Supplementary Information

Identification and Antimicrobial Susceptibility Testing of *Campylobacter* Using A Microfluidic Lab-on-a-chip Device

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FIGURES

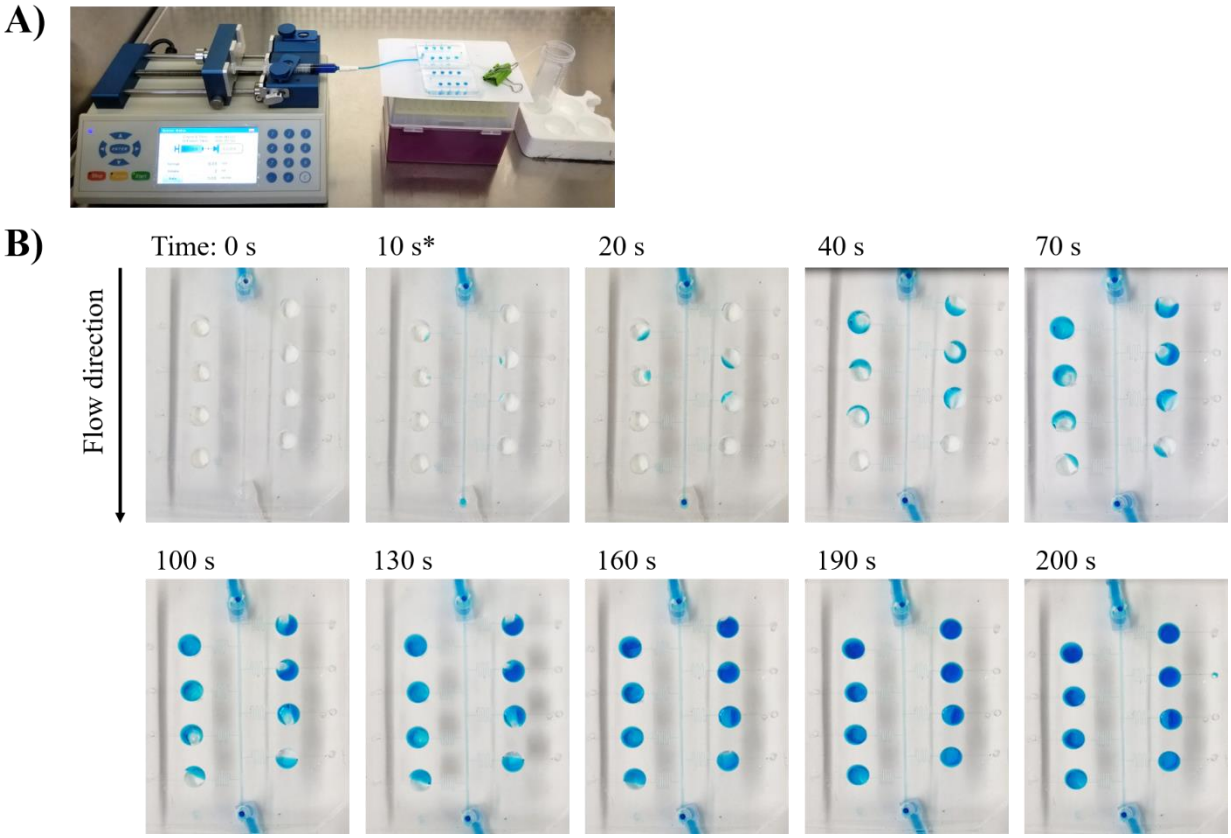

Figure S1. Demonstration of sample injection into the microfluidic device. (A) Image of sample injection setup that consists of a syringe pump, syringe, capillary PVC tubing, waste container and outlet blocker (*i.e.*, metal clip). (B) Real-time monitoring of sample injection for up to 200 s. Blue food dye was used to demonstrate the distribution of sample fluids in the microfluidic device. The flow rate was set at 0.05 mL/min. Asterisk (*) indicates the timepoint when the outlet port was blocked by using a metal clip.

