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Campylobacter spp. Susceptibility Testing: A Multi Site Evaluation of Broth Microdilution Methods Compared to the Agar Dilution Method.

C. C. Knapp[3], S. Bodeis[1], T. R. Fritsche[2], N. Holliday[3], R. N. Jones[2], T. Kelley[3], S. B. Killian[3], P. McDermott[1], P. R. Rhomberg[2], J. M. Streit[2]

1. FDA-CVM, Laurel, MD, 2. JMI Laboratories, Inc., North Liberty IA, 3. TREK Diagnostic Systems, Cleveland, OH

TREK DIAGNOSTIC SYSTEMS

982 Keynote Circle, Suite 6
Cleveland, OH 44131
800.871.8909 • www.trekds.com

ABSTRACT

Background: The standard NCCLS recommended method for determining the antimicrobial susceptibility (S) of *Campylobacter spp.* is agar dilution (AD). Currently, most laboratories are not staffed or equipped to routinely offer this testing. A multi site study was undertaken to compare the performance of reference broth microdilution (BMD) method, the Sensititre Dried Microdilution (DMD) method (TREK Diagnostic Systems, Cleveland, OH) and the NCCLS AD method for *Campylobacter jejuni* and *Campylobacter coli*.
Methods: The BMD method used in this study was the NCCLS M7-A6 method for *S. pneumoniae*; using Mueller Hinton broth with 2.5% lysed horse blood and incubating for 48hr in a microaerophilic atmosphere at 36°C. The AD testing was performed according to NCCLS M31-A 3.2.8 method. Each site tested 10 common (including ATCC 33560 QC strain) and 50 unique wild type isolates consisting of 131 *Campylobacter jejuni* and 29 *Campylobacter coli*. The isolates were tested against the 6 antimicrobials with NCCLS QC ranges: Ciprofloxacin, Doxycycline, Erythromycin, Gentamicin, Nalidixic acid and Tetracycline -.
Results: Percent agreement of the MIC results for BMD and DMD compared to AD were as follows: AD vs. BMD +/- 2 log dilutions: 96%, AD vs. DMD +/- 2 log dilutions: 96% and BMD vs. DMD +/- 1 log dilution: 96%.
Conclusions: These multi site MIC results indicate BMD and DMD methods are comparable to the standard AD method. This provides potential for practical and economical methods that can be used in the clinical laboratory for testing the S of *Campylobacter jejuni* and *Campylobacter coli* isolates.

INTRODUCTION

Campylobacter spp. is one of the most common bacterial causes of gastro-intestinal infections in humans in the United States. In most cases the handling or ingesting of raw and undercooked poultry has been implicated as the source of infection with *Campylobacter jejuni* being responsible for 95% of infections. Generally the disease is self-limiting, however in severe cases treatment with a fluoroquinolone or macrolide may be required. Resistance to commonly used antimicrobial agents has been recognized for some years, and may be the result of increased utilization of fluoroquinolones and macrolides in human and veterinary medicine, and in animal husbandry. Fluoroquinolones (approved by FDA in 1995) are frequently used to treat upper respiratory infections in poultry. This may explain a correlation with the handling and consumption of poultry containing fluoroquinolone resistant *C. jejuni* strains. While resistance to ciprofloxacin was unknown before the early 1990's, it increased to 13-19% in late 1997- 2001 according to some surveys. Resistance to macrolides, also considered as first-line agents in the treatment of *C. jejuni*, is also recognized, but occurs at a lower rate than does fluoroquinolone resistance. To date there has been no resistance detected to chloramphenicol or gentamicin. For epidemiological purposes a standardized MIC test method that is easy to perform and interpret is needed to detect resistance and track longitudinal changes in the antibiograms of *Campylobacter spp.*

PURPOSE OF THE STUDY

Presently the recommended standard method for MIC determination is agar dilution (NCCLS, M31-A2), which is not easily nor routinely performed in most laboratories because of the technical expertise required. This multi-site study was undertaken to develop a broth microdilution method based upon the NCCLS M7-A6 standard, testing both frozen reference and Sensititre® dried plates that both veterinary and clinical laboratories could utilize routinely for susceptibility testing of *Campylobacter spp.* and comparing results to the standard agar dilution method.

MATERIALS & METHODS

Organisms Tested:

- 10 common isolates of *Campylobacter spp.*, including the ATCC 33560 QC strain *Campylobacter jejuni*, were tested between 3 sites.
- 50 unique wild type strains of *Campylobacter spp.* were tested at each of the 3 testing sites.

