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Cost analysis and antimicrobial susceptibility testing comparing the E test and the agar dilution method in *Campylobacter jejuni* and *Campylobacter coli*☆

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Abstract

Although numerous reports have compared the antimicrobial susceptibility of *Campylobacter* spp., controversy still exists about the use of the E test as an alternative to the agar dilution method suggested by the Clinical and Laboratory Standards Institute. MICs of 8 antimicrobials were determined using the E test and agar dilution methods for 103 *Campylobacter jejuni* and *Campylobacter coli* isolates from fresh chicken randomly purchased from stores in 3 southern Ontario counties. Overall, 72.6% of E test MIC values were within 1 $\log_2$ dilution and 95.7% within 2 $\log_2$ dilutions of the corresponding agar dilution MICs. For individual antimicrobials, agreement within 1 $\log_2$ dilution and 2 $\log_2$ dilutions was as follows: ampicillin (n = 103), 90.3% and 98.1%, respectively; chloramphenicol (n = 104), 85.6% and 99%; ciprofloxacin (n = 99), 51.5% and 97.0%; clindamycin (n = 99), 26.3% and 78.8%; erythromycin (n = 99), 52.5% and 96.0%; gentamicin (n = 99), 100% and 100%; nalidixic acid (n = 98), 91.8% and 99.0%; and tetracycline (n = 86), 82.6% and 97.7%. Relative to agar dilution, the E test underestimated the MIC value by a mean of 0.74 (ampicillin), 0.82 (chloramphenicol), 1.44 (ciprofloxacin), 1.94 (clindamycin), 1.40 (erythromycin), 0.21 (gentamicin), 0.94 (nalidixic acid), and 0.20 (tetracycline) $\log_2$ dilutions and by a median of 1 $\log_2$ dilution for all antimicrobials except clindamycin (2), gentamicin (0), and tetracycline (0). Cost analysis, including materials and labor, showed a 39.0% higher cost per analyte for the agar dilution method as compared with the E test. The most relevant advantage of the E test over the agar dilution method is the turnaround time because testing 99 strains by the agar dilution method takes 3.6 times longer compared with the E test using the same number of strains. The E test is an acceptable alternative for antimicrobial susceptibility testing in *Campylobacter* because it corresponds well with the agar dilution method although being considerably less expensive, is less labor intensive, and is more rapid. However, the relationship between E test and agar dilution MICs must be considered when interpreting E test results.

Keywords: Cost analysis; *Campylobacter*; Antimicrobial susceptibility testing; E test; Agar dilution

1. Introduction

Antimicrobial resistance is a major emerging threat to public health (Cohen, 2000). Infections due to antibiotic-resistant bacteria cause considerable mortality as well as significant health care costs and productivity losses (Maragakis et al., 2008). The worldwide increase in bacterial resistance to antibiotics comes from a variety of environments, such as hospitals, communities, and agricultural and aquaculture settings. In animal production, the widespread use of antimicrobials for both therapeutic and subtherapeutic use at the farm and in aquaculture facilities creates selective pressure that fosters development of resistance to human

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pathogens (Hamer and Gill, 2002; Threlfall et al., 2000). Regulatory agencies and public health officials need to detect emerging resistance issues by implementing surveillance programs to track and monitor antimicrobial resistance trends. The resistance status is determined using the MIC and interpretive criteria using standardized antimicrobial susceptibility tests. The MIC is the lowest concentration of the antibiotic needed to inhibit the growth of the microorganism. The appropriate antimicrobial susceptibility test and cost are important factors to consider in the design of these surveillance programs because of the long-term application and the need to compare results with those generated by similar international programs. Although a number of tests for measuring antimicrobial susceptibility exist, they all follow 1 of 2 fundamental principles (diffusion or dilution of the antimicrobial agent), and these are available in a variety of formats (Walker, 2006). Tests generating quantitative data are the only ones currently used in countries with active surveillance programs (Canadian Integrated Program for Antimicrobial Resistance Surveillance [CIPARS], 2005; Danish Integrated Antimicrobial Resistance Monitoring and Research Programme, 2006; National Antimicrobial Resistance Monitoring System [NARMS], 2005). For *Campylobacter* antimicrobial susceptibility testing, the agar dilution and microbroth dilution are considered the gold standard (Tenover et al., 1992); yet, the agar dilution method was not standardized until recently (McDermott et al., 2004), and the microbroth dilution, although a very attractive alternative (Halbert et al., 2005; Luber et al., 2003), needs to be standardized by the Clinical and Laboratory Standards Institute (CLSI). Other methodologies for determination of MICs in *Campylobacter* are also available, namely, the E test (Ge et al., 2002; Luber et al., 2003). This quantitative test, like the agar dilution method, uses a combination of dilution and agar diffusion principles in 1 single inert, nonporous thin plastic strip. The strip contains a predefined continuous concentration gradient of antimicrobial agent dried and stabilized in 15 2-fold dilutions on 1 side of the strip. The other side of the strip is labeled with a scale for reading the corresponding MIC values. The MIC reading is where the inhibition of microbial growth intersects the E test strip.

