

Antibiotic Resistance Profile of Gram-Positive Bacteria Isolated from Inpatients of an Academic and a Private Hospital in Arak, Central Iran, 2013-2014

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ABSTRACT

Introduction: Our country's per capita consumption of antibiotics and especially third-generation cephalosporins is significant. It has resulted in emergence of new drug resistant strains. The aim of this study was to investigate antibiotic resistance profile of Gram-positive bacteria isolated from inpatients of an academic and a private hospital in the Arak, central Iran.

Methods: This cross-sectional study was conducted in 2013-2014, and included all cases referred to Amir Almomemini (Academic Hospital) and Imam Khomeini Hospital (Private hospital) microbiology laboratories. Gram-positive organisms pattern, were tested for sensitivity to penicillin, oxacillin, ampicillin, TMP-SMX (trimethoprim-sulfamethoxazole), rifampin, vancomycin, clindamycin, ciprofloxacin and gentamicin by the disk diffusion method.

Results: From the 846 specimens of patients in both hospitals, 690 (81.6%) samples were from Amir

Almomemini Hospital and 156 (18.4%) cases from Imam Khomeini Hospital, of which, 326 samples (38.5%) were Gram-positive and 520 samples (61.5%) were Gram-negative. Of the 326 samples of Gram-positive bacteria, 61.3% were coagulase-negative *Staphylococcus*, 13.5% enterococci and 5.5% *Staphylococcus aureus*. The prevalence of gram-positive organisms in Amir Almomemini Hospital was 84.4% and in Imam Khomeini Hospital 15.6% ($P=0.34$). In addition, the specific resistance components in the public hospital were significantly higher than the private hospital ($P<0.001$).

Conclusion: Antibiotic resistance profile of Gram positive organisms isolated from the two hospitals were significantly different. The profile of specific resistances in two academic and non-academic hospitals showed significant differences. Therefore, a care program for reviewing and monitoring of the regional resistance patterns is recommended.
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INTRODUCTION

Our country's per capita consumption of antibiotics is significant and sometimes irrational and the consumption has increased considerably in recent years. On the other hand, the types of antibiotics being prescribed in hospitals are usually third-generation cephalosporins that are effective on *Pseudomonas aeruginosa* and MRSA (Methicillin-resistant *Staphylococcus aureus*); thus, the

pattern of antimicrobial resistance in hospitals is changing. Consumption of over-the-counter antimicrobial agent has been increased especially among high-risk groups such as IV drug abusers and immune compromised patient [1].

The antibiotics used for patients admitted to different hospitals, are diverse; thus, the pattern of hospital antimicrobial resistance should be defined separately [3, 4]. In fact, patterns of antimicrobial resistance in any hospital, or in any geographic area can be different and for this

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reason it should be reported separately and monitoring antimicrobial resistance in various organisms in the community and in hospitals is imperative [4]. Among Gram-positive bacteria, in terms of the resistance, MRSA (methicillin-resistant *S. aureus*) and VRE (vancomycin-resistant *Enterococci*) are important and surveillance programs must be designed by nosocomial infection control committees of hospitals for their monitoring [5].

Each region has its own type of drug resistant bacteria and in our area (Markazi Province of Iran); it seems that the first line and second-line drug-resistant strains are growing. In daily practice, we are faced with multi-drug resistant organisms, both in hospitalized cases and outpatients that cause many clinical dilemmas. This descriptive was designed to identify these organisms and their antimicrobial resistance patterns, so that future interventions can be designed to reduce this trend.

MATERIALS AND METHODS

This cross-sectional study was conducted for a year (Jun 2013 to Jun 2014) in all cases referred to Amir Almomemini Hospital (academic referral hospital) and Imam Khomeini Hospital, Arak, central Iran.

Examples referral to hospitals' microbiology labs included: urine, blood, nasal discharge, ulcer discharge, sputum, catheters, endotracheal tube, abscesses, ascites, synovial fluid, pleural effusion, and CSF. Upon the request of the attending physician, different samples were collected from the main site of infection with the appropriate methods (swab, aspiration, needle biopsy, etc.) and, if necessary, were placed in transport media and along with demographic data questionnaire were sent to the hospital laboratory [5].

First direct smears were studied and then the samples were cultured in suitable medium including blood agar, chocolate agar and other selective media. In case of a positive culture of potential pathogenic bacteria, initial direct smear, and Gram stain, Gram-positive specimens were examined for sensitivity to penicillin, oxacillin, ampicillin, TMP-SMX (trimethoprim-sulfamethoxazole), rifampin, vancomycin, clindamycin, ciprofloxacin and gentamicin by the disk diffusion method according to 2010 Clinical Laboratory Standards Institute (CLSI M100-S20) (MAST Company, UK).

