Short Communication

Comparison of Two Methods for the Recovery of *Campylobacter* from Chicken Carcasses

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Abstract

Campylobacteriosis, one of the most common food-borne diseases in humans, is mainly caused by the consumption of chicken meat contaminated with *Campylobacter.* In this study, we examined the recovery rate of *Campylobacter* from carcass rinse using direct plating method and selective enrichment method. *Campylobacter* genotypes obtained from both methods were also determined using *flaA*-short variable region (*flaA*-SVR) sequencing. Results showed that *Campylobacter* recovery rates by the direct plating method and selective enrichment method were 40.00% and 46.86%, respectively. However, when a combination of the two methods was used, the recovery rate increased to 64.57%. For genetic characterization of *Campylobacter*, 9 *flaA*-SVR types were found by both methods. The most common allele types identified among *Campylobacter* isolates from these isolation methods were *flaA*-SVR allele 208, 769 and 783. High Simpson's index of diversity (SID) was observed for both direct plating method (SID = 0.843) and selective enrichment method (SID = 0.820). Additionally, good strength of agreement between *flaA*-SVR types obtained from these two methods was noticed (Kappa = 0.629). In conclusion, this study reveals that the direct plating method may be used as an alternative method for *Campylobacter* isolation from chicken carcass rinse since the recovery rate and *Campylobacter* genotypes obtained from this method was quite similar to those obtained from the selective enrichment method. Moreover, the direct plating method is also simple, less labor-intensive and more cost-effective than the selective enrichment method.

Keywords: *Campylobacter*, chicken carcasses, direct plating method, selective enrichment method

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Introduction

Campylobacter is one of the most common causes of diarrhea in many countries worldwide (CDC, 2015; FSA, 2015). Diarrhea caused by *Campylobacter* is mainly associated with the consumption of undercooked poultry meat or cooked food cross-contaminated with raw chicken meat juice via kitchen tools (Humphrey et al., 2001; Luber et al., 2006). Since the contamination of this organism in chicken meat mostly occurs at slaughterhouses, several attempts are made to recover *Campylobacter* from chicken carcasses during slaughter in order to effectively control and reduce *Campylobacter* contamination in retail chicken meat. However, the isolation of *Campylobacter* from carcass rinse during slaughter is quite difficult as *Campylobacter* might appear in sub-lethally injured form under harsh conditions such as high temperature of scalding process or low temperature of chilling water (Jasson et al., 2007). Therefore, various approaches to recover *Campylobacter* from chicken carcass at slaughter level have been conducted in recent years (Line et al., 2001; Richardson et al., 2009).

Currently, the selective enrichment method with nutrient supplemented broth is widely used for *Campylobacter* isolation from food samples (Jorgensen et al., 2002; Richardson et al., 2009). According to the Advisory Committee on the Microbiological Safety of Food (ACMSF) Surveillance Working Group (2010) and several studies (Jorgensen et al., 2002; Allen et al., 2007), the selective enrichment method with Exeter broth provided good results for *Campylobacter* recovery from different types of sample including carcass rinse. Although nutrient broth used in the selective enrichment method enhances the growth of *Campylobacter*, some contaminating bacteria might be promoted as well. Consequently, the isolation of *Campylobacter* is not totally successful (Musgrove et al., 2001; Kiess et al., 2010). In addition to the selective enrichment method, a direct plating method can also reduce the amount of contaminating bacteria on chicken carcasses (Ugarte-Ruiz et al., 2012). However, only limited information on the application of direct plating method for isolation of *Campylobacter* from chicken carcass rinse is available (Line et al., 2001; Richardson et al., 2009). Therefore, this study aimed to determine whether or not the direct plating method can be used as an alternative method for *Campylobacter* isolation from chicken carcass.

Materials and Methods

Sample collection: One hundred and seventy-five carcass rinses were collected during June 2012 to April 2013. Carcass rinses were obtained by shaking whole carcass with 400 ml of buffer peptone water for 1 min (USDA FSIS, 2013). Samples were transferred to sterile bottle and kept on ice and brought back to the laboratory within 4 h.

