Interactions of organic acids with *Campylobacter coli* from swine

Ross C. Beier¹ *, Roger B. Harvey¹, Charles A. Hernandez¹, Michael E. Hume¹, Kathleen Andrews¹, Robert E. Droleskey¹, Maureen K. Davidson², Sonia Bodeis-Jones², Shenia Young², Sara E. Duke¹, Robin C. Anderson¹, Tawni L. Crippen¹, Toni L. Poole¹, David J. Nisbet¹

¹ United States Department of Agriculture, Agricultural Research Service, Southern Plains Agricultural Research Center, Food and Feed Safety Research Unit, College Station, Texas, United States of America, ² United States Food and Drug Administration, Office of Research, Center for Veterinary Medicine, Laurel, Maryland, United States of America

* ross.beier@ars.usda.gov

**Abstract**

*Campylobacter coli* is a bacterial species that is a major cause of diarrheal disease worldwide, and *Campylobacter* spp. are among the top 5 foodborne pathogens in the United States. During food production organic acids (OAs) are often used to remove bacteria from animal carcasses. The interactions of six OAs with 111 *C. coli* strains obtained from swine and retail pork chops were studied by determining the molar minimum inhibitory concentrations (MICₘₐₓ) of the *C. coli* strains, and the pH at the MICₘₐₓ. The Henderson-Hasselbalch equation was used to calculate the concentrations of the undissociated and dissociated OAs at the MICₘₐₓ of the *C. coli* strains. The results for the 111 different *C. coli* strains obtained from different locations were treated as a single group for each OA since many of the *C. coli* strains behaved similarly to each different OA. Inhibition of *C. coli* was not dependent on pH or on the undissociated OA species, but *C. coli* inhibition correlated with the dissociated OA species. Therefore, if the concentration of the dissociated OAs decreases from optimum, one may then expect that *C. coli* bacteria would escape disinfection. The concentration of the dissociated OA should be carefully controlled in a carcass wash. We suggest maintaining a concentration of the dissociated acetic, butyric, citric, formic, lactic and propionic acids at 29, 23, 11, 35, 22 and 25 mM, respectively, when using a carcass wash with these OAs to remove *C. coli* bacteria. However, due to *C. coli* utilization of acetate, formate, lactate and propionate, these four OAs may not be the best choice to use for a carcass wash to remove *C. coli* contamination. Of the six OAs, citric acid was the most efficient at inhibiting *C. coli*.

**Introduction**

*Campylobacter* spp. are Gram-negative, non-spore forming bacterial rods [1,2] that are a major cause of diarrheal disease in the United States [3] and throughout the world [1,4–8]. The Centers for Disease Control and Prevention (CDC) has estimated that each year there are...
9.4 million domestically acquired foodborne illnesses, 55,961 hospitalizations, and 1,351 deaths due to 31 major pathogens in the United States [3,9]. Campylobacter spp. are among the top 5 foodborne pathogens in the United States, and they are estimated to be responsible for 845,024 illnesses, 8,463 hospitalizations, and 76 deaths each year [3,9]. Campylobacter jejuni and C. coli are the two main species most often associated with human foodborne illness in this genus [10–13], and they have a high % of DNA homology [14] and possess identical or highly related antigens [15]. In 2016, the CDC reported that Campylobacter and Salmonella caused the most reported bacterial foodborne illnesses in the United States [16]. In England during 2002 C. jejuni accounted for 93% of the reported cases and C. coli accounted for 7% of the reported cases [4]. Campylobacteriosis was the most often reported zoonosis in the European Union (E.U.) in 2015 [7]. The type and number of organisms in the E.U. illnesses during 2015 caused by Campylobacter spp. were primarily divided between C. jejuni and C. coli at 81.0% and 8.4%, respectively [7], although in France C. coli had a higher percentage of cases at 15.25% [13]. Therefore, C. jejuni, which is commonly found in poultry and poultry products, causes the most campylobacteriosis, and low levels of C. jejuni are also found in swine [17]. However, in some areas of the world the percentage of campylobacteriosis caused by C. coli may be as high as 35–40% [18]. Campylobacter coli is the predominant Campylobacter species found in the intestines of pigs and on pork products [19,20]. The impact of C. coli on infectious intestinal disease in humans has largely been ignored, even though C. coli is the second most common cause of human campylobacteriosis [21]. Most likely C. coli have been neglected as a human pathogen because of the predominance of C. jejuni campylobacteriosis [21]. Trace back investigations of C. coli foodborne outbreaks in Belgium (1995) [22], in Poland (2006) [23] and in Alaska (2013) [24] have all resulted in not determining the source of contamination. Epidemiologic and microbiologic data compiled by the Great Britain Public Health Laboratory Service (PHLS) Communicable Disease Surveillance Centre determined that risk factors for transmission of C. coli to humans are different compared to those for C. jejuni [4]. Therefore, this data shows a need to carry out species-specific studies, and develop separate strategies for control of these different organisms [21].

Comprehensive strategies to control foodborne pathogens throughout the food chain from the farm to the table are important [25]. A critical step in processing animals into food products is to wash the animal carcasses with organic acids (OAs) to remove surface bacteria. The OAs often used are acetic [26–28], citric [26], formic [27], lactic [26–31] and propionic acids [27,28]. Bacteria that are not removed from the carcass during the acid wash may later be found on the processed meat. Therefore, the efficacy of the acid wash step should be carefully evaluated.

It is believed that bacterial inhibition by OAs is dependent on pH or the undissociated acid species [32–35]; however, the specific mechanisms by which pH and OAs inhibit bacteria are not understood [36]. In our previous studies, molar values have been used for minimum inhibitory concentrations (MICs) when comparing pH, undissociated or dissociated acid forms because it allows an equivalent comparison of MIC results for acids with different molecular weights [37]. Previous studies evaluated Escherichia coli O157:H7 [37], Pseudomonas aeruginosa [38], non-O157 Shiga toxin-producing E. coli (non-O157 STECs) [39] and Salmonella enterica serovars [40] against OAs and clearly show that pH and levels of undissociated acids do not correlate with the MICs. However, levels of dissociated acids do closely correlate with the MICs. Also, a fully dissociable acid has been shown to cause the disintegration of the bacterial LPS layer [41]. During our previous studies it was observed that a decrease in the concentration of the dissociated acids may result in a large number of bacteria escaping disinfection [37–40].
In this present study, we describe the interactions of six different OAs with 111 C. coli strains, which were obtained in earlier studies that evaluated the pathogens in market age pigs [42], and food animals and retail meat [43]. Susceptibility studies of 111 C. coli strains to the OAs, acetic, butyric, citric, formic, lactic and propionic acids were conducted here. Comparisons are shown of the pH, undissociated acid species and dissociated acid species at the MICs of the C. coli strains.

