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Selective Medium for Aerobic Incubation of *Campylobacter*

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Abstract

The objective of this study was to examine the efficacy of three antibiotic mixtures for use as supplements in a selective medium that could be used to isolate *Campylobacter* from mixed bacterial cultures during aerobic incubation. A non-selective, basal broth medium was prepared and supplemented with Bolton, Cefex, or Skirrow antibiotic mixtures. The ability of pure cultures of *Campylobacter coli, Campylobacter fetus, Campylobacter jejuni*, and *Campylobacter lari* to grow in basal broth and basal broth supplemented with each one of the antibiotic mixtures after aerobic incubation for 24 and 48 h at 37°C was determined. Also, the ability of pure cultures of *Escherichia coli, Enterococcus faecalis, Listeria monocytogenes, Pseudomonas aeruginosa, Salmonella Kentucky*, and *Staphylococcus aureus* to grow in basal and supplemented broth medium was determined. Additionally, the recovery of bacteria from mixed cultures containing one *Campylobacter* isolate with the other 6 non-*Campylobacter* isolates after aerobic incubation for 48 h at 37°C in basal broth and broth supplemented with the Bolton antibiotic mixture was examined. Results indicated that there was significant ($p \leq 0.05$) growth of most *Campylobacter* isolates in basal broth and in broth supplemented with Bolton, Cefex, or Skirrow antibiotic mixtures after 24 or 48 h of aerobic incubation. Although, there was significant growth non-*Campylobacter* isolates in basal broth, growth of most these bacteria was inhibited in media supplemented with antibiotic mixtures. Also, *Campylobacter* growth was generally significantly less than other bacteria in mixed bacterial cultures grown in basal broth for 48 h at 37°C, but significantly more of most *Campylobacter* isolates were recovered from mixed bacterial cultures grown in media supplemented with the Bolton antibiotic mixture. Findings indicate that basal medium containing this supplement should be studied as a selective medium that may be utilized with aerobic incubation to isolate *Campylobacter* from environmental samples containing other bacteria.

Keywords : *Campylobacter*; Selective medium; Aerobic incubation

Introduction

Campylobacter is classified as a microaerophile that is generally isolated and cultured under microaerobic atmospheres composed of 5% oxygen, 10% carbon dioxide, and 85% nitrogen [1,2]. Media in primary containers such as test tubes, flasks, or petri dishes, are inoculated with cultures of the bacterium and incubated in an artificially generated, microaerobic atmosphere. The production of this microaerobic atmosphere requires additional technical training for the use of specialized equipment and supplies, such as microaerobic glove boxes, tanks of special gas mixtures, or GasPak jars [3]. Furthermore, most media utilized in the isolation of *Campylobacter* include blood, charcoal, and other substances [4] to support the growth of this enteropathogen. Due to the status of *Campylobacter* as a major human foodborne pathogen [2], simplifying current methods for isolating and culturing this microbe could stimulate additional research on this bacterium [5].

Recent findings indicate that media supplemented with optimal concentrations of amino acids and organic acids associated with the Krebs cycle can support the growth of *Campylobacter* in primary containers incubated in aerobic atmospheres [6,7]. Some meat and plant extracts routinely used in the formulation of bacteriological media contain high concentrations of these organic and amino acids. These extracts have been used in the formulation of non-selective media for culturing Campylobacter under aerobic conditions [8].

Campylobacter can be isolated from fresh poultry, red meats, milk, eggs, and other food products; however, other bacteria are also found in the native microflora of these foods. For example, fresh poultry may contain other human foodborne pathogens, such as *Salmonella* and *Listeria* [9]; spoilage bacteria, such as *Pseudomonas* [7]; indicator bacteria, such as *E. coli* and *Enterobacter* [9]; and other bacteria such as *Staphylococcus* [10]. In order for the recently described medium to be used to isolate *Campylobacter* from foods and other environmental samples, the medium must be supplemented with selective agents that permit the growth of *Campylobacter* while inhibiting the growth of other microorganisms found in the samples [11]. The purpose of the present study was to examine the efficacy of some *Campylobacter* selective antibiotic supplements for use in this medium. Since this is the first step in the development of the selective medium, the ability of the selective supplements to inhibit the growth of known bacterial isolates associated with poultry products was examined. A newly formulated selective medium may be used to isolate *Campylobacter* from mixed bacterial cultures in primary containers under aerobic incubation.

