Evaluation of ISO 10272:2006 standard versus alternative enrichment and plating combinations for enumeration and detection of *Campylobacter* in chicken meat

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Abstract— **In the present study, we evaluate the recommended ISO 10272:2006 versus alternative procedures for *Campylobacter* enumeration and enrichment in naturally contaminated chicken meat samples (*n*= 49). Three enrichment media were evaluated; Bolton broth, Preston broth and CampyFood broth® (bioMérieux SA, Marcy l'Etoile, France). In addition, three selective plating agars were compared; modified charcoal cefoperazone deoxycholate agar (mCCDA), CampyFood agar® (CFA; bioMérieux SA) and Brilliance CampyCount agar® (BCC; Oxoid, Basingstoke, England). Direct plating on CFA provided the highest number of *Campylobacter* positive samples (17/49); however this was not statistically different (*P* > 0.05) from numbers of positive samples recovered by direct plating on mCCDA (15/49) or BCC agars (14/49). Also, there was no significant difference between *Campylobacter* counts on the three compared media (*P* > 0.05). Enrichment of chicken meat samples in Bolton broth for 48 h and subsequent plating on CFA provided significantly higher (*P* < 0.05) *Campylobacter* detection compared to the other broth-agar combinations. Enrichment in Preston broth for 24 h followed by plating on mCCDA gave a higher number of positive samples (20/49) than 48 h enrichment in Bolton broth and plating on mCCDA (15/49). Enrichment in Bolton broth for 48 h followed by plating on CFA recovered 35% of samples below the limit for quantifications (< 10 CFU/g, *n*= 34), as identified by direct plating on mCCDA. Compared to the current ISO method, some alternative combinations of enrichment and agar media could provide significantly better detection and enumeration of *Campylobacter* in chicken meat.**

Keywords— ***Campylobacter*, methods evaluation.**

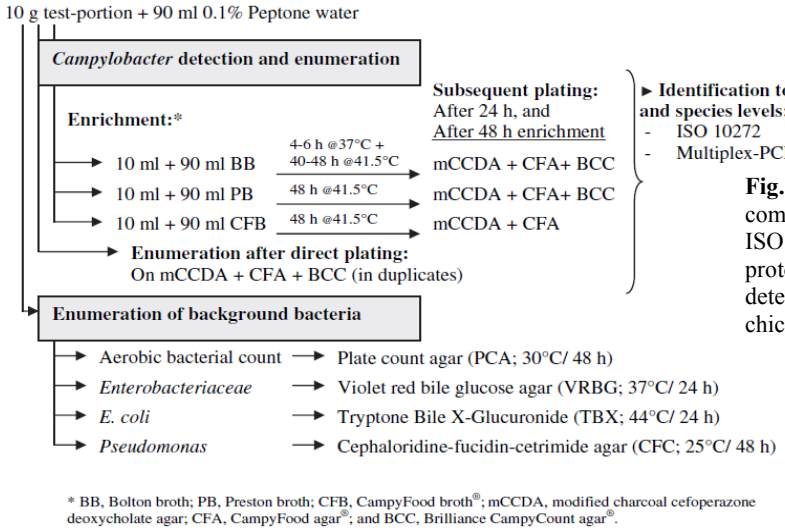
I. INTRODUCTION

Campylobacter jejuni and *Campylobacter coli* are among the most common bacterial causes of human gastroenteritis worldwide. The majority of human campylobacteriosis cases are sporadic, and consumption or mishandling of contaminated raw or undercooked poultry meat is believed to be an important vehicle of infection [1].

Effective monitoring and risk assessment of *Campylobacter* contamination in chicken meat is largely dependent on the availability of reliable detection and enumeration methods. In the present study, we evaluate the recommended ISO 10272:2006 procedures for *Campylobacter* enrichment and enumeration versus alternative combinations of enrichment and plating media. The evaluation was performed on naturally contaminated chicken meat samples and included evaluation of two new selective agars and a new enrichment broth.

II. MATERIALS AND METHODS

The evaluated procedures involved testing the performances of three enrichment media; Bolton broth ((CM983 with supplement SR183, Oxoid, Basingstoke, England), recommended in the current ISO standard method), Preston broth (CM689 with supplement SR117, Oxoid), recommended in the former version of the ISO standard method) and a new broth medium, CampyFood broth® (CFB; bioMérieux SA, Marcy l'Etoile, France). CFB is commercialized as a liquid in ready-to-use plastic bags, and its patented composition is currently confidential. In addition, three selective plating agars were evaluate; modified charcoal cefoperazone deoxycholate agar (mCCDA, Oxoid), which is recommended in the current ISO standard method, the chromogenic-like agar CampyFood agar® (CFA, bioMérieux SA) and the chromogenic agar Brilliance CampyCount agar® (BCC, Oxoid).



