Research Note

Evaluation of a New Chromogenic Medium for Direct Enumeration of Campylobacter in Poultry Meat Samples

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ABSTRACT

The present study was conducted to compare Campylobacter counts obtained by three selective media: modified charcoal cefoperazone-deoxycholate agar (mCCDA), Campy Food agar (CFA), and a novel agar RAPID’Campylobacter agar. Analysis of 12 artificially and 36 naturally contaminated samples indicated no significant differences in Campylobacter counts obtained with all three selective media. Lin’s concordance correlation coefficient (CCC) and the Bland-Altman plot revealed a high level of agreement between Campylobacter counts when evaluating RAPID versus mCCDA and CFA plates. RAPID agar was the only medium tested that could effectively suppress the growth of the background microflora with naturally contaminated samples. Results of this study clearly indicated that RAPID agar is highly selective without loss of sensitivity for recovering Campylobacter. Results obtained are in agreement with those for other commonly used media; therefore, RAPID medium is suitable for Campylobacter enumeration in poultry meat samples.

Even though the number of confirmed reported human campylobacteriosis cases decreased in European Union in 2012, Campylobacter remains the most frequently reported cause of bacterial gastroenteritis (5). Chicken broiler meat is the main vehicle for campylobacteriosis in humans and accounts for approximately 30% of all cases in Europe (4, 7). Slaughter and further processing of broiler carcasses carrying Campylobacter leads to contamination and cross-contamination of broiler meat along the food supply chain (12, 14).

A decrease of the prevalence of Campylobacter at the primary production stage would significantly reduce the public health risk, although at present eradication of Campylobacter infection in broiler flocks at the end of the rearing period is not feasible (10, 15).

The risk of campylobacteriosis might also be limited by interventions later in the food chain, based on reduction or control of the level of Campylobacter carcass contamination. Following a successful intervention in New Zealand (16), where a mandatory quantitative target for Campylobacter on poultry carcasses was introduced, both national and international authorities have considered implementation of quantitative criteria for Campylobacter in fresh poultry meat (4, 17). Nevertheless, such regulations require a reliable microbiological method for Campylobacter quantification. The most commonly applied method for enumeration of Campylobacter is the ISO standard 10272-2 (11), which uses modified charcoal cefoperazone-deoxycholate agar (mCCDA) plates. However, quantification of colorless Campylobacter colonies on black agar is difficult and time consuming. In contrast, working with Campy Food agar (CFA) plates is less laborious and leads to the same results (9, 18). Nevertheless, when analyzing naturally contaminated samples, background microflora may grow on CFA and mCCDA plates, which may result in overestimation of Campylobacter colonies (1).

The aim of the present study was to evaluate a newly developed agar (RAPID’Campylobacter agar) for quantification of Campylobacter in poultry samples and to compare results with those from the widely used mCCDA and CFA plates.

MATERIALS AND METHODS

Samples overview. Both artificially inoculated (n = 12) and naturally contaminated (n = 36) poultry samples were used within this study.

The artificially inoculated samples were spiked by a third party laboratory. Three of these samples did not contain Campylobacter cells, six were contaminated with Campylobacter jejuni, and three were contaminated with Campylobacter coli, both strains of poultry origin. Inoculum levels were 103 to 105 CFU/g. All samples were analyzed on the day of delivery.

Naturally contaminated samples (n = 36) were collected during six visits in broiler slaughterhouses when Campylobacter-positive birds were slaughtered. The Campylobacter-positive status of these birds was established previously at the farm by examination of cecal droppings for Campylobacter. During every slaughterhouse visit, six carcasses were collected aseptically after chilling, transported to the laboratory under chilled conditions, and analyzed on the same day.

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**TABLE 1. Comparison of Campylobacter counts from artificially and naturally contaminated samples plated on three media**

<table>
<thead>
<tr>
<th>Medium</th>
<th>No. of samples</th>
<th>Campylobacter counts (log CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Below detection limit</td>
<td>Uncountable(^a)</td>
</tr>
<tr>
<td>Artificially inoculated samples (n = 12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mCCDA</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>CFA</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>RAPID</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Naturally contaminated samples (n = 36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mCCDA</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>CFA</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RAPID</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

\(^a\) Campylobacter colonies were uncountable because of swarming.

**RESULTS**

Campylobacter was detected in 9 of the 12 artificially inoculated samples. Additionally, 36 % of the naturally contaminated breast skin samples were positive when the limit of detection (0.1 CFU/g) was applied (Table 1). Figure 1 shows Campylobacter colonies on RAPID (Fig. 1A), mCCDA (Fig. 1B), and CFA (Fig. 1C) plates. As expected, no Campylobacter growth was observed on mCCDA, CFA, and RAPID plates. However, on mCCDA and RAPID plates, Campylobacter was not quantified for four and three of the naturally contaminated samples, respectively, because of Campylobacter swarming. In one of these samples, Campylobacter swarmed on both mCCDA and RAPID plates; thus, six samples were excluded from the analysis.