Materials and Methods

Non-selective basal broth medium

The non-selective basal broth was prepared by mixing Remel™ beef extract (Thermo Fisher Scientific, Lenexa, KS), 50 g; Bacto Tryptose (Becton, Dickson, and Co., Sparks, MD), 10 g; soluble starch (Sigma-

Aldrich Chemical, Co., St. Louis, MO), 10 g; sodium lactate (syrup, 60% w/w, Thermo Fisher Scientific), 3.0 g; and Bacto agar (Becton, Dickson, and Co.), 0.5 g in 900 ml of distilled water. The mixture was heated to boiling while stirring to dissolve the agar and starch. Nine ml aliquots of the medium were transferred to screw capped test tubes and sterilized by autoclaving at 121°C for 15 min. Tubes of the cooled medium were supplemented with 1 ml of a 1.5% (w/v) filter sterilized solution of sodium bicarbonate (Spectrum Chemical Mfg. Corp., Gardena, CA) immediately before inoculation with bacteria.

Bacterial isolates

Campylobacter fetus ATCC® 27374, *Campylobacter coli* ATCC® 33559, *Campylobacter jejuni* ATCC® 33560, and *Campylobacter lari* ATCC® 35221 were obtained from the American Type Culture Collection (Manassas, VA). *Campylobacter jejuni* 127-2, *Campylobacter jejuni* 129-25, *Campylobacter jejuni* 1997-8, *Campylobacter jejuni* 1999-1, and *Campylobacter jejuni* 48100 were obtained from Dr. Richard J. Meinersmann of the U. S. National Poultry Research Center, Athens, GA. The gentamicin resistant ($gent^r$) *Campylobacter coli* isolate was previously described [12]. Stock cultures of the *Campylobacter* isolates were grown on slants of Remel™ Blood Agar Base #2 (Thermo Fisher Scientific) supplemented with 7% sheep blood (Lampire Biological Laboratories, Inc., Pipersville, PA) and Blaser-Wang antibiotic mixture (Oxoid Limited, Hampshire, England). The cultures of the isolates were incubated in BD BBL™ GasPak™ jars (Becton, Dickson, and Co.) with activated BD GasPak™ EZ Campy paper sachets (Becton, Dickson, and Co.) at 37°C for 48 h. Isolates were stored at 4°C, and cultures were maintained by transferring to slants of fresh media at 1-2-week intervals.

Non-*Campylobacter* bacteria associated with poultry were isolated from rinsates of whole poultry carcasses obtained from a local, commercial processing facility. Carcasses were rinsed for 2 min on a multi-unit mechanical bird rinser [13] in 200 ml of 0.1% peptone (w/v), and serial dilutions of the carcass rinsates were plated on the appropriate differential, selective medium. *Enterococcus faecalis* was isolated on Difco™ m-Enterococcus agar (Becton, Dickson, and Co.). *Escherichia coli* was isolated on Remel™ Levine EMB agar (Thermo Fisher Scientific). *Listeria monocytogenes* was isolated on *Listeria* Selective Agar base (Oxford Formulation) with modified Listeria Selective Supplement (Oxoid). *Pseudomonas aeruginosa* was isolated on *Pseudomonas* agar base with *Pseudomonas* C-F-C supplement (Oxoid). *Salmonella* Kentucky was isolated on XLT4 agar base (Difco™) with XLT4 Agar Supplement (Becton, Dickson, and Co.). *Staphylococcus aureus* was isolated on Remel™ Mannitol Salts agar (Thermo Fisher Scientific). All inoculated media were incubated aerobically at 37°C for 24-48 h, and selected colonies from the agar media were identified using the Biolog OmniLog Microbial ID Identification System with GEN III database (Biolog, Hayward, CA). Stock cultures of the isolates were stored on Tryptic Soy agar (Becton, Dickson, and Co.) slants at 4°C and maintained by transferring to fresh media at 4-6 week intervals.

For each experiment, fresh cultures of each bacterial isolate were prepared by using a bacteriological loop to add the stock cultures to 10 ml of the basal broth in 12.5 cm^2 tissue culture flasks with plugged caps (Corning, Co., Corning, NY). Flasks inoculated with *Campylobacter* were incubated aerobically at 37°C for 48 h, while flask inoculated with the other bacterial isolates were incubated aerobically at 37°C for 18-24 h. *Campylobacter* and non-*Campylobacter* isolates in the fresh cultures were enumerated by plating serial dilutions of the cultures on the appropriate selective medium, incubating as described above, and counting the number of CFU on the plates.

