Detection of *cambylobacter* spp. in stool samples by new methods in comparison to culture

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Abstract: Campylobacter enteritis is a food-borne or waterborne illness caused by *Campylobacter jejuni* and, to a lesser extent, by *Campylobacter coli*. The aim of this study to compare *Campylobacter* spp. detection by molecular method (multiplex polymerase chain reaction) and by 2 immunoenzymatic methods (Premier CAMPY and ImmunoCard Stat Campy to the culture on Skirrow's medium . **Subjects and methods:** In this study 350 stool specimens were collected from patients suffering from gastroenteritis manifestations with a mean age of 58.5 years. Faecal samples were subjected to culture on Skirrow's selective media, multiplex PCR and 2 immunoenzymatic methods, Premier CAMPY ELISA and ImmunoCard Stat Campy. **Results:** Out of 350 stool specimens tested, 23 (6.6%) fulfilled the positivity criteria, i.e., they were positive by culture method or, in case of a negative culture, by a positive molecular method and a positive immunoenzymatic method ,16 were positive by culture and 7 were culture negative but positive by both a molecular method (multiplex PCR) and an immunoenzymatic method. The *Campylobacter* species identified by culture were 10 (62.5%) *C. jejuni* and 6 (37.5%) *C. coli* while multiplex PCR identified 13 (56.5%) *C. jejuni* , 8 (34.8%) *C. coli* and 2 (8.7%) as mixed *C. jejuni and C. coli*. The sensitivity of the multiplex PCR was higher than the ELISA and ImmunoCard Stat Campy tests (100%, 95.6**%** and 86.9% respectively) while the ImmunoCard Stat Campy had higher specificity than the ELISA and multiplex PCR (98.7%, 98.1**%** and 97.9 % respectively). ***Conclusion:*** Multiplex PCR is attractive as it enables the detection and speciation of *campylobacter*. Also the procedure of the multiplex PCR had a rapid turnaround time of 6 h. The Premier CAMPY ELISA was rapid and had acceptable performance sensitivity of 95.6**%**.
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1. Introduction

Campylobacter spp. is a major cause of bacterial gastroenteritis worldwide [1,2]. In the USA, the incidence of *C. jejuni* infections is the second largest after *Salmonella* cases [3]. The relatively low infective dose, the potentially serious squeals [4], as well as the association between certain *Campylobacter* virulence genes and the pattern of clinical infection confirm the importance of this zoonotic infection as a significant health hazard [5,6].

Campylobacter jejuni and *C. coli* colonize the gastrointestinal tracts of poultry, cattle, sheep, pigs and domesticated pets, such as dogs and cats. Most human enteric infections result from the ingestion of undercooked chicken [7]. Contaminated water or unpasteurized milk may also be sources for sporadic cases of disease or outbreaks of infection [8].

Campylobacter enteritis usually develops within 1 to 7 days after ingestion of a contaminated food, water or raw milk, with presenting symptoms of fever, abdominal pain, and mild to severe diarrhea. The disease is self-limited and does not usually require medical or therapeutic intervention except in severe cases. On rare occasions, serious post infection squeals ranging from a transient reactive arthritis to Guillain-Barré syndrome [2], may develop due to the production of cross-reacting antibodies. Deaths from *Campylobacter* enteric infection are rare and occur primarily in infants, the elderly, or patients with underlying diseases [9].

The use of a selective medium is recommended for the optimal recovery of *Campylobacter* from stool samples [10]. Some of these selective media are Skirrow's medium [11], charcoal cefoperazone deoxycholate agar (CCDA) [12], and Campy-CVA medium [13]. Once inoculated, the medium is placed in a microaerophilic growth environment, incubated at 42°C for 72 h, and observed daily for the *Campylobacter* growth [12].

Direct detection of *Campylobacter* antigens in stool specimens by enzyme immunoassays (EIAs) has been developed such as the Premier CAMPY EIA (Meridian Bioscience, Cincinnati, OH), the ProSpecT Campylobacter EIA (Remel), and the ImmunoCard stat Campy test (Meridian Bioscience, Cincinnati, OH) [12].

Recently, DNA sequences of the *Campylobacter* genome were detected using real-time PCR, while the standard culture methods were

negative [14]. Also an in-house real-time PCR and a multiplex PCR named Seeplex Diarrhea ACE detection were studied by Bessède et al., [15] for detection of *Campylobacter* species, especially *Campylobacter jejuni* and *Campylobacter coli* in stool samples, as a major cause of human bacterial enteritis.