Materials and methods

Ethics statement
No animals were utilized in this study. All C. coli strains were obtained from frozen stocks in glycerol as prepared by researchers in previous studies.

Campylobacter coli and media
Previously, C. coli was isolated from cecal contents (n = 7), rectal swabs (n = 51) and feces (n = 5) of market age pigs [42], and C. coli also was previously isolated from cecal contents (n = 16) of market age pigs, from cecal contents of sows (n = 20) and from retail pork chops (n = 12) [43]. The above 111 C. coli strains were grown in our laboratory for 48 hours at 42°C on trypticase soy agar w/5% sheep blood BBL Stacker Plates (Becton, Dickinson and Company, Sparks, MD, USA) in a microaerobic atmosphere of 10% CO2, 5% O2, and 85% N2. For cryopreservation, the 111 C. coli strains were transferred from the BBL Stacker Plates and placed in FBP medium [44]. Briefly, FBP medium was made with Nutrient Broth (234000, Difco, Franklin Lakes, NJ, USA), Bacto™ Agar (214010, BD, Franklin Lakes, NJ, USA) at a final concentration of 0.12% (w/v), glycerol (49769, Fluka, Sigma-Aldrich, St. Louis, MO, USA) at a final concentration of 15% (v/v), and Bacto™ Yeast Extract (212750, BC, Franklin Lakes, NJ, USA) at a final concentration of 0.1% (w/v). The prepared FBP mixture was then autoclaved for 15 min at 121°C and 15 PSI and allowed to cool to 50°C in a water bath. Per label directions, Campylobacter Growth Supplement (SR0232E, Oxoid, Basingstoke, United Kingdom) was added to the cooled mixture. The prepared medium (1 ml) was added to each sterile cryogenic vial (5000–0020, Thermo Fisher Scientific, Houston, TX, USA). Campylobacter cells were added to the FBP medium at a turbidity of McFarland 3 to 4. The cells were then placed in a –80°C freezer for long term storage.

Organic acid susceptibility testing
The OA MICs against the C. coli strains were determined by broth microdilution testing of fastidious bacteria according to the Clinical and Laboratory Standards Institute (CLSI) [45], and the methods presented by TREK Diagnostic Systems for susceptibility using Campylobacter sensitiitre plates [46]. Briefly, The C. coli strains were grown for 48 hours at 42°C, as described earlier. All Campylobacter susceptibility studies required incubation for 48 hours at 42°C either on trypticase soy agar w/5% sheep blood or in 96-well plates (U-bottom microplate, Greiner bio-one North America Inc., Monroe, North Carolina, USA) for broth microdilution testing because there were some strains that did not grow a sufficient amount in 24 hours to run the test. Several C. coli colonies were selected from the trypticase soy agar plates and diluted in 5 ml of Sensitiitre™ cation adjusted Mueller-Hinton broth w/TES (Remel Lenexa, KS, USA) to a 0.5 McFarland standard in a Nephelometer (TREK Diagnostic Systems Ltd., East Grinsted, UK). Since our experiments have a final total liquid volume of 100 μl in each well, to maintain a consistent bacterial concentration as suggested by the TREK Diagnostic Systems sensitiitre susceptibility test for Campylobacter, 200 μl of the 0.5
McFarland suspension was placed in tubes containing 11 ml of Sensititre™ cation adjusted Mueller-Hinton broth w/TES w/Lysed horse blood to provide 1 × 10⁶ CFU/ml. Following the proper dilution of OAs to 50 μl in each well of the 96-well plates [40], 50 μl of the lysed horse blood diluted bacteria was layered in all 96-wells of the microplate. Briefly, the OA dilutions consisted of 50 μl of each OA solution placed in wells 1 and 2, and the well 2 solution was diluted 1:2 across a 96-well U-bottom Greiner bio-one microplate through column 11, and column 12 was used as the positive control [40]. The bacteria filled microplates were covered with a perforated plastic adhesive cover sheet (YG522EA, Remel, Lenexa, KS, USA) and placed in a BD GasPak™ EZ standard or small incubation container (BD #260671 or BD #260002, respectively, Becton, Dickinson and Company, Sparks, MD, USA). BD GasPak™ EZ Campy Container System Sachets (BD #260680, Becton, Dickinson and Company, Sparks, MD, USA) were placed inside the incubation containers and the sealed containers were allowed to incubate for 48 hours at 42˚C. MICs were determined as the lowest concentration of a compound that showed no visible growth of the organism [47] on a SensiTouch imaging system (TREK Diagnostic Systems Ltd., East Grinsted, UK). Campylobacter jejuni ATCC 33560 was used as a control organism for the OA susceptibility testing in the microaerobic atmosphere. These results were compared with results obtained from testing Escherichia coli ATCC 25922 in aerobic conditions, as ATCC 25922 was previously used as the control organism during aerobic OA testing [37–40].

Acetic acid was obtained from EM Science (Gibbstown, NY, USA). Butyric, citric, formic and propionic acids were obtained from Sigma-Aldrich (Milwaukee, WI, USA). Lactic acid was obtained from Alfa Aesar (Wad Hill, MA, USA). To make working solutions, the OAs were diluted with reverse osmosis water and then filter-sterilized using a 0.2 μm × 25 mm syringe filter (No. 431224, Corning Inc., Corning, NY, USA). The following concentrations of OAs were tested: acetic acid, 32–32,768 μg/ml; butyric acid, 16–16,384 μg/ml; citric acid, 16–16,384 μg/ml; formic acid, 16–16,384 μg/ml; lactic acid, 8–8,192 μg/ml; and propionic acid, 32–32,768 μg/ml.

Determination of solution pH in 96-well plates at the C. coli MICs

Determination of pH was conducted as previously described [40]. Briefly, the pH was determined in three separate samples at each MIC for each OA, and then the means and standard deviations were determined. The solutions from 16-wells (1,600 μl) at the same MIC value for each OA were combined in a sterile 5 ml microtube (Argos Technologies, Inc., Vernon Hills, IL, USA). An Orion 3 STAR benchtop pH meter was used to measure the pH with a ROSS Ultra, glass combination pH electrode (Thermo Fisher Scientific, Chelmsford, MA, USA). Each pH determination at each MIC was conducted in triplicate.

