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Comparison of Three Different Methods for *Campylobacter* Isolation from Porcine Intestines

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Using 200 porcine colon tissues, the efficiencies of three isolation methods of *Campylobacter* from porcine intestines were compared: Method 1, direct streaking of colon mucosa; Method 2, direct inoculation of intestinal contents with a swab; Method 3, inoculation of pre-enriched medium. A total of 460 *Campylobacter* isolates were obtained from 178 samples (89%) by direct streaking of colon mucosa, 142 samples (71%) by direct streaking of a swab, and 94 samples (47%) by pre-enrichment of intestinal contents in Preston broth. Direct streaking of colon mucosa was superior to the other two isolation methods, in terms of rapidity and higher efficiency. When isolates were identified with various biochemical tests and PCRs specific to 16s rRNA, *mapA*, and *ceuE*, *C. coli* was the predominant species (87%) in porcine, whereas the rest of the isolates were identified as *C. lanienae*.

Keywords: Thermophilic *Campylobacter*, porcine, comparison of isolation method

Many animal species harbor *Campylobacter* in their intestinal tracts, which are considered as potential sources of *Campylobacter* for humans [6, 22]. *C. jejuni* appears predominantly among cattle and broiler chickens, but is found only at a low frequency among pigs, in which *C. coli* predominates [1, 3]. In recent years, human campylobacteriosis has shown a dramatic increase in industrialized countries and currently represents one of the principal causes of bacterial foodborne diseases [20, 21]. *Campylobacter* infections tend to be self-limiting and do not normally require treatment. However, such infections can result in bacteremia in immunocompromised patients. *Campylobacter* bacteremia can have a fairly high mortality rate (>30%), and fatalities due to treatment failures have also been reported [15]. There is a demand for a rapid and sensitive detection method for *Campylobacter* spp. from food animals [14]. Rapid isolation and accurate identification of these organisms are thus desirable objectives to provide important clinical and epidemiological information [12]. Although the fecal-based method [2] is less sensitive and laborious, it is still the most widely used and considered as the reliable detection method of *Campylobacter* spp. within animals.

This study was performed to choose the best isolation method for thermophilic *Campylobacter* species from porcine intestines among three methods: Method 1, direct streaking of colon mucosa; Method 2, direct streaking with a swab of intestinal contents; Method 3, inoculation of pre-enriched medium [7].

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Materials and Methods

Sample Collection

Two hundred colon tissues (approximately 20 g) with contents were taken from a slaughterhouse located in Kyunggi Province from two visits during June and July 2007. Samples were obtained shortly after slaughter, and transferred into a sterile disposable bottle. Samples were transported to the laboratory in a cold bag (4°C) in 1 h distance and treated immediately.

Isolation of *Campylobacter*

Campylobacter spp. were isolated with the following three methods. Colonies with different morphology (grey, flat, irregular, and spreading) were isolated and stored separately, even though they were originated from the same sample.

Method 1: Direct Streaking of Colon Mucosa

Colon tissue was aseptically picked from the sample bottle and washed twice with sterile phosphate-buffered saline (PBS, pH 7.4) to remove intestinal contents. The inner mucous membrane of the washed colon tissue was streaked onto a Campy blood-free selective medium (mCCDA; Acumedia Manufacturers, Baltimore, MD, U.S.A.) with supplement (LC30-05; Dalynn Biologicals, Calgary, Canada) [9, 11]. Colonies were examined after incubation under microaerobic condition at 42°C for 48 h. Presumptive *Campylobacter* colonies were picked and subcultured onto mCCDA plates. Gram-negative and curved rods were chosen and stored at −70°C for further study.

Table 1. PCR primer sets used in this study.

