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Revision of Linezolid Disk Diffusion Quality Control Guidelines for Testing Staphylococcus aureus ATCC 25923: An Independent Seven Laboratory Trial

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ABSTRACT

Background: The increased utilization of linezolid (LZD) for treating serious Gram-positive infections requires that accurate QC ranges be established. Preliminary data (Stevens et al., ICAAC Abstr. D-167, 2001) supporting the need to revise the QC zone diameter ranges recommended by the NCCLS for *S. aureus* ATCC 25923 has prompted this multicenter study to determine if errors do exist.

Methods: Seven laboratories participated in this evaluation according to NCCLS M23-A2 (2001) guidelines. Each site tested 2 disk lots of LZD on 3 different Mueller-Hinton (MH) agar lots from commercial vendors for 10 days. Vancomycin (VANC) was used as the QC comparator control. All replicates utilized a 0.5 McFarland standard inocula and plates were incubated for 16-18 hr in 35ºC ambient air by NCCLS guidelines. A total of 420 LZD and 210 VANC zone diameter results were generated.

Results: Both LZD disk lots and all 3 MH media lots produced identical median zone diameters of 28 mm. Inter- and intra-laboratory comparisons showed LZD zone diameters from 3 to 10 mm and median zone diameters of 26 (1 occurrence), 27 (1), 28 (2), 29 (2) and 30 (1) mm. Overall, the evaluation of LZD results (420) yielded a 12 mm range (21 - 32) with only 82.4% of generated results within the current recommended range (27 - 31 mm). All VANC zone diameters were with NCCLS QC ranges (control). Using NCCLS statistical procedures for QC ranges, a range modification to 25 - 31 mm was calculated.

Conclusions: These results support revision of FDA and NCCLS QC ranges for LZD when testing *S. aureus* ATCC 25923, the only available control for LZD disk testing. Utilizing the expanded range of 25 - 31 mm produces acceptable results (95.7%) for this valuable oxazolidinone and minimizes the current high-level of "out-of-control" results without being too lax.

INTRODUCTION

The use of linezolid has been increasing since early 2000 to treat serious, antimicrobial-resistant Gram-positive infections. Since this time, many hospitals have added linezolid to their formulary to combat infections caused by vancomycin-resistant *Enterococcus* spp., methicillin- (oxacillin-) resistant *Staphylococcus* spp., penicillin-resistant *Streptococcus* spp., and the multi-drug co-resistances often associated with these species. It is important to note that resistance to other novel Gram-positive focused agents such as everninomicins and streptogramin combinations emerged soon after their introduction into clinical and even veterinary practice. Likewise, the development of linezolid-resistant Gram-positive pathogens has been documented, including cases during early compassionate use trials and those resistant isolates emerging in patients without prior drug exposure. Therefore, it is essential to accurately detect the susceptibility profile of bacterial pathogens which are targeted by newly introduced antimicrobial agents as the selective pressure increases. To accomplish this, routine quality control (QC) procedures using standardized strains is essential in the determination of reagent and technical quality. Preliminary data supporting the need to revise the QC zone diameter range recommended by the National Committee for Clinical Laboratory Standards (NCCLS) for *Staphylococcus aureus* ATCC 25923 and linezolid has prompted this independent multi-laboratory study to determine if an error does exist as suggested by others.

MATERIALS AND METHODS

Seven laboratories were recruited to determine if significant inter- and intra-laboratory problems were occurring when testing linezolid (30-µg) disks against *S. aureus* ATCC 25923. The study design followed NCCLS M23-A2 recommendations. Consistent with these guidelines, all laboratories tested three agar lots of media (Mueller-Hinton agar) and two linezolid disk lots (Remel, Lenexa, KS; BD Microbiology Systems, Cockeysville, MD) over a 10 day period. This generated 60 values from each site resulting in 420 determinations from all participants combined. Agar media were prepared commercially by Remel. Vancomycin disks were tested concurrently to provide internal QC at each site.

All test procedures adhered to NCCLS recommendations using a 0.5 McFarland standard inoculum which was applied to the agar surface. Plates were allowed to dry before disks were applied, and then incubated overnight (16 - 18 hours) prior to reading/measuring zone diameters. The data was analyzed by site using median zone diameters and the range of zone diameter results to determine if the currently recommended, narrow 5 mm QC range for linezolid and *S. aureus* ATCC 25923 was functional.

These results were compared to the QC zone diameters reported by the participants in the KB-ZAPS in vitro surveillance trial for linezolid published in 2001.

Table 1. Inter- and intra-laboratory comparisons of the linezolid zone diameter results versus *Staphylococcus aureus* ATCC 25923 for an seven medical center protocol complying the study design guidelines found in NCCLS M23-A2 [2001].