Calculation of the ratio of undissociated to dissociated acids

The Henderson-Hasselbalch equation can be used to calculate the concentration of conjugate base and undissociated weak acid [48]:

$$\text{pH} = \text{pK}_a + \log \left( \frac{[A^-]}{[HA]} \right)$$

(1)

Where the pKₐ is –log₁₀ of the acid dissociation constant (Kₐ), [A⁻] is the molar concentration of the conjugate base (or dissociated weak acid), and [HA] is the molar concentration of the undissociated weak acid [48]. The Henderson-Hasselbalch equation can be rearranged to
provide the ratio of undissociated to dissociated acid [33]:

\[
\text{ratio} = \frac{[HA]}{[A^-]} = \frac{1}{10^{pH - pK_a}} \tag{2}
\]

Therefore, when the pK_a of a particular acid and the pH of the solution are known, then the ratio of the undissociated to dissociated acid can be calculated. The pK_a for acetic, butyric, citric, formic, lactic and propionic acid is 4.75, 4.82, 3.14, 3.75, 3.86 and 4.87, respectively. If the molar concentration of the acid is known, then the concentrations of the undissociated and dissociated acid species can be calculated from the ratio [37–40].

**Statistics**

A contingency table association analysis was conducted on the data in Table 1 between the MIC_M values and sources. A Fishers Exact test (due to the small sample size) was used to assess for patterns requiring greater OA concentrations for control of *C. coli* strains from different sources.

Table 1. Organic acid MICs and MIC_Ms* for 111 *Campylobacter coli* strains isolated from cecal contents, feces and rectal swabs of market age pigs, cecal contents of sows and from retail pork chops.

| MIC (µg/mL) | MIC_M (mM) | Market Age Pigs | Number of Bacteria from Swine |
|-------------|------------|----------------|-------------------------------|
|             |            | Cecal | Feces | Rectal Swabs | Cecal (sows) | Pork Chops |
| Acetic Acid |            |       |       |              |              |           |
| 4096        | 68.2       | –     | –     | –            | –            | 1         |
| 2048        | 34.1       | 19    | 4     | 40           | 14           | 5         |
| 1024        | 17.05      | 4     | 1     | 11           | 6            | 6         |
| Butyric Acid|            |       |       |              |              |           |
| 2048        | 23.24      | 22    | 5     | 48           | 15           | 10        |
| 1024        | 11.62      | 1     | –     | 3            | 5            | 2         |
| Citric Acid |            |       |       |              |              |           |
| 2048        | 10.66      | 14    | 2     | 27           | 14           | 10        |
| 1024        | 5.33       | 9     | 3     | 24           | 6            | 2         |
| Formic Acid |            |       |       |              |              |           |
| 2048        | 44.5       | –     | 4     | 24           | 3            | –         |
| 1024        | 22.25      | 23    | 1     | 26           | 17           | 12        |
| 512         | 11.12      | –     | –     | 1            | –            | –         |
| Lactic Acid |            |       |       |              |              |           |
| 4096        | 45.47      | 1     | –     | 1            | 3            | 4         |
| 2048        | 22.74      | 4     | 3     | 17           | 8            | 5         |
| 1024        | 11.37      | 18    | 2     | 32           | 9            | 3         |
| 512         | 5.68       | –     | –     | 1            | –            | –         |
| Propionic Acid|          |       |       |              |              |           |
| 2048        | 27.65      | 16    | 5     | 36           | 13           | 8         |
| 1024        | 13.82      | 7     | –     | 13           | 7            | 4         |
| 512         | 6.91       | –     | –     | 1            | –            | –         |
| 256         | 3.45       | –     | –     | 1            | –            | –         |

*aMIC_Ms = Molar MICs.

b'–' = No observed MIC at this acid concentration.

https://doi.org/10.1371/journal.pone.0202100.t001
Results

The MICs and MIC_Ms obtained for C. coli strains against the OAs tested here are shown in Table 1. The C. coli MIC_Ms for acetic, butyric, citric, formic, lactic and propionic acids are similar for each individual acid whether the bacterial strains were obtained from market age pigs, sows or pork chops. Campylobacter coli strains from feces and rectal swabs of market age pigs required differential levels of OAs for control. The highest level of formic acid (44.5 mM) was required for inhibition of 50% of the feces and rectal swab strains. But a citric acid level of only 10.66 mM inhibited these same C. coli strains, which also was a lower acid concentration than the other OAs, acetic, butyric, formic, lactic, and propionic acids, except for lactic and propionic acids which inhibited 1 and 2 strains at levels of 5.68 and 6.91 mM, respectively. The highest level of an OA required for control of C. coli strains was for retail pork chop samples, which required 45.47 mM of lactic acid, and one strain required 68.2 mM acetic acid for inhibition. The lowest OA levels required for control of all strains was for citric acid (10.66 mM).

Interplay of the six organic acids with respect to differential association for inhibition of Campylobacter coli from different isolation sources

Using Fishers Exact test, acetic and butyric acids have a weak differential association with respect to the control of C. coli strains from the different isolation sources, P = 0.107 and P = 0.097, respectively. Citric acid has no differential association with respect to the control of C. coli from the different isolation sources, P = 0.24.

Formic acid has differential control of C. coli strains from different isolation sources, P = 0.0001. Eighty percent of the strains from fecal samples required the highest formic acid concentrations (44.5 mM) for control, and 77.4% of the strains from rectal swab samples from market aged pigs required the highest formic acid concentration (44.5 mM) for control (Table 1).

Lactic acid also has differential control of C. coli strains from different isolation sources, P = 0.012. Thirty-three percent of the C. coli strains from retail pork chops required the highest lactic acid concentration (45.47 mM) for bacterial control (Table 1). Also, 41.7% of the C. coli strains from retail pork chops and 40% of the C. coli strains from cecal sow samples required the 2nd highest concentration of lactic acid (22.74 mM) for bacterial control (Table 1). While 78.3% of the C. coli strains from cecal samples of market age pigs were controlled at 11.37 mM lactic acid (Table 1). Propionic acid showed no differential control of C. coli from different sources, P = 0.91, but required 27.65 mM to inhibit 73.2% of the C. coli strains from fecal and rectal swab samples (Table 1).

Table 2 presents the median, mode, range and 90th percentile of the C. coli MICs and MIC_Ms for each OA.

