GLYCOPEPTIDE SUSCEPTIBILITY AMONG STAPHYLOCOCCI AND ENTEROCOCCI ISOLATES FROM SULAIMANI HEALTH LABORATORIES

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ABSTRACT

Background

Glycopeptide antibiotics are bactericidal agents that inhibit late stage bacterial cell wall peptidoglycan synthesis in Gram-positive bacteria. They are used for multiresistant Gram-positive cocci infection such as staphylococci and enterococci.

Objectives

To determine glycopeptide susceptibility among staphylococci and enterococci isolates from Sulaimani city health laboratories and find combined methicillin and vancomycin resistance among *S. aureus* isolates.

Materials and Methods

Isolates of staphylococci and enterococci were collected from different hospital laboratories and community health laboratories in Sulaimani city. Staphylococci were differentiated based on coagulase test while enterococci species were determined using Vitek 2 ®automated system. Antimicrobial susceptibility test was preformed according to the Bauer-Kirby disk diffusion method using vancomycin, teicoplanin, amoxicillin-clavulanate, cefoxitin, methicillin, erythromycin, amikacin, gentamicin and netilmicin disks. The susceptibility was determined according to the Performance Standards for Antimicrobial Disk Susceptibility Tests.

Results

A total of 207 isolates of staphylococci and enterococci were collected from six hospital laboratories and two community health laboratories in Sulaimani city. The isolates were 146 *Staphylococcus aureus*, 36 coagulase-negative staphylococci (CoNS) and 25 enterococci isolates. The isolates were from inpatients 182 (87.9%), the other 25 (12.1%) were from outpatients submitting their specimens to community health laboratories. Resistance to vancomycin was detected in one isolate of each *S. aureus* (0.7%), CoNS (2.8%) and enterococci species (4%). For teicoplanin, one *S. aureus* isolate showed intermediate response (0.7) and two isolates (1.4%) were resistant. One isolate of each CoNS (2.8%) and enterococci spp. (4%) was resistant to teicoplanin. One MRSA isolate (1.25%) was resistant to vancomycin and combined resistance to both vancomycin and teicoplanin was detected in three isolates.

Conclusion

Glycopeptide resistance was reported in staphylococci and enterococci but was uncommon. The reported resistance advises regular observation and monitoring the antibiotic susceptibility and strict antibiotic policy. Glycopeptide alternative still can be used in treatment of some Gram-positive infections, this may delay the emergence and spread of glycopeptide resistance.

Keywords: *Glycopeptide susceptibility, S. aureus, CoNS, enterococci, Sulaimani.*

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INTRODUCTION

Glycopeptide antibiotics are large, bactericidal molecules that inhibit late stage bacterial cell wall peptidoglycan synthesis in Gram-positive bacteria by binding directly to the terminal amino acid of the peptide chain, they include vancomycin and teicoplanin [1, 2]. Glycopeptide are primarily active against Gram-positive cocci but are reserved for multiresistant Gram-positive cocci infection caused by staphylococci or enterococci [3].

Vancomycin was the first discovered glycopeptide in early 1950s. It acts by irreversible binding to the terminal D-alanyl-D-alanine of cell wall disaccharide-pentapeptide precursors, thus inhibiting the synthesis of bacterial cell wall [4, 5]. Resistance to vancomycin was first reported in *Staphylococcus epidermidis* in 1987 [6], while the first clinical isolate of *S. aureus* with reduced susceptibility to vancomycin was reported in 1995 from a French child who was receiving vancomycin for methicillin-resistant *Staphylococcus aureus* (MRSA) line infection [7]. In 1996, a wound infection caused by vancomycin-intermediate resistant *Staphylococcus aureus* (VISA) was reported in Japan in a child receiving vancomycin for a MRSA infection [8]. In 1997, the first VISA isolate was reported in the United States [9].

Vancomycin resistance in enterococci (VRE) was first described in Europe in the late 1980s and spread to much of the developing world [10]. Vancomycin-resistant enterococci have emerged as important nosocomial-pathogens in the past two decades all over the world and have seriously limited the choice available for treating infections caused by these agents [11].

