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Review

Antimicrobial resistance in *Campylobacter*: Susceptibility testing methods and resistance trends

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ABSTRACT

Most *Campylobacter* infections are self-limiting but antimicrobial treatment (e.g., macrolides, fluoroquinolones) is necessary in severe or prolonged cases. Susceptibility testing continues to play a critical role in guiding therapy and epidemiological monitoring of resistance. The methods of choice for *Campylobacter* recommended by the Clinical and Laboratory Standards Institute (CLSI) are agar dilution and broth microdilution, while a disk diffusion method was recently standardized by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Macrolides, quinolones, and tetracyclines are among the common antimicrobials recommended for testing. Molecular determination of *Campylobacter* resistance via DNA sequencing or PCR-based methods has been performed. High levels of resistance to tetracycline and ciprofloxacin are frequently reported by many national surveillance programs, but resistance to erythromycin and gentamicin in *Campylobacter jejuni* remains low. Nonetheless, variations in susceptibility observed over time underscore the need for continued public health monitoring of *Campylobacter* resistance from humans, animals, and food.

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1. Introduction

Campylobacter is among the most common causes of bacterial foodborne illness in the United States and worldwide. According to the U.S. Centers for Disease Control and Prevention (CDC), *Campylobacter* infections accounted for approximately 35% of laboratory-confirmed bacterial and parasitic infections that occurred in FoodNet surveillance areas in 2012 (CDC, 2013). Two thermotolerant species, *Campylobacter jejuni* and *Campylobacter coli*, are responsible for the vast majority of human infections, among which 80–90% are due to *C. jejuni* (CDC, 2013; Nachamkin et al., 2000). Contaminated raw or undercooked poultry has been identified as an important transmission vehicle for human campylobacteriosis (Mughini Gras et al., 2012; Neimann et al., 2003; Stafford et al., 2007).

Most *Campylobacter* infections cause acute, self-limiting diarrheal disease; however, severe, prolonged, or relapsing campylobacteriosis does occur, especially in the very young, the elderly, and people with underlying diseases (Blaser and Engberg, 2008). Extraintestinal infections are rare and include bacteremia, reactive arthritis, hemolytic uremic syndrome, meningitis, and following septicemia, infections of nearly any organ (Blaser and Engberg, 2008; FDA, 2012). Additionally, *C. jejuni* is the most commonly identified antecedent infection in patients with Guillain–Barré syndrome, an acute inflammatory polyneuropathy. Among the subset of patients with Guillain–Barré syndrome whose symptoms are triggered by an infection, 20–50% are attributed to a *C. jejuni* infection (Jacobs et al., 2008).

When antimicrobial therapy is indicated, macrolides (primarily erythromycin, or alternatively one of the newer macrolides, such as clarithromycin or azithromycin) remain the frontline agents for treating culture-confirmed *Campylobacter* cases (Blaser and Engberg, 2008). Fluoroquinolones (e.g., ciprofloxacin) are also commonly used because they are the drugs of choice for empirical treatment of undiagnosed diarrheal illness, such as travelers' diarrhea (Aarestrup et al., 2008; Guerrant et al., 2001). A meta-analysis indicated that early treatment with macrolides or fluoroquinolones shortened the duration of *Campylobacter* intestinal symptoms by 1.32 days (Ternhag et al., 2007). Tetracycline, doxycycline, and chloramphenicol are alternative drugs that can be used for treatment (Skirrow and Blaser, 2000). Serious systemic infections should be treated with an aminoglycoside such as gentamicin or a carbapenem such as imipenem (Okada et al., 2008; Skirrow and Blaser, 2000). Third-generation cephalosporins are used widely as alternatives to fluoroquinolones for empirical treatment of community-acquired bacterial diarrhea, but they have not been proven effective for treating bacteremia due to *Campylobacter* species other than *Campylobacter fetus* (Pacanowski et al., 2008).

Antimicrobial susceptibility testing continues to play a critical role in guiding therapy and epidemiological monitoring of resistance. The emergence of antimicrobial resistance in *Campylobacter*, particularly to fluoroquinolones, has underscored the importance of in vitro antimicrobial susceptibility testing. This article reviews the current knowledge on susceptibility testing methods and resistance trends in *Campylobacter* with a focus on *C. jejuni* and *C. coli*.

