

Efficiency of Mini VIDAS and Culture Technique for Detection of *Campylobacter* Spp. In Minced Beef

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Abstract: This study was performed to evaluate the efficiency of the mini VIDAS automated immunoassay system and conventional culture technique to detect *Campylobacter* species from minced beef enriched in Bolton and Preston broths. A total of 92 minced beef samples were collected from different local markets in Erzurum province in Turkey. Minced beef samples were tested for *Campylobacter* spp. by the mini VIDAS (bioMérieux, France) in parallel with the culture technique using supplemented agar plates following the enrichment at 42°C for 48 h. Positive results were verified by conventional culture technique with quality control strains (ATCC 33559, ATCC 33560) on selective solid media. Out of 92 minced beef samples, 11 (11.95%) and 10 (10.86%) were positive by the mini VIDAS and culture technique respectively. The sensitivity of mini VIDAS was higher, and provided more accurate results than the conventional culture technique. In addition, both broths can be used efficiently for the enrichment.

Keywords: *Campylobacter* spp., conventional culture, Mini VIDAS, minced beef

Sığır Kıymalarında *Campylobacter* Türlerinin Tespitinde Mini VIDAS ve Konvansiyonel Kültür Tekniğinin Etkinliği

Özet: Bu çalışma, Bolton ve Preston brothlarda zenginleştirilmiş sığır kıymalarından *Campylobacter* türlerinin tespitinde, mini VIDAS otomatik immunoassay system ve konvansiyonel kültür tekniğinin etkinliklerinin değerlendirilmesi amacıyla gerçekleştirildi. Erzurum ilindeki (Türkiye) yerel marketlerden toplam 92 sığır kıyma örneği toplandı. *Campylobacter* türlerinin tespiti için sığır kıyması örnekleri, 42°C'de 48 h zenginleştirmeyi takiben mini VIDAS (bioMérieux, France) ve paralel olarak supplement içeren besi yerlerinde test edildi. Pozitif sonuçlar, kontrol suşları (ATCC 33559, ATCC 33560) kullanılarak selektif besiyerlerinde konvansiyonel kültür tekniği ile doğrulandı. İncelenen 92 sığır kıymasının 11 (% 11.95)'i ve 10 (%10.86)'u sırası ile mini VIDAS ve konvansiyonel kültür tekniği ile pozitif bulundu. Konvansiyonel kültür tekniğine göre Mini VIDAS'ın duyarlılığının daha yüksek olduğu ve daha doğru sonuçlar verdiği saptandı. Ayrıca, her iki broth'un zenginleştirme amacıyla etkin olarak kullanılabilceği kanaatine varıldı.

Anahtar kelimeler: *Campylobacter* spp., konvansiyonel kültür, Mini VIDAS, sığır kıyması

Introduction

Campylobacter is a genus of Gram-negative, spiral-shaped bacteria consisting of at least 16 different species. *Campylobacter* spp. are recognized as the most common bacterial cause of foodborne disease in human worldwide (Friedman et al., 2000; Lawley, 2013). Domestic pets, wild birds and wild animals are potential sources of *Campylobacter* infections in human (Aydın et al., 2001a). The agent can be transmitted either through the consumption of contaminated water, raw milk and undercooked animal products or direct contact with infected animals or handling contaminated poultry (Friedman et al., 2000; Giesendorf et al., 1993; Pearson et al., 1993). Although *Campylobacter* species are commonly present in the gastrointestinal tracts of domestic and wild animals as commensal organism (Aydın et al., 2001a), predominantly *C. jejuni* and in a lesser

extent *C. coli* and occasionally *C. lari*, *C. hyointestinalis* and *C. upsaliensis* cause gastroenteritis in human (Lawley, 2013; Zhao et al., 2001). *Campylobacters* can also lead to Reiter's syndrome and Guillain-Barré syndrome as the complications (Ghast et al., 2013; Lawley, 2013; Leonard et al., 2004). In Turkey, *Campylobacteriosis* due to *C. jejuni* were found as 2.25% by Aktaş and Tuncel (1987), 7.5% by Işık et al. (1996) and 1.43% by Aydın et al. (2007) in human with enteritis. Most of the outbreaks encountered in many countries associated with poultry meats, because the poultry carcasses are frequently and heavily contaminated with *Campylobacter* species. On the other hand, lack of isolation (Madden et al., 2001) or lower frequency of isolation of *Campylobacter* species from beef, pork and lamb meat have been reported (Aydın et al., 2007; Lake

