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Oxazolidinone-Resistant Enterococci Emerging in 2001: Patient Reports from the SENTRY Antimicrobial Surveillance Program (USA)

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AMENDED ABSTRACT

Background: The oxazolidinone (OXA) class has demonstrated remarkable activity against Gram-positive cocci (GPC), and the initial clinical candidate, linezolid (LZD) has proven to be a welcome therapeutic option. LZD clinical trial results and subsequent published case reports cite rare LZD-resistance (R) emerging in patients receiving prolonged therapy. Since the abstract submission, we have identified a total of 7 GPC isolates demonstrating linezolid resistance. We report the initial OXA-R cases obtained through the SENTRY Program after screening >40,000 GPC without R in 1998-2000.

Methods: In 2001, 7 patients from 5 different states and 6 different institutions in the USA (WA, 3-NY, KY, TX, IA) were identified among positive bloodstream infection isolates of *E. faecalis* (EF, 2), *E. faecium* (EFM, 3), *S. epidermidis* (SEPI, 1), and *S. oralis* (SOR, 1) . Patient ages were 11 months, (female, EF), 32 years (male, EFM), 36 years (female, EFM), 64 years (male, EF), 76 years (male, SEPI), 72 years (male, EFM), and 27 years (female, SOR). R was detected by NCCLS broth microdilution methods, confirmed by identical results with Etest and disk diffusion (DD) tests plus confirmed by detection of a G2576U ribosomal mutation in all isolates and in 1 isolate (SEPI) another C2534U mutation.

Results: The table lists the MICs for 6 agents along with a novel OXA (AZD2563 [AZD], AstraZeneca). MIC and DD tests showed elevated MICs (8 - > 8 µg/ml) for all 7 strains to LZD and AZD, as well as small zones of inhibition (12-16 mm; R at ≤ 20 mm) eg. co-R. Vancomycin-R was found in 2 strains. All EF/EFM, and SOR strains showed R to erythromycin (≥1 µg/ml), respectively. EFM strains were R to penicillins (≥ 32 µg/ml), but S to quinupristin/dalfopristin (Q/D). See chloramphenicol (CHLOR) and doxycycline (DOXY) results below:

	EFM	EF	EF	EFM	SEPI	EFM	SOR
	11-4103	21-6943	15-534	15-4011	82-1645	24-1575	27-2832
LZD MIC/Zone	>8/14	8/14	>8/13	8/16	8/13	8/15	8/19
AZD MIC/Zone	>8/13	>8/12	>8/12	8/16	8/12	8/16	8/15
Vancomycin MIC	>16	1	2	8	2	1	0.5
Teicoplanin MIC	>16	0.25	0.25	0.25	4	1	0.12
Q/D MIC	0.5	8	8	1	0.12	1	0.5
CHLOR MIC	16	8	>16	16	16	>16	8
DOXY MIC	0.5	>4	>4	1	0.5	>4	>4

Conclusions: Occurrence of OXA-R remains rare, but no longer is limited to enterococci. Clinical laboratories should routinely test LZD to monitor for emerging OXA-R (MIC, ≥ 8 µg/ml). Longitudinal surveillance programs continue to be warranted in order to monitor development of OXA-R, determine the mechanism and recommend alternative therapies or epidemiological interventions.

INTRODUCTION

Therapeutic problems created by vancomycin-resistant enterococci (VRE) were first recognized in the 1980's. In a report from the Centers for Disease Control covering the period of 1989 to 1993, the incidence of nosocomial infections due to VRE increased from 0.3% to 7.9%, most apparent in the intensive care unit. The release of linezolid, though a welcome addition as an effective therapy for VRE (specifically *Enterococcus faecium*), also created a growing need for careful, prospective surveillance of this class in order to limit further expansion in resistance patterns.

Resistance to linezolid had been reported only rarely during treatment of complicated VRE infections. However, reports from the linezolid clinical trials, and subsequent published case reports of enterococci and *staphylococcus aureus* cite emerging resistance patterns in patients who have received prolonged courses of oxazolidinone therapy. A recent report describes the isolation of an *E. faecium* strain from a blood stream infection that was resistant to linezolid, but unassociated with OXA treatment.

The SENTRY Antimicrobial Surveillance Program was initiated in 1997, with the primary purpose of monitoring antimicrobial resistance trends of both nosocomial and community-acquired pathogens over large geographic areas, and currently includes, North America (NA), Latin America (LA), Europe (EU), and the Asia-Pacific (APAC) regions. In this report we present the initial OXA-R cases obtained through the SENTRY Program after screening more than 40,000 GPC without OXA-R during 1998-2000.

MATERIALS AND METHODS

Isolates in this study were recent clinical strains obtained from the SENTRY Antimicrobial Surveillance Program (1997-2001) which were from blood stream infections. A total of 46,449 Gram-positive isolates were evaluated and included; *S.aureus* (22,920 strains), coagulase-negative staphylococci (CoNS, 8,401 strains) *Enterococcus* spp. (6,693 strains), *Streptococcus pneumoniae* (2,899 strains), beta-haemolytic streptococcus (3,799 strains) and viridans-group streptococcus (1,321 strains).

Strains were tested against a panel of eight or ten Gram-positive focused agents (among 50 total antimicrobials). These included; linezolid, AZD2563, quinupristin/dalfopristin (Q/D), vancomycin, teicoplanin, oxacillin, ampicillin, penicillin, erythromycin (staphylococci and streptococci), rifampin, doxycycline, clindamycin (staphylococci), ciprofloxacin, gatifloxacin (staphylococci and streptococci), and high-level gentamicin and streptomycin (*enterococcus* only).