Although numerous reports exist comparing the agar dilution method and the E test (Ge et al., 2002; Luber et al., 2003; Oncul et al., 2003), no cost comparisons have been conducted with these 2 methods. In this communication, we report cost analysis and correlation of MICs obtained in 103 *Campylobacter* strains using the agar dilution method and the E test.

2. Materials and methods

2.1. Microorganisms and antimicrobial agents

One-hundred three *Campylobacter* isolates were obtained by inoculation of 25 g of chicken skin in 100 mL of Rosef's enrichment broth using temperature ramping procedure. After 2 days of incubation, 200 μL was placed on a paper filter disc that was on a hydrophobic grid-membrane filter sitting on semisolid media containing ferrous sulfate, sodium metabisulfite, and sodium pyruvate and no antibiotics as described previously (Valdivieso-Garcia et al., 2007). *Campylobacter* isolates were identified by dark-field microscopy for motility and morphology, ELISA reactivity to specific monoclonal antibodies to thermophilic *Campylobacter* (Valdivieso-Garcia et al., 1998), hydrolysis of sodium hippurate, and polymerase chain reaction using specific primers for *Campylobacter jejuni* and *Campylobacter coli* as described by Linton et al. (1997). The isolates were stored at −70 °C in glycerol and fetal bovine serum (1:1) until subcultured on a blood agar plate before antimicrobial susceptibility testing. Reference strains used as controls for the antimicrobial susceptibility testing were *Staphylococcus aureus* ATCC 29213, *Escherichia coli* 25922, and *C. jejuni* ATCC 33560. Ampicillin and chloramphenicol were purchased from Sigma-Aldrich (St. Louis, MO), and ciprofloxacin, clindamycin, erythromycin, gentamicin, nalidixic acid, and tetracycline were obtained from United States Pharmacopeia (USP, Rockville, MD).

2.2. Antimicrobial susceptibility testing

Campylobacter strains were subcultured onto Mueller–Hinton blood agar plates for 24 h at 42 °C under microaerobic conditions. Colonies were suspended in 2 mL of Mueller–Hinton broth to obtain turbidity equivalent to a 0.5-McFarland standard using a Dade Behring reader (West Sacramento, CA). This procedure was followed for both the agar dilution method and the E test.

2.2.1. Agar dilution method

Agar dilution MICs were determined according to the CLSI guidelines. Briefly, Mueller–Hinton agar plates supplemented with 5% sheep blood and with antimicrobial agents at concentrations ranging from 0.02 to 256 μg/mL (ampicillin, chloramphenicol, clindamycin, erythromycin, gentamicin, nalidixic acid, and tetracycline) and from 0.002 to 32 μg/mL (ciprofloxacin) in doubling dilutions were used. Plates were inoculated with a Cathra replicator with 1-mm-diameter inoculating pins and incubated at 37 °C for 48 h under microaerobic conditions.

2.2.2. E test

The E test was performed using E test strips containing ampicillin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, nalidixic acid, and tetracycline according to the manufacturer's instructions in plates with Mueller–Hinton agar supplemented with 5% lysed horse blood. Plates were inoculated and incubated under the same conditions described for the agar dilution method, and MICs were determined.