C2-135

Linezolid Resistance Mechanisms among Non-susceptible *Staphylococcus* spp. and *Enterococcus* spp. Pathogens Collected from Global Surveillance Programs (2001-2008)

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ICAAC 2009
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ABSTRACT

Background: Linezolid resistance has been generally associated with mutations in the 23S ribosomal RNA. We assessed the linezolid non-susceptibility rates over an eight year period and provided the molecular basis for the non-susceptible phenotypes.

Methods: *Staphylococcus aureus* (69,778), coagulase-negative *Staphylococcus* spp. (CoNS; 19,674) and *Enterococcus* spp. (23,488) were tested for susceptibility by CLSI broth microdilution methods and interpretive criteria. Non-susceptible phenotypes were confirmed by Etest. Isolates were collected from 223 hospitals located in 33 countries in North America (45.6%), Europe (EU; 30.1%), Latin America (LA; 12.2%) and Asia (12.1%) during 2001-2008. Isolates displaying linezolid MIC values at ≥8 μg/mL were screened for 23S rRNA, L4 and L22 mutations and *cfr* using PCR and sequencing. Clonality was assessed by PFGE.

Results: Linezolid showed MIC_{90} values of 1, 2 and 2 μg/mL when tested against CoNS, *S. aureus* and *Enterococcus* spp. in each year evaluated and overall non-susceptibility rates were only 0.4, <0.1 and 0.3%, respectively. A total of 157 strains were non-susceptible and the vast majority (84.7%; 133/157) showed the G2576T mutation. The remaining isolates carried G2447T (3.8%) or T2504A mutation (0.6%) or *cfr* (3.2%). Mutations at L4 and L22 were not observed. Linezolid resistance mechanisms could not be determined in one *S. aureus,* seven CoNS and four *Enterococcus* spp. Isolates with G2576T alteration displayed linezolid MIC ranging from 8 to >256 μg/mL, while those with G2447T or T2504A had linezolid MIC at >256 μg/mL. The *cfr*-carrying *S. aureus* and CoNS showed linezolid MIC values of 8 μg/mL and 16 to >256 μg/mL, respectively. Clonal dissemination was noted primarily among CoNS and *E. faecium* within several hospitals.

Conclusions: Linezolid has exhibited consistent MIC_{90} potencies (1-2 μg/mL) against common Gram-positive pathogens and resistant isolates remained rare and not escalating outside of local outbreaks. rRNA mutations (mainly G2576T) were the dominant resistance mechanisms, and *cfr*-carrying isolates remain very uncommon. rRNA mutation previously found only in laboratory derived mutants were detected in this study (T2504A).

INTRODUCTION

Staphylococcus aureus, coagulase-negative staphylococci (CoNS) and *Enterococcus* spp. were the most frequent species recovered from nosocomial bloodstream infections (BSI) in the United States (USA) from 1995 through 2002, with an incidence of 31.3, 20.2 and 9.4%, respectively. *S. aureus* and CoNS are also leading causes of skin and skin structure infections (SSSI) in USA and European hospitals. In addition, *S. aureus* has long been considered an important cause of nosocomial pneumonia, and currently accounts for 15–35% of all hospital-acquired bacterial pneumonia (HAP) cases.

Linezolid is the only oxazolidinone approved for the treatment of cSSSI and nosocomial pneumonia caused by Gram-positive pathogens including methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant enterococci (VRE). Linezolid inhibits protein synthesis by interfering with the formation of the 70S initiation complex. Although, linezolid resistance remains very rare, sporadic staphylococci and enterococci non-susceptible isolates have been detected and are usually associated with mutations in the 23S rRNA, mostly G2576T. This report sought to determine the linezolid non-susceptibility rates over an eight year period and provide the molecular basis for the resistance phenotypes.

MATERIALS AND METHODS

Bacterial Strain Collection: Clinically-relevant *S. aureus* (69,778), CoNS (19,674) and *Enterococcus* spp. (23,488) isolates collected through several surveillance programs (2001 – 2008) were selected for this study. Isolates originated from 223 medical sites located in 33 regionally dispersed countries in North America (45.6%), Europe (30.1%), Latin America (12.2%) and Asia-Pacific region (APAC; 12.1%).

