Antibiotic Susceptibility Pattern of Gram-positive Cocci Cultured from Patients in Three University Hospitals in Tehran, Iran during 2001-2005

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Abstract- Bacterial resistance to antibiotics is a serious problem and is increasing in prevalence world-wide at an alarming rate. The antimicrobial susceptibility patterns of 1897 gram-positive bacterial Isolates were evaluated. The minimum inhibitory concentration (MIC) of isolates which comprised *Staphylococcus aureus* (927 isolates), coagulase-negative staphylococci (CNS; 425 isolates), *Enterococcus faecalis* (320 isolates), *Enterococcus faecium* (157 isolates), and pneumococci (50 isolates) collected from 3 teaching hospitals in Tehran were determined by agar dilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The presence of *mecA* gene was investigated in methicillin-resistant staphylococci by PCR method and *vanA* and *vanB* genes were targeted in enterococcal isolates by Multiplex PCR method. The resistance rate to methicillin among *S. aureus* and CNS isolates were 33% and 49%, respectively. All *S. aureus* isolates were susceptible to vancomycin .The lowest rate of resistance in all *S. aureus* isolates was found for rifampicin (<4%). The vancomycin resistance rate in enterococci isolates was 11% which was more frequent among *E. faecium* (19%) than *E. faecalis* (4%), all resistant isolates carrying *van*A. High-level resistance to gentamicin and streptomycin, were detected in 47% and 87% of enterococcal isolates respectively. The rate of penicillin resistance in pneumococci was 3% and about 27% of isolates had reduced susceptibility to penicillin. The prevalence of erythromycin resistant among pneumococci was 58%. All pneumococcal isolates were susceptible to ceftriaxone, rifampicin and vancomycin. Our data highlight the importance of access to updated bacterial susceptibility data regarding commonly prescribed agents for clinicians in Iran.

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Key words: Antimicrobial susceptibility patterns, gram-positive Cocci, MRSA, VRE, Pneumococci

Introduction

Gram-positive cocci still predominate as a cause of nosocomial- and community-acquired infections. *Staphylococcus aureus* is the most common cause of wound infections, whereas coagulase-negative staphylococci (CNS) is the most common in nosocomial blood stream infections. Enterococci has appeared as the second or third most commonly isolated organisms from nosocomial infections. In community-acquired infections, one of the most important agents is pneumococci as a common cause of upper (sinusitis, otitis media) and lower (pneumonia) respiratory tract infections, bacteremia, meningitis and other suppurative infections (1). Furthermore, antimicrobial resistance in gram-positive cocci in particular the emergence of methicillin resistance in staphylococci, glycopeptides resistance in enterococci and *Streptococcus pneumoniae* resistant to penicillins are recognized as global problems with serious implications at the clinical level (2). The dramatic reduction of therapeutic options to treat patients infected with these microorganisms is of great concern.

Regards to this situation there is an agreed need for more effective surveillance of resistance. In developed countries, nationwide surveillance programs such as the National Nosocomial Infections Surveillance (NNIS) System monitor the antimicrobial resistance patterns of bacterial pathogens (3). Unfortunately, in many parts of the world, including Iran, such national surveillance programs are absent and information regarding the antimicrobial susceptibility patterns of pathogens is scarce. Epidemiological studies have demonstrated that data

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regarding susceptibility patterns of bacteria from a geographical region are essential for controlling the local spread of bacterial resistance as well as preventing the spread of resistance in a geographical region (4). Moreover, updated bacterial susceptibility data are particularly crucial to physicians and infection control practitioners in countries such as Iran where over-the-counter antimicrobial consumption and abuse of prescribed antibiotics are widespread . In this study we assessed the antimicrobial susceptibility profiles of gram-positive cocci isolates from patients in teaching Hospitals in Tehran, Iran.

Patients and Methods

Patient specimens and bacterial strains

A total of 1897 isolates were taken from patients who were admitted to three teaching hospitals of Tehran University of Medical Sciences. All bacterial isolates were identified in the department of Microbiology using standard biochemical methods (5, 6). Staphylococci were identified by Grams stain and coagulase test, using rabbit plasma. *Streptococcus pneumoniae* strains were characterized on the basis of bile solubility and optochin susceptibility. Enterococci were identified by hydrolysis of esculin in the presence of bile and by growth in 6.5% NaCl. The enterococcal species was identified by motility test, arginine decarboxylation in Moeller decarboxylase media, pyruvate utilization, and fermentation of carbohydrates (Arabinose, Raffinose , Mannitol, Ribose).

Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MIC) and determination of MIC breakpoints performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (7), by using agar dilution method. MICs of oxacillin were determined on Mueller-Hinton agar supplemented with 2% NaCl. Plates were inoculated with 10^5 CFU/spot and incubated for 24 h at 35°C. The antimicrobial evaluated were ampicillin, ciprofloxacin, ceftriaxone, erythromycin, gentamicin, penicillin, rifampicin, streptomycin, teicoplanin, and vancomycin. The antimicrobial agents were obtained from the following manufacturers: vancomycin, oxacillin and streptomycin from Sigma Chemical Co. (Steinheim, Germany), teicoplanin, erythromycin, ceftriaxone, and gentamicin from Mast Group Ltd (Merseyside, UK), and penicillin G and rifampicin from Merck (Darmstadt, Germany).

Quality control strains included *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, and *S. pneumoniae* strain ATCC 49619.

Since ciprofloxacin CLSI interpretive categories do not exist for *S. pneumoniae*, we used the MIC of ≤4 µg/ml, which have been used in other studies, as the criterion for categorizing isolates as having reduced ciprofloxacin susceptibility (8).

All antimicrobial susceptibility results were rounded down if they were <0.5, and were presented as whole numbers if they were ≥0.5.

Detection of *mecA*, *vanA*, and *vanB* Genes

The *mec*A gene was detected by a PCR-based method, using a set of previously designed primers (9). All enterococci with vancomycin MICs >4 µg/ml were evaluated for *van*A and *van*B genes by multiplex-PCR.

E. faecalis E206 (*vanA* positive), *E. faecium* E2781 (*vanB* positive), courtesy of Dr. Edet Udo *and* methicillin-resistant *S. aureus* ATCC 43300 were used as control strains.

Results

In total, 1897 clinical strains of gram-positive cocci include 927 isolates of *S. aureus*, 425 isolates of CNS, 495 isolates of Enterococcus Spp and 50 isolates of *S. pneumoniae* were evaluated in this study.

Resistance pattern

The antimicrobial susceptibility patterns of staphylococci species are summarized in Table 1.

The prevalence of resistance to oxacillin was 33 % in *S. aureus* isolates and 49% in CNS isolates. These isolates were confirmed as methicillin resistant by detection of the *mecA* gene.

High rates of resistance in MRSA isolates were found with erythromycin (73%), gentamicin (67%) and ciprofloxacin (51%). Resistance rate for rifampicin was 7 %. In methicillin susceptible *S. aureus* (MSSA) strains, 65% and 88% of isolates were susceptible to erythromycin and gentamicin, respectively. No vancomycin-intermediate *S. aureus* isolates were detected (MIC_{90} <2 µg/mL.

The lowest percentage of susceptibility amongst CNS isolates was observed in methicillin (51%) and erythromycin (58%) followed by ciprofloxacin (89%) and gentamicin (80 %). All CNS isolates were susceptible to vancomycin.

Of 495 enterococci isolates, 320 (67%) were *E. faecalis*, 157 (32%) were *E. faecium* and 18 (3 %) were other enterococcal species include *E. mundtii* and *E. durans*.

Table1. MIC distribution of staphylococcal isolates

Organism (N)	Antibiotic	Break point	% of isolates MIC (µg/mL)			
			Range	50%	90%	R
MRSA (310)	Ciprofloxacin	≥ 4	≤ 1-16≤	8	16	51
	Erythromycin	≥ 8	≤ 4-1024	512	512	73
	Gentamicin	≥ 8	≤ 8-512≤	128	512	67
	Rifampicin	≥ 4	≤ 1-128≤	1	1	7
	Vancomycin	≥ 32	≤ 0.5-2	1	2	0
	Oxacillin	≥ 4	8-1024 ≤	128	512	100
MSSA (617)	Ciprofloxacin	≥ 4	≤ 1-16 ≤	0.5	1	4
	Erythromycin	≥ 8	≤ 4-1024	8	8	35
	Gentamicin	≥ 8	≤ 8-512 ≤	4	128	12
	Rifampicin	≥ 4	≤ 1-128 ≤	0.5	1	1
	Vancomycin	≥ 32	≤ 0.5-2	0.5	1	0
	Oxacillin	≥ 4	≤ 0.25-2	0.5	0.5	0
CNS (425)	Ciprofloxacin	≥ 4	≤ 1-16 ≤	1	16	11
	Erythromycin	≥ 8	≤ 4-1024	4	512	42
	Gentamicin	≥ 8	≤ 8-512 ≤	8	64	20
	Rifampicin	≥ 4	≤ 1-128 ≤	1	1	6
	Vancomycin	≥ 32	≤ 0.5-2	1	2	0
	Oxacillin	≥ 0.5	≤ 0.25-1024≤	0.25	256	49