Campylobacter isolation*: Campylobacter* isolation from the carcass rinses was performed by both selective enrichment method and direct plating method. The selective enrichment method used in this study was modified from USDA FSIS (2013). Briefly, 25 ml of carcass rinse were added to 25 ml of double strength modified Exeter nutrient broth (Allen et al., 2007) and incubated at 37°C for 48 h under a microaerobic condition (85% N_2, 10% O_2 and 5% CO_2). Following enrichment, 100 µl of the enrichment broth were spread onto modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) in duplication and the inoculated plates were incubated under a microaerobic environment at 42°C for 48 h. For the direct plating method, 100 µl of the carcass rinse were directly spread onto duplicate mCCDA plates. The inoculated plates were then incubated at 42°C under microaerobic conditions as previously described. The presence of *Campylobacter*, characterized by metallic, grayish and flat colonies, on the agar plates was determined and presumptive *Campylobacter* colonies were collected for further identification.

Campylobacter identification: DNA extraction was carried out by conventional boiling method. To confirm *Campylobacter* genus, primers targeting at *Campylobacter* 16S rRNA gene were used. The PCR condition was composed of denaturation at 94°C for 12 min followed by 30 cycles of 94°C for 30 sec, 55°C for 60 sec and 72°C for 60 sec (Linton et al., 1996). Multiplex PCR with primers specific for *hipO* gene and *glyA* gene was used to identify *C. jejuni* and *C. coli* as previously described by Wang et al. (2002). The PCR condition for species identification comprised denaturation at 95°C for 15 min, followed by 30 cycles of 94°C for 20 sec, 55°C for 20 sec and 72°C for 30 sec and then a final extension step at 72°C for 5 min. The PCR product was analyzed by electrophoresis in 1.2% agarose gel. The gel was stained with ethidium bromide and visualized in a UV gel document system. The PCR product of *Campylobacter* genus was 816 bp in length, while a 323-bp amplicon and a 126-bp amplicon were generated for *C. jejuni* and *C. coli*, respectively.

Genotyping of Campylobacter isolates: *Campylobacter* isolates from carcass rinse that was positive by both direct plating method and selective enrichment method were selected for genotyping. Thirty-three *Campylobacter* isolates obtained from each method were genotyped by *flaA*-short variable region (*flaA*-SVR) sequencing according to Meinersmann et al. (1997). A 35-cycle reaction was carried out with denaturation at 96°C for 30 sec, annealing at 52°C for 45 sec and extension at 72°C for 1 min. The PCR product, approximately 425 bp, was purified by Nucleospin® Gel and PCR clean-up kit (MACHEREY-NAGEL, Duren, Germany) and sent to First BASE Laboratories, Malaysia for nucleotide sequencing. The nucleotide sequences were submitted to *flaA*-SVR database to identify *flaA* allele number at http://pubmlst.org/*campylobacter/flaA*.

Data and statistical analysis: Chi Square test was used for comparing differences in *Campylobacter* recovery rate between the direct plating method and selective enrichment method. Differences were considered significant at $p \leq 0.05$. Kappa statistic was used to determine agreement in *Campylobacter* genotypes recovered by both methods. Kappa value was interpreted as described by Altman (1991). Statistical analyses were conducted using SPSS

Statistics Software version 22.0, IBM, NY, USA. Furthermore, diversity of *Campylobacter* genotypes recovered by the direct plating method and selective enrichment method was measured using Simpson's index of diversity available at http://darwin.phyloviz.net/ComparingPartitions/index.php?link=Tool (Phasipol et al., 2013).