Growth of *Campylobacter* and other bacterial isolates in basal broth and basal broth supplemented with antibiotics

The effect of the antibiotic mixtures on the growth of these bacteria was determined by comparing bacterial growth after aerobic incubation of the isolates in culture flasks containing the basal broth with and without antibiotic supplements. Antibiotic supplementation consisted of adding the Bolton antibiotic mixture containing cefoperazone (20 mg/l), vancomycin hydrochloride (20 mg/l), trimethoprim lactate, (20 mg/l), and cycloheximide (50 mg/l) [14]; the Campy-Cefex antibiotic mixture containing cycloheximide (200 mg/l) and cefoperazone (33 mg/l) [15], or the Skirrow antibiotic mixture containing vancomycin hydrochloride (10 mg/l), trimethoprim lactate, (20 mg/l), and polymyxin B (2500 IU) [16] to 10 ml of basal broth. Concentrations listed are the final concentration of antibiotics after addition to the medium. All antibiotics were obtained from Sigma-Aldrich Co. (St. Louis, MO). Basal broth that was not supplemented with antibiotics served as the control.

Campylobacter growth studies were conducted to determine the ability of the *C. coli*, *C. fetus, C. jejuni*, and *C. lari* strains to grow in basal broth and in basal broth supplemented with Bolton, Cefex, or Skirrow antibiotic mixtures. Separate aliquots of 10 ml of media were inoculated with 0.1 ml of the ± fresh cultures to produce a concentration of approximately 10^4 CFU/ml of each *Campylobacter* isolate. The actual number of *Campylobacter* that could be recovered from the media immediately after inoculation was then determined by plating serial dilutions of the inoculated control media on the selective blood agar media with Blaser-Wang antibiotic mixture described above. Inoculated agar plates were incubated microaerobically at 37°C for 48 h, and CFU's on the agar were counted. Growth of the *Campylobacter* inocula was determined by placing the flasks containing inoculated basal broth medium and supplemented basal broth medium into 37°C aerobic incubators and enumerating CFU in the media after 24 and 48 h on selective blood agar media incubated micro aerobically at 37°C for 48 h.

Studies were then conducted to examine the growth of the other bacterial isolates in the basal broth and in basal broth supplemented with of each of the 3 antibiotic mixtures. Separate flasks of basal medium and supplemented basal medium were inoculated with 0.1 ml of the fresh cultures to produce a concentration of approximately 10^4 CFU/ml of pure cultures of *E. coli*, *E. faecalis, L. monocytogenes, P. aeruginosa, Salmonella Kentucky*, or *S. aureus*. The actual number of CFU/ml that could be recovered from the inoculated media immediately after inoculation and number of CFU/ml that could be recovered from the inoculated media after 24 and 48 h of incubation at 37°C was determined by plating on m-Enterococcus agar for *E. faecalis*, on Levine EMB agar for *E. coli*, on *Listeria* Selective Agar base with modified *Listeria* Selective Supplement for *L. monocytogenes*, on *Pseudomonas* agar base with *Pseudomonas* C-F-C supplement for *P. aeruginosa*, on XLT4 agar base with XLT4 Agar Supplement for *Salmonella Kentucky*, and on Mannitol Salts agar for *S. aureus*. All plates were incubated aerobically at 37°C for 24-48 h.

Recovery of *Campylobacter* from mixed bacterial cultures

Fresh cultures of the *Campylobacter* isolates and the other 6 bacterial isolates were prepared in separate flasks as described above.

Mixed bacterial cultures were grown by inoculating basal broth media and media supplemented with the Bolton antibiotic mixture with 0.1 ml of a mixed bacterial suspension consisting of 1 of the *Campylobacter* isolates in addition to *E. faecalis*, *E. coli, L. monocytogenes, P. aeruginosa, Salmonella Kentucky*, and *S. aureus* isolates in 0.1% peptone water. The final concentration of bacteria in the inoculated media was approximately10^4 CFU/ml of each organism. The inoculated media were incubated aerobically in culture flasks for 48 h at 37°C. After incubation, presumptive CFU/ml of *Campylobacter* and the other bacterial isolates were determined by counting colonies exhibiting the typical appearance of each isolate on the appropriate selective, differential media described above. Furthermore, the identity of *Campylobacter* isolates was also confirmed by selecting random colonies from the selective *Campylobacter agar*, and microscopically observing wet mount slides of the colonies for spiral shaped bacteria with darting motility using the phase contrast lens on the Olympus BX53 microscope (Olympus America, Inc., Center Valley, PA).