Aim of the work

This study aimed to compare *Campylobacter spp.* detection by molecular method (multiplex polymerase chain reaction) and by 2 immunoenzymatic methods (Premier CAMPY and ImmunoCard Stat Campy to the culture method on Skirrow's medium.

2. Subjects and methods

This study was conducted on 350 patients (270 males and 80 females) with a mean ± SD age of (58.5± 4.5yearss) attending the Internal Medicine outpatient clinic, Ain Shams University Hospitals, Cairo, Egypt in the period from March 2011 to February 2012. Patients presented with symptoms of gastroenteritis in the form of diarrhea, colics, nausea and vomiting.

All patients were subjected to the following after their written consent:

- Full history taking and thorough clinical examination.
- Chest plain X- ray.
- Abdominal ultrasound.
- Collection of stool specimens:

Fecal specimens of the patients were subjected to microbiological examination, multiplex PCR, antigen ELISA and ImmunoCard Stat Campy .

Sample collection:

Stool specimens obtained from patients with gastrointestinal illness were sent to the laboratory for culture, antigen ELISA and ImmunoCard Stat Campy within 4 hrs. The remaining part of the stool samples was then frozen at –80°C for multiplex PCR.

I- Microbiological examination of fecal specimens [16]**:**

* Wet preparation by saline and eosin to exclude *Entamoeba histolytica*, *giardia lamblia* and other cysts or ova of parasites.
* Basic fuchsin smears for *cambylobacter.*
* Methylene blue preparation to detect pus cells.
* Gram stained film and motility to exclude vibrio.
* Culture on the following media:

a- MacConkey agar.

b- Xylose lysine deoxycholate agar to exclude *salmonella* and *shigella*.

c- Sorbitol MacConkey agar to exclude *E.coli* O157:H7 responsible for haemorrhagic colitis.

d- Alkaline peptone and thiosulphate citrate bile sucrose (TCBS) media to exclude *cholera.*

e- Culture on selective media for *Campylobacter* detection:

A stool sample was directly inoculated on Skirrow's medium (Oxoid, Basingstoke, Hampshire, United Kingdom) and incubated at 37°C and 42°C in a microaerobic atmosphere. Colonies were observed 24 hrs and 48 hrs after culture. Isolates that were oxidase positive and Gram-negative curved rods by Gram staining were identified as *Campylobacter spp.* [17]. Hippurate hydrolysis was used as a confirmatory test for the identification of *C. jejuni*.

Hippurate *hydrolysis test*:

Suspend a loopful of *Campylobacter* growth in 400 µl of a 1% Hippurate solution. Incubate at 37 °C for 2 hours. Then slowly add 200 µl 3.5% ninhydrin solutions to the side of the tube to form an overlay. Reincubate at 37°C for 10 min, and read the reaction. Positive blue reaction indicating *Campylobacter jejuni*.

II- Antigen enzyme linked immunosorbent assay:

The Premier CAMPY (Meridian Bioscience, Inc., Cincinnati, OH) based on the use of specific monoclonal antibodies for a common antigen called *Campylobacter*-specific antigen (SA), that is shared by *C. jejuni* and *C. coli*. It can detect both species of *Campylobacter* in stool specimens but cannot differentiate them. Briefly: Fifty microliters of a well-mixed stool sample was transferred to the test tube containing 200 µl of sample diluent, and then the tube was vortexed for 15 s. One hundred microliters of the diluted stool sample was transferred to the microwell plate coated with specific monoclonal antibodies. After 60 min of incubation at room temperature, the microwell plate was washed with the washing buffer 5 times, and 2 drops of enzyme conjugate was added to each microwell and incubated for 30 min at room temperature. The microwell was washed 5 times before 2 drops of substrate was added and incubated for 10 min at room temperature. Then, 2 drops of stop solution was added, and the absorbance was read at 450 to 630 nm. Samples with an optical density greater than 0.1 were considered positive.

