Antimicrobial Susceptibility of Gram-Positive Bacteria Isolated in Brazilian Hospitals Participating in the SENTRY Program (2005-2008)

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We report the antimicrobial susceptibility patterns of the most frequently isolated Gram-positive bacteria in the Brazilian hospitals participating in the SENTRY Antimicrobial Surveillance Program. The strains were consecutively collected (one per patient) between January 2005 and September 2008 and susceptibility tested by reference broth microdilution methods at the JMI Laboratories (North Liberty, Iowa, USA). A total of 3,907 Gram-positive cocci were analyzed. The Gram-positive organisms most frequently isolated from bloodstream infections were *Staphylococcus aureus* (2,218 strains; 20.2% of total), coagulase-negative staphylococci (CoNS; 812 strains [14.7%]), and *Enterococcus* spp. (754 strains; 5.0%). *S. aureus* ranked first (28.1%) and *Enterococcus faecalis* ranked 7th (4.5%) among cases of skin and soft tissue infections. *S. aureus* was also the second most frequently isolated pathogen from patients with lower respiratory tract infections (24.9% of cases) after *Pseudomonas aeruginosa* (30.5%). Resistance to oxacillin was observed in 31.0% of *S. aureus* and the vast majority of oxacillin-resistant (MRSA) strains were also resistant to clindamycin, ciprofloxacin and levofloxacin. Vancomycin, linezolid and daptomycin were all very active against *S. aureus* strains tested (>99.9-100.0% susceptible), but daptomycin (MIC_{50}, 0.25 μg/mL and MIC_{90}, 0.5 μg/mL) was four- to eight-fold more potent than vancomycin (MIC_{50} and MIC_{90} of 1 μg/mL) and linezolid (MIC_{50}, 1 μg/mL and MIC_{90}, 2 μg/mL). Vancomycin resistance increased significantly among enterococci during the study period, but it was restrict to only one medical center until 2007 and emerged in a second medical center in 2008. Daptomycin was the most active antimicrobial tested against enterococci in general (100.0% susceptible), followed by linezolid (99.9% susceptible), ampicillin (87.4%) and vancomycin (84.6%). In conclusion, daptomycin and linezolid showed excellent *in vitro* activity against contemporary Gram-positive organisms (3,907) collected in Brazilian hospitals monitored by the SENTRY Program, including MRSA, vancomycin-resistant enterococci (VRE) and other multidrug-resistant organisms. Although vancomycin resistance rates in Brazil appears to be relatively low compared to those reported in the USA, VRE has emerged and rapidly disseminated in some Brazilian medical centers.

Key-Words: Antimicrobial resistance, SENTRY, nosocomial infections, Brazil.

Among the Gram-positive bacteria, staphylococci, streptococci and enterococci are important causes of both community- and hospital-acquired infections. *Staphylococcus aureus* is particularly important as a frequent cause of sepsis and many other types of nosocomial-acquired infections. This organism represents the first or second most frequently isolated pathogen from bloodstream infections, skin and skin structure infections (SSSI), and pneumonia in hospitalized patients [1,2]. Although the prevalence of methicillin-resistant *S. aureus* (MRSA) may vary significantly, it is usually high in Brazilian hospitals, especially in intensive care units (ICU). Furthermore, MRSA are usually resistant to most antimicrobial agents available for clinical use [3,4].

Coagulase-negative staphylococci (CoNS) has being also recognized as an important cause of nososcomial infections and this organism is usually more resistant to antimicrobial agents than *S. aureus* [5]; while *Enterococcus* spp., mainly *E. faecalis* and *E. faecium*, are among the most frequently isolated pathogens from nosocomial infections in the United States (USA) [1]. Vancomycin-resistant enterococci (VRE) emerged in the early 80's in USA hospitals and rapidly disseminated throughout the country [6]. More recently, the occurrence of VRE is increasing in many European hospitals while data from Latin American countries are still scarce [7]

The SENTRY Antimicrobial Surveillance Program was designed to monitor antimicrobial resistance among various types of infection [2,8,9]. The Program was initiated in early 1997 and today it includes more than 120 medical centers in North America, South America, Europe, Asia, and Western Pacific regions. Data generated by large multicenter programs are of great importance especially in developing regions such as Latin America, where extensive surveillance studies are not routinely conducted. We report the antimicrobial susceptibility of the most frequently isolated Gram-positive cocci in the Brazilian hospitals that participated in the SENTRY Program in the 2005-2008 period.

Material and Methods

In Brazil, four institutions participate in the SENTRY Program: Hospital São Paulo / UNIFESP, São Paulo, SP (A.C. Gales, A.C. Pignatari and S. Andrade), Hospital de Clínicas de Porto Alegre, Porto Alegre, RS (A. Barth), Hospital de Base do Distrito Federal, Brasília, DF (J. Ribeiro) and Laboratório Médico Santa Luzia, Florianópolis, SC (C. Zoccoli) which collects bacterial isolates from 4 regional smaller public and/or private hospitals (40 to 240 beds).

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Each institution collects approximately 500 consecutive, non-duplicate bacterial isolates every year. All isolates are identified at the participating institution by routine methodologies in use at each laboratory. Upon receipt at the central monitor (JMI Laboratories, North Liberty, IA, USA), isolates were subcultured to ensure viability and purity. Confirmation of species identification was performed with the Vitek system (bioMérieux Vitek, St Louis, MO) or conventional methods, as required.