et al., 2007; Lawley, 2013; Zhao et al., 2001). Several culture methods have been assessed for the detection of *Campylobacter* spp. in food and other samples. The culture methods mainly consist of spreading the samples onto selective media and incubating under microaerophilic conditions with or without preceding enrichment (Aydin et al., 2001b; Baylis et al., 2000; Chon et al., 2011, 2012; Kim et al., 2009; Reiter et al., 2005; Zhao et al., 2001). However, culture methods are time consuming. Thus, there is a growing necessity for rapid and reliable methods for detecting pathogens such as thermophilic *Campylobacter* spp. in food and stool samples. Therefore, rapid methods including latex agglutination, enzyme immunoassays and polymerase chain reaction have been developed for detecting *Campylobacter* spp. in clinical and food samples. Some of these tests are designed for confirmation of the culture results and the others are not culture dependent and some of them have some limitations (Aras, 2011; Borck et al., 2002; Hoorfar et al., 1999; Omar et al., 2012; Reiter et al., 2010). In recent years, the VIDAS/mini VIDAS CAM, which is an automated enzyme immunoassay received attention (Borck et al., 2002; Paulsen et al., 2005). In previous studies, mini VIDAS was evaluated for the efficiency of the detection of different microorganisms in several food species. However, some authors found the mini VIDAS superior to conventional culture (Oktay & Heperkan, 2006) some others did not (Liu et al., 2009; Chon et al., 2011). Furthermore, the studies evaluating the efficacy of the mini VIDAS to detect *Campylobacter* spp. in beef meat is very limited. To the author's knowledge, there is only a study conducted by Chon et al. (2011) who evaluated the efficacy of three different selective media and VIDAS for the detection of *C. jejuni* from artificially contaminated ground beef. In the study of these authors, the enriched samples in BB were used in the VIDAS *Campylobacter* assay and the VIDAS showed a recovery rate similar to medium combinations.

In the present study, the mini VIDAS and conventional culture technique were compared to determine the efficiency regarding the detection of *Campylobacter* spp. in minced beef enriched in two different enrichment broths, and all of the samples, regardless of enrichment protocol, were tested by mini VIDAS in order to evaluate the performance of BB and PB for beef samples.

Materials and Methods

Samples

A total of 92 minced beef samples (50 g) were collected from local retail in Erzurum province in

Turkey. Samples were stored at 4°C until transferring to the laboratory. All samples were protected from sunlight and processed within two hours after purchase.

Enrichment Protocols and Culture Techniques

After arrival of the samples to the laboratory, each sample was aseptically divided into two portions of approximately 25 g each. One portion was placed in 225 ml PB (Merck, Darmstadt, Germany) in a sealed stomacher bag and the other portion was enriched in 225 ml BB (Merck, Darmstadt, Germany) in sterile glass containers. The enrichment ratio was 1:9. Samples in PB were stomached, whereas the samples in BB were manually shaken to mix the contents. The bottles were then incubated in an anaerobic jar under microaerophilic conditions (5% O₂, 10% CO₂ and 85% N₂) generated by Anaerocult C (Merck, Darmstadt, Germany) at 42°C for 48 h.

Enriched samples in both broths (0.1 ml) were passed through a cellulose membrane filter of 0.65 µm pore size (Millipore Corp., Billerica, MA). All samples were spreaded onto blood free selective modified charcoal-cefoperazone-deoxycholate agar (mCCDA) with antibiotic supplements as described previously (Aydin et al., 2007; Liu et al., 2009). The plates were incubated under microaerophilic conditions (5% O₂, 10% CO₂ and 85% N₂) generated by Anaerocult C (Merck, Darmstadt, Germany). Following the incubation at 42°C for 48 hour, the plates were examined for the presence of either small, gray and drop-like or small and shiny or slimy colonies that are typical for the *Campylobacter* genus. The presumptive isolates on plates (spiral shaped and gram negative) were collected immediately according to the method described by Office International Epizootica Manuel (2008). The subcultures for verification of positive test results were carried out by conventional culture technique with quality control strains (ATCC 33559, ATCC 33560) on selective solid media at 42°C for 48 h under microaerophilic conditions.

Mini VIDAS

The Mini VIDAS *Campylobacter* kit was used according to the manufacturer's instructions (bioMérieux, France). The enriched samples in BB (1 ml) and in PB (1 ml) were used in the VIDAS *Campylobacter* assay. All samples were incubated in BB and PB under microaerophilic conditions (5% O₂, 10% CO₂ and 85% N₂) at 42°C for 48 h, and 10 ml of the sample then heated to 100°C in a water-bath for 15 minutes. The boiled samples were cooled to room temperature and vortexed to break any clots and 0.5 ml transferred to the sample well

of CAM reagent strip (bioMérieux, France). A maximum of 12 strips were loaded onto mini VIDAS and samples were tested automatically. The assay period was approximately 70 minutes.

Analysis of the Data

The difference between two methods was determined with Pearson Chi-Square test using Statistical Package for the Social Sciences version 16.0. Medcalc programme was used for the

sensitivity and specificity (<http://www.medcalc.org/calc/diagnostictest.php>).

Results

Campylobacter spp. was isolated from 10 (10.86%) samples by the conventional culture method whereas the agent was isolated from 11 (11.95%) samples by Mini VIDAS out of 92 samples. There was no statistically significant difference between two methods ($P>0.05$) (Table 1).

