



## Appearance of ciprofloxacin/chlortetracycline-resistant bacteria in waters of Québec City in Canada

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### ABSTRACT

Most of the waterborne fecal pathogens belong to the family of Gram-negative bacteria. Hence, minimal inhibitory concentrations of chlortetracycline and ciprofloxacin antibiotics towards Gram-negative representative, *Enterobacter aerogenes* were estimated, which were 7 µg/ml and 0.125 µg/ml, respectively. The combined antimicrobial effect of chlortetracycline and ciprofloxacin against *E. aerogenes* was also investigated to establish their potential interaction towards the pathogens present in water. Eventually, the water samples obtained from various drinking water treatment plants from Québec municipality were tested for the occurrence of chlortetracycline-, ciprofloxacin- and chlortetracycline/ciprofloxacin-resistant strains.

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### Introduction

Waterborne fecal pathogens usually belong to Enterobacteriaceae family that cause gastrointestinal illnesses. Between 1974–2001, *Shigella* and *Salmonella* caused around 56% drinking water outbreaks in Canada [1]. At the same time, the *Campylobacter* waterborne outbreak was ranged as a second causative agent (24 outbreaks), following *Giardia* (51 outbreaks) [1]. These bacterial infections can be treated with antibiotics. However, their frequent usage has resulted in their high concentration in various environmental compartments, which have led to selective pressure and the occurrence of antibiotic-resistant bacteria [2].

The goal of this study was to investigate the combined antimicrobial activity of two widely used antibiotics, chlortetracycline (CTC) and ciprofloxacin (CIP), towards Gram-negative representative bacteria – *Enterobacter aerogenes*. The objective of this work was to investigate the appearance of CTC-resistant, CIP-resistant and CIP-CTC-resistant strains in the water compartments from different areas of the Québec City. The presence of three pathogenic

bacteria that were potential resistant-gained species, *Campylobacter* sp., *Shigella* sp. and *Salmonella* sp. was investigated.

### Methodology

#### Minimal inhibitory concentration

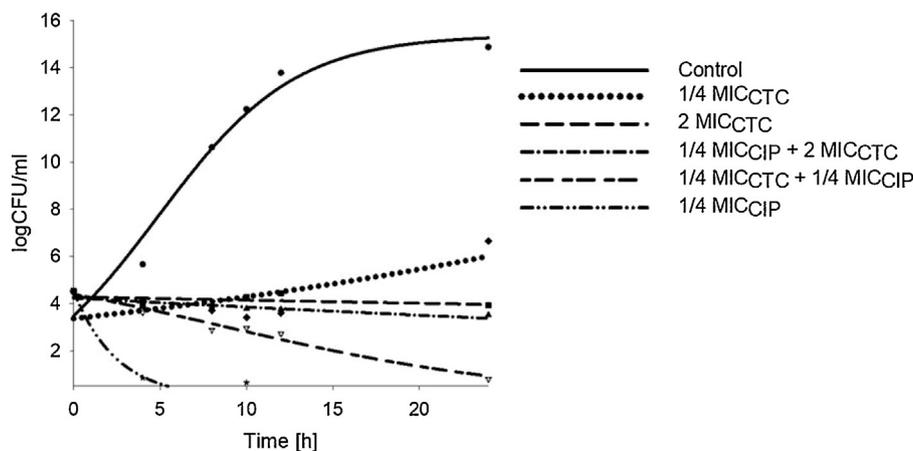
Minimal inhibitory concentration (MIC) was defined as the minimal concentration of antimicrobial agent, which exerts the visible bacterial growth inhibition. *E. aerogenes* (NRRL B-407) growth and MIC estimation were conducted as described previously [3].

#### Time-kill method

Time-kill method was used to determine the killing rate of bacteria by antimicrobial agents. The procedure was carried out by broth microdilution technique, as described in the National Committee for Clinical Laboratory Standards guidelines [4]. *E. aerogenes* was examined against CTC, CIP alone and in their combinations (1/4MIC and 2MIC). The pairwise t-test ( $p > 0.05$ ) was performed via SigmaPlot 12.3 to determine significant differences in the effectiveness of different antibiotic mixtures based on triplicate measurements.