Statistical analysis of data

Three replicates of each experiment were performed. The exponential CFU/ml values were converted to logarithms, and GraphPad InStat, version 3.0 for Windows (GraphPad Software, San Diego, CA), was used to perform statistical analysis of the data. One-way analysis of variance of group means of the values were performed, and the Tukey-Kramer multiple comparison test was used to determine which means differed significantly at $P \leq 0.05$.

Results and Discussion

Growth of pure bacterial cultures in media with and without antibiotic supplements

After aerobic incubation in the culture flasks, there was significant ($p \leq 0.05$) growth of most *Campylobacter* isolates in basal broth medium and in basal broth medium supplemented with the antibiotic mixtures (Table 1). In the basal broth media that was not supplemented with antibiotics, there was a significant increase in the number of CFU/ml of 7 of the 10 *Campylobacter* isolates after 24 h of aerobic incubation and for 9 of the 10 isolates after 48 h of incubation.

		Antibiotic Supplement							
Campylobacter Isolate	CFU/ml in inocula[2]	Control[3]		Bolton		Cefex		Skirrow	
		24h	48h	24h	48h	24h	48h	24h	48h
C. jejuni 127-2	4.59+0.49^{a}	7.45+0.98^{b}	9.09+0.13^{b}	6.72+1.50^{ab}	8.69+0.12^{b}	7.09+1.01^{b}	8.91+0.76^{b}	7.11+1.19^{b}	9.15+0.07^{c}
C. jejuni 129-25	4.75+0.28^{ab}	4.49+0.83^{a}	7.76+0.79^{ab}	6.21+1.86^{ab}	8.15+0.48^{b}	5.78+1.96^{ab}	8.10+0.66^{b}	5.20+1.84^{ab}	8.05+0.76^{b}
C. jejuni 1997-8	4.66+0.44^{a}	6.02+1.71^{ab}	9.09+0.69^{c}	6.88+1.56^{abc}	9.02+0.16^{c}	6.16+1.32^{abc}	9.14+0.24^{c}	6.71+1.43^{bc}	8.96+0.04^{bc}
C. jejuni 1999-1	5.04 ± 0.17^{a}	7.51 ± .21^{b}	9.01 ± 0.15^{c}	6.97 ± 0.52^{b}	9.25 ± 0.06^{c}	7.23 ± 0.25^{b}	9.13 ± 0.26^{c}	7.10 ± 0.13^{b}	9.23 ± 0.13^{c}
C. fetus 27374	4.96 ± 0.24^{a}	8.16 ± 0.20^{bc}	8.31 ± 1.19^{bc}	6.30 ± 2.21^{ab}	8.16 ± 0.65^{bc}	7.87 ± 0.41^{bc}	8.88 ± 0.28^{c}	7.98 ± 0.48^{bc}	9.03 ± 0.17^{c}
C. coli 33559	4.72 ± 0.44^{ab}	7.87 ± 0.66^{bc}	8.78 ± 0.11^{c}	8.10 ± 0.18^{bc}	8.44 ± 0.07^{bc}	8.08 ± 0.07^{bc}	8.58 ± 0.11^{c}	4.04 ± 2.25a	7.42 ± 2.69^{abc}
C. jejuni 33560	4.20 ± 0.16^{a}	6.47 ± 0.68^{b}	9.13 ± 0.26^{e}	6.48 ± 0 .74^{b}	8.21 ± 0.22^{cd}	6.08 ± 0.93^{b}	8.49 ± 0.17^{de}	6.77 ± 0.73^{bc}	9.07 ± 0.06^{de}
C. lari 35221	4.98 ± 0.55^{ab}	7.22 ± 0.75^{ab}	9.04 ± 0.39^{b}	8.10 ± 0.35^{b}	8.50 ± 0.22^{b}	7.80 ± 0.54^{b}	8.62 ± 0.19^{b}	1.98 ± 2.61^{a}	5.65 ± 4.92^{ab}
C. jejuni 48100	4.69 ± 0.52^{a}	7.53 ± 1.56^{b}	9.20 ± 0.26^{b}	7.41 ± 0.99^{b}	9.41 ± 0.12^{b}	7.39 ± 1.29^{b}	9.21 ± 0.14^{b}	7.28 ± 0.77^{b}	9.15 ± 0.13^{b}
C. coli gentr	4.40 ± 0.22^{a}	7.88 ± 0.24^{bc}	8.67 ± 0.07^{c}	7.77 ± 0.37^{bc}	8.57 ± 0.05^{c}	7.87± 0.33b^{c}	8.63 ± 0.18^{c}	7.30 ± 0.56^{b}	8.53 ± 0.09^{c}

