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Antimicrobial Susceptibility of Gram-positive Organisms Isolated in Latin American Hospitals: Evaluation of the Emergence and Dissemination of Vancomycin-resistant Enterococci

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ABSTRACT

Background: We report the antimicrobial susceptibility (S) patterns of the most frequently isolated Gram-positive (GP) bacteria in selected Latin American (LA) hospitals (2007-2008).

Methods: Consecutive, non-duplicate bacterial isolates (4,201) were collected from patient infections in 10 medical centers in Argentina (ARG; 21% of strains), Brazil (BRA; 39%), Chile (CHI; 21%) and Mexico (MEX; 20%), and tested for S by CLSI broth microdilution methods.

Results: Isolates were mainly from bacteremia (47%). VRE/MRSA rates were 6/47, 20/31, 5/59 and 0/42% in ARG, BRA, CHI and MEX, respectively. VRE was 0-3% in 6, 10-13% in 3 and 59% in 1 site. 81% of VRE were from 2 BRA sites, 32% were *E. faecalis* and 96% VanA. Rapid increase of VRE in BRA sites was related to dissemination of multiple clones. VRE showed high resistance (R) to ampicillin (64%) and gentamicin (GEN; high-level R, 36%). MRSA exhibited high R to levofloxacin (LEV; 82%), clindamycin (80%), GEN (59%) and TMP/SMX (17%), and 100% S to daptomycin (DAP), vancomycin (VAN), linezolid (LZD) and teicoplanin (TEI). TMP/SMX R among MRSA was high in BRA (53%) compared to other countries (2-4%). 4% of CoNS were TEI non-S. LEV-R BHS was only detected in ARG (3%). All strains were S to DAP except for 1 *S. aureus* (MEX), 1 CoNS (BRA) and 1 VGS (MEX), which had DAP MICs of 2 µg/ml, only 1 doubling dilution above S breakpoint (Table). LZD R was observed in 2 enterococci from BRA (MIC, 8 and >8 µg/ml).

	% cumulative inhibited at DAP MIC (µg/ml) of:					
Organism (no tested)	≤0.12	0.25	0.5	1	2	4
MSSA (1,317)	3.7	82.2	99.2	>99.9	100.0	-
MRSA (998)	1.7	48.9	98.3	100.0	-	-
CoNS (654)	10.6	59.2	96.0	99.9	100.0	-
VAN-S enterococci (710)	0.1	1.6	32.3	82.0	98.3	100.0
VAN-non-S enterococci (83)	0.0	3.6	14.5	56.6	98.8	100.0
β-haemolytic streptococci (BHS; 404)	85.6	99.3	100.0	-	-	-
Viridans group streptococci (VGS; 35)	51.4	82.9	88.6	97.1	100.0	-

Conclusion: DAP and LZD showed greatest in vitro activity against contemporary GP organism (4,201) collected in LA hospitals, including MRSA, VRE and other multidrug-resistant organisms. Emergence and rapid dissemination of VRE was documented in some hospitals, especially in BRA.

INTRODUCTION

Vancomycin-resistant enterococci (VRE), which emerged in late 1980's in United States (USA) and United Kingdom hospitals, are now endemic in many institutions, particularly in the USA of that nation. Furthermore, the prevalence of VRE has recently shown a dramatic increase in other geographic regions, especially in some European countries.

Staphylococcus aureus is a leading cause of infections in hospitalized patients and possesses or can acquire a remarkable number of mechanisms for producing antimicrobial resistance. *S. aureus* infections are usually associated with complicated skin and skin structure infections (cSSSI) and bacteremias, but are also related to pulmonary infections. Although vancomycin retains a good antimicrobial spectrum against *S. aureus* based on current susceptible breakpoints, there has been increasing reports of clinical failure, especially with methicillin-resistant (MRSA) isolates for which vancomycin MIC values were elevated (>1 µg/ml), but still within the CLSI susceptible range (≤2 µg/ml). Other Gram-positive organisms of importance in the hospital environment are coagulase-negative staphylococci (CoNS), which are usually more resistant to antimicrobial agents compared to *S. aureus* and β-haemolytic or viridans group streptococci.

Vancomycin remains as the mainstay for treatment for Gram-positive infections. However, the increasing incidence of VRE and the growing reports of suboptimal outcomes when treating MRSA infections with vancomycin have prompted the escalated use of other agents with broad-spectrum of activity against Gram-positive organisms, mainly linezolid and daptomycin. In the present study, we evaluated the antimicrobial susceptibility of the most frequently isolated Gram-positive bacteria collected in 2007 and 2008 from selected Latin American medical centers.