A total of 3,907 Gram-positive bacteria collected between January 2005 and September 2008, were analyzed in the present study. The organisms were consecutively collected according to the type of infections, which included mainly bloodstream infections (57% of strains), skin and skin structure infections (17%) and pneumonia in hospitalized patients (10%). The Gram-negative organisms were analyzed separately and results reported in another publication [9]. The organism collection evaluated in this study included *S. aureus* (2,218 strains), CoNS (812 strains), *Enterococcus* spp. (754 strains), β-haemolytic streptococci (99 strains) and viridians group streptococci (24 strains).

Antimicrobial susceptibility testing was performed by the broth microdilution method, following recommendations of the Clinical and Laboratory Standards Institute [10]. Antimicrobial powders were obtained from the respective manufacturers and microdilution plates were prepared by TREK Diagnostics (Cleveland, OH, USA). Susceptibility results were interpreted according to CLSI document M100-S18 [11]. Quality control was performed by testing *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212 and *Streptococcus pneumoniae* ATCC 49619.

Results

Approximately 60% of isolates evaluated in the present study were from bloodstream infections and the frequency of occurrence of organisms isolated from this type of infection is summarized in Figure 1. *S. aureus* (20.2% of total) was the most common cause of bloodstream infection, followed by CoNS (14.5%). *Enterococcus* spp. ranked 8th and was isolated from 5.0% of bloodstream infection cases. *S. aureus* was also the most common cause of SSSI (28.1%; Figure 2) and was isolated from 24.9% of patients with pneumonia (Figure 3). *Enterococcus* spp. was responsible for 4.5% of SSSI cases (Figure 2).

In general, 31.0% of *S. aureus* strains were resistant to oxacillin (MRSA) and the vast majority of MRSA strains were also resistant to clindamycin, ciprofloxacin and levofloxacin (Table 1). Furthermore, 68.1% of MRSA strains were resistant to trimethoprim/sulfamethoxazole. Daptomycin and vancomycin were active against all *S. aureus* strains tested (100.0% susceptible). Linezolid was also very active against *S. aureus* with only one strain being non-susceptible (MIC, 8 μg/mL) to this antimicrobial. Daptomycin (MIC_{50}, 0.25 μg/mL and MIC_{90}, 0.5 μg/mL) was four- to eight-fold more potent than vancomycin (MIC_{50} and MIC_{90} of 1 μg/mL) and linezolid

Figure 1. Frequency of occurrence of pathogens causing bloodstream infections in Brazilian hospitals (3,807 strains; 2005-2008).

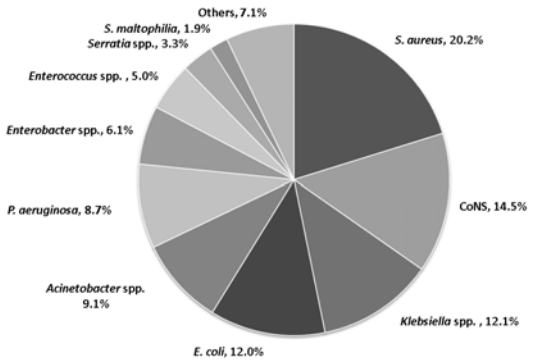

Figure 2. Frequency of occurrence of pathogens causing skin and soft tissue infections in Brazilian hospitals (605 strains; 2005-2008).

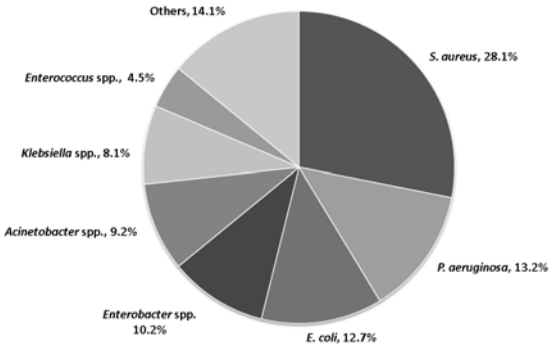

Figure 3. Frequency of occurrence of pathogens isolated from patients hospitalized with pneumonia in Brazilian hospitals (875 strains; 2005-2008).

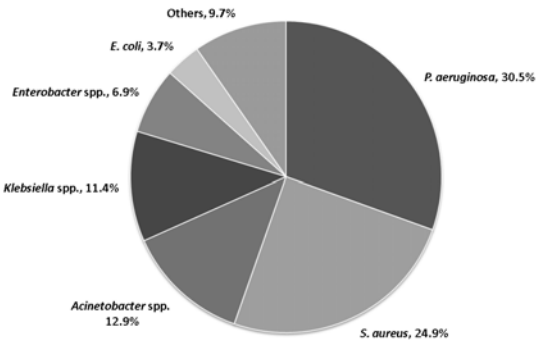

Table 1. Antimicrobial susceptibility of staphylococci isolated in Brazilian hospitals (2005-2008).