[1]Values are average log CFU/ml+standard deviation. n=3; [2]Log CFU of Campylobacter/ml recovered from basal medium immediately after inoculation; [3]Basal medium that was not supplemented with antibiotics; $^{a-c}$Within rows, values with different subscripts are significantly different ($p<0.05$).

Table 1: Log colony-forming-units/ml of *Campylobacter spp.* recovered from basal medium and basal medium supplemented with antibiotics after aerobic incubation at 37°C for 24 or 48 h.

There was approximately a 3-4 log increase in the number of *Campylobacter* recovered after incubation for 48 h. The primary source of Krebs Cycle amino acids and organic acids in the basal medium was the 5% concentration of beef extract in the medium [7], and the growth of *Campylobacter* in this medium is similar to previous findings [6,8]. These amino acids and organic acids may be able to support the growth of *Campylobacter* in secondary containers incubated aerobically because the bacterium has an incomplete Krebs cycle, and optimal concentrations of these metabolites included in the medium might provide substrates required for growth under these conditions [6,8].

There was significant growth of all 10 *Campylobacter* isolates after 48 h of incubation in media supplemented with Bolton or Cefex antibiotic mixtures. However, the log CFU/ml of the *C. lari* recovered in the media supplemented with the Skirrow antibiotic mixture decreased by approximately 3 log after 24 h of incubation before increasing by approximately 4 log CFU/ml after 48 h of incubation. The initial decrease in the population of the *C. lari* culture might

demonstrate sensitivity of a portion of the population of cultures of this isolate to the Skirrow antibiotic mixture followed by growth of portion of the population that could grow in the presence of this mixture of antibiotics. Also, although there was approximately a 3 log increase in the number of *C. coli* 33559 recovered after 48 h of incubation in the media supplemented with the Skirrow antibiotic mixture, the number of bacteria recovered was not significantly ($p<0.05$) greater than the number recovered immediately after inoculation.

All of the non-*Campylobacter* isolates were able to grow in the basal medium in the absence of the antibiotic supplements, but the addition of the antibiotic mixtures reduced the growth of most these isolates (Table 2). The utilization of selective, differential agar in these studies to enumerate known bacterial species in the samples facilitated the enumeration of presumptive cfu/ml recovered from each experiment.

Antibiotic Supplement									
Isolate	CFU/ml in inocula[2]	Control[3]		Bolton		Cefex		Skirrow	
		24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Enterococcus faecalis	4.04 ± 0.28^{cd} ±	8.98 ± 0.03^{e}	9.13 ± 0.04^{e}	0.49 ± 0.85^{a}	1.53 ± 0.56^{a}^{b}	5.21 ± 1.10^{d}	7.39 ± 1.01^{e}	2.97 ± 0.28^{bc}	2.67 ± 0.31^{bc}
Escherichia coli	4.23 ± 0.31^{b}	8.96 ± 0.20^{d}	9.12 ± 0.05 d	NR 4,a	NRa	NR a	NR a	7.72 ± 0.78^{c}	8.23 ± 0.63^{cd}
Listeria monocytogenes	3.21 ± 0.93^{b}	8.93 ± 0.24^{c}	8.86 ± 0.22^{c}	NR a	NRa	0.90 ± 0.85^{a}	0.33 ± 0.58^{a}	1.18 ± 1.27^{a}	NRa
Pseudomonas aeruginosa	3.90 ± 0.33^{a}	8.93 ± 0.20^{b}	9.07 ± 0.29^{b}	1.80 ± 0.51^{a}	2.16 ± 2.38^{a}	1.22 ± 1.06^{a}	1.56 ± 2.71^{a}	9.06 ± 0.08^{b}	8.90 ± 0.18^{b}
Salmonella Kentucky	3.41 ± 0.05^{b}	8.85 ± 0.25^{d}	8.48 ± 0.38^{c}^{d}	NR a	NRa	NR a	0.33 ± 0.58^{a}	7.94 ± 0.37^{c}	8.42 ± 0.44^{cd}
Staphylococcus aureus	3.51 ± 0.23^{b}	8.97 ± 0.09^{c}	8.97 ± 0.16^{c}	0.33 ± 0.58^{a}	NRa	0.43 ± 0.75^{a}	0.33 ± 0.58^{a}	2.22 ± 0.64^{b}	3.56 ± 0.67^{b}