Finally, all data were extracted by the sheet chart and the data obtained, according to research objectives were analyzed by chi-square

and Mann-Whitney test in SPSS software version 18 (Chicago, IL, USA).

Necessary training to hospital infection control committee on the implementation and coordination of the laboratories was provided and clinical isolates were matched with clinical manifestations. Cases recorded as index and those that merely suffered from mild infection were excluded.

This study was approved by Medical Ethics Committee of Arak University of Medical Sciences (approval code 88-75-9).

RESULTS

Of total 846 culture-positive samples taken from patients in two hospitals, 690 (81.6%) samples were from the Amir Almomemini Hospital and 156 samples (18.4%) from Imam Khomeini Hospital. In total, 326 samples (38.5%) contained Gram-positive and 520 (61.5%) Gram-negative bacteria. Of 326 gram-positive samples, 275 samples (84.4%) were from the Amir Almomemini Hospital and 51 samples (15.6%) were related to Imam Khomeini Hospital. The incidence of gram-positive organisms were significantly different between two hospitals ($P= 0.009$), and the prevalence of these organisms in Amir Almomemini Hospital which an academic referral hospital was more than Imam Khomeini Hospital. In Amir Almomemini Hospital, the most prevalent organisms were coagulase-negative *Staphylococcus* (69.8%), *Enterococci* (14.2%) and *S. aureus* (4.4%). In Imam Khomeini Hospital, the prevalences were similar to the results of Amir Almomemini Hospital (Table 1).

Of 275 strains isolated from patients of Amir Almomemini Hospital, most strains of Gram-positive isolates were from samples of urine, blood, and nasal secretions in descending order. Moreover, of 51 strains of gram-positive bacteria isolated from patients in Imam Khomeini Hospital, most strains were from blood samples followed by urine and wound samples (Table 2).

Antibiotic Resistance of Gram-positive Bacteria in Imam Khomeini and Amir Almomemini hospitals are shown in table 3 and 4, respectively.

Comparison of specific resistance tests among Gram-positive organisms between the Amir Almomemini and Imam Khomeini hospitals revealed that, specific resistances were significantly different in the two hospitals ($P=0.001$), and the components of the specific resistance in Amir Almomemini Hospital

(Academic Hospital) was higher than Imam Khomeini Hospital (Table 5).

Table 1. Gram-positive bacteria isolated from samples at each hospital of Arak, Iran

Hospital	Number of Samples	<i>S. aureus</i> N (%)	CONS N (%)	Enterococci N (%)	Other gram positives N (%)
Amir Almomenin	275	12 (4.4)	192 (69.8)	39 (14.2)	32 (11.6)
Imam Khomeini	51	8 (15.7)	30 (58.8)	10 (19.6)	3 (5.9)
Total	326	20 (6.1)	222 (68.1)	49 (15)	35 (10.7)

Key: CONS=coagulase-negative *staphylococcus*, NB: Values in parenthesis are percentage occurrence of isolates.

Table 2. Gram-positive bacteria isolated from samples at each source of infection in two hospitals of Arak, Iran

Location of samples	Amir Almomenin Hospital	Imam Khomeini Hospital
	N (%)	N (%)
Urine	105 (38.1)	17 (33.3)
Blood	70 (25.4)	24 (4.7)
Wound discharge	14 (5.09)	7 (13.7)
Nasal discharge	22 (8)	1 (1.9)
Sputum	22 (8)	0
Catheter	8 (2.9)	0
Tracheal aspirate	20 (7.2)	2 (3.9)
Ascetic fluid	6 (2.1)	0
Synovial fluid	1 (0.36)	0
pleura fluid	6 (2.1)	0
CSF	1 (0.36)	0
Total	275 (100)	51 (100)

Key: NB: Values in parenthesis are percentage occurrence of isolates

Table 3. Antibiotic Resistance of Gram Positive Bacteria from Imam Khomeini Hospital, Arak, Iran

Antibiotic	<i>S. aureus</i>				Coagulase-negative <i>Staphylococcus</i>				Enterococci				Other gram positives			
	*	Su	R	Int	*	Su	R	Int	*	Su	R	Int	*	Su	R	Int
Oxacillin	8	7	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Ampicillin	3	1	2	0	4	3	0	1	2	1	1	0	0	0	0	0
TMP-SMX	4	1	2	1	18	1	13	4	6	1	4	1	1	1	0	0
ciprofloxacin	2	1	0	1	2	2	0	0	3	0	3	0	1	0	1	0
clindamycin	5	4	1	0	24	11	9	4	3	1	1	1	3	0	3	0
gentamicin	3	2	1	0	2	1	1	0	1	1	0	0	6	4	1	1
Rifampin	1	1	0	0	9	5	4	0	1	0	1	0	1	0	1	0
Penicillin	0	0	0	0	8	2	6	0	1	0	1	0	1	0	1	0
Vancomycin	8	6	2	0	26	26	0	0	8	8	0	0	3	3	0	0