Organism (no. tested)/ Antimicrobial agent	MIC (µg/mL) 50%	MIC (µg/mL) 90%	% susceptible[a]	% resistant[a]
MSSA (1,531)				
Erythromycin	≤0.25	>2	86.2	13.0
Clindamycin	≤0.25	≤0.25	98.1	1.7
Ciprofloxacin	0.25	0.5	95.2	2.2
Levofloxacin	≤0.5	≤0.5	97.8	2.0
Tetracycline	≤2	>8	83.9	14.5
TMP/SMX[c]	≤0.5	≤0.5	98.6	1.4
Linezolid	2	2	100.0	-[b]
Vancomycin	1	1	100.0	0.0
Daptomycin	0.25	0.5	100.0	-
MRSA (687)				
Erythromycin	>2	>2	6.0	94.0
Clindamycin	>2	>2	11.2	87.9
Ciprofloxacin	>4	>4	7.6	91.4
Levofloxacin	4	>4	8.6	90.2
Tetracycline	≤2	>8	52.8	46.7
TMP/SMX[c]	>2	>2	31.9	68.1
Linezolid	1	2	99.9	-
Vancomycin	1	1	100.0	0.0
Daptomycin	0.5	0.5	100.0	-
All *S. aureus* (2,218)				
Oxacillin	0.5	>2	69.0	31.0
Erythromycin	≤0.25	>2	61.3	38.1
Clindamycin	≤0.25	>2	70.7	28.4
Ciprofloxacin	0.5	>4	68.1	29.9
Levofloxacin	≤0.5	>4	70.2	29.3
Tetracycline	≤2	>8	74.3	24.5
TMP/SMX[c]	≤0.5	>2	77.9	22.1
Linezolid	1	2	>99.9	-
Vancomycin	1	1	100.0	0.0
Daptomycin	0.25	0.5	100.0	-
CoNS (812)				
Oxacillin	>2	>2	21.3	78.7
Erythromycin	>2	>2	35.4	69.3
Clindamycin	>2	>2	47.4	50.9
Ciprofloxacin	4	>4	42.2	55.7
Levofloxacin	2	>4	43.5	46.0
Tetracycline	≤2	>8	82.8	16.0
TMP/SMX[c]	2	>2	50.3	49.7
Linezolid	1	1	99.8	-
Vancomycin	1	2	100.0	0.0
Daptomycin	0.25	0.5	99.8	-

a. According to CLSI breakpoints [11]; b. - = no breakpoint has been established by the CLSI or USA-FDA; c. TMP/SMX = trimethoprim/sulfamethoxazole.

(MIC_{50}, 1 µg/mL and MIC_{90}, 2 µg/mL) when tested against *S. aureus* (Table 1).

Almost 80% of CoNS strains were resistant to oxacillin. This organism showed high rates of resistance to most antimicrobial agents tested (Table 1). Vancomycin (MIC_{50}, 1 µg/mL and MIC_{90}, 2 µg/mL) was active against all CoNS strain at the susceptible breakpoint while two strains (0.2%) showed decreased susceptibility to daptomycin (MIC of 2 µg/mL, one doubling dilution above the susceptible breakpoint) and two other strains showed high level of resistance to linezolid (MIC, >8 µg/mL).

Approximately 83% of enterococci were *E. faecalis* and 7.7% showed vancomycin resistance (Table 2). Daptomycin (MIC_{50} and MIC_{90} of 1 µg/mL) was active against all *E. faecalis*

strains (100.0% susceptible) while linezolid (MIC_{50}, 1 μg/mL and MIC_{90}, 2 μg/mL) was active against 99.8%, ampicillin (MIC_{50}, ≤ 1 μg/mL and MIC_{90}, 4 μg/mL) was active against 98.9% and teicoplanin (MIC_{50} and MIC_{90} of ≤ 2 μg/mL) inhibited 92.3% of strains at the susceptible breakpoint.

Vancomycin resistance increased from 4.4% in 2005 to 12.2% in 2008 among *E. faecalis* and 94% of vancomycin-resistant strains (46 of 49) were isolated in one medical center. On this particular medical center, vancomycin resistance increased from 30.4% in 2005 to 50.0% in 2008 among *E. faecalis*. All vancomycin-resistant *E. faecalis* strains were susceptible to daptomycin and linezolid, and 95.9% were susceptible to ampicillin. Daptomycin was the most potent agent tested against vancomycin-resistant *E. faecalis* (MIC_{50} and MIC_{90} of 1 μg/mL; Table 2).

Among *E. faecium* strains, 65.7% of strains were resistant to vancomycin (Table 2). Again, the vast majority of vancomycin-resistant strains (47 of 59; 80%) were from one medical center. Vancomycin-resistant *E. faecium* was not observed in any medical center in 2005 and in only one medical center in 2006 (at a rate of 74.2%) and 2007 (68.4%). In 2008 vancomycin-resistant *E. faecium* emerged in a second medical center and both medical centers had high rates of vancomycin-resistance among *E. faecium* strains (64.7 and 78.6%; data not shown). Only daptomycin was active against all vancomycin-resistant strains. Linezolid was also very active against vancomycin-resistant *E. faecium* (98.5% susceptible), while quinupristin/dalfopristin was active against 92.5% of strains. Although high-level streptomycin resistance was observed in 94.0% of vancomycin-resistant *E. faecium* strains, only 9.0% showed high-level resistance to gentamicin. In contrast, streptomycin resistance was observed in 16.3% of vancomycin-resistant *E. faecalis* strains, while 63.5% of these strains showed high-level resistance to gentamicin (Table 2).

