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Efficacy of supplemented buffered peptone water for the isolation of *Campylobacter jejuni* and *C. coli* from broiler retail products

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Abstract

Broiler retail samples ($n=113$) were analyzed to determine (i) the effectiveness of buffered peptone water (BPW) supplemented with blood and antibiotics for the isolation of *Campylobacter jejuni* and *C. coli*, (ii) if a 1:4 enrichment ratio performs similarly as a 1:9 ratio, and (iii) if BPW is similar to Bolton broth for enumeration of *Campylobacter* spp. in retail broiler meat using the most probably number (MPN) procedure. Chi-square comparison showed that BPW performed similarly as Bolton broth ($P \leq 0.05$) for *Campylobacter* isolation in breast tenders, boneless breasts, split breasts and skin samples. However, BPW showed a lower detection rate ($P \geq 0.05$) for thighs and boneless thighs. When the results were combined, BPW performed similarly as Bolton broth for the isolation of *Campylobacter* spp. ($P \leq 0.05$). BPW at an enrichment ratio of 1:4 was statistically similar to Bolton broth or BPW at a ratio of 1:9. No differences were observed between the MPN data from Bolton broth and the MPN data from BPW ($P \leq 0.50$). A multiplex PCR assay revealed that ca. 48% of the isolates obtained from Bolton broth and 59% of the isolates obtained with BPW were *C. coli*. Both Bolton broth and BPW allowed for the growth of *C. jejuni* and *C. coli* from the same sample. Remarkably, a large genomic variability was observed by PFGE analysis of the isolates collected from the same sample with Bolton broth or BPW, which confirms that more than one genotype can successfully multiply during enrichment and be recoverable on agar plates. These findings suggest that BPW could be used as an enrichment medium for isolation of *Campylobacter* from retail broiler samples. The implications of the high number of *C. coli* isolates found in this study is discussed.

Keywords: Broiler retail meat; Buffered peptone water; *Campylobacter coli*; *Campylobacter jejuni*

1. Introduction

In the US, retail poultry meat samples are highly contaminated with *Campylobacter* spp., reported to be 69 (Willis and Murray, 1997), 71 (Zhao et al., 2001) and 82% (Dickins et al., 2002). As an alternative to enrichment, filtration and centrifugation techniques are commonly used to concentrate *Campylobacter* spp. cells in samples where the numbers of bacteria are low (<1 log), such as retail broiler meat samples, and where direct quantification on agar plates is limited (Dickins et al., 2002; Willis and Murray, 1997). These limitations have been recently highlighted for the new quantitative method for *Campylobacter* by the International Organization for Standardization by the European Union (Hutchison et al., 2006).

Presently, a useful enrichment procedure of a retail broiler sample is needed to determine if the sample was contaminated with *Campylobacter* cells or not (Baylis et al., 2000; Kramer et al., 2000; Willis and Murray, 1997). Several enrichment broths have been developed for the isolation of *Campylobacter* from retail poultry meat (Corry et al., 1995; Doyle and Roman, 1981; Hunt et al., 2001). In general, enrichment broths contain some of the components used originally in the development of

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plate media for isolation of *Campylobacter* spp. from fecal samples in humans. The typical composition of an enrichment broth comprises a rich, basal medium, such as Brucella broth or Nutrient broth (Bolton and Robertson, 1982; Corry et al., 1995), antimicrobials, and the addition of lysed horse or sheep blood. The basal medium is added under the assumption that thermotolerant *Campylobacter* spp. are "fastidious" pathogens that require complex media to grow (Corry et al., 1995). However, we have an incomplete understanding of how other basal media, such as buffered peptone water (BPW), could perform for the isolation of *Campylobacter* spp. from retail meat. Bolton and Robertson (1982) used nutrient broth No. 2, peptone and sodium chloride, to develop Preston enrichment broth. However, poultry meat samples enriched in Preston broth exhibited less *Campylobacter* growth than Bolton broth and *Campylobacter* enrichment broth (Baylis et al., 2000). A blood-free enrichment medium with a composition similar to that of Bolton broth has been tested for the growth and isolation of *Campylobacter* spp. under aerobic condition and different food samples. The efficacy of this medium was affected by the food type and bacterial strain (Tran, 1998), which highlighted our lack of understanding of the impact of each component of a medium on the efficacy to isolate *Campylobacter* from food samples.