Measured pH at the MICs of the Campylobacter coli against organic acids

Since the C. coli strains behaved similarly against many of the individual different OAs, the pH determined at the C. coli MIC_Ms for all strains (n = 111) against each individual OA were combined into a single group for each OA. The pH values obtained at the C. coli MIC_Ms for the six OAs are graphically presented in Fig 1. Each data point is the mean and standard deviation of triplicate samples, and next to each data point on the graph is depicted the number of strains at each MICM. The pH at the MIC_M for 100% of the strains against butyric, citric and propionic acids was 6.34, 5.79 and 5.84, respectively, an average pH of 5.99 ± 0.304. But the pH at the MIC_M for 100% of the strains against acetic, formic and lactic acids was 4.60, 4.29 and 3.80,
respectively, an average pH of 4.23 ± 0.403. The pH difference for 100% of the *C. coli* strains against these two groups of acids is on average 1.76 pH units.

Graphical presentations showing the pH at the MICMs of the *C. coli* strains isolated from the individual sources, cecal contents, feces and rectal swabs of market age pigs, cecal contents of sows, and from retail pork chops against the six OAs are shown for each source in S1–S5 Figs, respectively.

Undissociated organic acid concentrations calculated at the *C. coli* MICMs

The results calculated by the Henderson-Hasselbalch calculation for the undissociated OA concentrations of acetic, butyric, citric, formic, lactic and propionic acids at the MICMs of 111 *C. coli* strains are shown in Fig 2. The undissociated acetic, formic and lactic acid concentrations at the MICM for 100% of the *C. coli* strains tested was 39.93, 9.96 and 24.3 mM, respectively. The undissociated butyric, citric and propionic acid concentrations at the MICM for 100% of the *C. coli* strains tested was 0.68, 0.024 and 2.68 mM, respectively. The MICM of all 111 strains occurred at an undissociated citric acid level of 0.024 mM. The MICM of all 111 *C. coli* strains occurred at an undissociated acetic acid concentration of 39.93 mM. A concentration of undissociated butyric and citric acids of 0.68 and 0.024 mM was observed at 100% of the *C. coli* at their MICMs. A difference of Δ = 39.91 mM OA levels between the MICMs of 100% of the strains against acetic and citric acids is shown by the shaded band in Fig 2.

Graphical presentations showing the undissociated acid species at the MICMs of the 111 *C. coli* strains isolated from the individual sources, cecal contents, feces and rectal swabs of market age pigs, cecal contents of sows, and from retail pork chops against the six OAs are shown for each individual source in S6–S10 Figs, respectively.

| Organic Acid | Median (μg/mL) | Mode (μg/mL) | Range | 90th Percentile (μg/mL) |
|--------------|----------------|--------------|-------|-------------------------|
| Acetic Acid  | 2048           | 2048         | 1024–4096 | 2048                     |
| MICM (mM)    | 34.1           | 34.1         | 17.05–68.1 | 34.1                     |
| Butyric Acid | 2048           | 2048         | 1024–2048 | 2048                     |
| MICM (mM)    | 23.24          | 23.24        | 11.62–23.24 | 23.24                    |
| Citric Acid  | 2048           | 2048         | 1024–2048 | 2048                     |
| MICM (mM)    | 10.66          | 10.66        | 5.33–10.66 | 10.66                    |
| Formic Acid  | 1024           | 1024         | 512–2048  | 2048                     |
| MICM (mM)    | 22.25          | 22.25        | 11.12–44.5 | 44.5                     |
| Lactic Acid  | 1024           | 1024         | 512–4096  | 2048                     |
| MICM (mM)    | 11.37          | 11.37        | 5.68–45.47 | 22.74                   |
| Propionic Acid | 2048        | 2048         | 256–2048  | 2048                     |
| MICM (mM)    | 27.65          | 27.65        | 3.45–27.65 | 27.65                    |

*MICMs = Molar MICs.

https://doi.org/10.1371/journal.pone.0202100.1002
Dissociated organic acid concentrations calculated at the C. coli MIC

The calculated concentrations of the dissociated OAs, acetic, butyric, citric, formic, lactic and propionic acids at the MIC of the 111 C. coli strains are shown in Fig 3. The molar dissociated OA concentrations required to produce MIC for 100% of the 111 C. coli strains by all six OAs are shown by the shaded band in Fig 3. The shaded band shows a Δ = 23.9 mM difference between the MIC of 100% of the 111 C. coli strains inhibited by citric acid and 100% of the 111 strains inhibited by the other five OAs. The MIC for 100% of the 111 strains occurs at a dissociated acid level of 10.64 mM citrate. The MIC for 100% of the 111 strains for all dissociated acids occurs at a level of 34.54 mM formate. However, only the results for the dissociated butyric and citric acids may not be affected by C. coli utilization. The concentration difference of these two dissociated acids for inhibition of 100% of the 111 C. coli results in a Δ = 11.92 mM.

Graphical presentations of the dissociated acid species at the MIC of the 111 C. coli strains isolated from the individual sources, cecal contents, feces and rectal swabs of market age pigs, cecal contents of sows, and from retail pork chops against the six OAs is shown for each individual source in S11–S15 Figs, respectively.

Discussion

Organic acids are regularly used to decontaminate meat surfaces. But many bacterial food pathogens have the ability to adapt to varying pH environments, and decontamination...
strategies are often based on pH [49]. We studied six different OAs, acetic, butyric, citric, formic, lactic and propionic acids against 111 *C. coli* strains to evaluate the effect that pH, the undissociated and dissociated acid species had on these bacteria at their MICs.

The median MIC for acetic and propionic acids required for disinfection of the same strains are the highest and the median MIC for inhibition of the *C. coli* strains by butyric and formic acids have an intermediate value, while the median MIC for inhibition by citric and lactic acids have the lowest values. However, acetic, formic and lactic acids have the highest MIC values for the range of disinfection of all six OAs, and 33.3% of *C. coli* from retail pork chops required the highest level of lactic acid for bacterial control. While the citric acid MICs demonstrate the lowest range, and the lowest 90th percentile value of 10.66 mM for inhibition of all the 111 *C. coli* strains. This suggests that citric acid may be the best OA for inhibiting *C. coli*. This is also confirmed by showing that citric acid has no differential association with respect to the control of *C. coli* from different isolation sources, *P* = 0.24. Conversely, citric acid has a common inhibition effect and lowest concentration required on *C. coli* no matter where the bacteria are isolated from.

