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Zyvox® Annual Appraisal of Potency and Spectrum (ZAAPS) Program: report of linezolid activity over 9 years (2004–12)

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Objectives: To summarize the activity and spectrum of linezolid and comparators tested against 7972 Gram-positive clinical isolates as part of the Zyvox® Annual Appraisal of Potency and Spectrum (ZAAPS) Program for 2012. Moreover, to provide molecular characterization for associated resistance mechanisms and epidemiological typing.

Methods: A total of 7972 isolates were collected from 73 medical centres (33 countries) on five continents. Isolates were tested for susceptibility by broth microdilution following the CLSI M07-A9 document. MIC interpretations were based on CLSI and EUCAST criteria.

Results: Linezolid showed MIC_{50} and MIC_{90} results of 1 and 2 mg/L, respectively, when tested against *Staphylococcus aureus*. These isolates were inhibited by linezolid at ≤2 mg/L, except for four *S. aureus* exhibiting higher MIC values (4–8 mg/L), which had *cfr* and/or target site mutations, including a first detection of *cfr* in an isolate from Brazil. Coagulase-negative staphylococci (CoNS) were susceptible to linezolid ($MIC_{50/90}$, 0.5/1 mg/L), with only eight isolates exhibiting high MIC results (16–32 mg/L). These CoNS had *cfr* and/or single or multiple target site alterations in 23S rRNA and/or ribosomal proteins (L3, L4). The same species of linezolid-resistant CoNS collected from the same hospital were clonally related to those observed in previously surveyed years. Linezolid exhibited stable modal MIC and MIC_{50} results when tested against enterococci, regardless of the species or vancomycin resistance phenotype; in addition, linezolid inhibited all streptococci at ≤2 mg/L.

Conclusions: This surveillance report documents stable linezolid activity and susceptibility rates against a large and longitudinal collection of clinical isolates worldwide.

Keywords: surveillance, oxazolidinones, resistance, *cfr*

Introduction

The oxazolidinones have become an important class of antimicrobial agents, clinically represented by linezolid, which has been the only in-class agent approved by the US FDA and other regulatory agencies. Linezolid has become an attractive alternative for treating respiratory tract and skin and soft tissue infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and other serious infections due to vancomycin-resistant enterococci (VRE).[1,2] The clinical and commercial success of linezolid has prompted several pharmaceutical companies to investigate and develop oxazolidinone-like compounds.[3] Numerous molecules have been developed, but few have succeeded and further advanced into clinical trials.[4,5]

The Zyvox® Annual Appraisal of Potency and Spectrum (ZAAPS) Program has surveyed and documented the spectrum and activity of linezolid tested against non-USA Gram-positive pathogens for nine consecutive years (2004–12). Table 1 summarizes the linezolid non-susceptibility rates obtained across the programme years, including those yet to be reported for 2012. Overall, linezolid has inhibited all tested isolates at their respective susceptible breakpoints, except for coagulase-negative staphylococci (CoNS) and enterococci collected from 2006 and 2012 (0.3%–1.2% non-susceptible) and one *S. aureus* each from 2007 and 2012 (<0.1%).[6–13] In addition to the non-susceptibility rate summary described above, this study reports on the *in vitro* activity and spectrum of linezolid (and comparator agents) by applying centralized testing using the broth microdilution method against 7972 clinical isolates recovered from 2012. Moreover, it provides molecular characterization for associated resistance mechanisms and epidemiological typing.

Table 1. Summary of non-susceptibility rates documented for linezolid when tested against a total of 52082 clinical isolates included in the 9 year ZAAPS Program (2004–12)

	Percentage linezolid non-susceptibility by year (number of isolates tested)[a]								
Organism (no. tested)	2004	2005	2006	2007	2008	2009	2010	2011	2012
S. aureus (25148)	0.0 (1422)	0.0 (1416)	0.0 (2276)	<0.1 (3000)	0.0 (3240)	0.0 (2958)	0.0 (2875)	0.0 (3884)	<0.1 (4077)
CoNS (6909)	0.0 (652)	0.0 (634)	0.5 (615)	0.3 (716)	0.4 (748)	0.5 (827)	0.8 (885)	1.2 (927)	0.9 (905)
Enterococci (6718)	0.0 (719)	0.0 (718)	0.9 (423)	0.7 (906)	0.7 (864)	0.5 (744)	0.5 (787)	0.4 (760)	0.8 (797)
S. pneumoniae (6691)	0.0 (796)	0.0 (853)	0.0 (395)	0.0 (452)	0.0 (216)	0.0 (636)	0.0 (926)	0.0 (1207)	0.0 (1210)
Viridans group streptococci (2463)	0.0 (196)	0.0 (218)	0.0 (209)	0.0 (155)	0.0 (216)	0.0 (214)	0.0 (325)	0.0 (530)	0.0 (400)
β-Haemolytic streptococci (4153)	0.0 (313)	0.0 (570)	0.0 (295)	0.0 (362)	0.0 (398)	0.0 (375)	0.0 (507)	0.0 (750)	0.0 (583)