Different terms were proposed to describe response to glycopeptides. In addition to VISA or VRSA, there are strains of *S. aureus* referred as "heteroresistant (hVISA)". These strains are susceptible to vancomycin (MIC, ⩽4 μg/mL); however, they contain subpopulations for which the MIC of vancomycin is in the intermediate range. These subpopulations become apparent when the original isolate was cultured on a plate containing vancomycin and the isolates started growing [12]. It has been proposed that GISA or GRSA also be used to cover strains that are resistant to teicoplanin, although this is an accurate description, not all teicoplanin-resistant strains are resistant to vancomycin [13].

The increased prevalence of MRSA infections and use of vancomycin has led to the emergence of *S. aureus* strains with reduced vancomycin susceptibility but VRSA remains extremely rare and is less common than VRE or even vancomycin-resistant coagulase-negative staphylococci (CoNS) [14].

As CoNS have been increasingly recognized in bloodstream infections, especially among immunocompromised patients and patients with indwelling medical devices, infection with glycopeptide resistant-CoNS were also reported [15]. In 1983, clinical methicillin-resistant *S. epidermidis* isolates with teicoplanin MIC of 12.5 μg/mL (vancomycin MIC <3.13 μg/mL) were noticed [16]. However, the first two cases of infections with glycopeptide resistant *S. haemolyticus* were reported in 1986 [17, 18]. In 1990, a bloodstream infection caused by vancomycin-resistant, methicillin-resistant *S. haemolyticus* strain was reported [19]. In 1991, isolation of a methicillin-susceptible *S. epidermidis* strain with vancomycin MIC of ~16 μg/mL (teicoplanin MIC > 16 μg/mL) from a continuous ambulatory peritoneal dialysis associated peritonitis patient who responded poorly to vancomycin therapy (25 μg /mL intraperitoneal) was described [20].

In *S. aureus*, cell wall thickening and, potentially, genetic exchange is currently thought to underlie the development of vancomycin resistance [21]. *S. aureus may* acquire *van* gene that code for vancomycin resistance form *Enterococcus species*; this process was successfully demonstrated in the laboratory [22]. The presence of sex pheromone in *S. aureus* had been demonstrated which promotes plasmid transfer from *Enterococcus* species. Release of these pheromones by *S. aureus* with proximity to VRE causes the transfer of plasmids encoding *van* gene to the *S. aureus* [23].

Studies of vancomycin resistance in CoNS have shown that altered cell wall precursors are produced, but in amounts that are likely too small to account for the degree of resistance observed. Analysis of the cell wall peptidoglycans from highly resistant CoNS has demonstrated the presence of altered cross-links compared to susceptible strains [24].

The first enterococcal isolates resistant to high levels of vancomycin and teicoplanin were reported in 1988 [25]. Glycopeptide-resistant enterococci evade the action of vancomycin by modifying the antibiotic's molecular target: D-Ala-D-Ala, to D-Ala-D-Lac or D-Ala-D-Ser. Vancomycin binds these peptidoglycan precursors with greatly reduced affinity. At least five glycopeptide resistance phenotypes (VanA, VanB, VanC, VanD and

VanE*)* can be distinguished in enterococci on the basis of the level and induciblity of resistance to vancomycin and teicoplanin. VanA and VanB are the most common resistance phenotypes [(26)].

The aim of this study was to determine glycopeptide susceptibility among staphylococci and enterococci isolates from Sulaimani city health laboratories and find combined methicillin and vancomycin resistance among *S. aureus* isolates.

MATERIALS AND METHODS

This is a prospective observational study. Staphylococci and enterococci isolates were collected in a period from March 2017 to February 2018 from different hospital laboratories and community health laboratories in Sulaimani city. The isolates were cultured on blood agar (Lab M™, UK), incubated at 37°C for 18-24 hours. The bacterial growth was confirmed by culture characteristics, Gram staining features and catalase test. *Staphylococcus* species were differentiated by their ability to ferment mannitol and by coagulase test [(27)]. Enterococci isolates were subjected to biochemical identifications using the Vitek 2® automated system (bioMerieux, France) according to the manufacturer's instructions.