When all enterococci strains are analyzed together, daptomycin was the most active antimicrobial (100.0% susceptible), followed by linezolid (99.9% susceptible), ampicillin (87.4%) and vancomycin (84.6%). Although ampicillin and vancomycin showed reasonable activity against enterococci in general (84.6%-87.4% susceptible), these compounds showed limited activity against *E. faecium* (15.7 and 34.3% susceptible respectively; Table 2).

Among viridans group streptococci, 87.5 and 91.7% were susceptible to penicillin and ceftriaxone respectively, while daptomycin (MIC_{50}, 0.12 μg/mL and MIC_{90}, 0.5 μg/mL), levofloxacin (MIC_{50}, ≤ 0.5 μg/mL and MIC_{90}, 1 μg/mL), linezolid (MIC_{50} and MIC_{90} of 1 μg/mL) and vancomycin (MIC_{50} and MIC_{90} of 0.5 μg/mL) were active against all strains tested (100% susceptible; Table 3). β-haemolytic streptococci exhibited high rates of susceptibility to all antimicrobial agents tested, except tetracycline (27.3% susceptible) and erythromycin (92.3% susceptible; Table 3).

Table 4 shows the comparison of the *in vitro* potency of the most active compounds, vancomycin linezolid and daptomycin, tested against staphylococci and enterococci. Daptomycin was generally four-fold more potent than linezolid and vancomycin against staphylococci (*S. aureus* and CoNS). All *S. aureus* and 99.8% of CoNS were inhibited at daptomycin MIC of 1 μg/mL or less. Daptomycin and linezolid showed similar *in vitro* activity against enterococci while vancomycin exhibited more limited *in vitro* activity against this organism, especially *E. faecium* (only 34.3% susceptible). MRSA strains exhibited daptomycin MIC values slightly higher (MIC_{50}, 0.5 μg/mL) than MSSA strains (MIC_{50}, 0.25 μg/mL; Table 5). In contrast, vancomycin-susceptible and -resistant enterococci showed similar susceptibility to vancomycin (Table 5). CoNS (MIC_{50}, 0.25 μg/mL), as well as viridans group and β-haemolytic streptococci (MIC_{50}, ≤ 0.12 μg/mL) showed very low daptomycin MIC values (Table 5).

Discussion

The high prevalence of MRSA in some Brazilian hospitals is a concern because these isolates are often resistant to multiple antimicrobial agents. The overall MRSA rate in the present study was 31.0%, which is comparable to that reported in previous studies [2]. However, MRSA rates may vary greatly among hospitals or even among units of a hospital. The emergence of MRSA is largely due to dissemination of clonal strains, and temporary hospital outbreaks are typically due to cross-transmission between patients of these strains. Furthermore, a direct correlation between antimicrobial usage and resistant rates has been difficult to establish due to a high number of variables involved [12,13].

Vancomycin-resistant *S. aureus* (VRSA) is a serious concern, but only very few isolates have been reported, all from the USA and most of them from the state of Michigan. In contrast, there are many reports of vancomycin-intermediate *S. aureus* (VISA), especially after the reduction of the CLSI vancomycin-susceptible breakpoint from 4 to 2 μg/mL [14]. Interestingly, we did not observe any *S. aureus* isolate with a vancomycin-intermediate MIC value (4 μg/mL) in the present study.

Another interesting finding of this study was the documented linezolid resistance (one strain with MIC of 8 μg/mL) and quinupristin/dalfopristin resistance (two strains with MIC of 2 μg/mL [intermediate] and one strain with MIC >2 μg/mL) among *S. aureus*. Although linezolid-resistant *S. aureus* has been previously reported from Brazil [15], it remains extremely rare in the Brazilian hospitals monitored by the SENTRY Program. Acquired quinupristin/dalfopristin resistance was reported in *E. faecium* from Brazilian hospitals before this antimicrobial became available for clinical use in this country [16]. The emergence and dissemination of this resistance phenotype may be related to the clinical use of natural streptogramin mixtures such as pristiniamycin and synergistin, orally and topically since the 1960s.

S. aureus was the most common Gram-positive organism recovered from bloodstream infections (20.2% of cases) and SSSI (28.1%), and the second most common from patients with pneumonia (24.9%). Furthermore, 31.0% of strains were

Table 2. Antimicrobial susceptibility of enterococci isolated in Brazilian hospitals (2005-2008).