III- ImmunoCard Stat Campy:

Immunocard Stat Campy (Meridian Bioscience) is an immunochromatographic rapid test. A small solid stool sample was suspended in 1,400 µl of diluent, or 50 µl of liquid stools was added to 1,400 µl of the sample diluent, depending on the stool consistency. The diluted specimen was vortexed for 15 s, and then 175 µl was transferred to the sampling port of the device. After 20 min of incubation at room temperature, the result was read and validated if the control line band was clearly visible. A positive result showed 2 pink-red bands, the control band and a test line band, whereas a negative result showed only the pink-red control band.

IV - Multiplex polymerase chain reaction (PCR):

***DNA extraction from stool.**

DNA from 180 mg stool specimens was extracted using a QIAamp DNA stool mini kit (Qiagen) according to the manufacturer's recommended procedures. The DNA obtained was stored at –20 °C.

***Multiplex PCR protocol.**

Three genes were targeted in the multiplex PCR protocol, namely *cadF* (genus-specific virulence gene), *hipO* (hippuricase gene for *C. jejuni*) and *asp* (aspartokinase gene for *C. coli*). The primer sets used, as described in table [1].

The multiplex protocol used a reaction mixture at a final volume of 50 µl, consisting of 25 µl multiplex master mix (Qiagen) contains HotStar *Taq* DNA polymerase, 0.5 µl *cadF* primer , 0.3 µl *asp* primer , 1.0 µl *hipO* primer , 0.5 µl 10 mg BSA ml^{-1} (Promega), 4.5 µl eluted DNA and sterile water. The PCR amplification cycle included initial denaturation at 95 °C for 15 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 49 °C for 1 min and extension at 72 °C for 1 min. The final stage was an extension cycle at 72 °C for 7 min. PCR cycles were carried out in a GeneAmp (Perkin-Elmer 9700; Applied BioSystems). *C. jejuni* ATCC 33291, *C. coli* strain, AGU 12305 were used as positive controls, and the negative control were included in every PCR run. Following gel electrophoresis, the amplified PCR product was stained with ethidium bromide and visualized with a UV transilluminator. The sizes of PCR products were estimated by comparison with 100 bp DNA molecular mass markers and the amplified control strains.

Table 1: The primer sets used in the multiplex PCR.

Primer	Sequence (5′–3′)	Product (bp)
For *cadF*		
(forward)	TTG AAG GTA ATT TAG ATA TG	
(reverse)	CTA ATA CCT AAA GTT GAA AC	400
For *hipO*		
(forward)	GAA GAG GGT TTG GGT GGT G	
(reverse)	AGC TAG CTT CGC ATA ATA ACT TG	735
For *asp*		
(forward)	GGT ATG ATT TCT ACA AAG CGA G	
(reverse)	ATA AAA GAC TAT CGT CGC GTG	500

Nayak *et al.* (2005) [18]

Definition of a *Campylobacter*-positive stool sample:

The following criteria were used to define a stool sample positive for *Campylobacter*: either culture method was positive, or in the case of a negative culture, a positive molecular method and an immunoenzymatic method were both positive [15].

Results

Out of the 350 stool samples collected from patients with gastroenteritis, 23 specimens were positive (6.6%) based on the case definition, i.e., they were positive by culture method or, in case of a negative culture, by a positive molecular method and a positive immunoenzymatic method: 16 were positive by culture and 7 were culture negative but positive by both a molecular method (multiplex PCR) and an immunoenzymatic method. A total of 29 specimens were positive by at least one method. The different combinations are presented in table [2].

All methods were positive in only 14 cases. Of the positive culture specimens, 3 were missed, (one) by the ELISA and (two) by ImmunoCard.

In 6 out of 7 samples that fulfilled the positivity criteria when culture was negative, all of the methods were positive.

Of the 6 cases that where positive by immunoenzymatic methods only, 4 samples were positive by 2 immunoenzymatic methods and 2 samples were positive by ELISA only.

The *Campylobacter* species identified by culture were 10 (62.5%) *C. jejuni*, 6 (37.5%) *C. coli*, while multiplex (PCR) revealed 13 (56.5%) *C. jejuni*, 8 (34.8%) *C. coli* and 2 (8.7%) specimens had mixed infection (*C. jejuni* and *C. coli*). The *Campylobacter* species identified by culture and multiplex PCR were shown in table [3]. The immunoenzymatic test did not allow differentiation between *C. jejuni* and *C. coli*.