We performed antimicrobial susceptibility test according to the Bauer-Kirby disk diffusion method [(28)] on Mueller-Hinton agar using the following antimicrobial disks (MASTDISCS ™ Mast Diagnostics, UK); vancomycin (VA, 30 μg), teicoplanin (TEC, 30 μg), amoxicillin-clavulanate (AMC, 20/10 μg), cefoxitin (FOX, 30 μg), methicillin (ME, 10 μg), erythromycin (E, 15 μg), amikacin (AK, 10 μg), gentamicin (CN, 10 μg) and netilmicin (NET, 30 μg). The antimicrobial disks were placed on the surface of a freshly inoculated Muller-Hinton agar (Lab M ™, UK) plate. Before adding the disks, the plate surface was inoculated using bacterial suspension standardized to match the turbidity of the 0.5 McFarland turbidity standard of the isolate [(28)]. The plates were allowed to dry and incubated at 35°C for 18-24 hours. Following incubation, the diameter of the zone of inhibition was measured in millimeters and the susceptibility was determined according to the CLSI standards [(29, 30)].

RESULTS

In this study, 207 isolates of staphylococci and enterococci were collected from different laboratories in Sulaimani city, including six hospital laboratories and two community health laboratories. The source of the isolates were from 111 females (mean age 32 year, range 5 to 73 year) and 96 males (mean age 38 year, range 2 to 95 year). The isolates were 146 *Staphylococcus aureus*, 36 coagulase-negative staphylococci (CoNS) and 25 enterococci isolates. Table 1 shows the isolates in regard to the patient's sex and age (P =0.525), the laboratory type (P =0.103), and the specimen source from which bacteria was isolated. Most of the isolates were from inpatients 182 (87.9%), the other 25 (12.1%) isolates were from outpatients submitting their specimens to community health laboratories. Only one CoNS and two enterococci isolates were from outpatients while 22 (15%) *S. aureus* isolates were from outpatients indicating that *S. aureus* infections occur in community and hospitals rather than hospitals as compared with CoNS and enterococci.

The source specimens were blood, swab and tissues from burn injury, pus, surgical wound, urine and others including, throat swab, endotracheal samples, skin infections from outpatients and seminal fluid.

Table 2 shows the enterococci species identified by Vitek 2® system. *E. faecalis* was the most frequent isolate followed by *E. faecium*. *E. faecalis* isolates were mostly from inpatients (94.1%) and from blood samples and urine samples (70.5% and 29.4% respectively). Two *E. gallinarum* isolates were from pus specimens from two patients admitted for septic arthritis.

Table 3 shows the antimicrobial susceptibility pattern of the isolates. Resistance to vancomycin was detected in one isolate of each S. *aureus* (0.7%), CoNS (2.8%) and enterococci species (4%). For teicoplanin, one *S. aureus* isolate showed intermediate response (0.7%) and two isolates (1.4%) were resistant. One isolate of each CoNS (2.8%) and enterococci spp. (4%) was resistant to teicoplanin.

For amoxicillin-clavulanate, resistance was detected in 80 (54.8%) of *S. aureus*, 26 (72.2%) of CoNS and in 14 (56%) of *Enterococci*. Cefoxitin resistance was 106 (58.2%) which was less than methicillin resistance 168 (92.3%) among all staphylococcal isolates. Among the 146 *S. aureus* isolates, MRSA were isolated from 80 (54.8%) samples based on cefoxitin resistance.

Erythromycin resistance was detected in the range of 28 (77.8%) in CoNS, 123 (84.2%) in *S. aureus* to 25 (100%) among the enterococci. Three aminoglycoside agents were tested on *S. aureus* and CoNS; netilmicin was most effective glycopeptide ranging from 109 (74.7%) among *S. aureus* to 29 (80.6%) among CoNS.

In all 207 isolates, only five isolates were found to be either resistant or intermediately- resistant to the glycopeptides; three isolates of *S. aureus*, and one isolate of each CoNS and enterococci species (Table 4). Three isolates showed resistance to both vancomycin and teicoplanin, including among them a MRSA isolate, while another MRSA isolate was resistant to teicoplanin.

Table 1. The bacterial isolates, sex and age of patients, laboratory, and the specimen source of the isolates.