[a]Linezolid susceptibility results were based on the CLSI breakpoint for susceptibility. Results were adapted from the following references: 2004,[7] 2005,[8] 2006,[9] 2007,[6] 2008,[13] 2009,[12] 2010,[10] 2011[11] and 2012 (this study).

Table 2. Linezolid MIC distribution when tested against species and groups of Gram-positive cocci isolated from five continents (ZAAPS, 2012)

	Number (cumulative %) of isolates inhibited at linezolid MIC (mg/L) of:								MIC (mg/L)	
Organism (no. tested)	≤0.12	0.25	0.5	1	2	4	8	>8	MIC_{50}	MIC_{90}
S. aureus (4077)	1 (0.0)	18 (0.5)	455 (11.6)	2847 (81.5)	752 (99.9)	1 (99.9)	3 (100.0)		1	2
oxacillin susceptible (2765)	1 (0.0)	7 (0.3)	239 (8.9)	1928 (78.7)	589 (100.0)	1 (100.0)			1	2
oxacillin resistant (1312)	0 (0.0)	11 (0.8)	216 (17.3)	919 (87.3)	163 (99.8)	0 (99.8)	3 (100.0)		1	2
CoNS (905)	0 (0.0)	73 (8.1)	594 (73.7)	221 (98.1)	9 (99.1)	0 (99.1)	0 (99.1)	8 (100.0)	0.5	1
Enterococcus spp. (797)	0 (0.0)	5 (0.6)	132 (17.2)	567 (88.3)	87 (99.2)	4 (99.7)	2 (100.0)		1	2
E. faecalis (434)	0 (0.0)	5 (1.2)	74 (18.2)	305 (88.5)	46 (99.1)	3 (99.8)	1 (100.0)		1	2
E. faecium (333)	0 (0.0)	0 (0.0)	49 (14.7)	244 (88.0)	39 (99.7)	0 (99.7)	1 (100.0)		1	2
VRE (141)	0 (0.0)	0 (0.0)	22 (15.7)	100 (87.1)	18 (99.3)	0 (99.3)	1 (100.0)		1	2
S. pneumoniae (1210)	3 (0.2)	32 (2.9)	427 (38.2)	714 (97.2)	34 (100.0)				1	1
Viridans group streptococci (400)	2 (0.5)	20 (5.5)	168 (47.5)	203 (98.3)	7 (100.0)				1	1
β-Haemolytic streptococci (583)	0 (0.0)	0 (0.0)	130 (22.3)	452 (99.8)	1 (100.0)				1	1

Eight *E. faecalis* isolates were vancomycin resistant (linezolid MIC range 0.5–2 mg/L).

Methods

Clinical isolates

This investigation included 7972 Gram-positive strains collected from 73 medical centres on five continents (33 countries). Isolates included in this study originated from the following countries (number of medical sites): North America: Canada (2); South America: Argentina (2), Brazil (4), Chile (2) and Mexico (2); Europe and surrounding countries: Belgium (1), Czech Republic (1), France (4), Germany (3), Greece (1), Hungary (1), Ireland (2), Israel (1), Italy (3), Poland (1), Portugal (1), Russia (3), Slovenia (1), Spain (3), Sweden (2), Turkey (2), Ukraine (1) and UK (3); Asia-Pacific (APAC) region: Australia (6), China (10), Hong Kong (1), Japan (2), Korea (2), Malaysia (1), New Zealand (2), Singapore (1), Taiwan (1) and Thailand (1). An additional 44110 isolates monitored in the ZAAPS Program for 2004–11 were also included (see Table 1).[6–13]

Participating centres selected consecutively collected unique isolates associated with documented infections, per local guidelines, in hospitalized patients. These isolates were recovered mostly from blood (25.2%), wound (33.3%) and lower respiratory tract (22.5%) specimens. Overall, each site submitted 250–500 isolates to reach a minimum target of 200 Gram-positive organisms per country, except for China (600) and Japan (400) that had different target minimums. JMI Laboratories and the Women's and Children's Hospital (Adelaide, Australia), which processed isolates only from Australia and New Zealand, confirmed the organism identification and performed MIC testing. Isolates were primarily identified by the participating laboratory and identifications were confirmed by the reference monitoring laboratory (JMI Laboratories or the Women's and Children's Hospital) by standard algorithms and the Vitek® 2 system (bioMérieux, Hazelwood, MO, USA), and supported by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonics, Bremen, Germany).

