Paper # **U-084**

Determination of MICs of Nocardia Using the TREK Vizion™ System (VIZ) Compared to Manual Readings

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UPDATED ABSTRACT

Background: Susceptibility testing of Nocardia is performed using the CLSI (Clinical and Laboratory Standards Institute) standardized broth microdilution method. Often, poor growth characteristics of Nocardia make reading of MICs difficult without special background lighting adjustments. The Vizion™ (TREK Diagnostic Systems, Cleveland, OH) has been designed to project a digital image of growth in each MIC well onto a touchscreen monitor with the antimicrobial template overlaid on the image when used in conjuction with the Sensititre® SWIN® computer system. Touching the MIC well provides instant categorical feedback, records MICs and stores images. The objective of this study was to compare MIC readings of Nocardia in the VIZ system with manual readings.
Methods: 123 isolates of Nocardia including 31 *Nocardia nova* complex, 7 *N. abscessus* complex, 10 *N. transvalensis* complex, 12 *N. farcinica*, 16 *N. cyriacigeorgica* complex, 14 *N. brasiliensis*, 1 *N. otitidiscaviarum* complex, and 34 *Nocardia* sp. were tested using 96-well MIC plates according to the CLSI procedure. After 3 days incubation at 35°C, plates were read manually using a mirrored light box. Subsequently, the same plates were read in blinded fashion using the VIZ. Comparisons were made between the two readings using CLSI susceptibility criteria for interpretation of susceptible (S), intermediate (I), and resistant (R) isolates. Major errors were those with susceptibility interpretive category change from S to R or R to S. Minor errors were those with interpretive category change for S to I, I to S, I to R, or R to I. Percentages of major and minor interpretive errors were calculated.
Results: 99% of the MICs were the same interpretive category for manual and VIZ readings. 1% had minor errors and 0% had major errors.
Conclusion: Although this was a small single center study, the VIZ demonstrated excellent correlation with manual MIC reads of Nocardia. The VIZ built-in lighting adjustment was advantageous for facilitating reading of MICs. The ability to store MIC readings for future review was an added convenience of the VIZ with the SWIN system. Additional larger studies with Nocardia are recommended to establish the utility of the system in the clinical laboratory.

ADDENDUM: Since the submission of the original abstract, additional isolates have been added.

INTRODUCTION

Nocardia are being increasingly recognized in the clinical laboratory as causes of various diseases. Currently more than 50 species of Nocardia have been characterized by phenotypic and molecular methods. Among the currently validated species, approximately one-half are human or animal pathogens. Susceptibility testing of the Nocardia is fraught with difficulties, including poor growth of some species that makes a standard read time of 72 hours difficult. Performance and interpretation of antimicrobial susceptibility testing requires skill acquired through experience with the test method and knowledge of various susceptibility patterns, which may predict species in some instances.

In 2003, the Clinical and Laboratory Standards Institute recommended antimicrobial susceptibility testing of Nocardia be performed using a broth microdilution method. Recently we reported 99% correlation of manual interpretations with interpretations using the Vizion in 189 isolates of nontuberculous mycobacteria (Abstract, ECCMID 2008). The TREK Vizion system is able to facilitate the reading of MICs by the broth microdilution method. This study details our laboratory experience with antimicrobial susceptibility testing of Nocardia using the Vizion.

MATERIALS & METHODS

MICs for 123 clinical isolates of Nocardia were performed in cation adjusted Mueller-Hinton broth using procedures published by the Clinical and Laboratory Standards Institute (CLSI) in the M-24A document. Briefly, the organisms were suspended in sterile distilled water to an inoculum turbidity which matched the 0.5 McFarland standard using a nephelometer. Appropriate dilutions were prepared and the 96-well panels were inoculated using a multichannel pipettor. Nocardia panels were incubated 72 hours. All panels were then read using a Sensititre® mirrored view box and overhead lighting to facilitate reading of the wells. The same panels were then read in a blinded fashion using the Vizion (TREK Diagnostics System, Inc.) and adjustments to lighting were made using the Vizion controls. Once the plate was loaded and the panel type identified on the SWIN® System computer, the appropriate template appeared on the monitor screen. The well which the user determines to be the MIC was then "touched" on the screen and SWIN automatically records the results and prints with interpretive categories printed on the report (susceptible S; intermediate I, resistant R).

