# Development of a Rapid Antimicrobial Susceptibility Testing Method for an Extensively Resistant Strain of *Campylobacter* Related to a Puppy Outbreak and Treatment with Imipenem

**Bodeis Jones, Sonya M, FDA/CVM; Ayers, Sherry L, FDA/CVM; Young, Shenia, FDA/CVM; Dheeraj, Goyal, MD/Mercy Fairfield Hospital/Ohio; Francois Watkins, Louise K, CDC; Montgomery, Martha P, CDC; Caidi, Hayat, CDC; Friedman, Cindy R, CDC; Zhao, Shaohua, FDA/CVM; McDermott, Patrick F, FDA/CVM**

## Abstract

**Background:** A multistate outbreak of campylobacteriosis linked to pet store puppies (2017-2019) was identified as being caused by a strain of *Campylobacter jejuni*. The strain was resistant to multiple antimicrobial classes, including macrolides and fluoroquinolones, both of which are routinely used for the treatment of *Campylobacter* infections in humans and animals. The Centers for Disease Control and Prevention (CDC) and the Ohio Department of Health identified patients who developed a severe illness due to a resistant strain of *Campylobacter jejuni* that was acquired through the handling of puppies purchased from pet stores.
**Purpose:** The isolated *Campylobacter* organisms were sent to the Center for Veterinary Medicine/Office of Research, Division of Animal and Food Microbiology, to determine susceptibility patterns to effective antimicrobial agents not traditionally used in the testing of *Campylobacter* species. Due to the treatment needs of patients infected in this outbreak, time was of the essence in finding a suitable antimicrobial. Agar dilution, the gold standard of antimicrobial susceptibility testing, required time-consuming testing and the availability of antimicrobial powders.
**Methodology:** Antimicrobial testing methods were designed based on supplies available to both clinical laboratories and the National Antimicrobial Resistance Monitoring System (NARMS). *Campylobacter* broth microdilution methods approved for use by the Clinical Laboratories Standard Institute (CLSI, M45) were applied to clinical and surveillance antimicrobial susceptibility testing (AST) panels (Fisher Scientific, Trek Diagnostics, Cleveland Ohio) in order to measure susceptibility to 14 classes of antibiotics. For method validation, quality control organisms and *Campylobacter* isolates with known susceptibility patterns were included.
**Results:** The *Campylobacter* outbreak strain was susceptible to imipenem/cilastatin, a carbapenem drug seldom used to treat *Campylobacter* infections in humans. Although clinical interpretive criteria for *Campylobacter* are only available for fluoroquinolones, macrolides, and tetracyclines, we demonstrated that outbreak isolates had low minimum inhibitory concentrations (MIC) to carbapenems.
**Conclusions:** This work highlights an excellent collaboration between government agencies, state agencies and hospital laboratories to effectively treat and improve human and animal health. It also highlights the need for laboratories to be able to identify approved methods that can be used in alternative systems to help aid in defining effective treatment options for clinicians and patients.

## Introduction

*Campylobacter* is a leading cause of diarrheal illness in the United States, causing an estimate of 1.3 million illnesses annually with approximately 187,000 illnesses due to animal contact. Human illness due to contact with domestic puppies has caused approximately 5% of these *Campylobacter* infections. In August of 2017, epidemiologists in Florida had identified an outbreak linked to puppies from a national pet store chain based in Ohio. The investigation revealed more than 100 patients from 18 U.S. states linked to the outbreak. The initial AST performed at CDC revealed the outbreak isolates were resistant to both macrolides and fluoroquinolones, which are normally used to treat *Campylobacter* infections in humans. Outbreak isolates were also resistant to macrolides, quinolones, lincosamides, ketolides, aminoglycosides, and tetracycline. A subset of outbreak isolates were sent to FDA/NARMS to determine antimicrobials that these isolates would be susceptible to in order to successfully treat human patients infected with this outbreak strain of *Campylobacter*. Two outbreak isolates from clinical patients were also tested using this extended AST method.

## Materials and Methods

**Outbreak Isolates:** Ohio and other state health department laboratories submitted a subset of outbreak-associated isolates to CDC/NARMS for AST. NARMS laboratories use a standard panel of nine antibiotics, including azithromycin, chloramphenicol, ciprofloxacin, erythromycin, florfenicol, gentamicin, nalidixic acid, telithromycin, and tetracycline (CAMPY Sensititre™ ThermoFisher Scientific, Cleveland, OH) for AST.

**Extended AST:** Fourteen outbreak isolates were submitted to FDA/NARMS for additional AST and standard *Campylobacter* broth microdilution methods were applied to six dehydrated Sensititre™ AST panels in accordance with current CLSI guidelines. Three of the panels (CMV4AGNF, CMV3AGPF, CMV2DW) are used routinely for NARMS testing. The other clinical AST panels (GN4F, STP6F, ANO2B) are available from Trek Diagnostics (Sensititre™ ThermoFisher Scientific).

