

Antibiotic Resistance Trends in *Campylobacter jejuni* isolates in Wisconsin, 2004-2005

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REVISED ABSTRACT

Background: *Campylobacter jejuni* is a significant cause of gastroenteritis worldwide. Numerous reports show an increase of *Campylobacter* resistance to fluoroquinolones in recent years. *C. jejuni* isolates submitted to the Wisconsin State Laboratory of Hygiene in the years 2004 and 2005 were tested for resistance against ciprofloxacin, erythromycin, and tetracycline. **Methods:** *C. jejuni* isolates were submitted to the Wisconsin State Laboratory of Hygiene through the Wisconsin Enteric Pathogen Surveillance (WEPS) program. Isolates represent 57 of 72 counties (79%) for 2004 and 52 of 72 counties (72%) in 2005. Both genders were represented equally. A 1 McFarland standard was created with 24-48 hour cultures and was seeded to Muller Hinton agar containing 5% sheep blood. E-test strips were applied and plates were incubated for 48 hours in a microaerophilic atmosphere at 42°C. Interpretive criteria were determined using guidelines set out by the National Antimicrobial Resistance Monitoring System (NARMS). Our definition of resistance was an MIC value of ≥4 mcg/ml for ciprofloxacin, ≥8 mcg/ml for erythromycin, and ≥16 mcg/ml for tetracycline. **Results:** Our data show similar resistance levels to ciprofloxacin (10.84% to 11.02%) and erythromycin (3.27% to 4.24%) in 2004 and 2005. Tetracycline resistance remains high but decreased slightly (55.83% to 53.67%) between 2004 and 2005. Ciprofloxacin resistance is less frequent in patients under the age of 14. We also describe a case where an isolate gained fluoroquinolone resistance after treatment with Levoquin®. **Conclusion:** Wisconsin has a significant level of resistance to ciprofloxacin in *C. jejuni* isolates. The results of PFGE analysis suggest that the *C. jejuni* from one patient acquired ciprofloxacin resistance after treatment with Levoquin®. These results implore laboratories to continue monitoring resistance trends in *C. jejuni*.

BACKGROUND

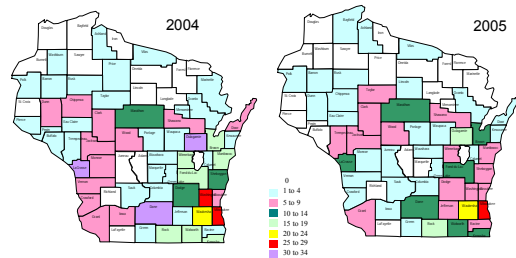
Campylobacter is the most common bacterial cause of diarrheal illness in the U. S. with more than 10,000 cases reported to CDC annually. It is estimated that 2.5 million people are effected each year. It is isolated from stool 2 to 7 times more frequently than *Salmonella* or *Shigella* species.

Campylobacteriosis is a zoonotic disease and domestic animals such as cattle, pigs, and poultry act as reservoirs for the organism. The most common sources of infection are consumption of unpasteurized milk, poultry or beef products, and contaminated water.

Campylobacter jejuni infections are characterized by diarrhea (sometimes bloody), abdominal cramps, fever, and headache. The typical incubation period for *C. jejuni* is 2 to 5 days. Most infections are resolved without the need for antibiotic treatment. Antibiotics are given when the patient has high fever, bloody diarrhea, illness lasting >1 week, pregnancy, or is immunocompromised (1).

Isolates were submitted through the Wisconsin Enteric Pathogen Surveillance (WEPS) program. WEPS purpose is to monitor trends in enteric pathogens and monitor for antimicrobial resistance through out Wisconsin.

Figure 1: Number of *Campylobacter jejuni* Isolates submitted to WSLH; by County



METHODS

Submission of Specimens

Campylobacter jejuni isolates were submitted to the Wisconsin State Laboratory of Hygiene through the Wisconsin Enteric Pathogen Surveillance (WEPS) program. Isolates represent 57 of 72 counties (79%) for 2004 and 52 of 72 counties (72%) in 2005.

METHODS

Susceptibility Testing Protocol

- 1 McFarland standard created with 24-48 hour cultures
- Seeded to Muller Hinton agar containing 5% sheep blood
- E-test strips applied
- Incubated for 48 hours in a microaerophilic atmosphere at 42°C
- Interpretive criteria were determined using guidelines set out by the National Antimicrobial Resistance Monitoring System (NARMS).
- Resistance was defined as MIC value of ≥4 mcg/ml for Ciprofloxacin, ≥8 mcg/ml for Erythromycin, and ≥16 mcg/ml for Tetracycline.