MATERIALS AND METHODS

Bacterial Isolates: A total of 4,201 consecutive, non-duplicate bacterial isolates were submitted from 10 Latin American SENTRY Antimicrobial Surveillance Program medical centers between January 2007 and December 2008. The centers were located in Argentina (2; Buenos Aires and Rosario); Brazil (4; Brasília, Florianópolis, Porto Alegre and São Paulo), Chile (2, Santiago) and Mexico (2; Guadalajara and Durango). All isolates were identified at the participating institution by routine methodologies in use at each clinical laboratory. Confirmation of species identification was performed by the central monitor (JMI Laboratories, North Liberty, Iowa, USA) using the Vitek 2 system (bioMérieux Vitek, St Louis, Missouri, USA) or conventional methods, as required.

Susceptibility Test Methods: All isolates were tested by the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method using validated commercially prepared panels (TREK Diagnostics, Ohio, USA) in cation-adjusted Mueller-Hinton broth (with 5% lysed horse blood added for testing of streptococci) against a variety of antimicrobial agents representing the most common classes and examples of drugs used in the empiric or directed treatment of the indicated pathogen. Testing was performed by the central laboratory monitor. Standard powders of antimicrobial agents were acquired from their respective manufacturers or from Sigma-Aldrich Chemical Co., (St. Louis, Missouri, USA). Categorical interpretations of MIC results were in accordance with current published CLSI criteria. Quality control (QC) strains utilized included *S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *Streptococcus pneumoniae* ATCC 49619.

RESULTS

- Approximately half of the isolates (47.0%) evaluated were from bloodstream infections and the second most common site of infection was skin and skin structure, including surgical wounds (24.6%).
- In general, 43.1% of *S. aureus* strains were resistant to oxacillin (MRSA) and the vast majority of MRSA strains were also resistant to erythromycin (85.4%), clindamycin (80.2%) and levofloxacin (81.9%; Table 1). Resistance to trimethoprim/sulfamethoxazole (TMP/SMX) was also relatively high among MRSA strains (17.2%), but varied greatly among the four countries evaluated (1.9-3.8% in Argentina, Chile and Mexico and 52.5% in Brazil; Table 2).
- Daptomycin was very active against *S. aureus* with a MIC_{90} of only 0.5 µg/ml for both oxacillin-susceptible *S. aureus* (MSSA) and MRSA (Table 1). Only one *S. aureus* strain (MSSA) was considered non-susceptible to daptomycin. This strain was collected in Mexico and exhibited a daptomycin MIC of 2 µg/ml, only one doubling dilution above the susceptible breakpoint of ≤1 µg/ml.
- Vancomycin (MIC_{50} and MIC_{90} of 1 µg/ml), teicoplanin (MIC_{50} and MIC_{90} of ≤2 µg/ml) and linezolid (MIC_{50}, 1-2 µg/ml and MIC_{90}, 2 µg/ml), were also very active against *S. aureus* strains, but generally two- to eight-fold less potent than daptomycin (Table 1).
- Among CoNS, >80% of strains were resistant to oxacillin and approximately 50% were resistant to levofloxacin, clindamycin and TMP/SMX. Daptomycin (MIC_{50}, 0.25 µg/ml and MIC_{90}, 0.5 µg/ml), vancomycin (MIC_{50}, 1 µg/ml and MIC_{90}, 2 µg/ml) and linezolid (MIC_{50} and MIC_{90}, 1 µg/ml) were the most active compounds tested against CoNS (99.7-100.0% susceptible; Table 1). Two *S. haemolyticus* isolates with decreased susceptibility to vancomycin (MIC, 8 µg/ml) were isolated in a pediatric unit of a Mexican medical center.
- Daptomycin (MIC_{50}, 1 µg/ml and MIC_{90}, 2 µg/ml; 100.0% susceptible), and linezolid (MIC_{50}, 1 µg/ml and MIC_{90}, 2 µg/ml; 98.8-99.9% susceptible) were the most potent agents tested against enterococci (Table 1). Overall, 9.7% of enterococci were resistant to vancomycin (VRE) and VRE strains also showed high resistance to ampicillin (63.9%), quinupristin/dalfopristin (36.1%) and gentamicin (high-level resistance, 36.1%).
- β-haemolytic streptococci exhibited high rates of susceptibility to all tested antimicrobial agents, except tetracycline (71.8% susceptible) and erythromycin (91.6% susceptible; Table 1). Tetracycline susceptibility varied from 83.3% in Mexico to as low as 22.9% in Brazil (Table 2).
- Among tested viridans group streptococci, 80.0 and 94.3% were susceptible to penicillin and ceftriaxone, respectively. Daptomycin (MIC_{90}, 0.12 µg/ml and MIC_{90}, 1 µg/ml), linezolid (MIC_{50} and MIC_{90}, 1 µg/ml) and vancomycin (MIC_{50} and MIC_{90}, 0.5 µg/ml) were very active against these organisms (Table 1).
- MRSA and VRE rates varied substantially among the countries evaluated. MRSA rates varied from 31.1% in Brazil to 59.3% in Chile. Furthermore, MRSA rates increased in Brazil and Chile, but decreased in Mexico in 2008 when compared to 2007 (Table 3).
- VRE rates were highest in Brazil (19.9%) and lowest in Mexico (0.0%). A significant increase of VRE was observed in Brazil (from 16.5 to 23.4%) and in Chile (from 0.0 to 8.5%) during the 2007-2008 study period (Table 3).