Out of the 350 stool specimens 15 (4.8%) specimens were reported as *Salmonella enterica serovar Typhi* positive and 7 (2%) were *Shigella sonii* positive.

Diagnostic validity test including sensitivity, specificity, predictive values of multiplex (PCR), Premier CAMPY ELISA and ImmunoCard Stat Campy were shown in tables [3, 4, 5]. The sensitivity of the multiplex PCR was higher than the ELISA and

ImmunoCard Stat Campy tests (100%, 95.6**%** and 86.9% respectively) while the ImmunoCard Stat Campy had higher specificity than the ELISA and multiplex PCR (98.7%, 98.1**%** and 97.9% respectively).

Table 2: Distribution of the positivity profiles of the cases using different techniques for detection of *campylobacter spp.*

	Culture	Mutiplex **PCR**	**Premier CAMPY ELISA**	**ImmunoCard Stat Campy**
Positive by culture (*n* = 16)				
14	+	+	+	+
1	+	+	+	-
1	+	+	-	-
Positive by other tests (*n* = 7)				
6	-	+	+	+
1	-	+	+	-
Positive by Ag tests only (*n* = 6)				
4	-	-	+	+
2	-	-	+	-

Table 3: The *Campylobacter* species identified by culture on Skirrows and multiplex PCR.

	C. jejuni **No (%)**	***C. coli*** **No. (%)**	**Mixed *C. jejuni* and *C. coli*** **No. (%)**	**Total**
Culture	10 (62.5%)	6 (37.5%)	-	16
Multiplex (PCR)	13 (56.5%)	8 (34.8%)	2 (8.7%)	23

Table 4: Diagnostic validity test of multiplex (PCR) using culture as the reference method.

	True positive	**False positive**	**True negative**	**False negative**
Multiplex (PCR)	16	7	327	0
	Sensitivity %	**Specificity %**	**Positive predictive %**	***Negative predictive %***
	100	97.9	69.6	100

Table 5: Diagnostic validity test of Premier CAMPY ELISA using multiplex (PCR) as the reference method.

	True positive	**False positive**	**True negative**	**False negative**
Premier CAMPY ELISA	22	6	321	1
	Sensitivity	**Specificity**	**Positive predictive value**	***Negative predictive** value*
	95.6**%**	98.1**%**	78.5%	99.7%

Table 6: Diagnostic validity test of ImmunoCard Stat Campy using multiplex (PCR) as the reference method.

	True positive	**False positive**	**True negative**	**False negative**
Immuno Card Stat Campy	20	4	323	3
	Sensitivity	**Specificity**	**Positive predictive value**	**Negative predictive value**
	86.9%	98.7%	83.3**%**	99.1%

1- Discussion

Campylobacter species, especially *Campylobacter jejuni* and *Campylobacter coli*, are a major cause of human bacterial enteritis. Current detection in stools is done essentially by culture on selective and nonselective media with filtration [14].

In our multiplex (PCR) protocol, three genes, namely *cadF* (genus-specific virulence gene), and *hipO* and *asp* (species specific for *C. jejuni* and *C. coli*, respectively), were targeted. The *cadF* gene, a virulence gene associated with adhesion, is 100 % conserved among isolates of diverse sources [19,12].The *hipO* gene is highly conserved in *C. jejuni* strains and represents the most widely validated gene for the identification of *C. jejuni* [20,7]. The *asp* gene encodes aspartokinase and is highly specific for *C. coli* [21].

Our results revealed that out of the 350 stool samples collected from patients with gastroenteritis, 23 specimens were positive (6.6%) based on the case