Interestingly, it only took a pH of 6.34, 5.79 and 5.84 to inhibit 100% of these bacteria with butyric, citric and propionic acids, respectively. But with acetic, formic and lactic acids it required a pH of 4.60, 4.29 and 3.80, respectively, to inhibit the same 111 *C. coli* strains. This is an average of 1.76 pH unit difference between the pH required for these two groups of acids to inhibit the same 111 *C. coli* strains. We have reported pH differences between OAs against other Gram-negative strains, but not this large a difference. Approximately 98% of 175

---

*Fig 2. Concentration (mM) of the undissociated acids at the MICs of the 111 *Campylobacter coli* strains. The shaded band depicts the difference between the undissociated acetic and citric acid concentrations required for disinfection of 100% of the strains; Δ = 39.91 mM. The number of strains is shown next to each data point.*

https://doi.org/10.1371/journal.pone.0202100.g002
P. aeruginosa strains showed a 0.98 pH unit difference when inhibited by different OAs [38]. A 0.56 pH unit difference was observed between the inhibition by different OAs for 98% of 344 E. coli O157:H7 strains [37], a 0.99 pH unit difference between different OAs was required to inhibit 100% of 138 non-O157 STEC strains [39], and a 1.1 pH unit difference was observed between four different OAs for inhibition of 95 to 100% of the same 145 Salmonella strains [40]. These data show that the inhibition of C. coli or the other Gram-negative bacteria are not primarily dependent on the pH of the acids, as has been suggested by others [33], but rather inhibition must be dependent on some other aspect of these acids. If indeed pH were the primary factor in bacterial inhibition, then one would expect that the MIC$_{50}$s for the same bacteria for all the different OAs would be at the same pH value; but that is not the case. Also, we saw more acid-tolerance in E. coli O157:H7 strains [37], since they have glutamate and arginine-dependent acid-resistance systems for protection against acid stress [50].

The inhibition range for 100% of the 111 C. coli strains by all six undissociated OAs, acetic, butyric, citric, formic, lactic and propionic acids extended from 0.024 mM citric acid to 39.93 mM acetic acid, which is an undissociated acid difference of 39.91 mM across the six different OA species for the same 111 strains. Also, undissociated citric acid shows an inhibition of C. coli strains at a very dilute acid concentration of 1 μM. There appears to be no correlation as to concentration of the undissociated OAs with the MIC$_{50}$s for the 111 C. coli strains. These results are in agreement with the four other Gram-negative foodborne pathogens we have
previously studied. In 175 *P. aeruginosa* strains the difference between undissociated citric acid (2.53 mM) and acetic acid (21.65 mM) for inhibition of 100% of the strains at the MIC$_{MS}$ was 19.12 mM [38]. In 344 *E. coli* O157:H7 the difference between undissociated citric acid (2.86 mM) and acetic acid (50.63 mM) for inhibition of 98.3% of the strains at the MIC$_{MS}$ was 47.77 mM [37]. In 138 non-O157 STECs the difference between undissociated citric acid (2.2 mM) and acetic acid (49.11 mM) for inhibition of 100% of the strains at the MIC$_{MS}$ was 46.91 mM [39], and in 145 *Salmonella* strains the difference between undissociated citric acid (2.29 mM) and acetic acid (19.0 mM) for inhibition of 100% of the strains at the MIC$_{MS}$ was 16.71 mM [40]. In all of these cases, the undissociated acid concentrations did not correlate with the MIC$_{MS}$.

Higher undissociated acid values were observed for *E. coli* O157:H7 and non-O157 STECs, but most likely this was a result of the glutamate and arginine–dependent acid-resistance systems inherent to those bacteria and used to protect themselves from extreme acid stress [50,51].

The inhibition of 100% of the 111 *C. coli* strains by the dissociated OAs was definitely a much smaller concentration range than that observed for the undissociated acids. But the inhibition concentration range shown for all six dissociated acids against *C. coli* is still large when compared to the dissociated OA concentration ranges against the other four Gram-negative foodborne pathogens that we previously studied. The inhibition of approximately 98% of 175 *P. aeruginosa* strains by dissociated citric acid (10.24 mM) and acetic acid (9.98 mM) had a concentration difference of 0.26 mM [38]. The inhibition of 98.3% of 344 *E. coli* O157:H7 strains by dissociated lactic acid (19.36 mM) and dissociated propionic acid (13.825 mM) had a concentration difference of 5.54 mM [37]. The inhibition of 100% of 138 non-O157 STEC strains by dissociated citric acid (19.12 mM) and lactic acid (12.93 mM) had a concentration difference of 6.19 mM [39], and the inhibition of 100% of 145 *Salmonella* strains by dissociated citric acid (19.03 mM) and propionic acid (13.67 mM) had a concentration difference of 5.36 mM [40]. The overall difference in dissociated acids required for inhibition of these four Gram-negative bacteria was from 0.26 mM to 6.19 mM. However with *P. aeruginosa*, we saw a large increase in the dissociated lactic acid concentration required for inhibition [38]. It is known that *P. aeruginosa* utilizes lactate [52,53], and the high inhibition concentration obtained for dissociated lactic acid could be expected [38]. Lactic acid is not an appropriate OA to use against *P. aeruginosa* [38].

Most *C. coli* strains from swine do not utilize citrate [54], and we see in this study the inhibition concentration for dissociated citric acid remains low, ≤ 10.64 mM. Also, *C. coli* were shown not to utilize butyrate [54]. This study corroborates earlier observations by demonstrating levels of dissociated butyric acid needed for inhibition of *C. coli* not widely different from the levels of other dissociated OAs against Gram-negative pathogens [37,39,40]. However, *C. coli* are known to utilize formate, lactate and propionate [55], and in a previous study approximately 13.5% of the *C. coli* strains utilized acetate [54]. The authors also noted the source of *C. coli* strains utilizing acetate was restricted to hogs [54]. Since all 111 strains are inhibited by both citric and butyric acid by ≤ 22.56 mM (knowing that *C. coli* does not utilize citrate or butyrate [54]), it is very interesting that 31 strains are not inhibited by dissociated formic acid until nearly 35 mM, 78 strains are not inhibited by dissociated propionic acid until about 25 mM, and 83 strains are not inhibited by dissociated acetic acid until about 28 mM. Based on our data for the dissociated acid species at the MIC$_{MS}$ of 111 *C. coli* strains from swine, perhaps as much as 83/111 strains (75%) of the *C. coli* analyzed from swine or swine products may utilize acetate.