Table 1. Antimicrobial susceptibility of gram-positive organisms isolated in Latin America (2007-2008).

Organism (no. tested)/ antimicrobial agent	MIC_{50}	MIC_{90}	Range	% susceptible[a]	% resistant[a]
S. aureus					
MSSA (1,317)					
Daptomycin	0.25	0.5	≤0.06 – 2	99.9	-[a]
Vancomycin	1	1	≤0.12 – 2	100.0	0.0
Teicoplanin	≤2	≤2	≤2	100.0	0.0
Linezolid	2	2	0.25 – 2	100.0	-
Levofloxacin	≤0.5	≤0.5	≤0.5 – >4	96.8	3.0
Erythromycin	≤0.25	>4	≤0.25 – >4	86.0	13.4
Clindamycin	≤0.25	≤0.25	≤0.25 – >2	97.7	2.1
Tetracycline	≤2	≤2	≤2 – >8	90.4	8.8
TMP/SMX[b]	≤0.5	≤0.5	≤0.5 – >2	99.7	0.3
MRSA (998)					
Daptomycin	0.5	0.5	0.12 – 1	100.0	-
Vancomycin	1	1	0.25 – 2	100.0	0.0
Teicoplanin	≤2	≤2	≤2 – 4	100.0	0.0
Linezolid	1	2	0.5 – 2	100.0	-
Levofloxacin	>4	>4	≤0.5 – >4	17.6	81.9
Erythromycin	>4	>4	≤0.25 – >4	14.4	85.4
Clindamycin	>2	>2	≤0.25 – >2	19.5	80.2
Tetracycline	≤2	>8	≤2 – >8	87.0	12.9
TMP/SMX[b]	≤0.5	>2	≤0.5 – >2	82.8	17.2
CoNS (654)					
Daptomycin	0.25	0.5	≤0.06 – 2	99.8	-
Vancomycin	1	2	≤0.12 – 8	99.7	0.0
Teicoplanin	≤2	8	≤2 – >16	96.2	0.6
Linezolid	1	1	≤0.06 – 2	100.0	-
Levofloxacin	4	>4	≤0.5 – >4	41.6	53.2
Erythromycin	>4	>4	≤0.25 – >4	32.9	67.1
Clindamycin	≤0.25	>2	≤0.25 – >2	52.3	46.3
Tetracycline	≤2	>8	≤2 – >8	87.0	11.8
TMP/SMX[b]	2	>2	≤0.5 – >2	54.0	46.0
Oxacillin	>2	>2	≤0.25 – >2	19.0	81.0
Enterococcus spp.					
Vancomycin-susceptible[c] (710)					
Daptomycin	1	2	0.12 – 4	100.0	-
Vancomycin	1	2	≤0.12 – 4	100.0	0.0
Teicoplanin	≤2	≤2	≤2 – 8	100.0	0.0
Ampicillin	≤1	>16	≤1 – >16	89.6	10.4
Linezolid	1	2	0.5 – >8	99.9	0.1
Q/D[b]	>2	>2	≤0.25 – >2	8.6	81.5
Levofloxacin	1	>4	≤0.5 – >4	67.0	30.8
Gentamicin (HL)[d]	≤500	>1000	≤500 – >1000	70.0	30.0
Streptomycin (HL)[d]	≤1000	>2000	≤1000 – >2000	63.5	36.5
Vancomycin-non-susceptible[e] (83)					
Daptomycin	1	2	0.25 – 4	100.0	-
Vancomycin	>16	>16	8 – >16	0.0	92.8
Teicoplanin	>16	>16	≤2 – >16	9.6	90.4
Ampicillin	>16	>16	≤1 – >16	36.1	63.9
Linezolid	1	2	0.5 – 8	98.8	1.2
Q/D[b]	1	>2	≤0.25 – >2	56.6	36.1
Levofloxacin	>4	>4	≤0.5 – >4	6.0	92.8
Gentamicin (HL)[d]	≤500	>1000	≤500 – >1000	63.9	36.1
Streptomycin (HL)[d]	>2000	>2000	≤1000 – >2000	36.1	63.9
β-haemolytic streptococci[f] (404)					
Daptomycin	≤0.06	0.25	≤0.06 – 0.5	100.0	-
Vancomycin	0.25	0.5	≤0.12 – 1	100.0	-
Penicillin	≤0.015	0.06	≤0.015 – 0.12	100.0	-
Ceftriaxone	≤0.25	≤0.25	≤0.25 – 0.5	100.0	-
Erythromycin	≤0.25	≤0.25	≤0.25 – >2	91.6	7.4
Levofloxacin	≤0.5	1	≤0.5 – >4	99.0	1.0
Linezolid	1	1	0.25 – 2	100.0	-
Tetracycline	≤2	>8	≤2 – >8	71.8	27.5