Key: TMP-SMX= trimethoprim-sulfamethoxazol, R= Resistant, Su = Susceptible, Int=Intermediate, *= Tested cases of every organism for each antibiotic

Table 4. Antibiotics Resistance of Gram Positive Bacteria from Amir Almomenin Hospital, Arak, Iran

Antibiotic	<i>S. aureus</i>				Coagulase-negative <i>Staphylococcus</i>				Enterococci				Other gram positives			
	*	Su	R	Int	*	Su	R	Int	*	Su	R	Int	*	Su	R	Int
Oxacillin	12	11	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Ampicillin	1	1	0	0	2	1	0	1	2	2	0	0	3	3	0	0
TMP-SMX	12	8	2	2	136	42	61	33	21	4	16	1	24	15	7	2
ciprofloxacin	0	0	0	0	15	11	3	1	18	2	14	2	11	7	3	1
clindamycin	12	11	1	0	177	86	86	5	2	2	0	0	18	9	8	1
gentamicin	0	0	0	0	5	3	2	0	6	3	2	1	1	0	1	0
Rifampin	6	5	1	0	75	60	11	4	12	1	8	3	9	5	2	2
Penicillin	5	3	2	0	35	13	22	0	6	0	5	1	5	1	3	1
Vancomycin	11	8	0	3	172	156	9	7	34	21	7	6	31	29	0	2

Key: TMP-SMX=trimethoprim-sulfamethoxazol, R= Resistant, Su = Susceptible, Int=Intermediate, *= Tested cases of every organism for each antibiotic

Table 5. Prevalence of antibiotics special resistances of Gram-positive bacteria from Amir Almomenin and Imam Khomeini hospitals, Arak, Iran

Hospital	MRSA N (%)	MSSA N (%)	VRE N (%)	VSSA N (%)	MRCONS N (%)	DZON N (%)	VISA N (%)
Amir Almomenin	1(8.3)	11(91.6)	6(15.3)	1(8.3)	77(40.1)	15(7.8)	3(25)
Imam Khomeini	1(12.5)	7(87.5)	1(10)	2(25)	12(40)	1(3.3)	0(0)
Total	2(10)	18(90)	7(14.2)	3(15)	89(40)	16(7.2)	3(15)

Key: NB: Values in parenthesis are % occurrence of isolates, MRSA: Methicillin-resistant *S. aureus*, MSSA: Methicillin-sensitive *S. aureus*, VRE: Vancomycin-resistant Enterococci, VSSA: Vancomycin-sensitive *S. aureus*, MRCONS: Methicillin-resistant coagulase-negative Staphylococci, VISA: Vancomycin-intermediate *S. aureus*, DZON: An indicator for evaluation of resistance induction from the erythromycin toward clindamycin in streptococci and staphylococci

DISCUSSION

A total of 846 bacteria were isolated in both hospitals, 38.5% were Gram-positive bacteria including *S. aureus*, coagulase-negative *Staphylococcus*, *Enterococcus* and others. Of these Gram-positive samples, 84.4% cases were from Amir Almomenin Hospital and 15.6% from Imam Khomeini Hospital. The incidence of gram-positive nosocomial infection was only 9.23% [6].

In our study, most strains isolated from Amir Almomenin and Imam Khomeini hospitals were from urine, blood, wound and nasal secretions that were similar to another study [7].

Most Gram-positive isolates were *Staphylococcus* coagulase negative. Gungal et al in a study in 2012 in Niger, performed on Gram-positive organisms isolated from wound

infections, reported that, of 500 samples, *S. aureus* was the most prevalent [8]. In a study performed in North America, the most pathogen isolated from blood stream infections was Enterococci [9]. In other studies, among the isolated Gram-positive organisms, the most common were *S. aureus*, coagulase-negative *Staphylococcus*, followed by *Enterococcus* [10]. Whereas, in our study the most common isolated gram-positive bacteria, were coagulase-negative *Staphylococcus*, *Enterococcus* and *S. aureus* respectively.

About the pattern of resistance to important antibiotics that are used in clinical practice, among the Gram-positive bacteria isolated from both hospitals of 52 gram-positive strains in two hospitals, 24 samples (46.1%) were resistant, 23 samples (44.2%) were sensitive and five samples (9.6%) were intermediate ciprofloxacin. In other studies, the sensitivity of

Gram-positive bacteria, especially *S. aureus* to ciprofloxacin is still considerable [1]. The rate of *S. aureus* resistant to ciprofloxacin at various hospitals of Niger were 10%, 14%, 11% and 28%, and it was found that the majority of isolated organisms were sensitive to ciprofloxacin [8].