Table 3. Comparative activity of linezolid tested against 7972 Gram-positive cocci from 33 nations in the ZAAPS Program (2012)

	MIC (mg/L)			Percentage susceptible/resistant[a]	
Organism (no. tested)/antimicrobial agent	50%	90%	range	CLSI	EUCAST
MRSA (1312)					
linezolid	1	2	≤0.12–8	99.9/0.1	99.9/0.1
clindamycin	≤0.25	>2	≤0.25 to >2	50.9/48.6	50.4/49.1
erythromycin	>16	>16	≤0.12 to >16	23.8/73.7	24.1/76.6
gentamicin	≤1	>8	≤1 to >8	60.3/38.1	58.8/41.2
levofloxacin	>4	>4	≤0.12 to >4	18.3/81.3	18.3/81.3
tetracycline	≤0.25	>8	≤0.25 to >8	69.0/29.5	68.2/31.4
trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5 to >4	95.0/5.0	95.0/4.4
teicoplanin	≤2	≤2	≤2–16	99.9/0.0	97.0/3.0
vancomycin	1	1	0.25–2	100.0/0.0	100.0/0.0
CoNS[b] (905)					
linezolid	0.5	1	0.25 to >8	99.1/0.9	99.1/0.9
oxacillin	>2	>2	≤0.25 to >2	23.5/76.5	23.5/76.5
clindamycin	≤0.25	>2	≤0.25 to >2	73.2/25.7	71.0/26.8
erythromycin	>16	>16	≤0.12 to >16	37.4/61.7	37.7/62.1
gentamicin	≤1	>8	≤1 to >8	56.1/36.5	50.8/49.2
levofloxacin	1	>4	≤0.12 to >4	51.3/46.7	51.3/46.7
tetracycline	0.5	>8	≤0.25 to >8	84.3/14.0	73.8/16.9
trimethoprim/sulfamethoxazole	≤0.5	>4	≤0.5 to >4	65.6/34.4	65.6/20.6
teicoplanin	≤2	8	≤2 to >16	94.5/0.7	80.2/19.8
vancomycin	1	2	0.25–4	100.0/0.0	100.0/0.0
E. faecalis (434)					
linezolid	1	2	0.25–8	99.1/0.2	99.8/0.2
ampicillin	1	2	0.5 to >8	99.8/0.2	96.8/0.2
erythromycin	>16	>16	≤0.12 – to >16	7.6/58.1	—/—
levofloxacin	1	>4	0.25 to >4	69.4/30.4	—/—
vancomycin	1	2	0.25 to >16	98.2/1.4	98.2/1.8
teicoplanin	≤2	≤2	≤2 to >16	98.8/1.2	98.4/1.6
E. faecium (333)					
linezolid	1	2	0.5–8	99.7/0.3	99.7/0.3
ampicillin	>8	>8	≤0.25 to >8	8.4/91.6	7.8/91.6
erythromycin	>16	>16	≤0.12 to >16	1.8/88.5	—/—
levofloxacin	>4	>4	0.5 to >4	6.3/89.2	—/—
vancomycin	1	>16	0.25 to >16	57.7/40.5	57.7/42.3
teicoplanin	≤2	>16	≤2 to >16	64.9/32.1	64.3/35.7
S. pneumoniae (1210)					
linezolid	1	1	≤0.12–2	100.0/—	100.0/0.0
amoxicillin/clavulanate	≤1	4	≤1 to >8	87.5/9.8	—/—
ceftriaxone	≤0.06	2	≤0.06 to >8	87.5/2.5	78.4/2.5
clindamycin	≤0.25	>2	≤0.25 to >2	75.7/23.9	76.1/23.9
erythromycin	≤0.12	>16	≤0.12 to >16	63.1/36.6	63.1/36.6
levofloxacin	1	1	≤0.12 to >4	98.3/1.3	98.3/1.7
penicillin[c]	≤0.06	4	≤0.06–8	63.7 (89.7)/19.6 (1.9)	63.7/10.3
tetracycline	0.5	>8	≤0.25 to >8	67.2/31.3	66.8/32.8
trimethoprim/sulfamethoxazole	≤0.5	>4	≤0.5 to >4	62.8/26.1	70.0/26.1
vancomycin	0.25	0.5	≤0.12–0.5	100.0/—	100.0/0.0
Viridans group streptococci[d] (400)					
linezolid	1	1	≤0.12–2	100.0/—	—/—
ceftriaxone	0.25	1	≤0.06 to >8	93.0/4.8	87.8/12.3

