

ORIGINAL ARTICLE

# Immuno-chromatography versus Conventional Culture Method for Detection of *Campylobacter jejuni* and *Campylobacter coli* in Gastroenteritis

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## ABSTRACT

**Key words:**

**Campylobacter jejuni (C.jejuni),  
Campylobacter coli (C.coli),  
Modified charcoal cefoperazol dextrose agar (MccDA)**

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**Background:** *Campylobacter* is an important food-borne diarrheal disease in the whole world. The use of culture-independent diagnostic tests, such as stool antigen tests, for the detection of *Campylobacter* in stool is of particular interest. **Objectives:** This study aimed to compare between conventional culture method and Immuno-chromatography (I.C) For Detection Of *C.jejuni* and *C. coli* in Gastroenteritis at Benha University hospitals, and determine their antibiotic susceptibility pattern. **Methodology:** This study was carried out on 100 patients who developed gastroenteritis like symptoms attending the Out Patient Clinic of Benha University Hospitals or admitted to the Pediatric and Internal medicine departments. Stool samples were cultured on modified charcoal cefoperazol dextrose agar (mCCDA) and were examined for Ag detection by I.C test using RIDA@QUICK kit. Antimicrobial susceptibility testing was performed for twelve different antibiotics by the Kirby–Bauer disc diffusion method. **Results:** *Campylobacter* was detected in 18% of samples by culture method. 72.2% of them were *C. jejuni*, while the other 27.8% were *C. coli*. Out of 18 positive by culture, 16(88.9%) were positive by I.C. However, out of 82 negative by culture, 2(2.4%) were positive by I.C. The diagnostic accuracy values of the RidaQuick *Campylobacter*® versus culture were: sensitivity of 88.9%, specificity of 97.6%, and positive and negative predictive values of 88.9% and 97.6%, respectively. **Conclusion:** RIDAQUICK *Campylobacter*® provide a rapid and reliable alternative for conventional culture in laboratory diagnosis of enteric infections with *C. jejuni* and *C. coli*.

## INTRODUCTION

Human infection by *Campylobacter* constitutes an important public health problem worldwide. The greatest threat comes from two species, *C. jejuni* and *C. coli*<sup>1</sup>.

*Campylobacter* is a major cause of human bacterial food-borne gastroenteritis and each year they are responsible for several 100 million cases of infection worldwide<sup>2</sup>.

The epidemiology of human *Campylobacter* infection is complex, with food, water, and environmental sources all having a role. Especially, the handling of raw poultry and eating undercooked chicken carry high relative risks of *Campylobacter* infection<sup>3</sup>.

*Campylobacteriosis* is an acute, self limiting gastrointestinal illness characterized by diarrhea, fever and abdominal cramps. Although in humans such infections are generally self limiting, complications can arise and may include bacteraemia, Guillain-Barré syndrome, reactive arthritis and abortion<sup>4</sup>.

Most patients infected with *Campylobacter* spp. will recover without any specific treatment other than replacing lost fluids and electrolytes. Antibiotics, generally macrolides, tetracycline and fluoroquinolones,

are reserved for more severe cases. However, the increasing resistance of *C. coli* and *C. jejuni* strains to these agents is increasing throughout the world and it is thought to be pushed by the frequent use of antibiotics in animals farmed for meat<sup>5</sup>.

The majority of studies of *Campylobacter* infection have used selective culture techniques designed to improve isolation of *C.jejuni* and *C. coli* which are thought to be the primary species associated with human disease<sup>6</sup>.

Cultural isolation, however, may fail since *Campylobacter* spp. are rather fastidious microorganisms, and the infection can be verified retrospectively in such cases by serology<sup>7</sup>.

Therefore, novel assays based on the detection of specific antigens have been developed, which can be performed rapidly, are independent from the bacterial viability and thus also suited for the analysis of frozen samples, and have consequently gained increasing attention in routine microbiology<sup>8</sup>.

The antigen detection methods are of particular interest because they yield results within hours or minutes and are easy to apply, especially using the immuno-chromatographic approach. In addition, *Campylobacter* antigens can persist in the clinical

sample in the absence of viable microorganisms, allowing primary infections to be detected by this method when the bacteria are not viable, which is a major potential clinical advantage. However, highly variable results have been obtained using commercialized kits<sup>9</sup>.

#### **Aim of the work:**

This study aimed to compare between conventional culture method and Immunochromatography. For Detection Of *C. jejuni* and *C. coli* in Gastroenteritis at Benha University hospitals, and determine their antibiotic susceptibility pattern.

## **METHODOLOGY**

This work was carried out in Medical Microbiology & Immunology department, Faculty of Medicine, Benha University in the period from May 2015 to April 2016. This study was approved by Benha University ethical committee and a consent was obtained from all patients and mothers of children under study.