2. Susceptibility testing methods

2.1. Overview

In vitro antimicrobial susceptibility testing involves measuring the antimicrobial's activity against the test microorganism by determining the minimum inhibitory concentration (MIC) or inhibition zone diameter. Interpretive criteria based on population MIC (or inhibition zone diameter) distributions, clinical outcome studies, and pharmacological parameters are used to classify the organism as susceptible, intermediate, or resistant. Some surveillance systems use population MIC (or inhibition zone diameter) distribution data alone for classification (see below in Section 2.2).

These phenotypic test methods include a number of diffusion (disk, tablet, and Etest®) and dilution (broth and agar dilution) methods. Variations of both types of methods have been used for *Campylobacter* susceptibility testing (Aarestrup et al., 2008). Fig. 1 is a schematic diagram showing the principles and procedures of four representative methods: disk diffusion, Etest®, agar dilution, and broth microdilution. Additionally, four automated susceptibility testing systems are currently approved by the U.S. Food and Drug Administration, namely, MicroScan WalkAway (Siemens Healthcare Diagnostics), Phoenix (BD Diagnostics), Sensititre ARIS 2× (Trek Diagnostic Systems), and Vitek 2 (bioMérieux) (Jorgensen and Ferraro, 2009). Due to the fastidious growth requirements of *Campylobacter* (e.g., microaerobic conditions, supplemented media, and slow growth), these systems have not yet been applied to routine *Campylobacter* susceptibility testing in clinical laboratories.

It is noteworthy that although *Campylobacter* was first recognized as an important human pathogen in 1972, standardized susceptibility testing methods were not available until 2004 (McDermott et al., 2004, 2005). Different laboratories have employed an array of protocols which vary widely in test medium, inoculum size, incubation condition (atmosphere, temperature, time), and quality control (QC) organism. These variations can impact the growth of *Campylobacter* and behavior of the drugs during testing. Consequently, the antimicrobial activity measured including MICs and inhibition zone diameters can vary greatly depending on the test parameters. There are also large variations in the criteria used to interpret results. In order to obtain accurate and comparable data, standardized methods must be validated and interpretive criteria harmonized for appropriate categorization of *Campylobacter* isolates.

Validation and standardization of susceptibility testing methods are conducted primarily by non-profit organizations developing consensus standards, such as the Clinical and Laboratory Standards Institute (CLSI; www.clsi.org) in the U.S. and the European Committee on Antimicrobial Susceptibility Testing (EUCAST; www.eucast.org) in Europe. Within EUCAST, national antimicrobial susceptibility testing committees from several European countries are represented, including the British Society for Antimicrobial Chemotherapy (BSAC; www.bsac.org.uk) and the Antibiogram Committee of the French Society for Microbiology (CA-SFM; www.sfm-microbiologie.org). Both CLSI and EUCAST develop guidelines for standardized antimicrobial susceptibility testing and publish interpretive criteria for categorizing organisms as susceptible, intermediate, or resistant. Table 1 lists interpretive criteria published by the two organizations as well as those of BSAC and CA-SFM. Table 2 summarizes current CLSI- and EUCAST-approved standardized susceptibility testing methods for *Campylobacter*, which consist of disk diffusion, agar dilution, and broth microdilution.

2.2. Clinical breakpoints and epidemiological cut-off values

There are two types of criteria used to interpret susceptibility testing results, one for clinical purposes (clinical breakpoints) and the other one for monitoring purposes (epidemiological cut-off values, ECOFFs). To develop clinical breakpoints, three sets of data are needed: antimicrobial susceptibility data generated by standardized in vitro susceptibility testing, pharmacokinetic and pharmacodynamic (PK/PD) information, and most importantly, outcome data from well-controlled clinical efficacy trials. ECOFFs, on the other hand, are based solely on the first parameter, antimicrobial susceptibility data of bacterial populations. This approach is focused on distinguishing susceptible isolates (wild-type) from those with reduced susceptibility (non-wild type) without taking into consideration pharmacological targets or clinical efficacy data (Aarestrup et al., 2008).

Since PK/PD analysis and clinical outcome data are lacking for *Campylobacter*, population MIC (or inhibition zone size) distributions and ECOFFs have been used to establish breakpoints by CLSI and EUCAST (Table 1). For example, CLSI resistance breakpoints for erythromycin and ciprofloxacin for disk diffusion (Table 1) are developed