[1]Values are average log CFU/ml+standard deviation. n=3; [2]Log CFU of Campylobacter/ml recovered from basal medium immediately after inoculation; [3]Basal medium that was not supplemented with antibiotics; [4]NR-None recovered; [a-e]Within rows, values with different subscripts are significantly different ($p<0.05$).

Table 2: Log colony-forming-units/ml of poultry bacterial isolates recovered from basal medium and basal medium supplemented with antibiotics after aerobic incubation at 37°C for 24 or 48 h.

There was a significantly increase in the number of CFU/ml of *E. faecalis, E. coli, L. monocytogenes, P. aeruginosa, Salmonella Kentucky*, and *S. aureus* recovered from the control media after 24 h with no further significant increase after 48 h of incubation. There was approximately a 4-5 log increase in the number of CFU/ml of these bacteria recovered from the media after 48 h of incubation. There was no significant increase in the number of either of the 6 isolates recovered from media supplemented with the Bolton mixture after 24 or 48 h of incubation, and no *E. coli, L. monocytogenes, Salmonella Kentucky,* or *S. aureus* were recovered from media containing this supplement after 48 h of incubation. There was also no significant increase in the number of *E. coli, L. monocytogenes, P. aeruginosa, Salmonella Kentucky*, or *S. aureus* recovered from media supplemented with the Cefex antibiotic mixture after 48 h of incubation, and no *E. coli* was recovered after 24 or 48 h of incubation in this medium. There was a significant increase in the number of *E. faecalis* recovered after 24 h with an additional significant increase after 48 h of incubation though. Also, there was a significant increase in the number of all isolates, except *L. monocytogenes*, recovered from media supplemented with the Skirrow antibiotic mixture after 48 h of incubation. Then, after 48 h of incubation, no *L. monocytogenes* was recovered from media supplemented with the Skirrow mixture.

Results from the experiments with basal medium supplemented with the 3 antibiotic mixtures indicated that the Bolton mixture allowed significant growth of all *Campylobacter* isolates while providing the broadest range of inhibition of growth of the non-*Campylobacter* bacteria used in this study.

Recovery of isolates from mixed bacterial cultures

Based on the ability of media supplemented with the Bolton antibiotic mixture to inhibit the growth of the *non-Campylobacter* isolates while not inhibiting *Campylobacter* growth, the Bolton mixture was selected for use in further studies. The use of known bacterial isolates in these studies in addition to the use of selective, differential agar that only support the growth known bacteria that produce colonies of distinctive morphology facilitated the enumeration of presumptive cfu/ml recovered in studies using mixed cultures of these bacteria. When mixed bacterial cultures containing one *Campylobacter* isolate with *E. faecalis, E. coli, L. monocytogenes, P. aeruginosa, Salmonella Kentucky*, and *S. aureus* were grown in for 48 h at 37°C in basal medium, significantly fewer *C. jejuni* 1999-1 and 48100 were recovered than the non-*Campylobacter isolate* in the bacterial culture (Table 3).