	S. aureus **No., %**	**CoNS No., %**	**Enterococci No., %**	**Total No., %**
Numbers of isolates	146 (70.5)	36 (17.3)	25 (12)	207 (100)
Sex of isolate source				
Female	82 (56.2)	17 (47.2)	12 (48)	111 (53.6)
Mean age (range), year	29.5 (5-72)	41 (8-73)	37.5 (8-67)	
Male	64 (43.8)	19 (52.8)	13 (52)	96 (46.4)
Mean age (range), year	36.2 (4-95)	38.5 (2-68)	45.2 (8-72)	
Laboratory				
Hospital	124 (84.9)	35 (97.2)	23 (92)	182 (87.9)
Community	22 (15)	1 (2.8)	2 (8)	25 (12.1)
Specimen				
Blood	16 (11)	11 (30.6)	13 (52)	40 (19.3)
Burn	44 (30.1)	6 (16.7)	0	50 (24.2)
Pus	8 (5.5)	3 (8.3)	2 (8)	13 (6.3)
Surgical wound	7 (4.8)	6 (16.7)	0	13 (6.3)
Urine	57 (39)	6 (16.7)	10 (40)	73 (35.3)
Other	14 (9.6)	4 (11.1)	0	18 (8.7)

Table 2. The source of different enterococci species tested in the study.

		E. faecalis **No., %**	***E. faecium*** **No., %**	***E. gallinarum*** **No., %**	***E. durans*** **No., %**	**Total No., %**
Number		17 (68)	5 (20)	2 (8)	1 (4)	25 (100)
Laboratory	Hospital	16 (94.1)	4 (80)	2 (100)	1 (100)	23 (92)
	Community	1 (5.9)	1 (20)	0	0	2 (8)
Specimen	Blood	12 (70.5)	1 (20)	0	0	13 (52)
	Urine	5 (29.4)	4 (80)	0	1 (100)	10 (40)
	Pus	0	0	2 (100)	0	2 (8)

Table 3. The antimicrobial susceptibility of the isolates tested by Bauer-Kirby disk diffusion method*.

Antimicrobials	*S. aureus* *n*=146			CoNS *n*=36			Enterococci *n*=25		
	S No., %	I No., %	R No., %	S No., %	I No., %	R No., %	S No., %	I No., %	R No., %
Vancomycin	145 (99.3)		1 (0.7)	35 (97.2)		1 (2.8)	24 (96)		1 (4)
Teicoplanin	143 (97.9)	1 (0.7)	2 (1.4)	35 (97.2)		1 (2.8)	24 (96)		1 (4)
Amoxicillin-clavulanate	66 (45.2)		80 (54.8)	10 (17.8)		26 (72.2)	11 (44)		14 (56)
Cefoxitin	66 (45.2)		80 (54.8)	10 (17.8)		26 (72.2)			
Methicillin	8 (5.5)	4 (2.7)	134 (91.7)	1 (2.7)	1 (2.7)	34 (94.4)			
Erythromycin	23 (15.8)		123 (84.2)	7 (19.4)	1 (2.8)	28 (77.8)	0 (0.0%)		25 (100)
Amikacin	83 (58.8)	10 (6.8)	53 (36.3)	25 (69.4)		11 (30.6)			
Gentamicin	81 (55.5)	3 (2.1)	62 (42.5)	20 (55.6)	1 (2.8)	15 (41.7)			
Netilmicin	109 (74.7)	1 (0.7)	36 (24.7)	29 (80.6)	1 (2.8)	6 (16.7)			

*** Response; S: Susceptible; I: Intermediate response; R: Resistance**

Table 4. The source and properties of glycopeptide-resistance and intermediate response isolates (*n*=5) *.

	S. aureus	*S. aureus*	*S. aureus*	CoNS	*E. gallinarum*
Sex, age in year	Female, 7	Female, 44	Female, 30	Male, 68	Male, 40
Specimen	Urine	Urine	Surgical wound	Surgical wound	Joint pus
Laboratory	Hospital	Community	Community	Hospital	Hospital
Antimicrobial response					
Vancomycin	S	S	R	R	R
Teicoplanin	R	I	R	R	R
Amoxicillin-clavulanate	R	S	R	R	R
Cefoxitin	R	S	R	R	
Methicillin	R	S	R	R	
Erythromycin	R	R	R	R	R
Amikacin	R	S	R	R	
Gentamicin	R	S	R	R	
Netilmicin	R	S	I	R	

*** Response; S: Susceptible; I: Intermediate response; R: Resistance**

DISCUSSION

Vancomycin has been the drug of choice for serious β-lactam-resistant Gram-positive infections for over three decades. However, the emergence and spread of resistance to this glycopeptide as well as other glycopeptide like teicoplanin among clinically important Gram-positive cocci like *enterococci*, *S. aureus*, and CoNS has made it difficult to manage serious infections caused by such pathogens [11].