Continued

Table 3. *Continued*

Organism (no. tested)/antimicrobial agent	MIC (mg/L)			Percentage susceptible/resistant[a]	
	50%	90%	range	CLSI	EUCAST
clindamycin	≤0.25	>2	≤0.25 to >2	85.0/14.3	85.7/14.3
erythromycin	≤0.12	>16	≤0.12 to >16	57.8/40.3	—/—
levofloxacin	1	2	≤0.12 to >4	95.7/3.3	—/—
penicillin	≤0.06	1	≤0.06 to >4	77.0/3.8	84.0/3.8
tetracycline	0.5	>8	≤0.25 to >8	61.9/36.6	—/—
vancomycin	0.5	1	≤0.12–1	100.0/—	100.0/0.0
β-Haemolytic streptococci[e] (583)					
linezolid	1	1	0.5–1	100.0/—	100.0/0.0
amoxicillin/clavulanate	≤1	≤1	≤1	—/—	100.0/0.0
ceftriaxone	≤0.06	0.12	≤0.06–0.5	100.0/—	100.0/0.0
clindamycin	≤0.25	≤0.25	≤0.25 to >2	91.9/8.1	91.9/8.1
erythromycin	≤0.12	4	≤0.12 to >16	82.3/16.7	82.3/16.7
levofloxacin	0.5	1	0.25 to >4	96.6/3.4	93.7/3.4
penicillin	≤0.06	≤0.06	≤0.06–0.12	100.0/—	100.0/0.0
tetracycline	0.5	>8	≤0.25 to >8	56.8/40.4	55.8/43.2
vancomycin	0.25	0.5	≤0.12–1	100.0/—	100.0/0.0

[a]Criteria as published by CLSI and EUCAST.
[b]Includes 20 staphylococcal species (898 isolates) and unidentified CoNS (7 isolates).
[c]CLSI 2013 susceptibility breakpoints for oral penicillin V (parenteral non-meningitis in parentheses).
[d]Includes 17 species (388 strains), *Streptococcus bovis* group (8 strains) and unidentified viridans group streptococci (4 strains).
[e]Includes group A (292 strains), group B (169 strains), group C (25 strains), group F (2 strains), group G (67 strains) and two other species (28 strains).

Susceptibility testing

Isolates were tested for susceptibility by broth microdilution following the CLSI M07-A9 document.[14] MIC testing was performed using panels manufactured by Thermo Fisher Scientific (Cleveland, OH, USA) containing cation-adjusted Mueller–Hinton broth (2.5%–5% lysed horse blood added for testing streptococci). The bacterial inoculum density was monitored by colony counts to ensure an adequate number of cells for each testing event. Validation of the MIC values was performed by the concurrent testing of CLSI-recommended quality control reference strains (*S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *Streptococcus pneumoniae* ATCC 49619).[15] MIC interpretations were based on the CLSI M100-S23 (2013) breakpoint criteria and EUCAST breakpoint criteria, as available.[15,16] Isolates processed by JMI Laboratories or the Women's and Children's Hospital with elevated linezolid MIC results at ≥4 mg/L were submitted for additional testing using customized frozen-form panels, molecular characterization of resistance mechanisms and epidemiological typing, as previously described.[17–19]