definition: 16 were positive by culture and 7 were culture negative but positive by both a molecular method (multiplex PCR) and an immunoenzymatic method. All methods were positive in 14 cases. The *Campylobacter* species identified by culture were 10 (62.5%) *C. jejuni*, 6 (37.5%) *C. coli*, while multiplex (PCR) revealed 13 (56.5%) *C. jejuni*, 8 (34.8%) *C. coli* and 2 (8.7%) specimens had mixed infection (*C. jejuni* and *C. coli*). Al Amri et al., [4] reported that of the 114 stool specimens (54 human and 60 chicken) evaluated by the multiplex PCR protocol, 70 (61.4 %) were identified as *C. jejuni*, 35 (30.7 %) as *C. coli* and 9 (7.9 %) as a mixed infection with both species. Among the stool specimens that were culture negative for *Campylobacter*, two (6.7 %) were *C. jejuni* positive. The ability of the multiplex PCR to detect such mixed infections has important therapeutic implications in view of the high level of resistance of *C. coli* to erythromycin, which is usually the drug of choice for *C. jejuni*. Bessède *et al.,* [15] reported that out of 242 stool specimens tested by 2 molecular methods, an in-house real-time PCR and a multiplex PCR named Seeplex Diarrhea ACE Detection, and 3 immunoenzymatic methods, Premier CAMPY, RidaScreen *Campylobacter*, and ImmunoCard Stat Campy, 23 (9.5%) fulfilled the positivity criteria: 16 were positive by culture and 7 were culture negative but positive by one molecular method and immunoenzymatic method. All methods were positive in only 7 cases. This could be explained by long contact of the bacteria with a normal atmosphere during stool processing, and the antibiotics incorporated into the selective media may inhibit certain *Campylobacter* strains.

Our study revealed that all culture-positive specimens were identified by multiplex PCR. In addition, 334 specimens identified as *Campylobacter* culture negative were examined and 7 (2%) were found to be *Cambylobacter* positive by the multiplex PCR. Six of them were positive by all methods (multiplex PCR Premier CAMPY ELISA and ImmunoCard Stat Campy). O'Leary *et al.,* [22] had studied 773 stool samples by routine culture and the EntericBio system (a multiplex PCR assay). He found that 42 samples had *Campylobacter* positive results by culture, and all of these were positive with the EntericBio system. This system detected an additional 12 positive samples *Campylobacter* spp.

Al Amri *et al.,* [4] reported that a total of 61 specimens were identified as *Campylobacter* culture positive and the remaining 30 were *Campylobacter* negative, all culture-positive specimens were identified by multiplex PCR protocol. In addition, 30 human specimens identified as *Campylobacter* negative were examined and 7 (6.7 %) were found to be *Cambylobacter* positive by the multiplex protocol. These findings are indicative of the usefulness of this protocol to correctly detect the presence of *Campylobacter* spp. in stools.

In our study the sensitivity of the multiplex PCR was higher than the ELISA and ImmunoCard Stat Campy tests (100%, 95.6**%** and 86.9% respectively) while the ImmunoCard Stat Campy had higher specificity than the Premier CAMPY ELISA and multiplex PCR (98.7%, 98.1**%** and 97.9 % respectively). Bessède *et al.,* [15] had reported that the specificities and negative predictive values (NPV) of the different methods were all in the range of 95 to 100% while the sensitivity of ELISA was higher than ImmunoCard Stat Campy and multiplex PCR it was (96%, 92% and 88.7% respectively) ***conclusion:*** multiplex PCR is attractive as it enables the detection and speciation of *campylobacter*. Also the procedure of the multiplex PCR had a turnaround time of 6 h. The Premier CAMPY ELISA was rapid and had acceptable performance sensitivity of 95.6%.

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References

1. Moore, J. E., Corcoran, D., Dooley, J. S., Fanning, S., Lucey, B., Matsuda, M., McDowell, D. A., Mégraud, F., Miller, B. C. & other authors (2005): *Campylobacter*. Vet Res, 36: 351-382.
2. Islam, Z., Gilbert, M., Mohammad, Q.D., Klaij, K., Li, J, et al, (2012) Aug 27. :Guillain-Barré Syndrome-Related Campylobacter jejuni in Bangladesh: Ganglioside Mimicry and Cross-Reactive Antibodies. PLoS One .7(8):e43976.
3. Gillis D., Cronquist A., Cartter M., Tobin-d'Angelo M., Blythe D., Smith K., Lathrop S., Cieslak P., Dunn J., Holt K. G., Guzewich J. J., Henao O. L., Mahon B., Griffin P., Tauxe R. V., Crim S. M. (2011). Vital signs: incidence and trends of infection with pathogens transmitted commonly through food – foodborne diseases active surveillance network, 10 U.S. sites, 1996-2010. MMWR Morb. Mortal. Wkly. Rep. 60, 749754.
4. Al Amri, A., Senok, A.C., Ismaeel, A.Y., Al-Mahmeed, A.E., Botta, G.A.(2007) Oct: Multiplex PCR for direct identification of *Campylobacter* spp. in human and chicken stools. J Med Microbiol ,56(Pt 10):1350-5.
5. Al-Mahmeed, A., Senok, A. C., Ismaeel, A.Y., Bindayna, K. M., Tabbara, K. S., Botta, G. A.