Table 1. Continued.

Antimicrobial agent/ organism (no. tested)	MIC_{50}	MIC_{90}	Range	% susceptible[a]	% resistant[a]
Viridans group streptococci[g] (35)					
Daptomycin	0.12	1	≤0.06 – 2	-	-
Vancomycin	0.5	0.5	≤0.12 – 1	100.0	-
Penicillin	0.03	0.5	≤0.015 – 8	80.0	2.9
Ceftriaxone	≤0.25	0.5	≤0.25 – 16	94.3	5.7
Erythromycin	≤0.25	>2	≤0.25 – >2	62.9	37.1
Levofloxacin	1	2	≤0.5 – >4	97.1	2.9
Linezolid	1	1	≤0.06 – 2	100.0	-
Tetracycline	≤2	>8	≤2 – >8	71.4	28.6

a. Criteria as published by the CLSI (2008). - = no breakpoints has been established by the CLSI.
b. TMP/SMX = trimethoprim/sulfamethoxazole; and Q/D = quinupristin/dalfopristin.
c. Includes: *Enterococcus avium* (8 strains), *Enterococcus casseliflavus* (1 strain), *Enterococcus durans* (1 strain), *Enterococcus faecalis* (597 strains), *Enterococcus faecium* (85 strains), *Enterococcus gallinarum* (9 strains), *Enterococcus hirae* (1 strain), and unspecified *Enterococcus* (8 strains).
d. HL = high level resistance.
e. Includes: *Enterococcus casseliflavus* (2 strains), *Enterococcus faecalis* (26 strains), *Enterococcus faecium* (52 strains), *Enterococcus gallinarum* (2 strains), and unspecified *Enterococcus* (1 strain).
f. Includes: *Streptococcus dysgalactiae* (8 strains), Group A *Streptococcus* (238 strains), Group B *Streptococcus* (114 strains), Group C *Streptococcus* (24 strains), Group F *Streptococcus* (5 strains), and Group G *Streptococcus* (15 strains).
g. Includes: *Streptococcus anginosus* (12 strains), *Streptococcus constellatus* (1 strain), *Streptococcus intermedius* (2 strains), *Streptococcus milleri* (1 strain), *Streptococcus mitis* (3 strains), *Streptococcus mutans* (1 strain), *Streptococcus salivarius* (6 strains), *Streptococcus sanguinis* (1 strain), and unspecified viridans group streptococci (8 strains).

Table 2. Susceptibility rates of Gram-positive organisms by country (2007-2008).