**Broth Microdilution Method:** For each *Campylobacter* isolate, a 0.5 McFarland ($10^8$ CFU/mL) bacterial suspension was prepared in 5 mL Sensititre™ Cation-Adjusted Mueller-Hinton broth (MHB). Suspensions were diluted to $10^5$ CFU/mL by transferring 100 µL of the above suspension into 11 mL MHB mixed with lysed horse blood. AST panels were inoculated at a volume of 100 µL, covered with a perforated seal, and incubated in a humid microaerophilic environment for 24 hours at 42°C.

**Method Validation:** Quality control (QC) strains were included for each test simultaneously under recommended standard conditions for each QC strain (*Campylobacter jejuni* ATCC 33560, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* ATCC 700603, and *Streptococcus pneumoniae* ATCC 49619). MICs were determined using the unaided eye. Five NARMS surveillance *C. jejuni* strains known to be susceptible to the antibiotics on the standard CAMPY panel were also included for validation. If multiple MICs resulted when the same antibiotic class was tested on more than one panel, we reported the more specific dilution range (e.g., ≤0.12 µg/mL rather than ≤0.5 µg/mL) or the higher MIC value. No result for the same antibiotic differed by more than a single dilution.

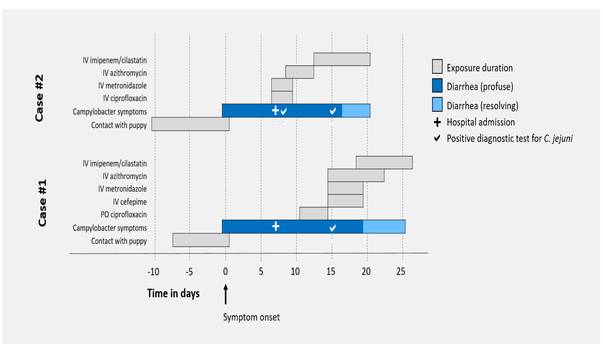


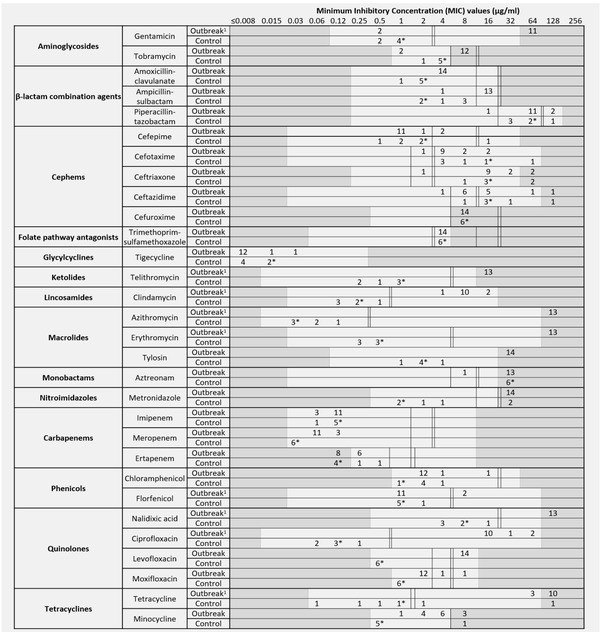

**Figure 1.** Clinical course of two patients showing the duration of diarrhea and key exposures in days.

## Results and Discussion

Due to the treatment needs of human patients infected with this outbreak strain, time was of essence in finding a suitable treatment agent. Agar dilution, the gold standard of AST, would have required time-consuming testing and the availability of antimicrobial powders. Therefore, we designed testing methods based on supplies available to both clinical and NARMS laboratories, applied CLSI guidelines, and used QC organisms, including known susceptible *Campylobacter* isolates, for validation. Outbreak isolates had MICs indicating resistance to most antibiotic classes; exceptions were phenicols, glycylcyclines, and carbapenems. (Table 1)

Two patients in Ohio with multi-drug resistant (MDR) *Campylobacter jejuni* infections linked to the multistate outbreak were also identified. Additional susceptibility testing was performed using the rapid extended method described here. Both patients developed severe illness requiring antibiotic therapy, and both had good response to treatment with imipenem/cilastatin. (Figure 1) All three isolates (from two patients and one puppy) underwent AST with 30 different antibiotics from 14 antibiotic classes and had susceptibility patterns that were similar to each other and consistent with outbreak strains (Table 2).

**Table 1.** Squashtogram showing minimum inhibitory concentration (MIC) values for 14 antibiotic classes and two populations of *C. jejuni* isolates: outbreak isolates (n=14)[1] and control isolates (n=6); the asterisk (*) indicates the MIC value of the commercially-available strain ATCC 33560.