Additionally, in the cultures containing *C. jejuni* 1997-8 or *C. jejuni* 33560, significantly fewer *S. aureus* and the *Campylobacter* isolates were recovered. Growth of the other *Campylobacter* isolates in mixed cultures generally indicated that the *Campylobacter* isolates, *S. aureus*, and *L. monocytogenes* were recovered in lower numbers than other bacteria included in the cultures. These results were probably due to the superior ability *of E. coli, E. faecalis*, *P. aeruginosa*, and *Salmonella Kentucky* to compete for nutrients and to grow in the basal medium at a faster rate than *Campylobacter*, *S. aureus*, and *L. monocytogenes.*

In basal broth supplemented with the Bolton antibiotic mixture growth of most *Campylobacter* isolates was not inhibited although growth of the other bacteria in the mixed bacterial cultures was inhibited. Significantly, more of each *Campylobacter* isolate, except for *C. jejuni* 1991-1, were recovered than the other bacteria from mixed bacterial cultures grown in media supplemented (Table 4). with the Bolton antibiotic mixture [17,18].

The finding for *C. jejuni* 1991-1 was probably related to 1 replicate of experiment in which there was little growth of the bacterium, resulting in a lower average number of *Campylobacter* recovered and the high standard deviation value for the 3 replicates of the experiment. In most cases, no *E. coli*, *Salmonella Kentucky*, or *S.*

aureus were recovered from the mixed cultures after 48 h of incubation at 37°C in the Bolton supplemented medium.

Campylobacter **isolate added to mixed bacterial culture**										
Bacterial Isolate Recovered	*C. jejuni* 127-2	*C. jejuni* 129-25	*C. jejuni* 1997-8	*C. jejuni* 1999-1	*C. fetus* 27374	*C. coli* 33559	*C. jejuni* 33560	*C. lari* 35221	*C. jejuni* 48100	*C. coli* gentr
Campylobacter spp.	5.98 ± 0.45^{a}	6.29 ± 0.70^{a}	6.42 ± 0.79^{a}	5.58 ± 0.69^{a}	5.76 ± 0.67^{a}	6.58 ± 0.62^{a}	6.34 ± 0.46^{a}	6.03 ± 0.51^{a}	5.40 ± 0.10^{a}	7.30 ± 0.58^{ab}
Enterococcus faecalis	8.62 ± 0.07^{cd}	8.81 ± 0.08^{c}	8.92 ± 0.03^{c}	8.72 ± 0.07^{c}	8.80 ± 0.05^{c}	9.09 ± 0.25^{b}	8.83 ± 0.11^{c}	8.88 ± 0.24^{c}	8.84 ± 0.35^{c}	8.44 ± 0.80^{b}
Escherichia coli	8.70 ± 0.17^{d}	8.74 ± 0.23^{c}	8.73 ± 0.11^{c}	8.89 ± 0.36^{c}	8.83 ± 0.24^{c}	8.69 ± 0.17^{b}	8.69 ± 0.21^{c}	8.72 ± 0.19^{c}	8.56 ± 0.14^{c}	8.69 ± 0.15^{c}
Listeria monocytogenes	7.40 ± 0.30^{bc}	7.30 ± 0.30^{b}	7.32 ± 0.18^{ab}	7.20 ± 0.21^{b}	7.24 ± 0.60^{b}	7.06 ± 0.18^{a}	7.36 ± 0.18^{b}	7.94 ± 1.17^{bc}	7.22 ± 0.33^{b}	6.79 ± 0.59^{a}
Pseudomonas aeruginosa	8.63 ± 0.06^{cd}	8.66 ± 0.27^{c}	8.71 ± 0.28^{c}	8.61 ± 0.06^{c}	8.65 ± 0.22^{c}	8.45 ± 0.27^{b}	8.65 ± 0.35^{c}	8.64 ± 0.17^{c}	8.40 ± 0.65^{c}	8.63 ± 0.11^{c}
Salmonella Kentucky	8.27 ± 0.15^{bcd}	8.34 ± 0.27^{c}	8.35 ± 0.03^{bc}	8.22 ± 0.06^{c}	8.36 ± 0.31^{c}	8.30 ± 0.17^{b}	8.50 ± 0.25^{c}	8.27 ± 0.08^{c}	8.29 ± 0.19^{c}	8.22 ± 0.10^{bc}
Staphylococcus aureus	7.08 ± 1.08^{ab}	6.69 ± 0.27^{ab}	6.51 ± 0.52^{a}	6.52 ± 0.18^{b}	6.52 ± 0.24^{ab}	6.64 ± 0.27^{a}	6.20 ± 0.35^{a}	6.56 ± 0.03^{ab}	6.50 ± 0.25^{b}	6.43 ± 0.44^{a}

[1]Values are average log CFU/ml+standard deviation. n=3; [2]Counts are presumptive CFU/ml based on appearance of colonies recovered on selective, differential bacteriological media; [a-d]Within columns, values with different subscripts are significantly different ($p<0.05$).

