both outpatients and inpatients seems to be the major cause of aminoglycoside resistance. In this regards, Vessal *et al*. reported that cefazolin plus gentamicin was the most commonly used preoperative antibiotic prophylaxis regimen at the surgical ward of Namazi Hospital in March 2010.[33] Apart from this, overuse of these medications in the agriculture as well as animal husbandry can also account for high resistance to aminoglycosides. High levels of gentamicin-resistant *Enterococcus faecalis* and *Enterococcus faecium* have been detected in the human food supply.[4,34] High resistance to aminoglycosides can challenge the combination treatment of Enterococcus spp. infections such as infective endocarditis because these agents are usually administered with beta lactams or glycopeptides as synergistic therapy.[16]

The present survey had 5 major limitations. First, the retrospective pattern of the study precluded comparing the results of patients' antibiogram with their clinical condition and response to antimicrobial treatment. Second, determination of antimicrobial susceptibility was performed by the classic disc diffusion method. Currently, more reliable and accurate methods such as microbroth dilution or E-test are recommended. Third, vanA and vanB genes were not determined by PCR to confirm isolates suspected to VRSA and VRE. Fourth, our hospital laboratory was not capable of distinguishing *Enterococcus faecium* from *Enterococcus faecalis* or *Streptococcus viridans* from *Streptococcus pneumoniae* isolates during the study period. Finally, some necessary antibiotic discs such as linezolid, teicoplanin, and rifampin were not routinely considered by the hospital laboratory for assessing antimicrobial susceptibility of isolated. *S. aureus* and Enterococcus spp.

S. aureus was the most frequent Gram-positive pathogen isolated from all samples. The rate of ORSA in our clinical setting was considerably high. In addition, 6.67% of *S. aureus* isolates were resistant to vancomycin. The sensitivity rate of CONS to vancomycin was high and did not change considerably during the 3 years period. Vancomycin can be remained as the first line empirical and definite treatment of CONS. The rate of VRE increased considerably (from 40.63% to 72.73%) during the study period. The resistance rate of CONS as well as Enterococcus spp. to aminoglycosides was also high. Lack of appropriate infection control activities and antimicrobial selection pressure due to antibiotic overuse may be the main reasons for our concerning findings about high rates of ORSA and VRE.

Antimicrobial stewardship programs including education and guideline implementation (e.g., active participation of clinical pharmacists and microbiologists in antibacterial therapy), preventive strategies (e.g., antimicrobial lock solution for prevention of catheter-associated infection), formulary and restriction strategies (dispensing linezolid or teicoplanin only after the verification of infectious disease service), review and feedback strategies (e.g., reviewing antimicrobial orders for catheter-associated infections), computer-assisted strategies (e.g., computerized physician order-entry and automatic stop orders), and antibiotic cycling strategies (e.g., using chloramphenicol instead of aminoglycosides for treatment of CONS or VRE) seems to be the last ditch to encounter and manage the antibacterial resistance dilemma in our center.

AUTHORS' CONTRIBUTION

Iman Karimzadeh contributed in study design, data analysis, and manuscript review. Mona Mirzaee contributed in data collection and manuscript drafting. Niloofar Sadeghimanesh contributed in data collection and manuscript drafting. Mohammad Mahdi Sagheb contributed in data interpretation and manuscript review.

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Conflicts of interest
There are no conflicts of interest.

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