Organism (no. tested)/ Antimicrobial agent	MIC (µg/mL) 50%	MIC (µg/mL) 90%	% susceptible[a]	% resistant[a]
E. faecalis				
Vancomycin-susceptible. (576)				
Ampicillin	≤1	4	99.1	0.0
Levofloxacin	1	>4	67.4	31.6
Gentamicin (HL)[c]	≤500	>100	74.3	25.7
Streptomycin (HL)	≤1000	>200	74.0	26.0
Linezolid	1	2	99.8	0.2
Teicoplanin	≤2	≤2	100.0	0.0
Daptomycin	1	1	100.0	-[b]
Vancomycin-resistant (49)				
Ampicillin	2	8	95.9	4.1
Levofloxacin	>4	>4	4.1	95.9
Gentamicin (HL)	>1000	>1000	26.5	63.5
Streptomycin (HL)	≤1000	>2000	83.7	16.3
Linezolid	1	2	100.0	0.0
Teicoplanin	>16	>16	2.0	98.0
Daptomycin	0.5	1	100.0	-
All *E. faecalis* (625)				
Ampicillin	≤1	4	98.9	1.1
Levofloxacin	1	>4	62.4	36.6
Gentamicin (HL)	≤500	>1000	70.6	29.4
Streptomycin (HL)	≤1000	>2000	74.7	25.3
Linezolid	1	2	99.8	0.2
Teicoplanin	≤2	≤2	92.3	7.7
Vancomycin	2	2	92.2	7.7
Daptomycin	1	1	100.0	-
E. faecium				
Vancomycin-susc. (35)				
Ampicillin	>16	>16	45.7	52.3
Levofloxacin	4	>4	48.6	42.9
Gentamicin (HL)	≤500	>1000	82.9	17.1
Streptomycin (HL)	≤1000	>2000	54.3	45.7
Quinupristin/dalfopristin	1	>2	57.1	20.0
Linezolid	1	2	100.0	0.0
Teicoplanin	≤2	≤2	100.0	0.0
Daptomycin	2	4	100.0	-
Vancomycin-resistant (67)				
Ampicillin	>16	>16	0.0	100.0
Levofloxacin	>4	>4	0.0	98.5
Gentamicin (HL)	≤500	≤500	91.0	9.0
Streptomycin (HL)	>2000	>2000	6.0	94.0
Quinupristin/dalfopristin	1	1	92.5	3.0
Linezolid	1	2	98.5	1.5
Teicoplanin	>16	>16	0.0	98.5
Daptomycin	2	2	100.0	-0
All *E. faecium* (102)				
Ampicillin	>16	>16	15.7	84.3
Levofloxacin	>4	>4	16.7	79.4
Gentamicin (HL)	≤500	>1000	88.2	11.8
Streptomycin (HL)	>1000	>1000	22.6	77.4

Quinupristin/dalfopristin	1	2	80.4	8.8
Linezolid	1	2	99.0	1.0
Teicoplanin	>16	>16	34.3	64.7
Vancomycin	>16	>16	34.3	65.7
Daptomycin	2	2	100.0	-
All enterococci (754)[d]				
Ampicillin	≤1	>16	87.4	12.6
Levofloxacin	2	>4	56.6	41.9
Gentamicin (HL)	≤500	>1000	73.3	26.7
Streptomycin (HL)	≤1000	>2000	67.9	32.1
Quinupristin/dalfopristin	>2	>2	12.3	80.8
Linezolid	1	2	99.9	0.1
Teicoplanin	≤2	>16	84.6	15.3
Vancomycin	2	>16	84.3	15.4
Daptomycin	1	1	100.0	-

a. According to CLSI breakpoints [11]; b.- = no breakpoint has been established by the CLSI or USA-FDA; c. HL = high level resistance; d. Includes *E. faecalis* (625), *E. faecium* (102), *E. avium* (9), *E. gallinarum* (4), *E. hirae* (2), *E. durans* (1) and *Enterococcus* spp. (11).

Table 3. Antimicrobial susceptibility of streptococci isolated in Brazilian hospitals (2005-2008).

Organism (no. tested)/ Antimicrobial agent	**MIC (μg/mL) 50%**	**90%**	**% susceptible**	**% resistant**
Viridans group streptococci (24)				
Penicillin	0.03	0.25	87.5	4.2
Ceftriaxone	≤0.25	0.5	91.7	8.3
Erythromycin	≤0.25	2	62.5	37.5
Clindamycin	≤0.25	≤0.25	95.8	4.2
Levofloxacin	≤0.5	1	100.0	0.0
Tetracycline	≤2	>8	83.3	16.7
Linezolid	1	1	100.0	-[b]
Vancomycin	0.5	0.5	100.0	0.0
Daptomycin	0.12	0.5	100.0	-
β-haemolytic streptococci (99)				
Penicillin	0.03	0.06	100.0	0.0
Ceftriaxone	0.25	0.25	100.0	0.0
Erythromycin	≤0.25	≤0.25	92.9	7.1
Clindamycin	≤0.25	≤0.25	98.0	2.0
Levofloxacin	≤0.25	1	100.0	0.0
Tetracycline	>8	>8	27.3	72.7
Trimethoprim/sulfa	≤0.5	=0.5	99.0	1.0
Linezolid	1	1	100.0	-
Vancomycin	0.5	0.5	100.0	0.0
Daptomycin	0.12	0.25	100.0	-

a. According to CLSI breakpoints [11]; b. - = no breakpoint has been established by the CLSI or USA-FDA.

resistant to oxacillin. This finding emphasizes the importance of the inclusion of an anti-MRSA drug in the initial antimicrobial regimen for these infections [18,19]. Three antimicrobial agents exhibited excellent potency and spectrum against MRSA and staphylococci in general: vancomycin, linezolid and daptomycin (Table 1). Vancomycin has been the standard antimicrobial therapy for serious MRSA infections since the early 1980s, when MRSA emerged as a significant nosocomial pathogen in the USA [20]. However, vancomycin has demonstrated slower *in vitro* bactericidal activity and clinical responses compared with antistaphylococcal beta-lactams. A reduction of efficacy of vancomycin against vancomycin-susceptible MRSA strains with elevated vancomycin MIC values (1-2 μg/mL) has also been extensively reported [14]. Furthermore, poor clinical response may also be related to the lack of bactericidal activity, which has been reported in approximately 20% of MRSA strains [21-24].