(2006): Clinical relevance of virulence genes in *Campylobacter jejuni* isolates in Bahrain. J Med Microbiol , 55: 839-843.
6. Rozynek, E., Dzierzanowska-Fangrat, K., Jozwiak, P., Popowski, J., Korsak, D., Dzierzanowska, D. (2005): Prevalence of potential virulence markers in Polish *Campylobacter jejuni* and *Campylobacter coli* isolates obtained from hospitalized children and from chicken carcasses. J Med Microbiol, 54: 615-619.
7. Stern, N. J., and J. E. Line. (1992): Comparison of three methods for recovery of *Campylobacter* spp. from broiler carcasses. J. Food Prot, 55:663-666.
8. Infectious Disease Epidemiology Section, Office of Public Health, Louisiana Department of Health and Hospitals. Revision date, 25 February (2008). Campylobacteriosis
9. Blaser, M., and B. M. Allos. (2005): *Campylobacter jejuni* and related species, p. 2548-2557. *In* G. Mandell, J. E. Bennet, and R. Dolin (ed.), Principles and practice of infectious disease, 6th ed. Elsevier/Churchill Livingstone, New York, NY.
10. Fitzgerald, C., and I. Nachamkin. (2007): *Campylobacter* and *Arcobacter*, p. 933-946. *In* P. R. Murray, E. J. Baron, J. H. Jorgensen, M.
11. Sinha, S., Prasad, K. N., Pradhan, S., Jain, D., Jha, S. (2004): Detection of preceding *Campylobacter jejuni* infection by polymerase chain reaction in patients with Guillain-Barre syndrome. Trans R Soc Trop Med Hyg, 98: 342-346.
12. Granato, P.A., Chen, L., Holiday. I., Rawling, R.A., Novak-Weekley, S.M., Quinlan, T., Musser, K.A .(2010) Nov: Comparison of premier CAMPY enzyme immunoassay (EIA), ProSpecT Campylobacter EIA, and ImmunoCard STAT CAMPY tests with culture for laboratory diagnosis of Campylobacter enteric infections. J Clin Microbiol, 48(11):4022-7.
13. Reller, L. B., S. Mirrett, and L. G. Reimer. (1983). Abstr. 85th Annu. Meet. Am Soc Microbiol, C-274: 357.
14. Nakamura, S., Maeda, N., Miron. I.M, Yoh, M., Izutsu, K, et al, (2008): Metagenomic diagnosis of bacterial infections. Emerg Infect Dis, 14:1784-1786.
15. Bessède, E., Delcamp, A., Sifré, E., Buissonnière, A., Mégraud, F. (2011) Mar: New methods for detection of campylobacters in stool samples in comparison to culture. J Clin Microbio , 49(3):941-4.
16. Cheesbrough,M. District laboratory practice in tropical countries,Cambridge University press, (2000) , part 2, page 104.
17. Hutchinson, D. N., and F. J. Bolton. (1984): Improved blood free selective medium for the isolation of *Campylobacter jejuni* from faecal specimens. J Clin Pathol, 37:956-957.
18. Nayak, R., Stewart, T. M., Nawaz, M. S. (2005): PCR identification of *Campylobacter coli* and *Campylobacter jejuni* by partial sequencing of virulence genes. Mol Cell Probes, 19: 187-193.
19. Datta, S., Niwa, H., Itoh, K. (2003): Prevalence of 11 pathogenic genes of *Campylobacter jejuni* by PCR in strains isolated from humans, poultry meat and broiler and bovine faeces. J Med Microbiol, 52: 345-348.
20. Skirrow, M. B. (1977): *Campylobacter* enteritis: a “new” disease. Br Med J, ii: 9-11.
21. Linton, D., Lawson, A. J., Owen, R. J., Stanley, J. (1997): PCR detection, identification to species level, and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* direct from diarrheic samples. J Clin Microbiol, 35: 2568-2572.
22. O'Leary, J., Corcoran, D and Lucey, B. (2009) Nov: Comparison of the EntericBio multiplex PCR system with routine culture for detection of bacterial enteric pathogens. J Clin Microbiol, 47(11):3449-53.

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