Results and Discussion

Recovery rate of Campylobacter from chicken carcass: Out of 175 samples tested, 70 samples (40.00%) were *Campylobacter* positive by the direct plating method, 82 samples (46.86%) were positive by the selective enrichment method and 39 samples (22.29%) were positive by both methods (Fig 1). When the combination of direct plating method and selective enrichment method was used, the recovery rate increased to 64.57%. Although the selective enrichment method had higher *Campylobacter* recovery rate than the direct plating method, the difference between these two methods was not statistically significant ($p = 0.21$). This finding was consistent with a previous study by Line et al. (2001) which found that the *Campylobacter* recovery rate from carcass rinse by the direct plating method and selective enrichment method was 100% similar. Likewise, Richardson et al. (2009) reported no significant difference in the *Campylobacter* recovery rate using the direct plating method (80%) and selective enrichment method with Bolton broth (73.57%). However, when different types of enrichment broth (e.g. TECRA broth) were used, Richardson et al. (2009) found difference in the *Campylobacter* recovery rate between the two methods. It is worth noting that the difference in *Campylobacter* recovery rate between the direct plating method and selective enrichment method might be dependent upon types of broth used for *Campylobacter* enrichment (Jorgensen et al., 2002; Paulsen et al., 2005; Allen et al., 2007).

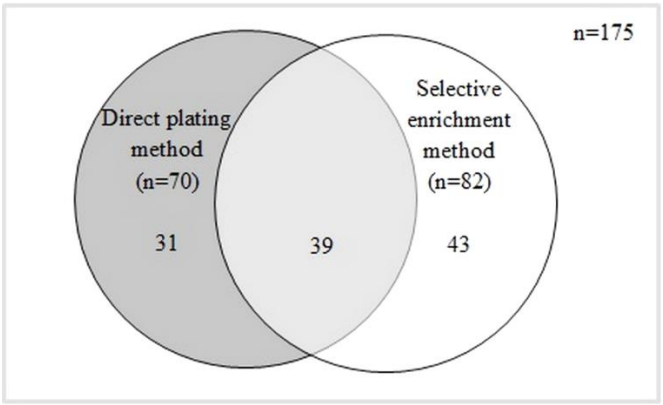

Figure 1 Number of *Campylobacter* positive carcasses by direct plating method and selective enrichment method

In this study, *C. jejuni* was the major species recovered by both methods. Sixty-two out of 70 (88.57%) and 8 out of 70 (11.43%) *Campylobacter* positive carcass rinses obtained from the direct plating method were contaminated with *C. jejuni* and *C. coli*, respectively. Similarly, 72 out of 82 (87.80%) *Campylobacter* positive carcass rinses determined by the selective enrichment method were contaminated with *C. jejuni* and 10 out of 82 (12.20%) carcass rinse samples were contaminated with *C. coli*.

Campylobacter genotypes present in chicken carcass rinse: To determine the genotypes of *Campylobacter* recovered by the direct plating method and selective enrichment method, a pair of isolates obtained from 33 samples that were positive by both methods was included for genotyping. Results showed that 9 *flaA*-SVR types were detected (Table 1). *FlaA*-SVR allele 208, 769 and 783 were the dominant allele types found among the *Campylobacter* isolates from both direct plating and selective enrichment methods. These genotypes accounted for two-thirds of the isolates. In addition to the dominant allele types, other *flaA*-SVR allele types derived from the direct plating method included *flaA*-SVR allele 22, 45, 57, 162, 287 and 581, while *flaA*-SVR allele 45, 236, 253, 287, 312 and 1527 were found among the isolates obtained from the selective enrichment method (Table 1). Good strength of agreement ($K = 0.629 \pm 0.085$) between the genotypes of *Campylobacter* isolates recovered by the direct plating method and selective enrichment method was noticed. Furthermore, a high Simpson's index of diversity was observed for both direct plating method (SID = 0.843 ± 0.034) and selective enrichment method (SID = 0.820 ± 0.037). These findings suggested that a wide variety of *Campylobacter* genotypes was recovered by the direct plating method as well as by the selective enrichment method.