Isolates Tested

Organism Species	Number Tested
Campylobacter jejuni	131
Campylobacter coli	29

Antimicrobials Tested

Antimicrobials	Range Tested	Supplied By
Ciprofloxacin	0.03-64µg/ml	Bayer
Doxycycline	0.12-64µg/ml	Sigma
Erythromycin	0.12-256µg/ml	Sigma
Gentamicin	0.12-64µg/ml	Sigma
Nalidixic Acid	4-64µg/ml	Sigma
Tetracycline	0.5-32µg/ml	Sigma

QC Ranges for Agar Dilution method for *Campylobacter jejuni* ATCC 33560 (M31-A2)

Antimicrobials	QC Range (µg/ml) at 36°C for 48h
Ciprofloxacin	0.12-1
Doxycycline	0.5-2
Erythromycin	1-8
Gentamicin	0.5-2
Nalidixic Acid	8-32
Tetracycline	1-4

METHODOLOGY

Test Method*	Media	Inoculum	36°C	48h
Agar dilution	Muller-Hinton agar with 5% defibrinated sheep blood	0.5 McFarland Direct colony suspension	+	+
Broth microdilution	Muller-Hinton broth with 2-5% lysed horse blood	0.5 McFarland adjusted to 10^5-10^6cfµ/ml	+	+
Sensititre® Dried microdilution	Muller-Hinton broth with 2-5% lysed horse blood	0.5 McFarland adjusted to 10^5-10^6cfµ/ml	+	+

*** All test methods were performed in a microaerophilic atmosphere (5%O_2,10%CO_2, and 85%N_2) using Pak-Microaero Sachets (Mitsubishi Gas Chemical America.,Inc., New York) and in a microaerophilic incubator, set to the same conditions as noted above.**

RESULTS

Percent (%) Essential Agreement for Wild Type Species

	Ciprofloxacin				Erythromycin				Gentamicin			
Methodology	Site 1	Site 2	Site 3	Total	Site 1	Site 2	Site 3	Total	Site 1	Site 2	Site 3	Total
Agar vs. 48 hrs. Dried +/- 2 Wells	97	83	97	92	98	95	94	96	100	100	98	99
Agar vs. 48 hrs. Frozen +/-2 Wells	97	85	97	93	98	93	60	84	100	100	100	100
48 Dried vs. 48 Frozen +/- 1 Well	98	85	100	94	97	96	87	93	98	98	98	98

Percent (%) Essential Agreement for Wild Type Species

	Nalidixic Acid				Tetracycline				Doxycycline			
Methodology	Site 1	Site 2	Site 3	Total	Site 1	Site 2	Site 3	Total	Site 1	Site 2	Site 3	Total
Agar vs. 48 hrs. Dried +/- 2 Wells	100	94	100	98	98	81	98	92	100	92	98	97
Agar vs. 48 hrs. Frozen +/-2 Wells	100	100	100	100	98	83	98	93	100	98	98	99
48 Dried vs. 48 Frozen +/- 1 Well	100	92	100	97	97	94	100	97	100	98	97	98

Percent (%) Essential Agreement for 10 Common Isolates Including QC

	Ciprofloxacin				Erythromycin				Gentamicin			
Methodology	Site 1	Site 2	Site 3	Total	Site 1	Site 2	Site 3	Total	Site 1	Site 2	Site 3	Total
Agar vs. 48 hrs. Dried +/- 2 Wells	100	100	100	100	100	100	100	100	100	100	90	97
Agar vs. 48 hrs. Frozen +/-2 Wells	100	100	100	100	100	70	70	80	100	100	100	100
48 Dried vs. 48 Frozen +/- 1 Well	100	100	100	100	100	80	100	93	100	90	100	97

Percent (%) Essential Agreement for 10 Common Isolates Including QC

	Nalidixic Acid				Tetracycline				Doxycycline			
Methodology	Site 1	Site 2	Site 3	Total	Site 1	Site 2	Site 3	Total	Site 1	Site 2	Site 3	Total
Agar vs. 48 hrs. Dried +/- 2 Wells	100	100	100	100	100	90	90	93	100	90	90	93
Agar vs. 48 hrs. Frozen +/-2 Wells	100	100	100	100	100	80	90	90	90	80	80	83
48 Dried vs. 48 Frozen +/- 1 Well	100	100	100	100	100	100	100	100	100	100	100	100

RESULTS

Overall Essential Agreement (60 isolates X 3 Sites)

- Agar dilution vs. reference broth microdilution ± two $\log_2$ dilution steps is 96%.
- Agar dilution vs. Sensititre dried broth microdilution ± two $\log_2$ dilution steps is 96%.
- Reference broth microdilution vs. Sensititre dried broth microdilution ± one $\log_2$ dilution step is 96%.