Linezolid also showed excellent anti-staphylococci spectrum, but this agent is predominantly bacteriostatic and has not been recommended for the treatment of some serious

Table 4. Comparison of the *in vitro* potency of the most active compounds tested against Gram-positive pathogens.

Organism (no. tested)/	No. of isolates (cumulative %) inhibited at MIC (µg/mL) of:								
Antimicrobial agent	**≤0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**>[a]**
S. aureus (2,218)									
Vancomycin	1(0.1)	2(0.1)	293(13.4)	1,867(97.5)	55(100.0)				
Linezolid	0(0.0)	1(0.1)	55(2.5)	1,133(53.6)	1,028(99.9)	0(99.9)	1(100.0)	-[b]	
Daptomycin	51(2.3)	1,403(65.6)	732(98.6)	32(100.0)	-[b]				
CoNS (812)									
Vancomycin	1(0.1)	5(0.7)	66(8.9)	444(36.6)	290(99.3)	6(100.0)			
Linezolid	3(0.4)	4(0.9)	209(26.6)	569(96.7)	25(99.8)	0(99.8)	0(99.8)	-[b]	2(100.0)
Daptomycin	65(8.0)	432(61.2)	282(95.9)	31(99.8)	2(100.0)			-[b]	
Enterococcus spp. (754)									
Vancomycin	0(0.0)	1(0.1)	38(5.2)	308(46.0)	283(83.6)	6(84.4)	2(84.6)	0(84.6)	116(100.0)
Linezolid	0(0.0)	0(0.0)	15(2.0)	411(56.5)	326(99.7)	0(99.7)	1(99.9)	-[b]	1(100.0)
Daptomycin	6(0.8)	12(2.4)	305(42.8)	322(85.5)	100(98.8)	9(100.0)		-[b]	

a. Greater than the highest concentration tested for this compound; b. Concentration not tested for this compound.

Table 5. Antimicrobial activity of daptomycin tested against Gram-positive organisms collected in Brazilian hospitals.

Organism (no. tested)/	No. of isolates (cumulative %) inhibited at daptomycin MIC (µg/mL) of:					
Antimicrobial agent	**≤0.12**	**0.25**	**0.5**	**1**	**2**	**4**
S. aureus (2,218)	51(2.3)	1403(65.6)	732 (98.6)	32 (100.0)	-	-
MSSA (1,531)	41(2.7)	1,211(81.8)	262(98.9)	17(100.0)	-	-
MRSA (687)	10(1.5)	192(29.4)	470(97.8)	15(100.0)	-	-
Enterococci spp. (754)	6(0.8)	12(2.4)	305(42.8)	322(855)	100(98.8)	9(100.0)
E. faecalis						
Vancomycin-susceptible(576)	5(0.9)	7(2.1)	268(48.6)	261(93.9)	34(99.8)	1(100.0)
Vancomycin-resistant (49)	0(0.0)	4(8.6)	21(51.0)	21(93.9)	2(98.0)	1(100.0)
E. faecium						
Vancomycin-susceptible. (35)	0(0.0)	0(0.0)	2(5.7)	5(20.0)	23(85.7)	5(100.0)
Vancomycin-resistant (67)	0(0.0)	0(0.0)	2(3.0)	29(46.3)	36(100.0)	-
Coagulase-negative staphylococci (812)	65(8.0)	432 (61.2)	282(95.9)	31(99.8)	2(100.0)	-
Viridans group streptococci (24)	14(58.3)	2(66.7)	7(95.9)	1(100.0)	-	-
β-haemolytic streptococci (99)	69(69.7)	27(97.0)	3(100.0)	-	-	-

infections, especially those in immunosuppressed patients [25]. Although the clinical importance of bactericidal activity in the treatment of most infections remains controversial, antimicrobial treatments that provide bactericidal therapy have been demonstrated to be superior to bacteriostatic regimens in the treatment of *S. aureus* bacteremia/sepsis and also in the treatment of systemic infections in immunosuppressed patients [26,27].

Although vancomycin resistance in enterococci is definitely increasing in the Brazilian hospitals participating in the SENTRY Program, the results of this and other studies indicate that this increase is medical center specific and probably related to clonal dissemination of resistant clones [6,28,29]. In Brazil, acquired vancomycin resitance among enterococci was initially reported in *E. faecium*. However, more recently *E. faecalis* became the predominant vancomycin-resistant *Enterococcus* species in Brazilian hospitals. The clonality of VRE has been evaluated in medical center (048) in a previously published study which found two predominant clones among vancomycin-resistant *E. faecalis* and a greater clonal variability among vancomycin-resistant *E. faecium* isolated in the intensive care units of that hospital [30].