The isolates included in this investigation were recovered from blood (55.7%), skin and skin structure (20.4%), respiratory tract (10.5%), urinary tract (4.4%), catheter (0.8%), bone/joint (0.6%) and other less prevalent or undetermined clinical specimen types (7.6%). Bacterial identification was confirmed by the central monitoring site (JMI Laboratories, Iowa, USA) using standard algorithms and an automated system, when needed (Vitek® 2; bioMerieux, North Carolina, USA).

Susceptibility Test Methods: The isolates were tested for susceptibility by the reference Clinical and Laboratory Standards Institute (CLSI) broth microdilution method using commercially prepared and validated panels (TREK Diagnostic Systems, Ohio, USA) in cation-adjusted Mueller-Hinton broth. Linezolid MIC values were confirmed by the Etest methodology, according to the manufacture's instruction (AB BIODISK, Solna, Sweden). Interpretation of linezolid MIC results was in accordance with published CLSI criteria (M100-S19). Quality control (QC) strains utilized were: *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212 and *Streptococcus pneumoniae* ATCC 49619; all MIC results were within CLSI published QC ranges.

Temporal trends for non-susceptibility rates were determined by the χ^2 test using the Epi Info™ Version 3.4.1 software package (Centers for Disease Control and Prevention, Georgia, USA). *P* values <0.05 were considered to be significant.

Molecular Screening for Resistance Mechanisms: Isolates showing a linezolid-non-susceptible phenotype (MIC, ≥8 μg/mL) were screened for mutations in the central loop of domain V region of 23S rRNA and presence of the *cfr*-encoding gene using standard PCR reactions, followed by sequencing. CoNS were also screened for mutations at L4 and L22 genes.

RESULTS

- Linezolid showed $MIC_{50/90}$ values of 2/2 μg/mL when tested against *S. aureus*, except during the 2005 and 2007 year sampling, where MIC_{50} values dropped to 1 μg/mL. When tested against CoNS, linezolid demonstrated constant $MIC_{50/90}$ values (1/1 μg/mL), except for the year of 2001 (1/2 μg/mL; Table 1).
- Linezolid showed stable MIC_{90} values (2 μg/mL) when tested against *E. faecalis* and *E. faecium* during the 2001 and 2008 years sampling (Table 1). However, a slight increase in the linezolid potency was noted against *E. faecalis* and *E. faecium* during 2005 and 2008 (MIC_{50}, 1 μg/mL) compared to previous years (MIC_{50}, 2 μg/mL).
- Overall non-susceptibility rates for linezolid among *S. aureus,* CoNS, *E. faecalis* and *E. faecium* were <0.1 (n=11), ≤0.8 (n=72), ≤0.3 (n=18) and ≤1.2% (n=56), respectively. A significant trend towards decreased susceptibility was noted only against CoNS (*P*<0.01), where clonal occurrences in a few medical centers influenced the non-susceptibility rates.
- A total of 157 (0.1%) strains displayed a linezolid-non-susceptible phenotype and the vast majority (84.7%; 133/157) of those showed the G2576T mutation. The remaining isolates carried G2447T (3.8%) or T2504A (0.6%) alteration or *cfr* (3.2%; Table 2). Mutations at L4 and L22 among CoNS were not observed.
- Linezolid resistance mechanisms could not be determined in one *S. aureus,* seven CoNS and four *Enterococcus* spp. Isolates with G2576T alteration displayed linezolid MIC ranging from 8 to >256 μg/mL, while those with G2447T or T2504A had linezolid MIC at >256 μg/mL. The *cfr*-carrying *S. aureus* and CoNS showed linezolid MIC values of 8 μg/mL and 16 to >256 μg/mL, respectively.
- Clonal dissemination of linezolid-non-susceptible isolates has been noted primarily among CoNS and *E. faecium* within several hospitals.

Table 1. Mode, MIC_{50}, MIC_{90} values (μg/mL) and percentage of linezolid-non-susceptible Gram-positive isolates selected for this study stratified by year.