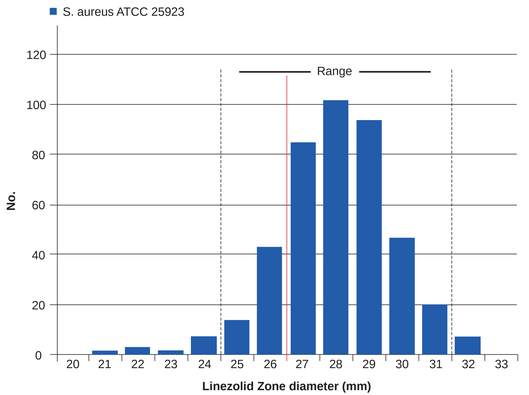
	Laboratory code (occurrences):								
Zone diameter (mm)	A	B	C	D	E	F	G	Total (%)[b]	KB-ZAPS QC[a]
21							1	1 (0.2)	
22							2	2 (0.5)	
23							1	1 (0.2)	
24							7	7 (1.7)	
25		1	2				10	13 (3.1)[b]	2 (0.6)
26		1	17		2	8	15	43 (10.2)[b]	0 (0.0)
27	2	13	19	27	3	6	14	84 (20.0)[b,c]	55 (17.7)
28	15	20	17	28	5	10	6	101 (24.0)[b,c]	79 (25.4)
29	28	19	5	5	13	21	3	94 (22.4)[b,c]	73 (23.5)
30	13	6			18	9	1	47 (11.2)[b,c]	65 (20.9)
31	2				14	4		20 (4.8)[b,c]	34 (10.9)
32					5	2		7 (1.7)	3 (1.0)d
Total	60	60	60	60	60	60	60	420	311
Median (mm)	29	28	27	28	30	29	26	28	28
Range (mm)	5	6	5	3	7	7	10	12	11

a. From reported QC zone diameters obtained from > 100 laboratories participating in the KB-ZAPS trial [Jones et al., 2001].
b. 95.7% in proposed QC range (25 - 31 mm).
c. 82.4% of qualified results in recommended NCCLS QC range (27 - 31 mm).
d. Range of reported zones included single occurrences at 32, 33, and 35 mm.

RESULTS

- Among the seven sites, median linezolid values for S. aureus ATCC 25923 ranged from 26 to 30 mm and the consensus median of 28 mm was only one mm higher than the lower limit of the currently recommended NCCLS range (27 to 31 mm).
- The results of all 420 determinations combined produced a 12 mm range and only 82.4% were within the currently recommended range (Table 1).
- Three sites (C, F, G) had values lower than the recommended range at unacceptable rates of 31.6, 13.3 and 60.0%, respectively.
- A graphical representation (Figure 1) shows that the current range bisects the normal distribution of linezolid zone diameters when testing *S. aureus* ATCC 25923 and an adjustment of 25 to 31 (broken vertical lines) seems more appropriate. This adjustment would contain 95.7% of participant results within this modified QC range.
- Table 1 also illustrates the QC zone diameter distribution for the KB-ZAPS participants (106 laboratories in 31 states, United States) using *S. aureus* ATCC 25923. The mode was 28 mm with 306 of 311 (98.4%) linezolid 30-µg disk zones within the published range. Using these results, a 28 ± 3 mm range also appears appropriate.

Figure 1: Bar graph showing the distribution of linezolid zone diameter results (mm) tested against *S. aureus* ATCC 25923. Broken lines suggest a revision by expansion of the lower limit by 2 mm (solid line indicates the previous QC range).



CONCLUSIONS

- Utilizing the current NCCLS published linezolid QC range for *S. aureus* ATCC 25923 creates unacceptable proportions of "out of range" zone diameter results.
- Clinical laboratories can not confidently report linezolid results with the current QC range recommendations.
- The interpretations of bacteristatic agents (linezolid) using measured zone diameter can be difficult and these technical issues can produce greater variation between technologists performing these tests (smaller zone diameters).
- The data generated in this study (confirmed by trends in a 106 laboratory trial) are consistent with previous findings reported at the 2001 ICAAC, and suggests that the more conservative, modified range (7 mm; 25 - 31 mm) should be considered to provide better linezolid QC range for *S. aureus* ATCC 25923.
- Note: Since the submission of this abstract, the NCCLS has modified this QC range for publication in 2003.

SELECTED REFERENCES

Gonzales RD, Schreckenberger PC, Graham MB, Kelkar W, DenBesten K, Quinn JP. (2001). Infections due to vancomycin-resistant *Enterococcus faecium* resistant to linezolid. *Lancet.* 357, 1179.

Jones RN, Ballow CH, Biedenbach DJ, the ZAPS Study Group Medical Centers. Multi-laboratory assessment of the linezolid spectrum of activity using the Kirby-Bauer disk diffusion method: Report of the Zyvox® antimicrobial potency study (ZAPS) in the United States. Diagnostic Microbiology and Infectious Disease 2001; 40:59-66.

Jones RN, Della-Latta P, Lee LV, Biedenbach DJ. Linezolid resistant *Enterococcus faecium* isolated from a patient without prior exposure to an oxazolidinone: Report from the SENTRY Antimicrobial Surveillance Program. *Diagnostic Microbiology and Infectious Disease* 2002; 42:137-139.

National Committee for Clinical Laboratory Standards. (2001). *Development of in vitro susceptibility testing criteria and quality control parameters. Approved guideline M23-A2.* Wayne, PA:NCCLS.

Stevens TM, Schreckenberger PC, Ristow KL, Eng-Wong LP, Johnson JL, Bouchillon SK. Problems with linezolid and *Staphylococcus aureus* ATCC 25923 disk diffusion quality control range. *In Program and Abstracts of the 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, abstr. 167, p. 151.* American Society for Microbiology, Washington, D.C.

Tsiodras A, Gold HS, Sakoulas G, Eliopoulos GM, Wennersten C, Venkataraman L, Moellering Jr RC, Ferraro MJ. Linezolid resistance in a clinical isolate of *Staphylococcus aureus*. *Lancet.* 2001; 358:207-208.

Zurenko GE, Todd WM, Hafkin B, Myers B, Kauffman C, Bock J. Development of linezolid-resistant *Enterococcus* faecium in two compassionate use program patients treated with linezolid. *In Program and Abstracts of the 39th Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, Canada,* Abstract 848, p.118. American Society for Microbiology, Washington, D.C. 1999.