Table 2. MIC distribution of enterococcal isolates

Organism (N)	Antibiotic	Break point	% of isolates MIC (µg/mL)			
			Range	50%	90%	R
E. faecalis (320)	Ampicillin	≥ 16	≤ 4-256	4	16	9
	Ciprofloxacin	≥ 4	≤ 2-16≤	2	8	36
	Erythromycin	≥ 8	≤ 1-1024	1	512	37
	Penicillin	≥ 16	≤ 2-64≤	4	4	9
	Rifampicin	≥ 4	≤ 1-128≤	8	64	57
	Gentamicin	> 500	≤ 125-4000 ≤	500	≥ 4000	42
	Streptomycin	> 2000	≤ 125-4000 ≤	≥ 4000	≥ 4000	85
	Vancomycin	≥ 32	≤ 2-512	8	128	4
	Teicoplanin	≥ 32	≤ 2-128 ≤	8	32	4
E. faecium (157)	Ampicillin	≥ 16	≤ 2-256 ≤	8	128	42
	Ciprofloxacin	≥ 4	≤ 2-16 ≤	4	16	51
	Erythromycin	≥ 8	≤ 1-512 ≤	256	512	57
	Penicillin	≥ 16	≤2-64≤	32	64	38
	Rifampicin	≥ 4	≤ 1-128 ≤	16	128 ≤	73
	Gentamicin	> 500	≤ 125-4000 ≤	2000	4000 ≤	59
	Streptomycin	> 2000	250-4000≤	4000 ≤	4000 ≤	90
	Vancomycin	≥ 32	≤ 2-512	4	512 ≤	19
	Teicoplanin	≥ 32	≤ 2-128 ≤	2	128 ≤	19
All Enterococci (495)	Ampicillin	≥ 16	≤ 2-128	4	128	20
	Ciprofloxacin	≥ 4	≤ 2-16 ≤	2	16	37
	Erythromycin	≥ 8	≤ 1-512 ≤	4	512	41
	Penicillin	≥ 16	≤ 2-64 ≤	4	32	19
	Rifampicin	≥ 4	≤ 1-128 ≤	8	64	57
	Gentamicin	> 500	≤ 125-4000 ≤	1000	4000≤	47
	Streptomycin	> 2000	250-4000 ≤	4000≤	4000≤	87
	Vancomycin	≥ 32	≤ 2-512	4	128	11
	Teicoplanin	≥ 32	≤ 2-128 ≤	4	32	11

Table 3. MIC distribution of *Streptococcus pneumoniae* isolates

Organism N)	Antibiotic	Break point	% of isolates			
			MIC (µg/mL)			
			Range	50%	90%	R
Pneumococci (50)	Ceftriaxone	≥ 4	≤ 0.25-0.5	0.25	0.5	0
	Ciprofloxacin	≥ 4	≤ 1-86 ≤	2	4	30
	Erythromycin	≥ 4	≤ 0.25-64	1	16	58
	Penicillin	≥ 2	≤ 0.06-2	0.06	0.5	3
	Rifampicin	≥ 4	1-1	1	1	0
	Vancomycin	≥ 2	0.5-1	0.5	1	0

Resistance rates and MIC of enterococci isolates are shown in Table 2. The highest susceptibility rate in *E. faecalis* was observed in vancomycin (96%), teicoplanin (96%), penicillin (91%) and ampicillin (91%), whereas for *E. faecium*, susceptibility rate for same antibiotics were 81%, 81%, 62% and 60% respectively. Resistance rate of *E. faecalis* to ciprofloxacin and erythromycin approximately was 16-21% lower than *E. faecium.* High-level resistance to the aminoglycosides, gentamicin (MIC >500 µg/mL) and streptomycin (MIC >2000 µg/Ml), were detected in 47% and 87 % of enterococcal isolates.

The overall rate of vancomycin resistance was 11%. The *van* determinants were targeted by multiplex- PCR method, fifty-four isolates carried the *van*A gene and their MIC value of vancomycin was ≥64 µg/mL.