[1]One isolate was not tested for susceptibility to azithromycin, ciprofloxacin, clindamycin, erythromycin, florfenicol, gentamicin, nalidixic acid, telithromycin, and tetracycline.

**Table 2.** Minimum inhibitory concentrations and interpretations for *C. jejuni* isolates from case-patient #1, case-patient #2, and the puppy belonging to case-patient #1.

| Antibiotic class | Antibiotic | Patient #1 MIC in µg/ml | Patient #2 MIC in µg/ml | Puppy MIC in µg/ml | Intermediate range (µg/ml) | Resistant range (µg/ml) |
|---|---|---|---|---|---|---|
| Aminoglycosides | Gentamicin[1] | >32 | >32 | >32 | N/A | ≥4 |
| | Tobramycin[2,3] | >4 | >4 | >4 | 8 | ≥16 |
| β-lactam combination agents | Amoxicillin-clavulanic acid[2] | 4/2 | 4/2 | 4/2 | 16/8 | ≥32/16 |
| | Ampicillin-sulbactam[2,3] | >8/4 | >8/4 | >8/4 | 16/8 | ≥32/16 |
| | Piperacillin-tazobactam[2] | 64/2 | 64/2 | 64/2 | 32/4–64/4 | ≥128/4 |
| Carbapenems | Ertapenem[2] | 0.25 | ≤0.12 | ≤0.12 | 1 | ≥2 |
| | Imipenem[2] | 0.12 | 0.12 | 0.12 | 2 | ≥4 |
| | Meropenem[2] | 0.06 | 0.06 | 0.06 | 2 | ≥4 |
| Cephems | Cefuroxime[2,3,4] | >4 | >4 | >4 | 8–16 | ≥32 |
| | Cefotaxime[2] | 4 | 4 | 4 | 2 | ≥4 |
| | Ceftazidime[2] | 16 | 8 | 8 | 8 | ≥16 |
| | Ceftriaxone[2] | 16 | 16 | 16 | 2 | ≥4 |
| | Cefepime[2] | 1 | 1 | 1 | 4–8 | ≥16 |
| Folate pathway antagonists | Trimethoprim-sulfamethoxazole | >4/76 | >4/76 | >4/76 | N/A | ≥4/76 |
| Glycylcyclines | Tigecycline | ≤0.008 | ≤0.008 | ≤0.008 | N/A | N/A |
| Ketolides | Telithromycin[1] | >8 | >8 | >8 | N/A | ≥8 |
| Lincosamides | Clindamycin[1] | 4 | 8 | 8 | N/A | ≥1 |
| Macrolides | Azithromycin[1] | >64 | >64 | >64 | N/A | ≥0.5 |
| | Erythromycin[1] | >64 | >64 | >64 | N/A | ≥8 |
| | Tylosin | >16 | >16 | >16 | N/A | N/A |
| Monobactams | Aztreonam[2] | >16 | >16 | >16 | 8 | ≥16 |
| Nitroimidazoles | Metronidazole[5] | >16 | >16 | >16 | 16 | ≥32 |
| Phenicols | Chloramphenicol[1] | ≤2 | ≤2 | ≤2 | N/A | ≥32 |
| | Florfenicol[1] | 1 | 1 | 1 | N/A | ≥8 |
| Quinolones | Ciprofloxacin[1] | 16 | 16 | 16 | N/A | ≥1 |
| | Levofloxacin[2] | >4 | >4 | >4 | 1 | ≥2 |
| | Moxifloxacin[5] | 2 | 2 | 2 | 4 | ≥8 |
| | Nalidixic acid[1] | >64 | >64 | >64 | N/A | ≥32 |
| Tetracyclines | Minocycline[2] | 2 | 2 | 4 | 8 | ≥16 |
| | Tetracycline[1] | >64 | >64 | >64 | N/A | ≥2 |

## Conclusions

Based on the extended AST, this extensively resistant (XDR) *C. jejuni* strain showed apparent in-vitro resistance to antimicrobials from 11 of 14 classes tested. With further collaboration between clinicians, state and federal agencies, imipenem was identified as the best course of treatment for this infection, resulting in saving human life. It has been suggested that MDR strains could be treated with glycylcyclines (tigecycline) or gentamicin in conjunction with carbapenem antibiotics; however, this XDR strain appeared resistant to gentamicin. Although AST indicated potential in-vitro susceptibility of the outbreak strain to cefepime, MICs of other third and fourth generation cephalosporins were relatively high. Also, Patient #1 did not improve with cefepime; therefore, cefepime was not used to treat Patient #2.

This work highlights an excellent collaboration between government agencies, state agencies and hospital laboratories to effectively identify XDR *Campylobacter* infections and improve human and animal health. It also highlights the need for laboratories to be able to identify approved methods that can be used in alternative systems to help aid in defining effective treatment options for clinicians and patients.