Among the Gram-positive bacteria isolated from both hospitals to clindamycin among the strains of *S. aureus* (coagulase positive and negative together) 218 strains underwent clindamycin disk susceptibility testing and 112 cases (51.3%) were susceptible, 97 cases (44.5%) were resistant and 9 cases (4.1%) were intermediate. Clindamycin is an antibiotic especially effective on anaerobic organisms; therefore, it can be postulated that this antibiotic continues to be effective in this group of organisms [1].

From 10 strains of *S. aureus* and coagulase-negatives tested for gentamicin at both hospitals, six cases (60%) were susceptible, three cases (30%) were resistant and one case (10%) was intermediate. Sani et al. reported similar rates of *S. aureus* resistant to gentamicin in a few hospitals (39%, 11%, 11% and 16%) [8].

From the 7 strains of *S. aureus* isolated from two hospitals 6 samples (85.7%) were susceptible to rifampin and 1 sample (14.2%) was resistant. While, in Hasani et al's study, the rate of *S. aureus* resistant to rifampin was 17.3% [11].

Of 43 strains of coagulase-negative staphylococci tested for sensitivity to penicillin, 15 cases (34.8%) were sensitive and 28 cases (65.1%) were resistant. As a result, resistance to coagulase negative Staphylococci to penicillin have increased. From the 7 strains of Enterococci tested for sensitivity to this antibiotic, 6 cases (85.7%) were resistant and 1 case (14.2%) was intermediate, As a result, similar to coagulase-negative *Staphylococcus*, enterococci resistant to penicillin was significant.

Yezli et al noted that, vancomycin resistant *S. aureus* were not reported in Saudi Arabia [12]. It should be noted that, the necessary elements for resistant to vancomycin required confirming MIC (Microbial Inhibition Concentration) [5]. In the case of coagulase-negative *Staphylococcus*, from the 172 strains in Amir Almomemini Hospital that were tested for vancomycin, 156 cases (90.6%) were sensitive, 9 cases (2.5%) were suspected of resistance and 7 cases (4.06%) were also suspected intermediate.

In Biedenhach et al. study, the rate of vancomycin-resistant enterococci was 17.7% and it was reported as the most resistant gram-positive organisms in North America [9]. From

the eight strains of enterococci isolated in Imam Khomeini Hospital, which were tested for vancomycin, all eight were sensitive, which indicates that the prevalence of VRE in academic hospital was much higher than private hospital.

About the patterns of specific resistance in Amir Almomemini hospital, from the 39 strains of Enterococci isolated, six cases were VRE, while in Imam Khomeini hospital from the 10 strains one case was VRE, which indicates the prevalence of this resistance pattern in teaching hospital was higher than the private hospital. Were conducted on bacterial resistance of Gram-positive bacteria, it was found that, types of antibiotic specific resistance in recent surveys is on the rise in both community-acquired and hospital-acquired cases [12].

Of the 12 *S. aureus* isolates from Amir Almomemini hospital, only 1 case was MRSA, and from the 8 strains of *S. aureus* isolated in Imam Khomeini hospital 1 case was MRSA. Louis et al. found that, 60% of hospital *S. aureus* infections was due to MRSA, and this trend is also rapidly increasing [10], which is different from our findings.

From the 192 strains of coagulase negative *Staphylococcus* isolated in Amir Almomemini Hospital, 98 strains (51.04%) were MRCONS negative and 77 strains (40.1%) were MRCONS positive. In contrast, from the 30 coagulase-negative Staphylococci isolated from Imam Khomeini Hospital, 12 strains (40%) were MRCONS positive and 18 cases (60%) were MRCONS negative.

From the 192 strains of coagulase negative *Staphylococcus* isolated In Amir Almomemini Hospital, 15 strains (7.8%) were positive for DZON test (An indicator for evaluation of resistance induction from the erythromycin toward clindamycin in streptococci and staphylococci), and from the 30 strains of coagulase-negative *Staphylococcus* isolated in Imam Khomeini Hospital, only one strain (3.3%) was positive for this test. Thus, the components of this resistance in teaching hospital were higher than the private hospital.

Generally, in this study, we found that the most common organisms isolated were coagulase-negative *Staphylococcus* followed by Enterococci, and *S. aureus*. Among the investigated antibiotic, resistance to penicillin was of considerable importance, and the prevalence of VRE (vancomycin-resistant enterococci) organisms has increased and is growing, subsequently this issue can be viewed as warning sign for a new trend in antibiotic resistance, especially in academic hospitals. The limitations of the study was the small sample size, it is recommended that future studies with

larger sample sizes to be done. It should be noted that this study did not evaluate the resistance pattern of linezolid. Nevertheless, in Potoski et al's study as a warning, resistant coagulase-negative *Staphylococcus* to linezolid were discussed [13].

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