**Conclusion**

Inhibition of *Campylobacter coli* strains in this study was not primarily dependent on pH or on the concentration of undissociated OAs. The concentration of dissociated OA, butyric, citric,
formic, lactic and propionic acids correlated with the MICs of 100% of the 111 *C. coli* strains. However, some *C. coli* can utilize acetate, formate, lactate and propionate, which most likely resulted in increased levels of these acids at the MICs in our studies. One may expect that a large number of bacteria could escape disinfection as a result of only a small drop in the concentration of a dissociated OA. Therefore, an OA carcass wash may not provide the expected elimination of surface bacteria if the concentration levels of the dissociated OA used is not carefully controlled. A concentration of dissociated acetic, butyric, citric, formic, lactic and propionic acids of 29, 23, 11, 35, 22 and 25 mM, respectively, should be maintained when disinfecting the *C. coli* strains studied here. However, due to the utilization of acetate, formate, lactate and propionate by *C. coli*, these four OAs would probably not be the best choice for control of *C. coli*. If these 4 acids are used for disinfection of *C. coli* bacteria the concentrations of these dissociated organic acids must be held at high enough levels to facilitate complete inhibition of the bacteria. Of the six OAs, citric acid is the most efficient at inhibiting *C. coli*.

**Supporting information**

S1 Fig. pH at the MICs of acetic, butyric, citric, formic, lactic and propionic acids for the 23 *Campylobacter coli* strains from the cecal contents of market age pigs. The number of strains is shown next to each data point. Each data point is the mean and standard deviation of triplicate samples.

(TIF)

S2 Fig. pH at the MICs of acetic, butyric, citric, formic, lactic and propionic acids for the 5 *Campylobacter coli* strains from the feces of market age pigs. The number of strains is shown next to each data point. Each data point is the mean and standard deviation of triplicate samples.

(TIF)

S3 Fig. pH at the MICs of acetic, butyric, citric, formic, lactic and propionic acids for the 51 *Campylobacter coli* strains from the rectal swabs of market age pigs. The number of strains is shown next to each data point. Each data point is the mean and standard deviation of triplicate samples.

(TIF)

S4 Fig. pH at the MICs of acetic, butyric, citric, formic, lactic and propionic acids for the 20 *Campylobacter coli* strains from the cecal contents of sows. The number of strains is shown next to each data point. Each data point is the mean and standard deviation of triplicate samples.

(TIF)

S5 Fig. pH at the MICs of acetic, butyric, citric, formic, lactic and propionic acids for the 12 *Campylobacter coli* strains from retail pork chops. The number of strains is shown next to each data point. Each data point is the mean and standard deviation of triplicate samples.

(TIF)

S6 Fig. Concentration (mM) of the undissociated acids at the MICs of acetic, butyric, citric, formic, lactic and propionic acids for the 23 *Campylobacter coli* strains from the cecal contents of market age pigs. The shaded band depicts the difference between the undissociated lactic and citric acid concentrations required for disinfection of 100% of the strains; Δ = 24.3 mM. The number of strains is shown next to each data point.

(TIF)
S7 Fig. Concentration (mM) of the undissociated acids at the MICₘₙₜₙ of acetic, butyric, citric, formic, lactic and propionic acids for the 5 Campylobacter coli strains from the feces of market age pigs. The shaded band depicts the difference between the undissociated formic and citric acid concentrations required for disinfection of 100% of the strains; Δ = 9.96 mM. The number of strains is shown next to each data point.

(TIF)

S8 Fig. Concentration (mM) of the undissociated acids at the MICₘₙₜₙ of acetic, butyric, citric, formic, lactic and propionic acids for the 51 Campylobacter coli strains from the rectal swabs of market age pigs. The shaded band depicts the difference between the undissociated lactic and citric acid concentrations required for disinfection of 100% of the strains; Δ = 24.3 mM. The number of strains is shown next to each data point.

(TIF)

S9 Fig. Concentration (mM) of the undissociated acids at the MICₘₙₜₙ of acetic, butyric, citric, formic, lactic and propionic acids for the 20 Campylobacter coli strains from the cecal contents of sows. The shaded band depicts the difference between the undissociated lactic and citric acid concentrations required for disinfection of 100% of the strains; Δ = 24.3 mM. The number of strains is shown next to each data point.

(TIF)

S10 Fig. Concentration (mM) of the undissociated acids at the MICₘₙₜₙ of acetic, butyric, citric, formic, lactic and propionic acids for the 12 Campylobacter coli strains from retail pork chops. The shaded band depicts the difference between the undissociated acetic and citric acid concentrations required for disinfection of 100% of the strains; Δ = 39.86 mM. The number of strains is shown next to each data point.

(TIF)

S11 Fig. Concentration (mM) of the dissociated acids at the MICₘₙₜₙ of acetic, butyric, citric, formic, lactic and propionic acids for the 23 Campylobacter coli strains from the cecal contents of market age pigs. The shaded band depicts the difference between the dissociated formic and citric acid concentrations required for disinfection of 100% of the strains; Δ = 16.96 mM. The number of strains is shown next to each data point.

(TIF)

S12 Fig. Concentration (mM) of the dissociated acids at the MICₘₙₜₙ of acetic, butyric, citric, formic, lactic and propionic acids for the 5 Campylobacter coli strains from the feces of market age pigs. The shaded band depicts the difference between the dissociated formic and citric acid concentrations required for disinfection of 100% of the strains; Δ = 23.9 mM. The number of strains is shown next to each data point.

(TIF)

S13 Fig. Concentration (mM) of the dissociated acids at the MICₘₙₜₙ of acetic, butyric, citric, formic, lactic and propionic acids for the 51 Campylobacter coli strains from the rectal swabs of market age pigs. The shaded band depicts the difference between the dissociated formic and citric acid concentrations required for disinfection of 100% of the strains; Δ = 23.9 mM. The number of strains is shown next to each data point.

(TIF)

S14 Fig. Concentration (mM) of the dissociated acids at the MICₘₙₜₙ of acetic, butyric, citric, formic, lactic and propionic acids for the 20 Campylobacter coli strains from the cecal contents of sows. The shaded band depicts the difference between the dissociated formic and
citric acid concentrations required for disinfection of 100% of the strains; Δ = 23.9 mM. The number of strains is shown next to each data point.