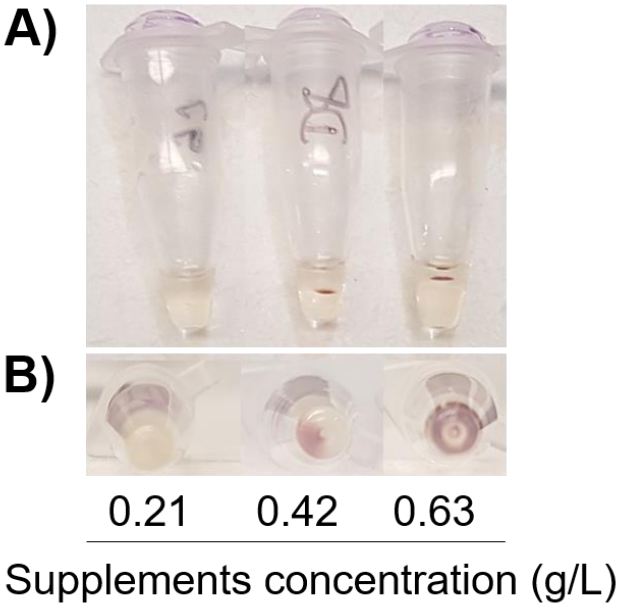

Figure S2. Optimization of the concentration of supplements. *C. jejuni* (10^6 CFU/mL) was incubated with the presence of different concentrations of supplements in *Campylobacter* chromogenic agar. After 48-h incubation, photos were collected from both front (A) and bottom views (B) of the incubation tubes.

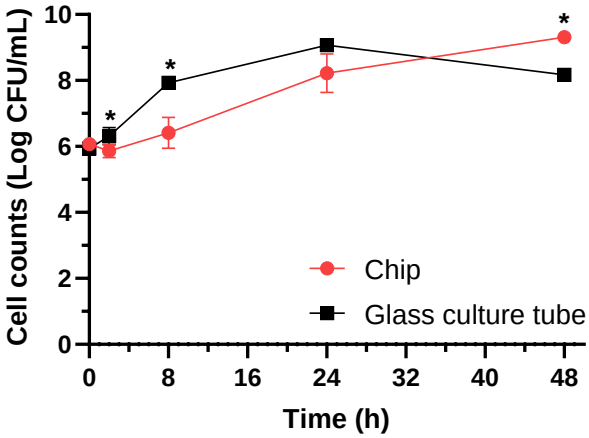

Figure S3. Growth curve of *C. jejuni* F38011 in the microfluidic device and conventional glass culture tube (n=4). Student's t-test was conducted to determine the significant differences between two cultivation platforms (* indicates $P < 0.05$).

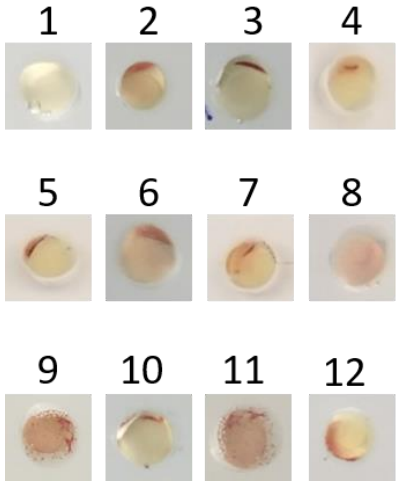

Figure S4. Growth of various *Campylobacter* isolates in the microfluidic chips. *Campylobacte*r isolates (initial concentration: ~10^8 CFU/mL) were incubated at 42°C for 48 h under microaerobic condition: 1. negative control (*i.e.*, Muller-Hinton broth only); 2. *C. jejuni* F38011; 3. *C. jejuni* ATCC 33560; 4. *C. jejuni* NCTC 11168; 5. *C. jejuni* 1143; 6. *C. jejuni* 1173; 7. *C. jejuni* 1329; 8. *C. coli* 171; 9. *C. coli* 314; 10. *C. coli* 1148; 11. *C. coli* 1330; 12. *C. lari* RM2818. Images were obtained by using iPhone 6.

