

# EUCAST standardised disk diffusion methodology for *Campylobacter jejuni* and *C. coli*

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## Introduction

Antimicrobial susceptibility testing of *Campylobacter* spp. in clinical laboratories is often performed using gradient MIC tests or disk diffusion, but standardised methodology and interpretive criteria are often lacking. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) is in the process of establishing clinical MIC breakpoints for *C. jejuni* and *C. coli*.

## Objective

The objective of this study was to investigate if the standard EUCAST MH-F medium and disk diffusion criteria can be used for reproducible antimicrobial susceptibility testing of *C. jejuni* and *C. coli*.

## Methods

Preliminary testing was performed on 15 *C. jejuni* and 15 *C. coli* from the Netherlands with known MIC values (broth microdilution) including isolates with and without resistance mechanisms. Additional testing was performed on 17 *C. jejuni* and 12 *C. coli* from Finland. All isolates were tested in triplicate for ciprofloxacin 5 µg, erythromycin 15 µg and tetracycline 30 µg. Investigated variables are shown in **Table 1**.

**Table 1**  
Investigated variables

Medium	Unsupplemented Mueller-Hinton agar (MH) and Mueller-Hinton agar with 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F)
Incubation	35, 37, 39 and 41°C 24 and 44 h
Reading of zones	Smallest and largest appearing zone when tilting the plate

All tests were performed with a 0.5 McFarland inoculum and microaerobic incubation in plastic jars.

## Results

The proposed EUCAST disk diffusion methodology for *C. jejuni* and *C. coli* is presented in **Table 2**. Both *C. jejuni* and *C. coli* grew best on MH-F plates at 41°C. All *C. jejuni* grew well after 24 h of incubation, whereas some *C. coli* required 44 h before inhibition zones could be read. Swarming was avoided by drying plates before inoculation.

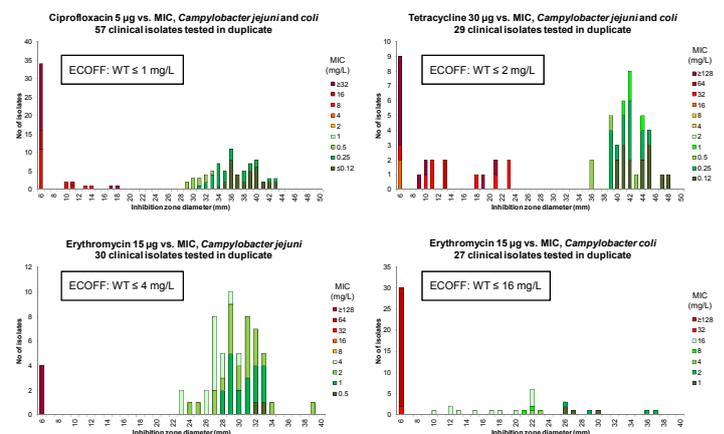
All isolates with MICs above the epidemiological cut off (ECOFF) were identified as non-wild type also with the disk diffusion test (**Figure 1**), irrespective of whether the smallest or largest zone was used. The results were reproducible within and between the sites. The standard deviation for triplicate tests ranged from 0.4–1.7 mm, with the largest variation for inhibition zones read after 44 h of incubation. Examples of combined inhibition zone distributions from several sites are shown in **Figure 2**.

## Conclusions

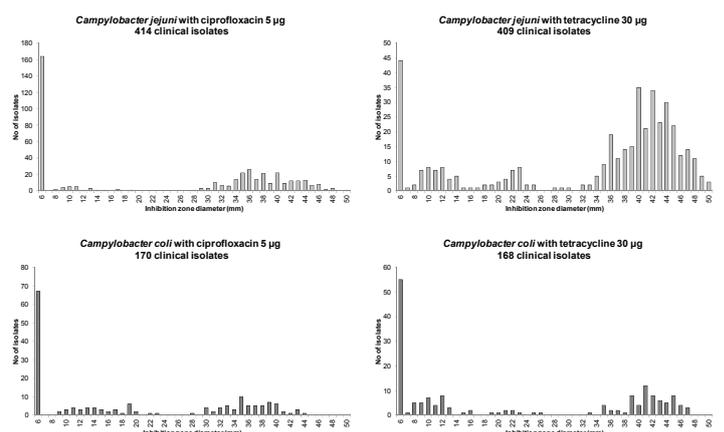
Reproducible disk diffusion testing of *C. jejuni* and *C. coli* can be performed using the EUCAST MH-F media. Swarming was effectively avoided by pre-drying of plates. Disk diffusion correlated well to MIC determination and reliably distinguished between wild-type and non-wild type isolates. Further evaluation of the methodology is currently performed at several laboratories in Europe.

**Table 2**  
EUCAST proposed disk diffusion methodology for *Campylobacter jejuni* and *Campylobacter coli*.

Medium	Mueller-Hinton agar with 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F) (Plates should be dried before incubation to avoid swarming, either at room temperature (20–22°C) overnight or at 35°C for 1–2 hours.)
Inoculum	McFarland 0.5
Incubation	Microaerobic environment 41°C 24 hours ± 30 minutes ( <i>C. coli</i> with non-sufficient growth after 24 h incubation are re-incubated immediately and read after totally 40–48 h incubation.)
Reading of zones	<b>EUCAST standard reading instructions:</b> Read MH-F plates from the front with the lid removed illuminated with reflected light. Zone edges should be read at the point of complete inhibition as judged by the unaided eye.



**Figure 1.** Inhibition zone distributions for *C. jejuni* and *C. coli* with MIC values as coloured bars. Inhibition zones were read according to EUCAST standard reading instructions at one site on MH from two manufacturers. Data for erythromycin are presented separately, due to different ECOFFs.



**Figure 2.** Combined inhibition zone distributions for ciprofloxacin and tetracycline from 4–6 sites for *C. jejuni* and *C. coli*, respectively.