PulseNet *Campylobacter* PFGE Protocol

- Cell suspension buffer (phosphate-buffered saline, pH 7.4)
 - Turbidity meter (0.35 to 0.45)
 - Proteinase K concentration = 0.1 mg/ml
 - Plug agarose 1% SeaKem Gold (No SDS)
- Lysis buffer: 50mM Tris; 50mM EDTA; pH8.0; 0.1mg/ml Proteinase K; 1% sarcosine
- Lysis at 54°C with constant agitation for 15 min
- Washes: 4 times (1X water and 3X TE washes at 50°C with constant agitation)
- Restriction digestion: Sma1 for 2-4 hours
- Electrophoresis conditions:
 - Sma1 (IST =6.75, FST =35.38, 18hr)

(We would like to acknowledge Collette Fitzgerald and Efrain Ribot of the CDC for their valuable assistance with the PFGE method.)

RESULTS

Figure 2: Comparison in Rates of Resistance to Antimicrobials; 2004 & 2005

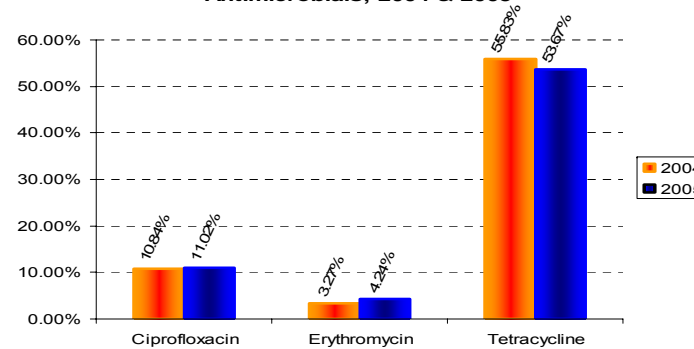


Figure 3: % Resistant to Ciprofloxacin in Age Group

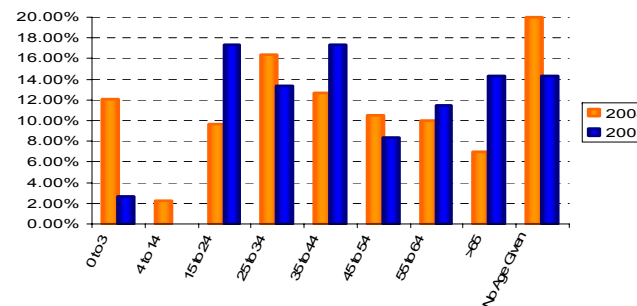


Figure 4: PFGE Patterns of Isolates From Two Patients



- Specimen on **Patient 1** collected 9/9/2004 (dated 9/14/2004) was pre Levoquin® treatment. MIC for Ciprofloxacin was 0.064 mcg/ml (Susceptible)
- Specimen on **Patient 1** collected 9/20/2004 (dated 9/24/2004) was post Levoquin® treatment. MIC for Ciprofloxacin was >32 mcg/ml (Resistant)
- The two isolates from **Patient 1** were indistinguishable by PFGE
- Specimen on **Patient 2** collected 8/30/2004 (dated 9/3/2004) had an MIC for Tetracycline of >256 mcg/ml (Resistant)
- Specimen on **Patient 2** collected 9/21/2004 (dated 9/24/2004) had an MIC for Tetracycline of 0.094 mcg/ml (Susceptible)
- PFGE patterns of the two isolates from **Patient 2** indicated they were different strains.

DISCUSSION AND CONCLUSIONS

Over the two year period of 2004-2005, there has been no significant change in the rate of resistance of *C. jejuni* isolates in Wisconsin to ciprofloxacin, erythromycin, and tetracycline.

Tetracycline resistance remains high at 53-56%. This compares to 38.3% in data from the CDC National Antimicrobial Resistance Monitoring System (NARMS) in 2003 (2).

Erythromycin resistance was stable at 3-4%. However, this is higher than the 0.3% rate seen in the 2003 NARMS isolates.

There is a low but significant rate of resistance to ciprofloxacin of about 11%. This is also reflected in data collected by NARMS. In 2003, NARMS reported 17.2% of *C. jejuni* isolates resistant to ciprofloxacin (2).

The results of PFGE analysis of isolates from patient 1 suggest that *C. jejuni* acquired resistance to ciprofloxacin during treatment with Levoquin®.

Patient 2 either was infected with multiple strains of *C. jejuni* or was infected with a different strain after the initial infection.

Because infection with ciprofloxacin-resistant *C. jejuni* has been shown to produce significantly longer diarrhea, it is important to continue to monitor resistance trends and make efforts to retain fluoroquinolone susceptibility (3).

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