Table 3: Log colony-forming-units/ml 1,2 bacteria recovered on selective agar from mixed cultures containing one *Campylobacter* and 6 other poultry bacterial isolates after 48 h aerobic incubation at 37°C in basal media not supplemented with Bolton antibiotic mixture.

Campylobacter **isolate added to mixed bacterial culture**										
Bacterial Isolate Recovered	*C. jejuni* 127-2	*C. jejuni* 129-25	*C. jejuni* 1997-8	*C. jejuni* 1999-1	*C. fetus* 27374	*C. coli* 33559	*C. jejuni* 33560	*C. lari* 35221	*C. jejuni* 48100	*C. coli gentr*
Campylobacter spp.	8.86 ± 0.77^{b}	8.71 ± 0.48^{c}	9.23 ± 0.99^{b}	3.13 ± 3.50^{a}	8.86 ± 0.77^{b}	8.23 ± 0.57^{b}	8.22 ± 0.63^{b}	8.37 ± 0.16^{b}	8.74 ± 0.16^{b}	7.08 ± 1.66^{c}
Enterococcus faecalis	1.07 ± 0.97^{a}	NR3,a	1.29 ± 1.17^{a}	0.84 ± 1.46^{a}	1.07 ± 0.97^{a}	0.72 ± 1.24^{a}	2.34 ± 0.44^{a}	0.70 ± 1.22^{a}	0.67 ± 1.16^{c}	0.78 ± 1.35^{a}
Escherichia coli	NRa	NRa	0.65 ± 1.13^{a}	NRa	NRa	0.43 ± 0.75^{a}	NRa	NRa	NRa	NRa
Listeria monocytogenes	1.00 ± 0.88^{a}	0.33 ± 0.58^{a}	0.77 ± 0.68^{a}	NRa	1.00 ± 0.88^{a}	0.95 ± 1.64^{a}	1.77 ± 1.55^{a}	0.83 ± 0.76^{a}	1.90 ± 1.67^{a}	1.24 ± 1.14^{a}
Pseudomonas aeruginosa	2.29 ± 1.97^{a}	3.71 ± 1.75^{b}	2.83 ± 1.91^{a}	0.33 ± 0.58^{a}	2.29 ± 1.97^{a}	2.56 ± 1.16^{a}	1.74 ± 2.00^{a}	1.65 ± 2.06^{a}	2.29 ± 3.14^{a}	3.63 ± 0.79^{b}
Salmonella Kentucky	NRa	NRa	1.54 ± 1.42^{a}	NRa	NRa	NRa	0.87 ± 1.50^{a}	NRa	NRa	NRa
Staphylococcus aureus	NRa	NRa	NRa	NRa	NRa	NRa	NRa	NRa	NRa	NRa

[1]Values are average log CFU/ml+standard deviation. n=3; [2]Counts are presumptive CFU/ml based on appearance of colonies recovered on selective, differential bacteriological media; [3]NR-None recovered; [a-c]Within columns, values with different subscripts are significantly different ($p<0.05$).

Table 4: Log colony-forming-units/ml bacteria 1,2 recovered from mixed cultures containing one *Campylobacter* and 6 other poultry bacterial isolates after 48 h aerobic incubation at 37°C in basal media supplemented with Bolton antibiotic mixture

Conclusion

These findings indicate that the addition of Bolton's antibiotic mixture to the basal medium produces a selective broth medium that can be used to isolate *Campylobacter* from mixed bacterial cultures. The use of the procedure will simplify methods for isolating this pathogen from environmental samples containing other bacteria. Additionally, since this procedure will not require the use of plastic bags, jars, microaerobic glove boxes; the equipment, supplies, technical

training, and cost required for isolating *Campylobacter* should be reduced. Studies are now being planned to examine the ability of the selective medium to isolate *Campylobacter* from environmental samples containing a more diverse microbial flora. These future studies will determine if further modifications to the broth are required before medium can be considered for use by industry to monitor contamination of processed foods by *Campylobacter*, by regulatory agencies in verification testing, and by academia in basic and applied research projects.

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