#### **Subjects and samples:**

This study was carried out on 100 patients who developed gastroenteritis like symptoms attending the Out Patient Clinic of Benha University Hospitals or admitted to the Pediatric and Internal medicine departments. A stool sample was collected from each patient. Each stool sample was divided into 2 parts:

-First part was cultured on a selective medium (mCCDA) for isolation and identification of *C. jejuni* and *C. coli*. The isolated stains will be subjected to antibiotic susceptibility testing by disc diffusion method.

The second part was store at -20°C until used for immunologic detection of Campylobacter antigens in human stool samples by immunochromatography method.

#### **A- Isolation and identification of Campylobacter**

Each stool sample was directly streaked onto a plate of modified CCDA media supplemented with CCDA selective supplement. Plates were incubated at 42°C under microaerophilic conditions generated by Oxoid microaerophilic gas packs (Oxoid, UK), and examined daily for 72 hours. Suspicious colonies were presumptively identified by their colony morphology, Gram stain, motility test, oxidase test, catalase test, H<sub>2</sub>S production on TSI agar, hippurate test and susceptibility to nalidixic acid and cephalothin.

#### **B- Antimicrobial susceptibility testing:**

Antimicrobial susceptibility testing was performed on Mueller Hinton Blood agar plates by Kirby-Bauer disc diffusion method and interpreted according to Clinical Laboratory Standards Institute recommendations (CLSI)<sup>10</sup>.

The discs used were: Ampicillin (10ug), Chloramphenicol (30ug), Tetracycline (30ug), Gentamycin (10ug), Doxycycline (30ug),

Trimethoprim-sulfamethoxazole (25ug), Ciprofloxacin (5 ug), Streptomycin (10ug), Erythromycin (15ug), Clindamycin (2ug), Cephalothin (30ug), Nalidixic acid (30 ug).

#### **C- Immunological detection of campylobacter Ag in stool by using RIDAQUICK® kit:**

The RIDAQUICK Campylobacter® was used according to the manufacturer's instructions, 50 µL of stool suspension mixed well in a tube containing 0.5 mL of reagent A and 0.5 mL of reagent B. The mixture was allowed to stand and incubated at room temperature for 5 min, and 150 µL of the supernatant was then placed in the sampling port of the device. The result was considered positive when violet red lines could be seen in the control (C) and test (T) bands, negative when the control line alone appeared, and invalid when no line could be observed.

#### **Statistical analysis**

Data were recorded and analyzed using the computer program SPSS (Statistical package for social science) version 16 to obtain descriptive data in the form of number and percent. Kappa test measure the level of agreement between Culture & Immunochromatography method.

## **RESULTS**

Of the total 100 patients who participated in the study, 54 (54%) were males and 46 (46%) were females and their ages ranged from 2 months to 65 years with mean age of 16 years.

Direct isolation and identification from culture and biochemical reactions Eighteen (18%) Campylobacter spp. out of 100 stool samples were isolated on modified CCDA media and are oxidase and Catalase positive (**Table 1**). 13 (72.2%) out of the 18 positive isolates are *C. jejuni* tested positive by hippurate hydrolysis while the other 5 (27.8%) samples are *C. coli*.

The incidence of Campylobacter infection was more in age group below 1 year than other groups and more common in males than females and in rural than urban areas. However, these results are statistically insignificant ( $P > 0.05$ ) (**Table 2**). There is a significant association between Campylobacter positive cases and both animal exposure ( $P < 0.05$ ) and decreased immunity ( $P < 0.05$ ) (**Table 3**).

Out of 18 positive by culture, 16(88.9%) were positive by I.C. However, out of 82 negative by culture, 2(2.4%) were positive by I.C. (**Table 1**). The diagnostic accuracy values of the RidaQuick Campylobacter® versus culture were: sensitivity of 88.9%, specificity of 97.6%, and positive and negative predictive values of 88.9% and 97.6%, respectively.

All the isolated 18 strains show 100% resistance to Ampicillin, Clindamycin and Cephalothin. There is also high resistance rate to Streptomycin 94.4%, Doxycycline 88.9%, tetracycline 77.8%, Gentamycin

72.2% and Trimethoprim, sulfamethoxazole 72.2%. About 10 of 18 isolated strains show resistance to Ciprofloxacin and Erythromycin. Most of the isolated

strains was sensitive to Nalidixic acid 88.9% and Chloramphenicol 66.7%.