Gene	Primer	Sequence (5' to 3')	Product length (bp)	Reference
16S rRNA	Forward	ATC TAA TGG CTT AAC CAT TAA AC	857	[10, 16]
	Reverse	GGA CGG TAA CTA GTT TAG TAT T		
mapA	Forward	CTA TTT TAT TTT TGA GTG CTT GTG	589	[18, 16]
	Reverse	GCT TTA TTT GCC ATT TGT TTT ATT A		
ceuE	Forward	ATT TGA AAA TTG CTC CAA CTA TG	462	[4, 16]
	Reverse	TGA TTT TAT TAT TTG TAG CAG CG		
16S rRNA (sequencing)	27F	AGA GTT TGA TCM TGG CTC AG	1,060	[19]
	1088R	GCT CGT TGC GGG ACT TAA CC		

Method 2: Direct Streaking of Intestinal Contents with a Swab on mCCDA Plate

Swabs of intestinal contents were directly inoculated onto a mCCDA plate [11]. After 48 h of incubation as described above, colonies were examined and stored as described above.

Method 3: Pre-Enrichment in Preston Broth

A portion (0.5 g) of the intestinal contents was aseptically transferred into a sterile closed screw-cap bottle (15 ml) containing 5 ml of Preston broth (Oxoid Nutrient No. 2; Oxoid, Basing Stoke, Hampshire, U.K.) containing supplements LC38-5, LC18-05, and HL60-100 (Dalynn Biologicals, Calgary AB, Canada) [7, 8]. The bottle was vigorously shaken and incubated at 42°C under microaerobic condition. After 24 h incubation, an aliquot (20 μl) of the enriched culture was streaked onto a mCCDA plate and incubated as described above. Colonies that appeared on the mCCDA plates were examined after 48 h incubation.

Identification of *Campylobacter* Isolates

Isolates were presumed as *Campylobacter* based on Gram-staining, oxidase-positive reaction, and catalase-positive reaction. Further identification was performed with multiplex PCR using primer sets specific to genes *mapA* in *C. jejuni* [16, 18], *ceuE* in *C. coli* [4], and 16S rRNA in the *Campylobacter* genus [10], as shown in Table 1. Template DNA for multiplex PCR was prepared by suspending one colony in 0.05 N NaOH and heating at 80°C in the Gene Amp PCR System 9700 (Applied Biosystems, Foster City, CA, U.S.A.) for 15 min. Supernatant after centrifugation was used as genomic DNA, and PCR was performed with one cycle for 5 min at 95°C, 35 cycles for 30 s at 95°C, 90 s at 58°C, and 1 min at 72°C, and a final extension for 10 min at 72°C. *C. jejuni* ATCC 33560 and *C. coli* ATCC 33559 were included in every test as controls. For sequencing of the 16S rRNA gene, a DNA fragment was purified using the QIAquick Gel extraction kit (Qiagen, Valencia, CA, U.S.A.), in accordance with the manufacturer's protocol. Sequencing PCR [8, 19] was performed with one cycle of predenaturation at 95°C for 5 min, 30 cycles at 95°C for 30 s, 57°C for 30 s, at 72°C for 45 s, and a final extension for 10 min at 72°C and sequenced using an ABI 3100 automated sequencer (Applied Biosystems, Foster City, CA, U.S.A.). DNA sequences were compared using the online BLAST algorithm at the National Center for Biotechnology Information Web server (http://www.ncbi.nlm.nih.gov).

Results

Isolation of *C. coli* and *C. lanienae* with Three Methods

A total of 460 *Campylobacter* isolates were obtained from 200 samples with the three methods, based on the biochemical and physiological characteristics of *Campylobacter* species. Among these isolates, 400 isolates (87%) produced DNA fragments with 857 bp and 462 bp from PCRs specific to the 16S rRNA gene for the *Campylobacter* genus and the *ceuE* gene in *C. coli*, respectively, but did not produce DNA fragments from PCR specific to *mapA* in *C. jejuni*. Based on PCR results, these 400 isolates were identified as *C. coli*. The remaining 60 *Campylobacter* isolates (13%) were identified as *C. lanienae* from sequence analysis of the 16S rRNA gene.