The prevalence of vancomycin-resistant *E. faecalis* has increased continuously, but is essentially restrict to one medical center (048). Regarding *E. faecium*, the results of this study showed that the prevalence of infections caused by *E. faecium* in general (vancomycin-susceptible or -resistant) remained very low until vancomycin-resistant *E. faecium* emerged. *E. faecium* represented only 1.7 and 5.3% of enterococcal strains in medical centers where vancomycin-resistant *E. faecium* was not observed, and only 3.6% in center 101 in the 2005-2007 period (vancomycin-resistant *E. faecium* emerged in 2008 in this medical center). In contrast, *E. faecium* represented 35.5% of enterococcal strains collected in medical center 048 in the period of this study and 27.4% of enterococcal strains isolated in medical center 101 in 2008. In summary, the occurrence of *E. faecium* infections increased drastically when vancomycin-resistant

E. faecium emerged and the majority of *E. faecium* infections are due to vancomycin-resistant strains.

Only two compounds showed acceptable activity against enterococci, those being daptomycin and linezolid. Linezolid had potent *in vitro* activity against vancomycin-resistant *E. faecalis* and *E. faecium*, as well as good therapeutic efficacy for VRE bacteremia in mice [25]. However, as previously discussed, linezolid has some limitations due to its predominantly bacteriostatic activity [25].

In general, daptomycin demonstrated excellent *in vitro* activity against recent clinical isolates of Gram-positive species (3,907 isolates). Daptomycin is a novel lipopeptide with potent *in vitro* activity against Gram-positive cocci [31-35]. Daptomycin has a unique mechanism of action and has demonstrated a rapid bactericidal activity against a wide spectrum of Gram-positive organisms, including multidrug-resistant (MDR) strains of staphylococci, enterococci and streptococci [36]. Furthermore, daptomycin monotherapy was shown to be superior to vancomycin monotherapy in the treatment of experimental endocarditis due to methicillin (oxacillin)-resistant *S. aureus* (MRSA)[26]. This agent was approved by the USA Food and Drug Administration (USA-FDA) and by the European Medicine Agency for the treatment of complicated SSSI using a dose of 4 mg/kg every 24 hours and *S. aureus* bacteremia, including right-sided endocarditis, at an increased dose of 6 mg/kg every 24 hours [31,37].

Although vancomycin resistance rates in Brazil appears to be relatively low compared to those reported in the USA [7], VRE has emerged and rapidly disseminated in some medical centers. Furthermore, the results of the present study confirmed previous reports by showing that daptomycin is active against many MDR Gram-positive strains and that vancomycin resistance does not significantly affect its *in vitro* activity.