Mean Campylobacter counts from artificially inoculated samples were 4.10 to 4.16 log CFU/g; and no significant differences between plates were found with the repeated measures general linear model (P = 0.971) (Table 1). When analyzing naturally contaminated samples, only RAPID agar completely inhibited the growth of the background microflora. With the repeated measures general linear model, no significant differences between agars were detected when comparing Campylobacter counts from naturally contaminated samples (P = 0.110) (Table 1). C. jejuni and C. coli were recovered from 27 and 9 naturally contaminated samples, respectively. No significant
difference in counts was found between \textit{Campylobacter} species \((P > 0.2)\). Differences in \textit{Campylobacter} counts were not associated with \textit{Campylobacter} species for any of tested media \((P > 0.1)\).

Close agreement in \textit{Campylobacter} counts was found between RAPID and the other two media. The CCC for overall (artificially and naturally contaminated samples) \textit{Campylobacter} counts on RAPID and mCCDA was 0.973 \((95\% \text{ confidence interval [CI], 0.949 to 0.986})\) (Fig. 2A). For CFA versus RAPID, the CCC was 0.978 \((95\% \text{ CI, 0.961 to 0.988})\) (Fig. 2B). The Bland-Altman plot (Fig. 3) shows little variation in \textit{Campylobacter} counts between RAPID and mCCDA (Fig. 3A) and CFA (Fig. 3B). The main difference between RAPID and CFA plates was 0.04 log CFU/g, and we estimated that 95\% of counts on RAPID will be situated between 0.38 log CFU/g below counts on CFA and 0.46 log CFU/g above the counts on CFA. Similar narrow intervals were indicated when plotting averages against differences in \textit{Campylobacter} counts on RAPID versus mCCDA. The main difference accounted for −0.05 log CFU/g, and the 95\% limits of agreement were −0.53 and 0.44 log CFU/g.

\textbf{DISCUSSION}

Implementation of quantitative criteria for \textit{Campylobacter} in fresh poultry meat requires reliable techniques for \textit{Campylobacter} quantification. Development of a highly selective medium that suppresses growth of background microflora might limit the number of confirmation tests and consequently increase the feasibility of cost-effective and simple \textit{Campylobacter} enumeration. The present study was conducted to compare the new chromogenic RAPID medium with mCCDA, recommended by ISO 10272-2, and another commercially available medium, CFA, for \textit{Campylobacter} enumeration in poultry samples.

No significant differences in \textit{Campylobacter} counts were detected on three agar types for artificially and naturally contaminated samples. Habib et al. \((9)\) and Ugarte-Ruiz et al. \((18)\) compared \textit{Campylobacter} counts on mCCDA and CFA but found no significant differences between these two media. However, Ahmed et al. \((1)\) found that \textit{Campylobacter} counts on CFA were significantly lower.
than those on mCCDA for artificially contaminated samples and that the recovery rate on different media differed with Campylobacter species. In contrast, in the present study no significant difference in Campylobacter counts was observed between the three media evaluated. One explanation for this difference is that the tested samples could have contained different Campylobacter strains.

Obtained results revealed a high level of agreement between results on RAPID plates and those on mCCDA and CFA plates, as demonstrated by the CCC values and the Bland-Altman plots. In agreement with our findings, a high concordance in Campylobacter counts recovered on CFA and mCCDA plates has been previously reported (7).

Similar to previous studies (1, 9), growth of non-Campylobacter colonies was observed on both mCCDA and CFA plates. Therefore, correct identification and quantification of Campylobacter on mCCDA and CFA plates requires trained and experienced personnel and time for confirmation tests (i.e., microscopic observation, biochemical testing, or PCR). In contrast, background microflora was absent on RAPID plates. As previously reported by other authors (6, 8), in the present study Campylobacter colonies swarmed on some mCCDA plates, making it impossible to obtain accurate colony counts. Similar swarming occurred on RAPID medium, but on CFA plates Campylobacter colonies from all samples had a regular round shape. This difference between media might be explained by the variability in the humidity of the plates. Although in the present study all plates were dried for the same amount of time (30 min) before inoculation, additional experiments revealed that a longer drying period can inhibit the spread of Campylobacter colonies on RAPID plates.

In conclusion, in the present study a high level of agreement in Campylobacter counts was found for RAPID plates and the other two media (mCCDA and CFA). RAPID agar also was the only medium of the three tested that effectively suppressed the growth of the background microflora in naturally contaminated samples. Consequently, this new chromogenic medium is a reliable alternative for less labor intensive Campylobacter quantification from poultry samples.

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**REFERENCES**