(TIF)

**S15 Fig. Concentration (mM) of the dissociated acids at the MICₘ of acetic, butyric, citric, formic, lactic and propionic acids for the 12 Campylobacter coli strains from retail pork chops.** The shaded band depicts the difference between the dissociated acetic and citric acid concentrations required for disinfection of 100% of the strains; Δ = 17.59 mM. The number of strains is shown next to each data point.

(TIF)

**Acknowledgments**

This work was funded by the USDA, Agricultural Research Service. Mention of trade names, proprietary products or specific equipment is solely for the purpose of providing specific information and does not constitute a guarantee, warranty or endorsement by the U.S. Department of Agriculture or by the U.S. Food and Drug Administration and does not imply its approval to the exclusion of other products that may be suitable. Additionally, the views expressed in this article are those of the authors and do not necessarily reflect the official policy of the U.S. Department of Agriculture, the U.S. Food and Drug Administration or the U.S. Government.

**Author Contributions**

**Conceptualization:** Ross C. Beier.

**Formal analysis:** Ross C. Beier, Sara E. Duke.

**Investigation:** Ross C. Beier, Charles A. Hernandez, Kathleen Andrews, Robert E. Droleskey, Sonia Bodeis-Jones, Shenia Young.

**Methodology:** Ross C. Beier, Maureen K. Davidson.

**Project administration:** Maureen K. Davidson, Robin C. Anderson, David J. Nisbet.

**Resources:** David J. Nisbet.

**Supervision:** Robin C. Anderson, David J. Nisbet.

**Validation:** Ross C. Beier, Charles A. Hernandez, Kathleen Andrews, Robert E. Droleskey.

**Visualization:** Ross C. Beier.

**Writing – original draft:** Ross C. Beier.

**Writing – review & editing:** Ross C. Beier, Roger B. Harvey, Charles A. Hernandez, Michael E. Hume, Kathleen Andrews, Robert E. Droleskey, Maureen K. Davidson, Sonia Bodeis-Jones, Shenia Young, Robin C. Anderson, Tawni L. Crippen, Toni L. Poole.

**References**

1. WHO. Media centre Campylobacter: fact sheet. 2016. [http://www.who.int/mediacentre/factsheets/fs255/en/](http://www.who.int/mediacentre/factsheets/fs255/en/) (Accessed: 19 July 2017).

2. Penner JL. The Genus Campylobacter: A decade of progress. Clin Microbiol Rev. 1988; 1:157–172. [http://dx.doi.org/10.1128/CMR.1.2.157](http://dx.doi.org/10.1128/CMR.1.2.157) PMID: 3069194

3. Centers for Disease Control and Prevention (CDC). Burden of foodborne illness: Findings. 2011. [https://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html](https://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html) (Accessed: 19 July 2017).

4. Gillespie IA, O’Brien SJ, Frost JA, Adak GK, Horby P, Swan AV, et al. A case-case comparison of Campylobacter coli and Campylobacter jejuni infection: A tool for generating hypotheses. Emerg Infect Dis.
24. Clark M. Foodborne Illness Outbreak Database. Campylobacter outbreak associated with consumption of raw milk, Alaska. January 2013. Final Report May 1, 2013. http://outbreakdatabase.com/details/campylobacter-outbreak-associated-with-consumption-of-raw-milk-alaska-2013?outbreak=Campylobacter+coli&organism=Campylobacter (Accessed: 10 July 2018).

25. Wachsmuth IK, Sparling PH, Barrett TJ, Potter ME. Enterohemorrhagic Escherichia coli in the United States. FEMS Immunol Med Microbiol. 1997; 18(4):233–239. https://dx.doi.org/10.1111/j.1574-695X.1997.tb01051.x PMID: 9348158

26. Departments of Pennsylvania State University, Texas Tech University, and Washington State University. Antimicrobial spray treatments for red meat carcasses processed in very small meat establishments, 2005. http://meathaccp.wisc.edu/validation/assets/acid_spray_intervention_booklet_from_penn_state_2005.pdf (Accessed: 12 September 2017).

27. Raftari M, Jallilian FA, Abdolamir AS, Son R, Sekawi Z, Fatimah AB. Effect of organic acids on Escherichia coli O157:H7 and Staphylococcus aureus contaminated meat. Microbiol J. 2009; 3(12):121–127. http://dx.doi.org/10.2174/1874285800903010121

28. Raftari M, Jallilian FA, Abdolamir AS, Ghafariun S, Radu S, Sekawi Z, et al. Optimized antibacterial measures against Escherichia coli O157:H7 and Staphylococcus aureus. Afr J Microbiol Res. 2011; 5(20):3113–3121. http://dx.doi.org/10.5897/AJMR10.099

29. Epling LK, Carpenter JA, Blakenship LC. Prevalence of Campylobacter spp. and Salmonella spp. on pork carcasses and the reduction effected by spraying with lactic acid. J Food Protect. 1993; 56(6):536–537. http://dx.doi.org/10.4315/0362-028X-56.6.536

30. Castillo A, Lucia LM, Roberson DB, Stevenson TH, Mercado I, Acuff GR. Lactic acid sprays reduce bacterial pathogens on cold beef carcass surfaces and in subsequently produced ground beef. J Food Protect. 2001; 64(1):58–62. http://dx.doi.org/10.4315/0362-028X-64.1.58

31. Reynolds AE, Jr. Utilization of spray wash with organic acids (peroxyacetic acid and lactic acid) and chlorinated wash in combination, utilizing direct application methods, for pathogen reduction on pork and beef carcasses in small and very small meat processing plants, 2005. http://www.fsis.usda.gov/wps/wcm/connect/acc8bddb-eb64-4ea2-82eb-565cf337691a/New_Technology_C29_Summary_FY2003.pdf?MOD=AJPERES (Accessed: 12 September 2017).

32. Sofos JN, Busta FF. Antimicrobial activity of sorbate. J Food Protect. 1981; 44(8):614–622. http://dx.doi.org/10.4315/0362-028X-44.8.614

33. Blocher JC, Busta FF, Sofos JN. Influence of potassium sorbate and pH on ten strains of type A and B Clostridium botulinum. J Food Sci. 1982; 47(6):2028–2032. http://dx.doi.org/10.1111/j.1365-2621.1982.tb12938.x

34. Ray B, Sandine WE. Acetic, propionic, and lactic acids of starter culture bacteria as biopreservatives. In: Ray B, Daeschel M, editors. Food biopreservatives of microbial origin. Boca Raton, FL: CRC Press, Inc.; 1992. pp. 103–136.