Tablo 1. Isolation rates of *Campylobacter* spp. in minced beef samples by Mini VIDAS and Conventional Culture Technique.

Samples	Mini VIDAS		Culture		P-value
	Positive (%)	Negative (%)	Positive (%)	Negative (%)	
92	11 (11.95)	81 (88.05)	10 (10.86)	82 (89.14)	0.817

No difference was found between the performance of BB and PB protocols concerning the detection rates in both conventional culture and mini VIDAS technique (Table 2).

The sensitivity of the mini VIDAS (100%) was higher than culture (90.91 %) and the specificities of both methods were similar (100 %).

Tablo 2. The Detection Rates of the *Campylobacter* spp. in Bolton Broth and Preston Broth by Mini VIDAS and Conventional Culture Technique.

Methods	Bolton Broth		Preston Broth	
	Positive	Negative	Positive	Negative
Culture	10	82	10	82
Mini VIDAS	11	81	11	81

Discussion and Conclusions

The prevalence of *Campylobacter* in retail beef varies widely in different countries. In a survey on beef carcasses conducted in 10 abattoirs in Northern Ireland, no *Campylobacter* was detected in 100 carcasses (Madden et al., 2001). Zhao et al. (2001) reported that 0.5% beef samples yielded *Campylobacter* in Greater Washington, D.C. A national survey in New Zealand revealed that 3.5% of beef and 10% of bobby veal samples were positive for *Campylobacter* (Lake et al., 2007), which is quite similar to our findings. The lower levels of *Campylobacter* in beef may be due to the lower incidence of the agent in cattle (Aydin et al., 2007).

The methods currently used for detecting *Campylobacter* spp. in food are often based on bacterial growth in several culture media. Although methods based on culture are the accepted methods by the authorities, they are time consuming because of the requirement of selective enrichment, selective media and biochemical tests (Baylis et al., 2000; Gharst et al., 2013; Omar et al., 2012). Simple, quick and reliable immunoassay instructions with the ability to detect pathogenic

thermophilic *Campylobacter* spp. are of importance for surveillance purposes in food production. In the recent years, VIDAS/mini VIDAS has been used in several countries (Liu et al., 2009; Reiter et al., 2010). A few studies indicated that this method performs similarly to culture methods for the identification of naturally occurring *Campylobacter* spp. in food. Gharst et al. (2013) and Liu et al. (2009) found that the number of *Campylobacter*-positive samples by mini VIDAS are similar to the results of traditional plating media for contaminated broiler meat. On the other hand, Chon et al. (2011) found more positive samples with the VIDAS than with the selective agars. In the present study, although the specificities of both methods were high (100 %) and the difference between two methods was not significant, we detected relatively more positive samples with the mini VIDAS in consistent with the results of Reiter et al. (2010). In the present study, the sensitivity of the mini VIDAS (100%) was higher than culture (90.91 %), which is consistent with the results of Hoorfar et al. (1999) and Borck et al. (2002) who reported higher sensitivity of mini VIDAS CAM than

the selective agar plates for turkey meat and fecal samples. Paulsen et al. (2005) has also indicated that the mini VIDAS is as good as the standard method with a sensitivity of 97.6% and with a reduction in assay time by 24 h for chilled and frozen meat.

The isolation of *Campylobacter* spp. from food would be difficult because of the existing at low numbers usually together with the higher numbers of competitor organisms. To obtain required sensitivity, it is necessary to enrich the samples in a broth containing selective agents to inhibit the growth of competing bacteria and to increase the concentration of target organism. In addition, enrichment is considered as an important step because of the random distribution nature of the *Campylobacter*, and their existence in clusters or aggregates that limits the direct streaking on solid media. Another reason for the enrichment procedure is to provide good environment for the stressed or injured bacteria for recovering (Omar et al., 2012). The duration of the enrichment may play a role in the detection rate. Reiter et al. (2005) incubated the samples at 30°C for 24 h to shorten the enrichment time but they obtained low isolation rate for *Campylobacter*s. Liu et al. (2009) determined more positive samples at 48 h of enrichment than those obtained at 24 h of enrichment regardless of the broth types. Therefore, in the present study, both BB and PB were incubated at 42 °C for 48 h, and the agent was determined equally (Table 2) in contrast to the studies indicating BB was better than PB for the isolation of *Campylobacter* from naturally-contaminated foods (Baylis et al., 2000), retailed chicken meat (Liu et al., 2009), ground pork (Kim et al., 2009). In our study, determination of no difference between the performance of BB and PB concerning the detection rates in both conventional culture and mini VIDAS techniques may indicate that the isolation of *Campylobacter* from samples is not related to the enrichment process.

In conclusion, Mini VIDAS is a simple and quick method for detecting *Campylobacter* in minced beef providing more sensitive results than conventional culture technique. Both enrichment broths provided satisfactory results for minced beef samples.

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