Buffered peptone water (BPW) is the medium recommended by the U. S. Department of Agriculture Food Safety and Inspection Services for the collection of microbiology samples with the rinse method from broiler carcasses (Anonymous, 1996). A successful enrichment with BPW could help with a direct enrichment of carcass rinses collected from processed broilers for the isolation of *Campylobacter* spp. Yet, such a medium must be validated with samples containing relatively low numbers of *Campylobacter* cells. The objectives of this study were: (i) to study the effectiveness of BPW supplemented with blood and antibiotics for the isolation of thermotolerant *Campylobacter* spp. from retail broiler meat; (ii) to determine if a 1:4 enrichment ratio performs similarly as a 1:9 ratio; and (iii) to assess if BPW is similar to Bolton broth for enumeration of thermotolerant *Campylobacter* spp. in retail broiler meat using the most probably number procedure. Isolates were identified with a multiplex polymerase chain reaction (PCR) assay and the extent of the DNA variability of isolates from the same sample was determined using pulsed field gel electrophoresis (PFGE).

2. Materials and methods

2.1. Sample collection

A total of 113 broiler retail samples were purchased from different retail stores in Auburn, Alabama, and kept at refrigeration temperatures (~4 °C) until processing within 5 h of purchase. Samples were grouped as breast tenders, boneless breasts, split breasts, thighs (including boneless thighs), and skin from breast and thighs. Samples were collected throughout 13 collection times.

2.2. Sample preparation and enrichment procedures

From each tray pack, one piece of meat was sampled by weighing 25 g of product twice (two sub-samples). The samples were placed in sterile Whirl-Pak® bags (Nasco, Fort Atkinson, WI). One sub-sample was enriched at a 1:9 ratio (w:v), where 25 g was dissolved in 225 ml of buffered peptone water (BPW, Acumedia, Baltimore, MD) supplemented with 5% lysed horse blood (School of Veterinary Medicine, Auburn University, Institutional Animal Care Committee Protocol PRN 2004–0623) and 2 mg/l of amphotericin B, 32 mg/l of cefoperazone, 10 mg/l of trimethoprim, and 10 mg/l of vancomycin (Sigma-Aldrich, St. Louis, MO). In parallel, the other sub-sample was enriched in Bolton broth following the specifications of the manufacturer (Oxoid Inc., New York, NY). Briefly, the Bolton basal medium (CM0983) was combined with Bolton broth selective supplements (SR183) and with 5% lysed horse blood. These sub-samples were enriched at a 1:9 ratio (w:v). Enriched samples were incubated at 42 °C for 24 h under a microaerophilic gas mixture containing 10% CO_2, 5% O_2, and 85% N_2 (Airgas, Radnor, PA) in sealed, plastic bags (Oyarzabal et al., 2005), or in anaerobic jars gassed with a MACSmics Jar Gassing System (Microbiology International, Frederick, MD).

2.3. Campylobacter isolation and presumptive identification

All enriched samples were plated out for isolation and identification of *Campylobacter* spp. A sterile cotton swab (Fisher Scientific, Pittsburgh, PA) was used to transfer ~0.1 ml of the enrichment broth onto modified Campy-Cefex (mCC) agar (Oyarzabal et al., 2005; Stern et al., 1992) plates. Plates were then incubated at 42 °C under microaerophilic conditions for 48 h and then screened for typical *Campylobacter* colonies. Colonies were considered presumptive positive when they showed the typical morphology and motility under phase contrast microscopy (Optiphot-2, Nikon, Tokyo, Japan) and when they were catalase and oxidase positive. All presumptive isolates were collected and individually stored at −80 °C in tryptic soy broth (TSB, Difco, Detroit, MI) supplemented with 30% glycerol (vol/vol) and 5% blood. These isolates were used for further identification with a multiplex PCR assay and characterization by PFGE.

2.4. PCR identification of Campylobacter isolates

For PCR analysis, stock cultures were transferred to mCC plates. Plates were incubated under microaerophilic conditions at 42 °C for 24 h. Bacterial DNA was extracted using PrepMan™ Ultra (Applied Biosystems, Foster City, CA). All the DNA was tested with a multiplex PCR that has been used for the identification of *C. jejuni* and *C. coli* from swine samples (Cloak and Fratamico, 2002) and processed broilers carcasses (Oyarzabal et al., 2005). With this multiplex PCR, isolates that react with primers targeting the *ceuE* gene are *C. coli* (Gonzalez et al., 1997), while those isolates that react with primers targeting an undefined portion of DNA from the genome of *C. jejuni* are