All strains of *S. pneumoniae* were isolated from a children hospital. The in vitro susceptibility data of selected antimicrobial agents tested against *S. pneumoniae* isolates are presented in Table 3. The proportion of penicillin-susceptible isolates among 50 evaluated isolates was 70 % (35/50) with MIC_{90} of 0.5µg/mL. One isolate had high level resistance to penicillin (MIC 2 µg/mL). Strains with intermediate penicillin resistance were 27 % of isolates. About 58% of isolates were resistant to erythromycin. No resistance to rifampicin, ceftriaxone and vancomycin was found. Fifteen isolates with reduced susceptibility to ciprofloxacin (MIC ≥ 4 µg/ml) were detected.

Discussion

Surveillance studies are extremely important component of any action designed to control the spread of antimicrobial resistance (1). Unfortunately, data regarding bacterial resistance to antimicrobial agents are scarce in Iran. The need for reliable and comprehensive data regarding antimicrobial susceptibility patterns of gram-positive cocci specific to Iran prompted this study. Our data bring to light the fact that a serious problem of antimicrobial resistance exists among gram-positive cocci in Tehran.

In this study the resistance rate of oxacillin among *S. aureus* isolates was 33% , which is slightly higher than similar reports (31.2%) from Spain and Saudi Arabia and almost 2 times lower than some reports from Egypt (10-12). However, MRSA rates vary greatly among different countries (13-15); this geographical variation may reflect differences in infection control policies and other factors. The oxacillin resistance rate of 49% among our CNS isolate was approximately 24% lower than the oxacillin resistance rate reported from CNS isolates in the European medical centres, Latin American medical centres and Egypt, but similar to the rate reported from France (12, 14-17). As found by others, higher resistance rates to other antibiotics were seen for methicillin resistant staphylococci than for methicillin susceptible staphylococci (15-18). In this study, same as other reports, most MRSA isolates were resistant to erythromycin (MIC_{90} 512µg/ml), gentamicin (MIC_{90} 512 µg/ml) and ciprofloxacin (MIC_{90} 16 µg/ml), whereas resistance to rifampicin remained rare amongst MRSA (7%) and CNS (63%) isolates (16-18).

In this study, *E. faecalis* was the predominant type of enterococcal species (67%). On the other hand, the *E. faecium* comprised of 32% of isolates 2). Like many reports from other countries, our *E. faecium* isolates exhibited higher resistance to evaluated antibiotics than *E. faecalis* isolates (19, 20). The finding that over 20% of enterococcal isolates were resistant to ampicillin (MIC_{90} 128 µg/mL) is of a great concern, since ampicillin is the drug of choice in the treatment of enterococcal infections (20).

Forty-two percent of *E. faecalis* and 59% of *E. faecium* isolates showed high-level resistance to gentamicin. High-level streptomycin resistance was seen in 87% (MIC_{90} ≥ 4000 µg/mL) of enterococcal isolates. The prevalence of high-level gentamicin resistance in our *E.*

faecalis and *E. faecium* isolates was more prevalent than to that reported in the SENTRY program in Europe, UK, Kuwait and Colombian hospitals (15, 18, 21, 22). Other studies have reported variable prevalence of isolates with high-level aminoglycoside resistance (19, 23).

Although vancomycin resistance rates in Iran, like Europe, are relatively low compared with those reported in the USA (24), VRE appears to become more prevalent in Iran in recent years, i.e. from 7% in 2005 to 11% in this study (25, 26). These findings indicate an alarming shift in vancomycin susceptibility among enterococci in Iran in recent years.

Like many reports, in our study VanA was the most prevalent of Van phenotypes (23, 27). On the other hand, 30 of the 157 *E. faecium* isolates were vancomycin resistant, consistent with other findings that show *E. faecium* are usually more resistant than *E. faecalis* (27, 28).

The frequency of penicillin-no susceptible pneumococcal isolates (30%) in our study is lower than that observed in other countries, such as UK and France (47.5%) (18, 29). It was, however, similar to those of several eastern European countries (30). Differences in the rates of pneumococcal penicillin resistance among countries have been shown to be associated with levels of antimicrobial consumption (31). In our study, predominance of high resistance of pneumococci to erythromycin (58%) is considerable.

Like reports from the USA and Canada in our study all *S. pneumoniae* isolates were susceptible to vancomycin (32).

In conclusion, our data highlight the importance of access to updated bacterial susceptibility data regarding commonly prescribed agents for clinicians in developing countries such as Iran. Continuous monitoring of changes in bacterial resistance will help set national priorities for local intervention efforts in Iran. The high risk of infections due to antibiotic-resistant pathogens, particularly Gram-positive cocci, emphasizes the importance of enforcing rational antibiotic prescription policies and new vaccination strategies in Iran.

Finally, the present study showed a moderate incidence of MRSA and VRE in the teaching Hospitals in Tehran.

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