35. Leeson S. Balancing science versus societal issues in poultry nutrition. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources. 2007; 2(71), 6 pp.

36. Presser KA, Ross T, Ratkovsky DA. Modelling the growth limits (growth/no growth interface) of Escherichia coli as a function of temperature, pH, lactic acid concentration, and water activity. Appl Environ Microbiol. 1998; 64(5):1773–1779. http://aem.asm.org/content/64/5/1773.full PMID: 9572950

37. Beier RC, Poole TL, Ehricha-Harhey DM, Anderson RC, Bischoff KM, Hernandez CA, et al. Disinfectant and antibiotic susceptibility profiles of Escherichia coli O157:H7 strains from cattle carcasses, face, and hides and ground beef from the United States. J Food Protect. 2013; 76(1):6–17. http://dx.doi.org/10.4315/0362-028X.JFP-12-253

38. Beier RC, Foley SL, Davidson MK, White DG, McDermott PF, Bodeis-Jones S, et al. Characterization of antibiotic and disinfectant susceptibility profiles among Pseudomonas aeruginosa veterinary isolates recovered during 1994–2003. J Appl Microbiol. 2014; 118(2):326–342. http://dx.doi.org/10.1111/jam.12707 PMID: 25431276

39. Beier RC, Franz E, Bono JL, Mandrell RE, Fratamico PM, Callaway TR, et al. Disinfectant and antimicrobial susceptibility profiles of the big six non-O157 Shiga toxin-producing Escherichia coli strains from food animals and humans. J Food Protect. 2016; 79(8):1355–1370. http://dx.doi.org/10.4315/0362-028X.JFP-15-600

40. Beier RC, Callaway TR, Andrews K, Poole TL, Crippen TL, Anderson RC, et al. Interactions of organic acids with Salmonella strains from feedlot water-sprinkled cattle. J Food Chem Nanotechnol. 2017; 3(2):60–66. https://dx.doi.org/10.17756/jfcn.2017-038

41. Alakomi H-L, Skyttä E, Saarela M, Mattila-Sandholm T, Latva-Kala K, Helander IM. Lactic acid permeabilizes Gram-negative bacteria by disrupting the outer membrane. Appl Environ Microbiol. 2000; 66(5):2001–2005. http://dx.doi.org/10.1128/AEM.66.5.2001-2005.2000 PMID: 10788373
42. Harvey RB, Anderson RC, Young CR, Hume ME, Genovese KJ, Ziprin RL, et al. Prevalence of *Campylobacter*, *Salmonella*, and *Arcobacter* species at slaughter in market age pigs, Chapter 25. In: Paul PS, Francis DH, editors. Mechanisms in the pathogenesis of enteric diseases 2. New York, NY: Kluwer Academic/Plenum Publishers; 1999. pp. 237–239. http://dx.doi.org/10.1007/978-1-4615-4143-1_25

43. National Antimicrobial Resistance Monitoring System (NARMS). NARMS isolates of *Campylobacter coli* from the FDA (retail meats) and USDA (food animals). 2015. https://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/default.htm (Accessed: 20 September 2017).

44. Gorman R, Adley CC. An evaluation of five preservation techniques and conventional freezing temperatures of −20˚C and −85˚C for long-term preservation of *Campylobacter jejuni*. Lett Appl Microbiol. 2004; 38(4):306–310. http://dx.doi.org/10.1111/j.1472-765X.2004.01490.x PMID: 15214730

45. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; Approved Guideline—2nd ed. M45-A2; Vol. 30, No 18. Wayne, PA: Clinical and Laboratory Standards Institute; 2010.

46. TREK Diagnostic Systems. TREK materials and methods for sensititre susceptibility plates for *Campylobacter*. http://www.uniscience.co.kr/data/trds/sensi Manuals/Campylobacter_panel.pdf (Accessed: 12 September 2017).

47. Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemother. 2001; 48 (Suppl. S1):5–16. https://doi.org/10.1093/jac/48.suppl_1.5

48. Helmenstine AM. Henderson Hasselbalch equation and example, 2014. http://chemistry.about.com/od/acidbase1/a/hendersonhasselbalch.htm (Accessed: 07 September 2017).

49. Shaheen BW, Miller ME, Oyarzabal OA. *In vitro* survival at low pH and acid adaptation response of *Campylobacter jejuni* and *Campylobacter coli*. J Food Saf. 2007; 27(3):326–343. http://dx.doi.org/10.1111/j.1745-4565.2007.00083.x

50. Bearson BL, Lee IS, Casey TA. *Escherichia coli* O157:H7 glutamate- and arginine-dependent acid-resistance systems protect against oxidative stress during extreme acid challenge. Microbiology. 2009; 155(3):805–812. http://dx.doi.org/10.1099/mic.0.022905-0

51. Large TM, Walk ST, Whittam TS. Variation in acid resistance among Shiga toxin-producing clones of pathogenic *Escherichia coli*. Appl Environ Microbiol. 2005; 71(5):2493–2500. http://dx.doi.org/10.1128/AEM.71.5.2493-2500.2005 PMID: 15870339

52. Gao C, Hu C, Zheng Z, Ma C, Jiang T, Dou P, et al. Lactate utilization is regulated by the FadR-type regulator LldR in *Pseudomonas aeruginosa*. J Bacteriol. 2012; 194(10):2687–2692. http://dx.doi.org/10.1128/JB.00679-12 PMID: 22408166

53. Gao C, Hu C, Ma C, Su F, Yu H, Jiang T, et al. 2012. Genome sequence of the lactate-utilizing *Pseudomonas aeruginosa* strain XMG. J Bacteriol. 2012; 194(17):4751–4752. http://dx.doi.org/10.1128/JB.00943-12 PMID: 22887660

54. Elharrif Z, Mégraud F. Characterization of thermophilic *Campylobacter*: I. Carbon-substrate utilization tests. Curr Microbiol. 1986; 13(3):117–122. http://dx.doi.org/10.1007/BF01568505

55. Wagley S, Newcombe J, Laing E, Yusuf E, Sambles CM, Studholme DJ, et al. Differences in carbon source utilization distinguish *Campylobacter jejuni* from *Campylobacter coli*. BMC Microbiol. 2014; 14:262, 10 pp. http://dx.doi.org/10.1186/s12866-014-0262-y PMID: 25348335