DISCUSSIONS

- Overall percent Essential agreement (± one $\log_2$ dilution step) with all 3 sites for the 10 common isolates by each method was:

1. Agar dilution: 81.7%
2. Reference broth microdilution: 97.2%
3. Sensititre dried microdilution: 99.0%

- Agar dilution showed the most variability in performance, particularly with erythromycin, which is pH sensitive and tends to display elevated MIC values in the presence of a CO_2 enriched environment.

CONCLUSIONS

- This multi-site MIC comparison indicates frozen broth microdilution and Sensititre® Dried broth microdilution methods are comparable to the standard agar dilution method within ± two $\log_2$ dilution steps.
- This provides a routine method for susceptibility testing that is both practical and economical for veterinary and clinical laboratories when testing *Campylobacter spp.*
- The subsequent establishment of QC ranges will further enhance the use of this methodology by providing clinical laboratories with a complete package of testing parameters for the accurate and reproducible susceptibility testing of campylobacters, permitting future studies aimed at establishment of interpretive criteria.

REFERENCES

1. National Committee for Clinical Laboratory Standards. 2003. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard-sixth edition.* Approved document M7-A6. Wayne, PA: NCCLS.
2. National Committee for Clinical Laboratory Standards. 2004. *Performance standards for antimicrobial susceptibility testing, 14th informational supplement* M100-S14. Wayne, PA: NCCLS.
3. National Committee for Clinical Laboratory Standards.2002. *Perfomance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; approved standard-second edition.* Approved document M31-A2. Wayne, PA: NCCLS.
4. Gupta A, Nelson JM, Barrett TJ, Tauxe RV, Rossiter SP, Friedman CR, Joyce KW, Smith KE, Jones TF, Hawkins MA, Shiferaw B, Beebe JL, Vugia DJ, Rabatsky-Ehr T, Benson JA, Root TP, Angulo FJ; NARMS Working Group. Antimicrobial resistance among *Campylobacter* strains, United States, 1997-2001. *Emerging Infectious Diseases* 2004 10:1102-9.
5. Nelson JM, Smith KE, Vugia DJ, Rabatsky-Her T, Segler SD, Kassenborg HD, Zansky SM, Joyce K, Marano N, Hoekstra RM, and Angulo FJ. Prolonged Diarrhea Due to Ciprofloxacin-Resistant *Campylobacter* Infection. *Journal of Infectious Dieases* 2004; 190: 1150-7.
6. McDermott PF, Bodeis SM, Aarestrup FM, Brown S, Traczewski M, Fedorka-Cray P, Wallace M, Critchley IA, Thornsberry C, Graff S, Flamm R, Beyer J, Shortridge D, Piddock LJ, Ricci V, Johnson MM, Jones RN, Reller B, Mirrett S, Aldrobi J, Rennie R, Brosnikoff C, Turnbull L, Stein G, Schooley S, Hanson RA, Walker RD. Development of a standardized susceptibility test for *Campylobacter* with quality-control ranges for ciprofloxacin, doxycycline, erythromycin, gentamicin, and meropenem. *Microbial Drug Resistance* 2004 10:124-31.
7. McDermott PF, Walker RD.Standardizing antimicrobial susceptibility testing of *Campylobacter* species. *Journal of Clinical Microbiology* 2003 41:1810.
8. National Antimicrobial Resistance Monitoring System (NARMS). Narms 2003 Annual Report, Center for Disease Control and Prevention [Available at the following web page: http:www.arru.saa.ars.usda.gov/campy.htm]
9. Gilligan PH. Cases in Medical Microbiology and Infectious Diseases. Third ED.Washington: ASM Press; 2003. p.149.
10. Fedorka-Cray PJ, Plumblee JR, Adams ES, Dillard LH, Ransom GM, Headrick ML. Changes in Antimicrobial Resistance Profile of *Campylobacter* Isolates-1994/1995 and 2000. [Available at the following web page: http:www.arru.saa.ars.usda.gov/campy.htm]