Year/ Mode/MIC_{50}/MIC_{90} % non-susceptible[a]	Organisms			
	S. aureus (69,778)	CoNS[b] (19,674)	*E. faecalis* (15,074)	*E. faecium* (7,441)
2001				
Mode	2	1	2	2
MIC_{50}/MIC_{90}	2/2	1/2	2/2	2/2
% non-susceptible (no.)	0.0 (0)	0.0 (0)	0.2 (2)	0.3 (1)
2002				
Mode	2	1	2	2
MIC_{50}/MIC_{90}	2/2	1/1	2/2	2/2
% non-susceptible (no.)	<0.1 (1)	0.1 (1)	0.0 (0)	0.7 (3)
2003				
Mode	2	1	2	2
MIC_{50}/MIC_{90}	2/2	1/1	2/2	2/2
% non-susceptible (no.)	<0.1 (1)	0.0 (0)	0.1 (2)	0.5 (6)
2004				
Mode	2	1	2	2
MIC_{50}/MIC_{90}	2/2	1/1	2/2	2/2
% non-susceptible (no.)	0.0 (0)	0.0 (0)	0.3 (4)	0.2 (1)
2005				
Mode	1	1	1	1
MIC_{50}/MIC_{90}	1/2	1/1	1/2	1/2
% non-susceptible (no.)	<0.1 (1)	0.1 (3)	0.1 (1)	0.9 (7)
2006				
Mode	2	1	1	1
MIC_{50}/MIC_{90}	2/2	1/1	1/2	1/2
% non-susceptible (no.)	<0.1(2)	0.6 (19)	0.2 (5)	1.2 (16)
2007				
Mode	1	1	1	1
MIC_{50}/MIC_{90}	1/2	1/1	1/2	1/2
% non-susceptible (no.)	<0.1 (3)	0.8 (28)	0.1 (2)	0.9 (14)
2008				
Mode	2	1	2	1
MIC_{50}/MIC_{90}	2/2	1/1	1/2	1/2
% non-susceptible (no.)	<0.1 (3)	0.8 (21)	0.1 (2)	0.7 (8)
Overall				
Mode	2	1	1	1
MIC_{50}/MIC_{90}	2/2	1/1	1/2	1/2
% non-susceptible (no.)	<0.1 (11)	0.4 (72)	0.1 (18)	0.8 (56)

a. *Staphylococcus* spp. and *Enterococcus* spp. with linezolid MIC values at ≥8 μg/mL.
b. CoNS - coagulase-negative staphylococci.

Table 2. Mechanisms of linezolid resistance detected among staphylococci and enterococci clinical isolates included in this study.

	Mechanism of linezolid resistance: no. of strains (%)									
Organism (no.)	G2576T	LZD[a] MIC Range (μg/mL)	G2447T	LZD MIC Range (μg/mL)	T2504A	LZD MIC Range (μg/mL)	*cfr*	LZD MIC Range (μg/mL)	Undetermined[b]	LZD MIC Range (μg/mL)
S. aureus (11)	8	8 - 32	-	-	-	-	2	8	1	8
CoNS[c] (72)	55	8 - >256	6	>256	1	>256	3	16 - >256	7	8 - 16
E. faecalis (18)	16	8 - >256	-	-	-	-	-	-	2	8 - 16
E. faecium (56)	54	8 - 128	-	-	-	-	-	-	2	64 - 128
Overall (157)	133 (84.7%)	-	6 (3.8%)	-	1 (0.6%)	-	5 (3.2%)	-	21 (13.4%)	-

a. LZD = linezolid, for which the MIC values were confirmed using the Etest methodology.
b. The investigated linezolid resistance mechanisms were not detected.
c. CoNS - coagulase-negative staphylococci and included *S. capitis* (2 strains), *S. epidermidis* (56 strains), *S. haemolyticus* (7 strains), *S. hominis* (2 strains), *S. simulans* (2 strains), *S. xylosus* (2 strains) and unspeciated (1 strain).

CONCLUSIONS

- Linezolid exhibited consistent MIC_{90} potencies (1-2 μg/mL) against commonly isolated Gram-positive pathogens and non-susceptible strains remained rare and not escalating outside of local outbreaks.
- The linezolid MIC values for those isolates carrying 23S rRNA gene mutations varied significantly, which may be explained by the proportion of mutated gene copies within the cell. In contrast, all CoNS harboring the G2447T or T2504A alteration had very high linezolid MIC values (>256 μg/mL).
- Isolates for which linezolid resistance mechanisms were not detected showed lower MIC values of 8 and 16 μg/mL, except for two *E. faecium* strains (64-128 μg/mL). Among CoNS with undetermined resistance mechanisms (7), five isolates were genetically or epidemiologically related.
- rRNA mutations (mainly G2576T) were the dominant linezolid resistance mechanisms, and *cfr*-carrying isolates remained very uncommon. rRNA mutation previously found only in laboratory derived mutants were detected clinically in this surveillance summary (T2504A).

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