* BB, Bolton broth; PB, Preston broth; CFB, CampyFood broth®; mCCDA, modified charcoal cefoperazone deoxycholate agar; CFA, CampyFood agar®; and BCC, Brilliance CampyCount agar®.

Fig. 1 Schematic summary of the comparison procedures of the ISO standard versus alternative protocols for *Campylobacter* detection and enumeration in chicken meat samples

In total, 49 samples of chicken meat were included in the present study. Samples were collected from February to June 2010, from local supermarkets and poultry processing companies. A schematic representation of the analysis protocol and different enrichment/plating combinations evaluated in this study is given in Figure 1. Confirmation of presumptive colonies to the genus level was conducted as described in the ISO 10272-1:2006 method [2].

III. RESULTS

Direct plating on CFA provided the highest number of *Campylobacter* positive samples (17/49); however this was not statistically different ($P > 0.05$) from numbers of positive samples recovered by direct plating on mCCDA or BCC agars. Also, there was no significant difference between the overall means of *Campylobacter* counts obtained with the three media ($P > 0.05$). Twenty samples were positive for *Campylobacter* by direct plating on one or more of the three selective agars. The positive detection agreement between the three agars was only in 10 of these 20 samples. For *Campylobacter* recovery by direct plating, false-negative findings varied between the three agars. The rate of false-negatives was significantly less ($P < 0.05$) on CFA (3/10) compared to mCCDA (5/10) and BCC (6/10).

Table 1 indicates that 31 samples (63.3%) were positive for *Campylobacter*, based on different combinations of enrichment-plating procedures. However, all combinations of enrichment and subsequent plating could lead to false-negative results. This varied from as low as 5/49 samples, using 48 h enrichment in Bolton broth and subsequent plating on CFA, to 18/49 samples when using 48 h enrichment in CFB and subsequent plating on mCCDA.

Table 1 Variation between enrichment broths-subsequent plating media[a] combinations of the number of samples in which *Campylobacter* was detected after 48 hr enrichment

No. of samples	Bolton broth			Preston broth			CampyFood broth	
	mCCDA	BCC	CFA	mCCDA	BCC	CFA	mCCDA	CFA
18	−	−	−	−	−	−	−	−
6	+	+	+	+	+	+	+	+
4	−	−	+	+	+	+	−	−
3	−	−	−	+	+	+	−	−
3	−	−	+	−	−	−	−	−
2	+	+	+	−	−	−	−	−
2	+	+	+	+	+	+	−	−
2	−	−	+	+	+	+	+	+
1	+	+	+	+	−	−	−	−
1	+	+	+	+	+	+	−	+
1	+	−	+	+	+	+	−	−
1	+	+	+	−	−	−	+	+
1	−	+	+	−	−	−	−	−
1	−	−	−	−	−	−	+	+
1	−	−	−	+	−	+	+	+
1	+	+	+	−	−	−	+	+
1	−	+	+	+	+	+	+	+

[a] mCCDA, modified charcoal cefoperazone deoxycholate agar; CFA, CampyFood agar; BCC, Brilliance CampyCount.

Enrichment of chicken meat samples in Bolton broth for 48 h followed by plating on CFA provided significantly higher ($P < 0.05$) *Campylobacter* detection compared to other combinations. The fastest enrichment recovery of *Campylobacter* was obtained

using Bolton broth and subsequent plating on CFA (Figure 2). However, Preston broth gave a consistent enrichment performance, regardless of the subsequent plating agar; as presented in Figure 2, number of *Campylobacter* positive samples after enrichment in Preston broth for just 24 h was significantly higher ($P < 0.05$) than the number of positive samples detected after the same period of incubation in Bolton broth or CFB. Added to that, extending the enrichment period to 48 h in Preston broth was not associated with a significant increase ($P > 0.05$) in the number of *Campylobacter* positive samples, compared to 24 h enrichment. Enrichment in Preston broth for 24 h followed by plating on mCCDA gave a higher number of positive samples than 48 h enrichment in Bolton broth and plating on mCCDA (Fig. 2).

To evaluate the potential of enrichment protocols in recovering low levels of *Campylobacter* contamination, samples (n= 34) identified as below the limit for quantifications (< 10 CFU/g) by direct plating on mCCDA were further examined for the correspondent results with enrichment. Enrichment in Bolton broth for 48 h followed by plating on CFA recovered *Campylobacter* from 35% (12/34) of the samples in which *Campylobacter* was below limit for quantification; while enrichment in Preston broth allowed recovery of *Campylobacter* from 29% (9/34) of the samples regardless of the subsequent plating agar.