Interpretive categories used were those described in the M-24A document for the following agents: amikacin, amoxicillin-clavulanic acid, ceftriaxone, ciprofloxacin, clarithromycin, imipenem, linezolid, minocycline, sulfamethoxazole, tobramycin, and trimethoprim sulfamethoxazole. For moxifloxacin, interpretive categories used were those outlined in the CLSI documents for susceptibility testing of bacteria (M100-S16).

Quality control was performed with the addition of in-house controls using *Mycobacterium peregrinum* ATCC 700686, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC 35218 (for quality control of amoxicillin-clavulanic acid).

RESULTS

Overall, there was 99% category or MIC agreement with the reads performed visually as compared to the Vizion reads (see Table 1). There was only one minor discrepancy (1%) (I S / S I, or I R / R I) among the total 123 isolates. This discrepancy was noted with the moxifloxacin MIC on 1/9 isolates of *Nocardia farcinica.* As defined by the CLSI, the error was minor (one dilution difference but a category change from S to I). There were no major errors or very major errors (S R, R S) noted in any of the results.

Quality controls were all within acceptable limits and no discrepancies (errors) were noted (see Table 3).

RESULTS cont.

Table 1. Comparison of standard MIC interpretive readings of the isolates of Nocardia with interpretive readings performed using the TREK Vizion System.

Nocardia Species or Complex	Interpretive Match Standard Read to VIZION™ ALL DRUGS	% Match	Interpretive Discrepant Standard Read to VIZION™ ALL DRUGS	% Error
N. abscessus complex	7 / 7	100	0 / 7	0
N. brasiliensis	14 / 14	100	0 / 14	0
N. cyriacigeorgica complex	16 / 16	100	0 / 16	0
N. farcinica	11 / 12	92	1 / 12	8
N. nova complex	31 / 31	100	0 / 31	0
N. otitidiscaviarum complex	1 / 1	100	0 / 1	0
N. transvalensis complex	10 / 10	100	0 / 10	0
Nocardia sp.	34 / 34	100	0 / 34	0

Table 2. MICs of isolates of Nocardia with discrepancies between standard readings, and readings performed using the TREK Vizion System.

Nocardia/ Antimicrobial	Interpretive Match	%	No. Discrepancies	%	Type of Discrepancy (Error)
N. farcinica					
Amikacin	12 / 12	100	0 / 10	0	-
Amoxicillin/ Clavulanic Acid	12 / 12	100	0 / 10	0	-
Ceftriaxone	12 / 12	100	0 / 10	0	-
Ciprofloxacin	12 / 12	100	0 / 10	0	-
Clarithromycin	12 / 12	100	0 / 10	0	-
Imipenem	12 / 12	100	0 / 10	0	-
Linezolid	12 / 12	100	0 / 10	0	-
Minocycline	12 / 12	100	0 / 10	0	
Moxifloxacin	11 / 12	92	1 / 10	8	Minor
Tobramycin	12 / 12	100	0 / 10	0	-
TMP-SMX	12 / 12	100	0 / 10	0	-

Table 3. Comparison of standard (visual) MIC interpretive readings of the quality control organisms with interpretive readings using the Vizion System.

QC Strains	Interpretive Match Standard Read to Vizion % ALL DRUGS	Interpretive Discrepant Standard Read to Vizion % ALL DRUGS
Enterococcus faecalis ATCC 29212	100	100
Pseudomonas aeruginosa ATCC 27853	100	100
Escherichia coli ATCC 35218	100	100
Mycobacterium smegmatis ATCC 700686	100	100

CONCLUSION

Although this was a small single center study, the Vizion system demonstrated excellent correlation to visual reads of MICs for the isolates of Nocardia. The Vizion built-in lighting adjustment was advantageous for facilitating the readings of MICs especially for isolates which had less than optimal growth. The ability to store MIC readings on the SWIN computer system for further review was an added convenience and also enabled the supervisor to check any questionable readings and also allowed for the generation of antibiograms. Laboratories must consider the cost of the system and training requirements prior to its use. However, specific training programs can be implemented in a relatively short period of time for most laboratories. Additional larger multicenter studies are recommended to establish the utility of the system in the clinical laboratory.

ACKNOWLEDGMENTS

This study was supported in part by a grant from TREK Diagnostics Systems Inc. We gratefully acknowledge Joan Lamprecht and Jenny Lorbach for their helpful discussions and review of the manuscript. The authors also thank Joanne Woodring for preparation of the manuscript.

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