Results and discussion

Linezolid showed high potency when tested against *S. aureus* isolated during the 2012 surveillance programme, with MIC_{50} and MIC_{90} results of 1 and 2 mg/L, respectively, regardless of oxacillin susceptibility (Tables 2 and 3). In addition, linezolid inhibited all tested *S. aureus* at ≤2 mg/L, except for four isolates exhibiting higher MIC values (i.e. 4–8 mg/L; Tables 2–4). These isolates originated from Italy, Hong Kong and Brazil, and showed the presence of *cfr* and/or target site mutations (Table 4). It is important to note that the presence of *cfr*-carrying *S. aureus* has previously been reported in Latin American countries, such as Colombia[20] and Mexico;[21] however, this surveillance programme (ZAAPS) is the first to report the detection of *cfr* from Brazil. The oxacillin resistance rate (MRSA) was higher in Latin America (45.9%), and lower in the APAC region (34.2%) and Europe (27.7%; data not shown). Except for teicoplanin, vancomycin and trimethoprim/sulfamethoxazole (≥95.0% susceptible), other comparators had limited *in vitro* activity (18.3%–85.5% susceptible) against all *S. aureus* (data not shown) or the MRSA subset (Table 3). In contrast, all comparators were active (≥92.9% susceptible) against oxacillin-susceptible *S. aureus*, except erythromycin (81.0%–81.3% susceptible; data not shown).

Linezolid had modal MIC, MIC_{50} and MIC_{90} results of 0.5, 0.5 and 1 mg/L, respectively, when tested against CoNS (Table 2). The vast majority of these CoNS isolates (99.1%) were inhibited by linezolid at ≤2 mg/L; otherwise, eight isolates showed MIC results between 16 and 32 mg/L (Table 4). These latter CoNS isolates exhibited a diverse array of linezolid resistance mechanisms, including *cfr* and/or single or multiple target site alterations. Except for linezolid, teicoplanin and vancomycin, other antimicrobial agents did not show satisfactory (>90%) coverage when tested against CoNS (Table 3). All linezolid-resistant CoNS detected during the 2012 sampling year were clonally related to the respective species collected from the same institution in previous years (Table 4). These results indicate that these CoNS belong to endemic clones, which have probably been established within these hospitals, causing sporadic infections.[19,21]

Table 4. Isolates with elevated or non-susceptible linezolid MIC values (≥4 mg/L) observed during the ZAAPS Program (2012)

Organism	Country[a]	Linezolid MIC[b] (mg/L)	Resistance mechanism	PFGE[c]
S. aureus	Italy	8	L3 (Q136H and H146Δ); L4 (G69A, T70P, G71S)	
S. aureus	Italy	8	G2576T	
S. aureus	Hong Kong	8	G2447T	
S. aureus	Brazil	4	*cfr*; L4 (V142I)	
Staphylococcus cohnii	Mexico	16	*cfr*; L4 (V155I, A133 T); L3 (S158F, D159Y)	SCO115A[d]
Staphylococcus haemolyticus	Brazil	16	G2576T	SH048A1[e]
S. epidermidis	Italy	16	C2319T; L4 (71G72ins); L3 (V154L, H146Q)	SEPI86A1[f]
S. epidermidis	Italy	16	L4 (71G72ins); L3 (V154L, H146Q, A157R)	SEPI86A1[f]
S. epidermidis	Italy	32	G2576T	SEPI86H[g]
S. epidermidis	Italy	32	*cfr*; L3 (F147L)	SEPI86A3[f]
S. epidermidis	Brazil	16	G2576T	SEPI048A[h]
S. epidermidis	Mexico	32	*cfr*; L3 (S158Y, D159Y)	SEPI115A[i]
E. avium	Poland	4	L4 (P171S)	
E. faecalis	Poland	8	G2576T	
E. faecalis	Ireland	4	none detected	
E. faecalis	China	4	none detected	
E. faecalis	Taiwan	4	none detected	
E. faecium	Germany	8	G2576T	