IV. DISCUSSION

Results from the present study indicate that the performances of CFA and BCC media are comparable to that of mCCDA for the purpose of enumeration of *Campylobacter* from naturally contaminated chicken meat. The ease of discerning (colored colonies on a transparent agar background) is important for reducing errors in counting. In recent years, there has been a growing demand for quantitative data to describe the occurrence and dynamics of *Campylobacter* in the broiler meat chain, especially to support quantitative risk assessment modelling [3]. Given the high level of correlation between counts on mCCDA and CFA or BCC media, the easier discernment of presumptive colonies on the new chromogenic agars, than on the ISO recommended mCCDA, is advantageous.

Another intriguing finding of this study was that the former ISO approach of enrichment in Preston broth and subsequent plating on mCCDA gave significantly higher recovery of *Campylobacter* than the current ISO approach of enrichment in Bolton broth and subsequent plating on mCCDA. Unlike the Bolton-mCCDA combination (in which cefoperazone is

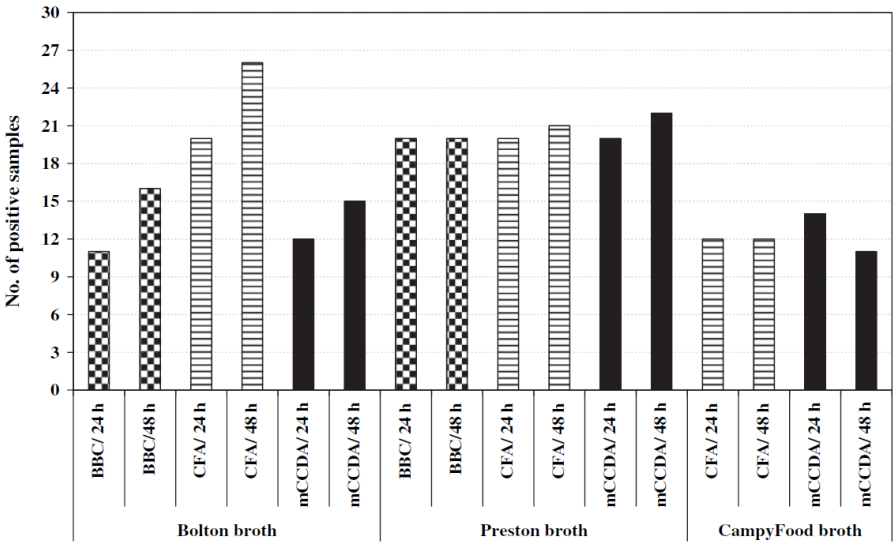


Fig. 2 Detection of *Campylobacter* from chicken meat samples (n= 49) in relation to enrichment broth, duration of incubation and subsequent plating agars (mCCDA, modified charcoal cefoperazone deoxycholate agar; CFA, CampyFood agar®; and BCC, Brilliance CampyCount agar®).

included in both the broth and the agar), the combination Preston-mCCDA is composed of a selective agar with an antimicrobial supplement (based on cefoperazone) that differs from that in the enrichment broth (which is based on rifampicin and polymxin). Theoretically, the later combination would be the best for isolating *Campylobacter*. Nevertheless, all of the evaluated combinations of enrichment broth and subsequent plating agars could give false-negative results.

Compared to Bolton broth and Preston broth, the newly released CFB medium showed lower recovery of *Campylobacter* from chicken meat samples. No reason for that can be suggested, as the medium formulation is currently under patent right. However, CFB medium is commercialized in a liquid format, in ready-to-use mini bags. This format is not common among food microbiology media, which mostly are provided in dehydrated powdered form. Whether the unusual format of CFB medium has an impact on its efficacy (e.g. the broth might be more exposed to light and susceptible to temperature fluctuations during delivery to and storage in the laboratory) needs to be evaluated further by the manufacturer.

In the present study, sample enrichment gave higher rates of *Campylobacter* recovery than direct plating. The number of *Campylobacter* in some samples may not have been large enough to allow for the recovery by direct plating. If this were the case, then enrichment would increase the rate of *Campylobacter* isolation from such samples, especially if the bacterial cells are injured [4]. Enrichment in Bolton broth and subsequent plating on CFA provided the highest recovery of *Campylobacter* from samples with low *Campylobacter* contamination levels (<10 CFU/g), with *Campylobacter* being recovered from almost twice as many samples as by the present ISO enrichment protocol. Proper choice of enrichment broths, depending on sample ecology and *Campylobacter* injury status, is important for the rapid and efficient enrichment of *Campylobacter* spp. in chicken meat samples [5].

V. CONCLUSIONS

The present study adds to the current knowledge on performance evaluation of detection methods for *Campylobacter* in chicken meat. New selective agars (CFA and BCC) showed an attractive performance for easy and precise *Campylobacter* enumeration. Successful recovery of *Campylobacter* after enrichment in Bolton broth is very dependent on the choice of subsequent plating agar, and our findings show that CFA performed significantly better than mCCDA medium when coupled with Bolton broth. On the other hand, enrichment in Preston broth provides fast and consistent recovery, regardless of the subsequent plating media.

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