**Table 1: Result of immunochromatography in comparison with culture on selective media (mCCDA).**

<i>Immunochro-Matography</i>	<i>Culture</i>	<i>Positive</i>	<i>Negative</i>	<i>Total</i>	<i>Kappa value</i>	<i>P value</i>
<b>Positive</b>		16(88.9%)	2(2.4%)	18	0.864*	<0.001
<b>Negative</b>		2(11.1%)	80(97.6%)	82		
<b>Total</b>		18	82	100		

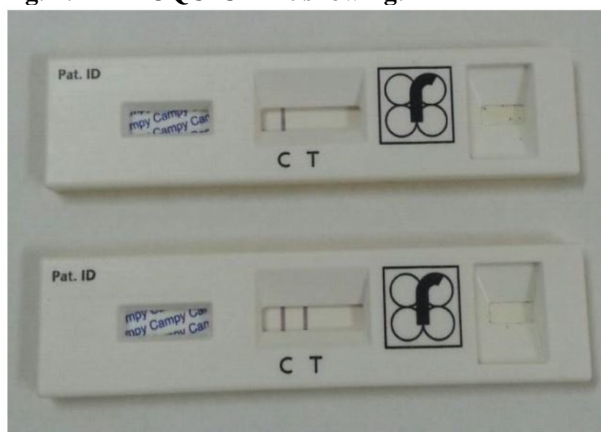
\*Strength of agreement between two tests is considered to be perfect.

**Table 2: Comparison between Campylobacter positive and negative cases as regards age , sex and residence.**

		<i>Campylobacter</i>				<i>P value</i>
		<b>Positive</b>		<b>Negative</b>		
		<b>N</b>	<b>%</b>	<b>N</b>	<b>%</b>	
<b>Age group</b>	Below 1 year	6	46.2	7	53.8	0.440
	1 to 11 years	3	13	21	87	
	12 to 24 years	5	20	35	80	
	25 to 34 years	1	9	17	91	
	35 to 45 years	2	20	2	80	
	Above 45	1	5.6	0	94.4	
<b>Sex</b>	Male	10	55.6	44	53.7	0.884
	Female	8	44.4	38	46.3	
<b>Residence</b>	Rural	14	77.8	48	58.5	0.128
	Urban	4	22.2	34	41.5	

**Table 3: Association of risk factors for diarrhea with Campylobacter positive and negative cases.**

		<i>Campylobacter</i>				<i>P value</i>
		<b>Positive</b>		<b>Negative</b>		
		<b>N</b>	<b>%</b>	<b>N</b>	<b>%</b>	
<b>Consumption of unpasteurized milk</b>	<b>No</b>	11	61.1	39	47.6	0.298
	<b>Yes</b>	7	38.9	43	52.4	
<b>Ingestion of undercooked chicken</b>	<b>No</b>	15	83.3	64	78.0	0.618
	<b>Yes</b>	3	16.7	18	22.0	
<b>Contact with animals&amp;birds</b>	<b>No</b>	7	38.9	55	67.1	0.026
	<b>Yes</b>	11	61.1	27	32.9	
<b>Immunosuppression</b>	<b>No</b>	11	61.1	69	84.1	0.027
	<b>Yes</b>	7	38.9	13	15.9	
<b>Season</b>	<b>Cold months</b>	6	33.3	32	39.0	0.652
	<b>Warm months</b>	12	66.7	50	61.0	

**Fig. 1: RIDA®QUICK kit showing:**

Upper one : -ve test  
 lower one: +ve test

## DISCUSSION

Campylobacteriosis is one of the most common bacterial causes of food-borne illness and leading cause of bacterial diarrheal disease in the world<sup>11</sup>.

There is evidence to suggest that there has been a rise in the global incidence of campylobacteriosis in the past decade. The numbers of cases of campylobacteriosis have increased in North America, Europe, and Australia. Although epidemiological data from Africa, Asia, and the Middle East are still incomplete, data indicate that Campylobacter infection is endemic in these regions<sup>12</sup>.

In Egypt, the incidence of *C. jejuni* within three year from 2012-2014 was highest in Minya Governorate followed by Fayoum then Cairo and finally Qalubia Governorate<sup>13</sup>.

In the present study, 18% (18/100) of stool samples were positive for Campylobacter culture on Charcoal Ceferaperazone Deoxycholate Agar (CCDA media). This result was in agreement with another study in Giza, Egypt by Hassanain<sup>14</sup> who reported that 8 (16.66%) out of the 48 human fecal samples were positive for Campylobacter and with Lengerh et al.<sup>15</sup> and Tafa et al.<sup>16</sup> in Ethiopia who reported that the isolation rate of campylobacter by culture was 15.4% and 16.7 % respectively.

In another studies in some areas in Egypt, a higher rate of isolation was found by Barakat et al.<sup>17</sup> as the prevalence of Campylobacter was (35%) and this finding is higher than that has been cited in their previous work (26%) in stool samples collected from children. This higher percentage could be attributed to the sampling of stool samples from human in contact with food animals.

In contrast, a lower rate of isolation by culture was found in Zagazig, Egypt by Awadallah et al.<sup>18</sup> only 2.7% of the stool samples were positive for Campylobacter and 5.3% by Abd ElTawab et al.<sup>19</sup> at Zagazig city.