	% susceptible (no. tested)			
Organism/antimicrobial agent	Argentina	Brazil	Chile	Mexico
S. aureus				
Oxacillin-susceptible	(238)	(628)	(240)	(211)
Daptomycin	100.0	100.0	100.0	99.5
Vancomycin	100.0	100.0	100.0	100.0
Teicoplanin	100.0	100.0	100.0	100.0
Linezolid	100.0	100.0	100.0	100.0
Levofloxacin	92.9	98.1	97.7	97.6
Erythromycin	84.0	84.9	89.2	88.2
Clindamycin	96.2	98.6	98.3	96.2
Tetracycline	94.5	86.3	97.1	90.5
TMP/SMX[a]	99.6	99.5	100.0	100.0
Oxacillin-resistant	(211)	(284)	(349)	(154)
Daptomycin	100.0	100.0	100.0	100.0
Vancomycin	100.0	100.0	100.0	100.0
Teicoplanin	100.0	100.0	100.0	100.0
Linezolid	100.0	100.0	100.0	100.0
Levofloxacin	65.4	7.4	1.1	8.4
Erythromycin	55.9	3.5	3.4	2.6
Clindamycin	66.4	7.4	4.9	11.0
Tetracycline	97.2	61.6	98.0	94.8
TMP/SMX[a]	96.2	47.5	96.6	98.1
Coagulase-negative staph.	(123)	(370)	(79)	(82)
Daptomycin	100.0	99.7	100.0	100.0
Vancomycin	100.0	100.0	100.0	97.6
Teicoplanin	96.6	96.8	97.5	91.5
Linezolid	100.0	100.0	100.0	100.0
Levofloxacin	51.2	43.5	34.2	25.6
Erythromycin	33.3	37.8	27.8	14.6
Clindamycin	72.4	50.5	46.8	35.4
Tetracycline	87.8	86.5	96.2	79.3
TMP/SMX[a]	66.7	54.6	51.9	34.1
Oxacillin	12.2	21.9	19.0	15.9
Enterococcus spp.	(116)	(316)	(151)	(210)
Daptomycin	100.0	100.0	100.0	100.0
Vancomycin	94.0	79.4	94.7	98.6
Teicoplanin	94.0	80.1	96.7	100.0
Ampicillin	81.9	83.2	84.8	85.7
Linezolid	100.0	99.4	100.0	100.0
Q/D[a]	6.0	17.1	15.9	11.0
Levofloxacin	69.8	55.1	64.2	61.4
Gentamicin (HL)[a]	67.2	75.3	70.2	61.0
Streptomycin (HL)[a]	62.9	62.0	76.2	46.2
β-haemolytic streptococci	(155)	(35)	(45)	(169)
Daptomycin	100.0	100.0	100.0	100.0
Vancomycin	100.0	100.0	100.0	100.0
Penicillin	100.0	100.0	100.0	100.0
Ceftriaxone	100.0	100.0	100.0	100.0
Erythromycin	92.9	85.7	77.8	95.3
Clindamycin	96.8	94.3	95.6	100.0
Levofloxacin	97.4	100.0	100.0	100.0
Linezolid	100.0	100.0	100.0	100.0
Tetracycline	73.5	22.9	60.0	83.4

a. TMP/SMX = trimethoprim/sulfamethoxazole; Q/D = quinupristin/dalfopristin; and HL = high level resistance.

Table 3. Yearly MRSA and VRE rates by country (2007-2008).

Pathogen/ Year	Resistance rate by country				
	Argentina	Brazil	Chile	Mexico	Overall
MRSA					
2007	47.9	28.2	57.4	50.6	42.8
2008	45.9	35.0	61.8	34.4	43.6
Overall	47.0	31.1	59.3	42.2	43.1
VRE					
2007	5.2	16.5	0.0	0.0	7.7
2008	6.9	23.4	8.3	0.0	11.6
Overall	6.0	19.9	4.6	0.0	9.7

CONCLUSIONS

- Daptomycin and linezolid demonstrated greatest in vitro activity against contemporary Gram-positive organisms (4,201) collected in Latin American hospitals, including MRSA, VRE and other multidrug-resistant organisms.
- Emergence and rapid dissemination of VRE was documented in some hospitals, especially in Brazil and Chile.
- The in vitro information presented here coupled with extensive clinical data available establish daptomycin as an important therapeutic option for serious Gram-positive infections in Latin American medical centers. Local resistance data, however, should be taken into consideration when prescribing empiric antimicrobial therapy or establishing national treatment guidelines.

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