Table 1 Comparison of *flaA*-SVR types among *Campylobacter* recovered by direct plating method and selective enrichment method

Isolation method	*flaA*-SVR allele (No. of isolates)		Simpson's index of diversity (95% CI)
	Dominant	Non-dominant	
Direct plating method	208 (10), 783 (7), 769 (5)	45 (3), 287 (3), 22 (2) 57 (1), 162 (1), 581 (1)	0.843 (0.776-0.910)
Selective enrichment method	208 (10), 783 (9), 769 (5)	287 (3), 1527 (2), 45 (1) 236 (1), 253 (1), 312 (1)	0.820 (0.748-0.892)

In conclusion, the direct plating method might be an interesting alternative for *Campylobacter* isolation from chicken carcass rinse as the recovery rate and *Campylobacter* genotypes obtained from this method were quite similar to those obtained from the selective enrichment method. Moreover, the direct plating method is rapid, simple and more cost-effective compared to the selective enrichment method.

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บทคัดย่อ

การเปรียบเทียบวิธีการเพาะแยกแคมไพโลแบคเตอร์จากซากไก่ 2 วิธี

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โรคติดเชื้อแคมไพโลแบคเตอร์จัดเป็นโรคอาหารเป็นพิษโรคหนึ่งที่พบได้บ่อยในมนุษย์ การติดเชื้อนี้โดยส่วนใหญ่มักเกิดจากการบริโภคเนื้อไก่ที่ปนเปื้อนด้วยเชื้อดังกล่าว การศึกษาครั้งนี้คณะผู้วิจัยได้ทำการตรวจสอบอัตราการเพาะแยกเชื้อแคมไพโลแบคเตอร์จากน้ำล้างซากไก่ด้วยวิธี direct plating และ วิธี selective enrichment รวมทั้งทำการศึกษาลักษณะทางพันธุกรรมของเชื้อที่แยกได้ด้วยวิธี *flaA*-short variable region (*flaA*-SVR) sequencing จากการศึกษาพบว่าร้อยละ 40.00 ของตัวอย่างทั้งหมดให้ผลบวกด้วยวิธี direct plating ในขณะที่ร้อยละ 46.86 ของตัวอย่างให้ผลบวกด้วยวิธี selective enrichment อย่างไรก็ตามหากทำการเพาะแยกเชื้อโดยใช้สองวิธีร่วมกัน พบว่าตัวอย่างที่ให้ผลบวกจะเพิ่มสูงขึ้นเป็นร้อยละ 64.57 เมื่อเปรียบเทียบลักษณะทางพันธุกรรมของเชื้อที่แยกได้ พบจำนวนลักษณะทางพันธุกรรมของเชื้อที่แยกได้จากแต่ละวิธี 9 แบบ โดยลักษณะทางพันธุกรรมหลักที่พบ ได้แก่ *flaA*-SVR allele 208 769 และ 783 หากวิเคราะห์ความหลากหลายทางพันธุกรรมของเชื้อที่แยกได้โดยพิจารณาจากค่า Simpson's index of diversity (SID) พบว่าค่า SID ของเชื้อที่แยกได้จากวิธี direct plating เท่ากับ 0.843 และค่า SID ของเชื้อที่แยกได้จากวิธี selective enrichment เท่ากับ 0.820 เมื่อพิจารณาความสอดคล้องของลักษณะทางพันธุกรรมของเชื้อที่แยกได้จากทั้งสองวิธีโดยการคำนวณค่า Kappa พบว่าให้ผลอยู่ในระดับที่ดี (ค่า Kappa เท่ากับ 0.629) การศึกษาครั้งนี้แสดงให้เห็นว่าวิธี direct plating อาจสามารถนำไปใช้ในการเพาะแยกเชื้อแคมไพโลแบคเตอร์จากน้ำล้างซากไก่ เนื่องจากวิธี direct plating มีอัตราการเพาะแยกเชื้อแคมไพโลแบคเตอร์ และลักษณะทางพันธุกรรมของเชื้อที่แยกได้ ใกล้เคียงกับวิธี selective enrichment นอกจากนี้การเพาะแยกเชื้อด้วยวิธี direct plating ยังทำได้สะดวกกว่า ไม่สิ้นเปลืองแรงงาน และมีความคุ้มค่ากว่าวิธี selective enrichment

คำสำคัญ: เชื้อแคมไพโลแบคเตอร์ ซากไก่ วิธี direct plating วิธี selective enrichment

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