References

1. NNIS. National Nosocomial Infections Surveillance (NNIS) Systems Report. 2004. Data summary from January 1992 through June 2004, issued October 2004. Am J Infect Control **2004**;32:470-85.
2. Sader H.S., Jones R.N., Gales A.C., et al. SENTRY antimicrobial surveillance program report: Latin American and Brazilian results for 1997 through 2001. Braz J Infect Dis **2004**;8:25-79.
3. Deresinski S. Methicillin-resistant *Staphylococcus aureus*: An evolutionary, epidemiologic, and therapeutic odyssey. Clin Infect Dis **2005**;40:562-73.
4. Rice L.B. Antimicrobial resistance in Gram-positive bacteria. Am J Med **2006**;119:S11-9; discussion S62-70.
5. Casey A.L., Lambert P.A., Elliott T.S. Staphylococci. Int J Antimicrob Agents **2007**;29 Suppl 3:S23-32.
6. Tacconelli E., Cataldo M.A. Vancomycin-resistant enterococci (VRE): transmission and control. Int J Antimicrob Agents **2008**;31:99-106.
7. Deshpande L.M., Fritsche T.R., Moet G.J., et al. Antimicrobial resistance and molecular epidemiology of vancomycin-resistant enterococci from North America and Europe: a report from the SENTRY antimicrobial surveillance program. Diagn Microbiol Infect Dis **2007**;58:163-70.
8. Biedenbach D.J., Moet G.J., Jones R.N. Occurrence and antimicrobial resistance pattern comparisons among bloodstream infection isolates from the SENTRY Antimicrobial Surveillance Program (1997-2002). Diagn Microbiol Infect Dis **2004**;50:59-69.
9. Andrade S., Sader H.S., Barth A., et al. Antimicrobial susceptibility of Gram-negative bacilli isolated in Brazilian hospitals participating in the SENTRY Program (2003-2008). Braz J Infect Dis **2008**;12(Suppl 2):3-9).
10. Clinical and Laboratory Standards Institute. *M7-A7, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard - seventh edition.* Wayne, PA: CLSI, **2006**.
11. Clinical and Laboratory Standards Institute. *M100-S18, Performance standards for antimicrobial susceptibility testing, 18th informational supplement.* Wayne, PA: CLSI, **2008**.
12. Muto C.A., Jernigan J.A., Ostrowsky B.E., et al. SHEA guideline for preventing nosocomial transmission of multidrug-resistant strains of *Staphylococcus aureus* and *enterococcus*. Infect Control Hosp Epidemiol **2003**;24:362-86.
13. Hsueh P.R., Chen W.H., Teng L.J., Luh K.T. Nosocomial infections due to methicillin-resistant Staphylococcus aureus and vancomycin-resistant enterococci at a university hospital in Taiwan from 1991 to 2003: resistance trends, antibiotic usage and *in vitro* activities of newer antimicrobial agents. Int J Antimicrob Agents **2005**;26:43-9.
14. Tenover F.C., Moellering R.C., Jr. The rationale for revising the Clinical and Laboratory Standards Institute vancomycin minimal inhibitory concentration interpretive criteria for *Staphylococcus aureus*. Clin Infect Dis **2007**;44:1208-15.
15. Gales A.C., Sader H.S., Andrade S.S., et al. Emergence of linezolid-resistant *Staphylococcus aureus* during treatment of pulmonary infection in a patient with cystic fibrosis. Int J Antimicrob Agents **2006**;27:300-2.
16. Sader H.S., Jones R.N., Ballow C.H., et al. Antimicrobial susceptibility of quinupristin/dalfopristin tested against Gram-positive cocci from Latin America: results from the global SMART (GSMART) surveillance study. Braz J Infect Dis **2001**;5:21-31.
17. Haroche J., Morvan A., Davi M., et al. Clonal diversity among streptogramin A-resistant *Staphylococcus aureus* isolates collected in French hospitals. J Clin Microbiol **2003**;41:586-91.
18. Ibrahim E.H., Sherman G., Ward S., et al. The influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting. Chest **2000**;118:146-55.
19. Hughes W.T., Armstrong D., Bodey G.P., et al. 2002 guidelines for the use of antimicrobial agents in neutropenic patients with cancer. Clin Infect Dis **2002**;34:730-51.
20. Kirst H.A., Thompson D.G., Nicas T.I. Historical yearly usage of vancomycin. Antimicrob Agents Chemother **1998**;42:1303-4.
21. Sader H.S., Fritsche T.R., Jones R.N. Daptomycin bactericidal activity and correlation between disk and broth microdilution method results in testing of *Staphylococcus aureus* strains with decreased susceptibility to vancomycin. Antimicrob Agents Chemother **2006**;50:2330-6.
22. Sader H., Jones R., Rybak M. Evaluation of the accuracy of the Etest macromethod for detection of heterogeneous vancomycin-intermediate *S. aureus* (hVISA) strains. 48th International Conference on Antimicrobial Agents and Chemotherapy (ICAAC); Washington, D.C.: American Society for Microbiology **2008**.
23. Sakoulas G., Moise-Broder P.A., Schentag J., et al. Relationship of MIC and bactericidal activity to efficacy of vancomycin for treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. J Clin Microbiol **2004**;42:2398-402.
24. Soriano A., Marco F., Martinez J.A., et al. Influence of vancomycin minimum inhibitory concentration on the treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. Clin Infect Dis **2008**;46:193-200.
25. Moellering R.C. Linezolid: the first oxazolidinone antimicrobial. Ann Intern Med **2003**;138:135-42.
26. Pankey G.A., Sabath L.D. Clinical relevance of bacteriostatic *versus* bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections. Clin Infect Dis **2004**;38:864-70.

27. Sakoulas G., Eliopoulos G.M., Alder J., Eliopoulos C.T. Efficacy of daptomycin in experimental endocarditis due to methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother **2003**;47:1714-8.
28. Cordeiro J.C., Silbert S., Reis A.O., Sader H.S. Inter-hospital dissemination of glycopeptide-resistant *Enterococcus faecalis* in Brazil. Clin Microbiol Infect **2004**;10:260-2.
29. Mascini E.M., Bonten M.J. Vancomycin-resistant enterococci: Consequences for therapy and infection control. Clin Microbiol Infect **2005**;11 Suppl 4:43-56.
30. d'Azevedo P.A., Furtado G.H., Medeiros E.A., et al. Molecular characterization of vancomycin-resistant Enterococci strains eight years apart from its first isolation in Sao Paulo, Brazil. Rev Inst Med Trop Sao Paulo **2008**;50:195-8.
31. Fowler V.G., Jr., Boucher H.W., Corey G.R., et al. Daptomycin *versus* standard therapy for bacteremia and endocarditis caused by *Staphylococcus aureus*. N Engl J Med **2006**;355:653-65.
32. Hair P.I., Keam S.J. Daptomycin: A review of its use in the management of complicated skin and soft-tissue infections and *Staphylococcus aureus* bacteraemia. Drugs. **2007**;67:1483-512.
33. Levine D.P., Lamp K.C. Daptomycin in the treatment of patients with infective endocarditis: Experience from a registry. Am J Med **2007**;120:S28-33.
34. Sader H.S., Jones R.N. The activity of daptomycin against wild-type *Staphylococcus aureus* and strains with reduced susceptibility to vancomycin. Clin Infect Dis **2006**;43:798-9; author reply 99-800.
35. Sauermann R., Rothenburger M., Graninger W., Joukhadar C. Daptomycin: a review 4 years after first approval. Pharmacology **2008**;81:79-91.
36. Sader H.S., Streit J.M., Fritsche T.R., Jones R.N. Antimicrobial activity of daptomycin against multidrug-resistant Gram-positive strains collected worldwide. Diagn Microbiol Infect Dis **2004**;50:201-4.
37. Package Insert. Cubicin (daptomycin for injection). Lexington MA. (Cubist Pharmaceuticals, Inc). Available at http://www.cubicin.com/pdf/PrescribingInformation.pdf. Accessed on January 13, **2008**.