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**Fig. 1.** Time-kill curves of ciprofloxacin (CIP) and chlortetracycline (CTC) at different concentration combinations against Gram-negative *Enterobacter aerogenes*. The curves representing  $2\text{MIC}_{\text{CIP}} + 2\text{MIC}_{\text{CTC}}$ ,  $2\text{MIC}_{\text{CIP}}$ ,  $2\text{MIC}_{\text{CIP}} + 1/4\text{MIC}_{\text{CTC}}$  are not shown due to high killing-rate of the solutions (all bacteria killed within 4 first hours); graphs represent the trend for a mean of the triplicate experiment.; MIC – minimal inhibitory concentration.

### Water samples and bacteria isolation

The sampling took place in November 2017. Water samples were obtained from upstream of the Drinking Water Treatment Plant (UTE) of Québec (UTE Québec), UTE Ste-Foy, UTE Charlesbourg and Desîlets. The treated water samples (UTE's downstream) were obtained from UTE Ste-Foy, UTE Québec, and UTE Charlesbourg. All samples were collected into the ethanol-washed containers after rinsing them twice with appropriate sample water. After transporting the samples to the laboratory on the same day, were kept in dark at  $4 \pm 1^\circ\text{C}$ .

Water samples were inoculated (1% v/v) in 3% w/v Tryptic Soy Broth (TSB) in triplicates to enrich present bacterial culture (48–72 h,  $42 \pm 1^\circ\text{C}$ ). Subsequently, they were plated onto solid media, with (CIP, CTC, CIP + CTC) and without antibiotics. Obtained colonies were harvested, DNA was isolated and polymerase chain reaction (PCR) was performed to confirm if the bacteria belong to *Shigella* sp., *Salmonella* sp., or *Campylobacter jejuni*. The detailed procedure has been described in Supplementary material.

### Results and discussion

To understand the possible repercussions of CTC/CIP presence in water, the combined antimicrobial activity tests were conducted. Obtained MIC values for Gram-negative representative, *E. aerogenes* were 0.125 and  $7 \mu\text{g}/\text{mL}$  for CIP and CTC, respectively. Fig. 1 shows the antimicrobial activity of both antibiotics alone and in combination.  $1/4\text{MIC}_{\text{CIP}}$  was able to eliminate all the cells in less than 6 h. *E. aerogenes* growth at  $1/4\text{MIC}_{\text{CTC}}$  was significantly slower than the control. The addition of CIP resulted in enhanced antimicrobial efficiency of CTC compared to  $1/4\text{MIC}_{\text{CTC}}$ , with hindering the activity of CIP ( $1/4\text{MIC}_{\text{CIP}}$ ). The increase of CTC concentration ( $1/4\text{MIC}_{\text{CIP}} + 2\text{MIC}_{\text{CTC}}$ ) resulted in similar killing-rate as  $2\text{MIC}_{\text{CTC}}$ . Hence, CIP and CTC interact in an antagonistic way.

Generally, there is a suppressive antibiotic interaction between inhibitors of DNA (CIP) and protein (CTC) synthesis, which applies in our case [5]. The bacterial cells can grow, even under the DNA stress, due to the regulation of ribosomal genes. Other studies confirm this interaction [5,6]. However, the antagonism between norfloxacin (fluoroquinolone) and tetracycline changed into synergy towards green alga at their higher concentration [7]. That would suggest that the antagonistic effect depends on the concentration and the targeted organism.

### Qualitative detection of bacteria in water samples

Fig. 2 A presents the detection of bacteria with possible resistant genes in water samples from Québec City municipality. The bacterial growth was observed in all seven sampling sites. The isolates from upstream samples of UTE Charlesbourg and Ste-Foy grew when CTC was present, suggesting the presence of potential CTC-resistant bacteria. The raw water from UTE Ste-Foy also contained bacteria that grew in the presence of CIP and CTC + CIP. This suggests the presence of CIP-resistant or even multi-resistant strains. The inference of bacteria not affected by both antibiotics is also possible. In Charlesbourg samples, PCR confirmed band corresponding to *IpaB* gene, specific for *Salmonella* sp. (Fig. 2B). The other targeted pathogens were not detected.