Isolation Efficiencies of Three Isolation Methods

The three isolation methods showed various efficiencies onto mCCDA (Table 2). Method 1 produced 209 isolates from 178 samples (89%), method 2 produced 154 isolates from 142 samples (71%), and method 3 produced 97 isolates from 94 samples (47%). When efficiencies were compared based on the number of isolates, method 1 produced the largest number of *Campylobacter* (209 isolates), method 2 produced 154 isolates, and method 3 produced 97 isolates.

Sixty-two samples rendered *Campylobacter* isolates with all three methods, and 9 samples did not produce any

Table 2. Numbers of samples that produced *Campylobacter* isolates from porcine intestines.

Inoculation method	Number of		Isolate number of		Total number of *Campylobacter*
	Positive sample	Negative sample	*C. coli*	*C. lanienae*	
Direct streaking of colon mucosa	178 (89%)	22 (11%)	182 (87%)	27 (13%)	209
Direct streaking of intestinal contents	142 (71%)	58 (29%)	129 (84%)	25 (16%)	154
Inoculation after pre-enrichment	94 (47%)	106 (53%)	89 (92%)	8 (8%)	97

Campylobacter with any method. *Campylobacter* could be detected in 21 samples only with Method 1 but not by the other two methods. Among samples that did not produce *Campylobacter* with Method 1, five samples produced *Campylobacter* with Method 2 and 4 samples produced *Campylobacter* with Method 3, and 3 samples produced *Campylobacter* with either of the other two methods.

Percentages of *C. coli* among total *Campylobacter* isolates were 87.1% by the colon mucosa streaking (Method 1), 83.7% by the direct swab streaking (Method 2), and 91.7% by the Preston pre-enrichment method (Method 3). In the case of *C. lanienae*, 16 isolates were obtained only with Method 1, 12 isolates by Method 2, and four isolates with Method 3. Compared with this result, 16 samples produced *C. coli* only with Method 1.

DISCUSSION

Although fecal-based methods are still the most widely used and considered to be reliable detection methods of *Campylobacter* in animals, their detection ranges are variable with each procedure [2]. The most widely used method for the surveillance of *Campylobacter* in animals is direct streaking of a fecal swab onto selective and nonselective media containing various combinations of antibiotics to suppress the growth of other bacteria, or blood and charcoal to protect *Campylobacter* against toxic oxygen derivatives, and incubation at 42°C [14]. Several media have been developed and evaluated for *Campylobacter* isolation from clinical and animal samples [9, 13]. In our previous study [17], both Brucella solid medium with supplemented 10% horse serum and antibiotic or Campy blood selective medium without antibiotic supplement were used. Madden *et al.* [11] reported a 100% isolation rate of *Campylobacter* from porcine with direct anal swab on mCCDA. When the Preston protocol and Park-Sanders protocol were compared, Preston protocol requires less labor and produces a better isolation rate [7, 8]. Therefore, mCCDA and the Preston protocol were used to isolate *Campylobacter* from porcine colon in this study.

In our previous study [17], only *C. coli* was detected from porcine colon when *Campylobacter* species were isolated with direct streaking of intestinal contents. In this study, *C. lanienae* was also isolated in 13% (8.3%–16.2%) of the samples. Detection of *C. lanienae* in this study may be due to the medium, mCCDA.

Pre-enrichment in Preston broth was less efficient than the other methods. This may be due to the presence of numerous microorganisms in the sample during pre-enrichment, which competitively out-grow, making it difficult to isolate *Campylobacter*.

Results showed that the direct streaking of colon mucosa on mCCDA is best for the isolation of *Campylobacter* species from porcine intestines. This method is fast and does not require the pre-enrichment step, with the best isolation efficiency. We think that this is due to the shorter preparation time, which lessens the damage of *Campylobacter* from oxygen.

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