In this study we found that glycopeptide resistance was more among enterococcal species (4%) rather than staphylococcal species (0.7-2.8%). The resistance to glycopeptide in enterococci is considerably higher than staphylococci; according to the National Healthcare Safety Network (NHSN), form 2009 to 2010, 35% of enterococcal hospital-associated infections were resistant to vancomycin, ranking the second most common cause of nosocomial infections in the US [31]. In Canada, a lower prevalence of VRE (6%) was reported from 2007 to 2011 [32]. In Europe, VRE is much less prevalent, but it is on rise. The European Antimicrobial Resistance Surveillance System reported 4% prevalence of VRE [33]. In comparison to our results, resistance was much less than in US, less than those from Canada and near the reported Europe figures, with a note that sample number in our study was much less than other studies. Another reason for lower resistance rates is that these antibiotics were introduced lately in our hospitals.

Regarding glycopeptide resistance among staphylo cocci, a study from India in the neonatal care unit of a tertiary hospital found that one isolate out of 55 methicillin-resistant CoNS was resistant to vancomycin [34]. Another study from Iran in 2008 revealed that out of 149 MRSA isolates, only two (1.3%) were resistant to vancomycin [35]. In Iraq, with limited available data on glycopeptide resistance, a research on staphylococci isolated from nasal swabs of 106 patients and health care workers revealed that four (3.8%) were resistant to vancomycin although teicoplanin was not included in their study [36]. These figures are near the figures for glycopeptide resistant staphylococci in the present study, indicating the occurrence of glycopeptide resistance but with limited prevalence.

In this study among staphylococci, vancomycin or teicoplanin resistance were detected in three *S. aureus* and one CoNS. Resistance rate was more in CoNS than *S. aureus* but they did not exceed 2.8% and resistance to both vancomycin and teicoplanin was detected in two of the four glycopeptide-resistant isolates and these two isolates were also resistant to methicillin. Although glycopeptide resistantce is less common in staphylococci, this represents a threat no less than glycopeptide resistance in enterococci [14].

Among the five glycopeptide-resistant or intermediate resistant isolates, three of which were resistant to both vancomycin and teicoplanin and were also found to be resistant to all other antibiotics used in the study, indicating multiresistant nature of these isolates. Most glycopeptide resistance in this study was detected from hospitalized patients which suggests that resistance is much more common in hospitals rather than in the community.

In this study, methicillin resistance was detected; 80 out of 146 (54.8%) in *S. aureus* and 26 out of 36 (72.2%) of CoNS. Among the 80 MRSA (74 hospital, 6 community) isolates, one (1.25%) VRSA isolate and two (2.5%) teicoplanin resistant was detected. These low figures was reported elsewhere, in USA, only 14 VRSA strains have arisen from MRSA that was reported to CDC until early 2015 [37] indicating that combined MRSA and VRSA are still low.

The high rate of MRSA in this study (54.8%) among *S. aureus* was also reported recently from our locality, at a rate of (65.85%) from burn patients [38]. High figure of MRSA was also reported in Iran (54.2%), but these are significantly higher than in Europe (20%) as reported in a study that included 50,759 isolates from several hundred laboratories in 27 European countries between January 1999 and December 2002 [39, 40]. Another European study from Austria was performed in 2017 in which 25 (19.8%) isolates out of 126 were MRSA [41]. The increasing prevalence of MRSA infections means more possibility of glycopeptide use and subsequent resistance against them.

Testing S. *aureus* for aminoglycoside susceptibility, we found that 109 (74.4%) were susceptible to netilmicin, so netilmicin or other aminoglycosides can be used as alternatives to vancomycin, this was also suggested previously [42].

This study showed that enterococcal and CoNS infections (or colonization by CoNS) are mostly hospital-acquired as opposed to *S. aureus* that was detected both in hospital-acquired and community infections. The reported glycopeptide resistance (although low in prevalence) was more in hospital

infections. This may lead to spread of resistance in the hospital environment among different bacterial species if these infections were not treated promptly.

In conclusion, glycopeptide resistance was reported in staphylococci and enterococci but was uncommon. The reported resistance advises regular observation and monitoring the antibiotic susceptibility and strict antibiotic policy. Glycopeptide alternative can be still used in treatment of some Gram-positive infections; this may delay the emergence and spread of glycopeptide resistance.

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