All strains were tested by reference broth microdilution methods recommended by the National Committee for Clinical Laboratory Standards (NCCLS) using dry-form panels supplied by TREK Diagnostics (Westlake, OH). Organisms from pure culture plates were suspended into a Mueller-Hinton broth media to equal a 0.5 McFarland standard and further diluted and inoculated into the antimicrobial containing wells to equal approximately $5x10^5$ CFU/ml. Panels were incubated in an ambient environment for 24 hours, depending on the species tested. Concomitant processing of ATCC quality control strains including *S.aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *S.pneumoniae* ATCC 49619.

RESULTS

- Seven bacterial isolates originating from 5 different states across the United States demonstrated oxazolidinone resistance; five isolates were enterococci (2 *E. faecalis*, 3 *E. faecium*), one *S. epidermidis*, and one *S. oralis* (Table 1).
- Vancomycin-resistance was present in one of the *E. faecium* isolates and quinupristin/dalfopristin resistance was intrinsically present in the two *E. faecalis* isolates. Both drugs demonstrated susceptible MIC results to the remaining isolates (Table 2).
- Penicillin demonstrated susceptible MICs against both *E. faecalis* isolates, and *S. oralis*, while ampicillin demonstrated activity against both *E. faecalis* strains, and intermediate susceptibilities against *S. oralis*.
- Alternative antimicrobials having susceptible MIC values against the *S. epidermidis* isolate included; vancomycin (MIC; 2 µg/ml), teicoplanin (4), quinupristin/dalfopristin (0.12), erythromycin (0.5), doxycycline (0.5), and rifampin (0.25).
- Vancomycin (MIC; 0.5 µg/ml), quinupristin/dalfopristin (0.5), clindamycin (0.06), and penicillin (0.015) were active against the *S. oralis*.
- The common ribosomal mutation, G2576U, was observed in all strains and one additional documented mutation was noted in the *S. epidermidis*.
- The oxazolidinone resistance in *S. epidermidis* and *S. oralis* were unique or the first reported cases.
- For the seven cases described, three were associated with the previous use of linezolid while four cases were not directly associated with linezolid use and warrants further evaluation.

Table 1. Demographic comparisons among seven patients with linezolid (LZD) resistant isolates

	Patient/Organism					*	*
	11-4103	15-4011	24-1575	21-6943	15-534	82-1645	27-2832
	E. faecium	*E. faecium*	*E. faecium*	*E. faecalis*	*E. faecalis*	*S. epidermidis*	*S. oralis*
Location (State)	Iowa	New York	Texas	Wash	New York	New York	Kentucky
Patient demographics							
Age (yrs)	36	32	72	64	11 mos.	76	56
Sex	Female	Male	Male	Male	Female	Male	Female
Prior LZD	Yes	No	No	No	No	Yes	Yes
Mutation(s) identified	G2576U	G2576U	G2576U	G2576U	G2576U	C2534U, G2576U	G2576U

Table 2. Antibiogram for seven patients with linezolid resistant isolates

	MIC (µg/ml)					*	*
	11-4103	15-4011	24-1575	21-6943	15-534	82-1645	27-2832
Antimicrobial Agent	*E. faecium*	*E. faecium*	*E. faecium*	*E. faecalis*	*E. faecalis*	*S. epidermidis*	*S. oralis*
Linezolid	>8	8	8	8	>8	8	8
AZD2563	>8	8	8	>8	>8	8	>8
Vancomycin	>16	8	1	2	2	2	0.5
Teicoplanin	>16	0.25	1	0.25	0.12	4	0.12
Quinupristin/dalfopristin	0.5	1	1	4	8	0.12	0.5
Erythromycin	>8	>8	>8	>8	>8	0.5	2
Clindamycin	>8	>8	>8	>8	>8	1	0.06
Chloramphenicol	16	16	>16	16	>16	16	8
Oxacillin	>8	>8	>8	>8	>8	>8	0.06
Penicillin	>32	>32	>32	8	2	8	0.015
Ampicillin	>16	>16	>16	2	<=2	8	2
Doxycycline	0.5	4	>4	>4	>4	0.5	>4
Ciprofloxacin	>2	>2	>4	>2	0.5	>4	>4
Rifampin	>2	>2	>2	>2	1	0.25	0.25
Gentamicin(HL)	>1000	>1000	>1000	>1000	≤500	NA	NA
Streptomycin (HL)	>2000	>2000	>2000	>2000	>2000	NA	NA

NA= Not Applicable

* = submitted as "Late Breaker"

CONCLUSIONS

- Oxazolidinone resistance has now extended beyond *enterococcus* spp., and has been reported within coagulase- negative staphylococci and viridans group streptococci (key mutation at G2576U).
- Oxazolidinone resistance should not preclude the use of older antimicrobials, and requires a careful examination of susceptibility patterns on a case-by-case basis in order to identify the agent of best choice.
- Routine monitoring for linezolid resistance needs to occur within the microbiology laboratory, along with timely reporting to practitioners to adequately select treatment alternatives.
- Longitudinal surveillance programs facilitate the discovery of resistance, and allow for the characterization of resistance with advanced epidemiological procedures (SENTRY Program).

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