According to the latest Canadian Antimicrobial Resistance Surveillance System (2017), the usage of antibiotics in Canada remained relatively stable over the last 10 years [8]. However, CIP was ranked as the most commonly purchased antibiotic for human medicine in Canada between 2010–2016. The usage of veterinary antibiotics, like CTC, follows decreasing trend in Canada due to concern of antimicrobial resistance development [8]. However, pathogens may attain the antibiotic resistance from other bacteria, thus the presence of bacteria insensitive to CIP/CTC was a concern [9]. The occurrence of CIP-resistant *Salmonella* strains in Canada has significantly increased from 0% (2003) to 14% (2014) [8]. *Campylobacter* sp. isolates were resistant to tetracycline (44–78%) and CIP (11%) in 2014 [8]. Moreover, CTC concentration ( $7\text{--}62 \mu\text{g}/\text{L}$ ) is much higher than CIP ( $<0.7 \mu\text{g}/\text{L}$ ) in Canadians waters [10,11]. Thus, one can suspect that in the aquatic environment, the possible scenario would be the antagonistic interaction of  $2\text{MIC}_{\text{CTC}} + 1/4\text{MIC}_{\text{CIP}}$ . This may lead to selective pressure from antimicrobials leading to bacterial mutation and antibiotic resistance. The occurrence of tetracycline-resistant genes is linked with a high concentration of tetracyclines in the environment, while fluoroquinolone resistance genes exhibited negative correlation [2]. Hence, this would explain the relatively high frequency of CTC-resistant bacteria presented in this study. And again another study showed that antagonism between CTC and CIP may suppress the evolution of resistance [12].

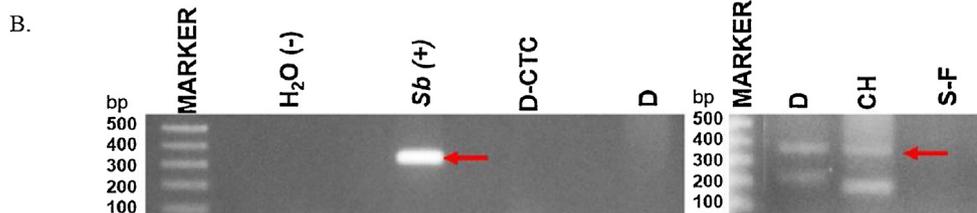
### Conclusion

Even though the pathogens contamination was found only in one water source, the presence of potentially resistant bacteria in the Quebec area is alarming. The time-kill studies showed the antagonistic relationship between CIP and CTC. Based on previous

A.

Media	RAW WATER				TREATED WATER		
	UTE Québec	UTE Charlesbourg	Desilets	UTE Ste-Foy	UTE Québec	UTE Charlesbourg	UTE Ste-Foy
TSB/TBA	+	+	+	+	+	+	+
TSA+CTC	-	+	-	+	-	+	-
TSA+CIP	-	-	-	+	-	-	-
TSA+CTC+CIP	-	-	-	+	-	-	-

TSB: Tryptic soya broth; TSA: Tryptic soya agar; CTC: chlortetracycline; CIP: ciprofloxacin; UTE: drinking water treatment plant



**Fig. 2.** (A) The presence of bacteria from water samples; (B) The 1% agarose gel electrophoresis of PCR products for detection of *Salmonella* sp.; Abbreviation of DNA samples: H<sub>2</sub>O (-) – negative control; Sb (+) – positive control (*Salmonella bongori*); D-CTC – colonies with potential chlortetracycline-resistance from raw water from Desilets; D – colonies from raw water from Desilets; CH – colonies from raw water from Charlesbourg Drinking Water Treatment Plant; S-F – colonies from raw water from Sainte-Foy Drinking Water Treatment Plant.

studies, such interaction may hinder the evolution of antibiotic-resistant strains. In fact, during the monitoring of drinking water, sanitary quality and improvement of the water plant are important and as well, the efficiency of antimicrobials removal should be the priority.

#### Ethical approval

Not required.

#### Conflict of interests

The authors declare no conflict of interests.

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#### Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jiph.2019.04.012>.

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