[a]Percentage of non-susceptible isolates by country: Italy 6/352, 1.7%; Taiwan 1/68, 1.5%; Poland 2/181, 1.1%; Hong Kong 1/93, 1.1%; Mexico 2/206, 1.0%; Brazil 3/435, 0.7%; Ireland 1/212, 0.5%; Germany 1/359, 0.3%; and China 1/523, 0.2%. Non-susceptible isolates were not observed in the following countries (number of isolates): Canada (199), Argentina (203), Chile (222), Belgium (198), Czech Republic (121), France (330), Greece (203), Hungary (89), Israel (54), Portugal (187), Russia (263), Slovenia (107), Spain (300), Sweden (298), Turkey (297), Ukraine (101), UK (426), Australia (813), Japan (389), Korea (240), Malaysia (100), New Zealand (256), Singapore (97) and Thailand (50). *S. aureus* from Italy originated from two medical sites.
[b]Preliminary elevated MIC values (≥4 mg/L) (Thermo Fisher Scientific; dry-form panels) were confirmed by using a customized frozen-form panel with an extended linezolid dilution range (1–128 mg/L). The *E. avium* isolate did not grow when retested in this customized panel, despite several attempts. Therefore, the MIC presented was obtained by a dry-form panel.
[c]PFGE types were assigned according to the organism code and origin of the isolate (medical site number), followed by a capital letter (type) and a number (subtype). PFGE was performed only when multiple same-species isolates were recovered from the same site. Comparisons of PFGE profiles followed the criteria established by Tenover *et al*.[26]
[d]One isolate exhibiting an elevated MIC value of linezolid (i.e. 32 mg/L) and associated with this PFGE type (SCO115A) was detected in this medical site in 2009.[21]
[e]One isolate exhibiting an elevated MIC value of linezolid (i.e. 16 mg/L) and associated with this PFGE type (SH048A) was detected in this medical site during the 2011 sampling year.
[f]Isolates exhibiting elevated MIC values of linezolid and associated with this PFGE type (SEPI86A) have been detected in this medical site in all sampled years since 2006, except for 2010.[11,12,19]
[g]PFGE profile indistinguishable from that observed in an isolate collected in 2010.[10]
[h]All linezolid-non-susceptible *S. epidermidis* recovered from this site and included in the ZAAPS surveillance programme during 2006 through 2012 (two strains in 2006, one strain in 2010, three strains in 2011 and one strain in 2012) have displayed this PFGE type.[9–11]
[i]Isolates exhibiting this PFGE type were detected at this site in 2009.[12]

Overall, linezolid exhibited stable modal MIC and MIC_{50} results when tested against enterococci, regardless of species or vancomycin resistance phenotype (Table 2). *E. faecalis* isolates were susceptible (≥98.2%) to linezolid, ampicillin and glycopeptides (vancomycin and teicoplanin), while *Enterococcus faecium* demonstrated multidrug resistance phenotypes (Table 3). Enterococcal isolates with linezolid MIC results of 4 mg/L did not show any alterations of 23S rRNA or ribosomal proteins, or the presence of *cfr*, results also observed in previous studies.[10,22] Previous studies have demonstrated that linezolid can be recognized as a substrate of efflux pump systems, which can extrude a wide range of structurally dissimilar substrates.[23,24] Although the isolates described above did not have the expression levels of efflux pumps evaluated, it is tempting to associate these low-level resistance phenotypes with the overexpression of such pumps. Further investigations should determine the role of efflux pump systems in these very rare but geographically diverse cases of decreased susceptibility to linezolid. An *Enterococcus avium* isolate had a substitution in P171 of L4; however, the implication of this alteration for the linezolid MIC result remains to be determined. Other linezolid-non-susceptible enterococci (MIC 8 mg/L) had mutations in the 23S rRNA (position G2576; *Escherichia coli* numbering).

Streptococcal clinical isolates exhibited linezolid MIC_{50}, MIC_{90} and MIC_{100} results of 1, 1 and 2 mg/L, respectively (Tables 2 and 3). Most worrisome were the susceptibility results obtained for the comparator agents tested against *S. pneumoniae*. Overall, tested agents (including ceftriaxone) showed low or marginal activity (62.8%–87.5% susceptible), and only linezolid (100% susceptible), levofloxacin (98.3%) and vancomycin (100%) were active

(Table 3). Similar results were observed for viridans group streptococci with the exception that ceftriaxone (93.0% susceptible; CLSI) was also active, but only when the CLSI breakpoint was applied (87.8% susceptible; EUCAST). In contrast, all agents tested against β-haemolytic streptococci had antimicrobial coverage, except for erythromycin (82.3% susceptible) and tetracycline (55.8%–56.8%).

These study results document the stable antimicrobial activity of linezolid throughout the surveillance programme (2004–12) when tested against a collection of worldwide (non-USA) isolates. In addition, all streptococci remained very susceptible to linezolid, while a very limited number of non-susceptible isolates were observed among enterococci and staphylococci, especially *Staphylococcus epidermidis*. Molecular analysis indicated that non-susceptible isolates of *S. aureus* and enterococci appear to be scattered across different surveyed sites and are likely to reflect random selection due to a previous and/or prolonged use of linezolid.[25] In contrast, the linezolid-resistant CoNS collected within each institution were invariably clonally related to one or more isolates observed in previous surveillance years, suggesting a persistence of endemic clones. Moreover, this study reports the first detection of a *cfr*-carrying *S. aureus* from Brazil, which was considered susceptible to linezolid (MIC 4 mg/L) when applying CLSI or EUCAST breakpoints, emphasizing the importance of active surveillance programmes.

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