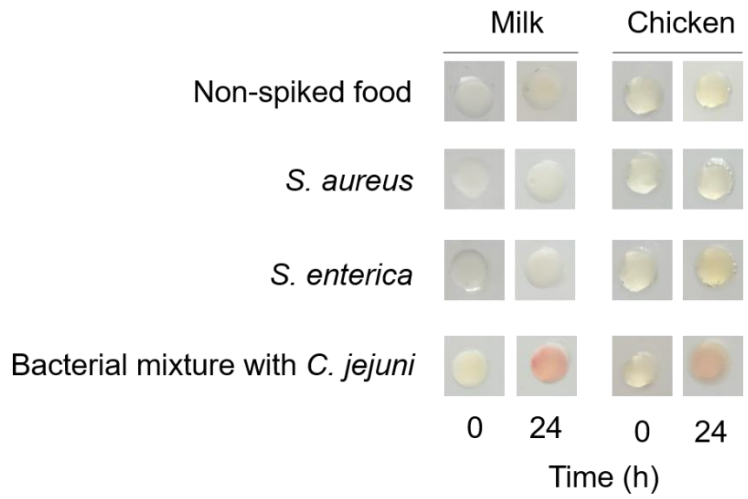

Figure S5. Specificity of on-chip identification for *Campylobacter* in pasteurized milk and raw chicken breast meat. Milk and chicken were purchased from local grocery stores in Vancouver and used without any further treatment. Food samples were spiked with *Staphylococcus aureus* MRSA-10 (1×10^8 CFU/mL), *Salmonella enterica* Enteritidis 43353 (1×10^8 CFU/mL), or the cocktail of *S. aureus* MRSA-10, *S.* Enteritidis 43353 and *C. jejuni* F38011 (1×10^8 CFU/mL for each). The spiked milk was injected into the microfluidic chip directly, while chicken samples (25 g) were rinsed with 25 mL of PBS before sample injection. Non-spiking samples were used as negative control. The microfluidic chips were incubated at 42°C for 24 h under microaerobic condition. Photos were obtained by using iPhone6.

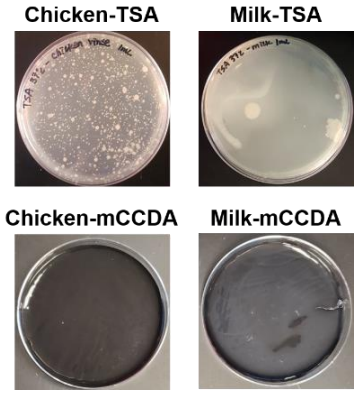

Figure S6. Natural microflora on raw chicken breast meat and pasteurized milk. Milk was tested without any pretreatment, while 25 g of chicken meat was rinsed with 25 mL of PBS. To check the presence of natural microflora, 1-mL aliquot of milk or chicken rinse water was streaked onto tryptic soy agar (TSA) plates, followed by incubation at 37°C for 24 h. To assess the presence of *Campylobacter*, 1-mL of food sample aliquots were streaked onto modified charcoal-cefoperazone-deoxycholate agar (mCCDA) plates and incubated at 42°C under microaerobic condition for 48 h.

TABLES

Table S1. Equations for categorical agreement in antimicrobial susceptibility testing (AST).

Terminology	Equation [a]	Equation terms
Categorical agreement (CA)	$N_{CA}/NT \times 100$	N_{CA} – number of isolates with a same categorical interpretation between new AST and reference method NT – number of isolates tested
Very major error (VME)	$N_{VME}/N_{RefR} \times 100$	N_{VME} – number of isolates that tested false-susceptible N_{RefR} – number of isolates resistant by the reference method
Major error (ME)	$N_{ME}/N_{RefS} \times 100$	N_{ME} – number of isolates that tested false-resistant N_{RefS} – number of isolates susceptible by the reference method
Minor error (mE)	$N_{mE}/NT \times 100$	N_{mE} - number of isolates having minor errors Nt - number of isolates tested

[a] Equations were retrieved from reference (1).

Table S2. Minimal inhibitory concentrations (MICs) of *C. jejuni* F38011 determined by the microfluidic device and conventional agar dilution method.

Antibiotic agents	MICs (mg/L)		Essential agreement	MIC breakpoints (mg/L) [a]	
	On chip	Agar dilution		S	R
Ampicillin	2	2	+ [b]	≤8	≥32
Tetracycline	0.25	0.5	+	≤4	≥16
Ciprofloxacin	0.125	0.125	+	≤1	≥4

[a] MIC breakpoints were obtained from Clinical Laboratory Standard Institute (2, 3). As no ampicillin breakpoint is available for *Campylobacter*, we use breakpoints for *Enterobacteriaceae* as alternatives (3). S: susceptible; R: resistant.

[b] Plus symbol (+) indicates that essential agreement is achieved between two antimicrobial susceptibility testing (AST) methods, in which MICs determined by two methods are within 2-fold dilution range.

REFERECES

1. Humphries RM, Ambler J, Mitchell SL, Castanheira M, Dingle T, Hindler JA, Koeth L, Sei K. 2018. CLSI methods development and standardization working group best practices for evaluation of antimicrobial susceptibility tests. J Clin Microbiol 56:e01934-17.
2. Clinical and Laboratory Standards Institute (CLSI). 2016. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria, 3rd edition. CLSI M45. Wayne, PA, USA.
3. Clinical and Laboratory Standards Institute (CLSI). 2013. Performance standards for antimicrobial susceptibility testing; twenty-third informational supplement. CLSI M100-S23. Wayne, PA, USA.