This different prevalence could be explained by the differences in targeted population (selected or randomly collected, different age), hygienic measures, methods used as well as identification techniques and possible geographic factors (location, climate factors) indicating different infection patterns in different population groups as well as the sample size<sup>20</sup>.

The good selection of cases with Campylobacter diarrhea like symptoms may explain the higher rates of Campylobacter isolation in our study than that recorded in other studies, in addition to bad hygiene level in handling of poultry meat and direct contact with birds and animal farms in rural areas.

In our study, the most commonly isolated Campylobacter species by culture was *C. jejuni* 72.2% (13 out of 18) followed by *C. coli* 27.8 % (5 of 18). Awadallah et al.<sup>18</sup> at Zagazig, Egypt also found that Campylobacter spp. were identified as *C. jejuni* and *C. coli* in 66.7% and 33.3%, of the examined human stool samples, respectively .

In our study, the infection was more common in males (53.7%) than females (46.3%) which agrees with that found by Girgis et al.<sup>21</sup> at Ain shams and Wang et al.<sup>22</sup> they both found that males were more affected than females and this revealed no significant association between the sex and Campylobacter infection ( $P > 0.5$ ). Similarly, Inns and associates<sup>23</sup> (UK) reported that there was no significant difference in age or gender between cases and controls.

However, Adekunle and associates<sup>24</sup>, Nigeria reported that the Campylobacter infection rate was significantly higher among males (0.82%; 3/368) than females (0%; 0/368). On the contrary, Hassanzadeh and Motamedifar<sup>25</sup> found that the infection was more common in females. But Feizabadi et al.<sup>26</sup> found that no difference in the isolation rates among both males and females. This variation may be explained by lifestyle, behavioral, and socioeconomic differences.

In the current study, Campylobacter isolation was the highest (46.2%) in the age group of less than 12 months followed by the group of 12 to 24 years and 35 to 45 years (20%) and the lowest (5.6%) in the age group Above 45 years. This difference within age groups was statistically insignificant.

Similarly, Tafa et al.<sup>16</sup> in Ethiopia, found that Campylobacter spp. isolation was the highest in the age group of less than 12 months.

Girgis et al.<sup>21</sup>, revealed significant association between the age group and Campylobacter infection being highest in the youngest age group between 3½-10 years of age. On the other hand, this is different from that carried by Al-Shamahy et al.<sup>27</sup> in Yemen who

found that the majority of cases were above 15 years old.

Usually as the age increases, the level of antibody tends to increase; this is associated with future protection to infection<sup>28</sup>. The organism is isolated from infants and young adults more frequently than from persons in other age groups and from males more frequently than females<sup>29</sup>.

Campylobacter infections have shown seasonality in occurrence. In the current study, Campylobacter-associated diarrhea was more common during warmer months (66.7%) and this is in agreement with Wierzba et al.<sup>30</sup> who found that Campylobacter associated diarrhea was more common during warmer months. In developed countries, more than 90% of human campylobacteriosis cases occur during the summer because of undercooked meats from outdoor cooking facilities<sup>31</sup>.

studies in Central Africa and Malawi showed a higher prevalence of campylobacter infection during the rainy season<sup>32</sup>.

Although the finding was not statistically significant, higher rates were observed in rural (77.8%) than urban (22.2%), which is in line with the findings in Ethiopia by Lengerh et al.<sup>15</sup> who found that residence in village and rural areas was a major risk factor for Campylobacter infection. This may be due to unprotected water source and presence of domestic animals in almost all rural house hold.

This study has shown animal exposure was the most common risk factor in Campylobacter positive cases (61.1%) followed by immune suppression (38.9%) and this was of statistical significant value ( $P < 0.05$ ).

These results are in agreement with Doorduyn et al.<sup>33</sup> who reported a highly significant association between Campylobacter infection and contact with farm animals and with Tafa et al.<sup>16</sup> who found that there was statistically significant association between contact with domestic animals and recovery rate of Campylobacter spp.

In Egypt, Girgis et al.<sup>21</sup> revealed statistically significant association between immune suppression and infection with Campylobacter.

We found that out of 18 cases diagnosed by culture, 16(88.9%) cases were positive by IC also, and out of 82 cases negative by culture, there are 2 cases (11.1%) were positive by IC.

As regard evaluation of the IC in this work, it has a sensitivity of 88.9%, specificity of 97.6%, PPV of 88.9% and NPV of 97.6%.

In a study by Granato et al.<sup>34</sup>, who reported an acceptable performance of IC sensitivity of 98% & specificity of 94.2%, its PPV was 92.6%, NPV was 98.8% giving its convenience in use and the short turnaround times for final test results. It provides early therapeutic decision, not affected by organism viability and can be used in routine use in small-volume laboratories.

In another study by Zaghoul et al.<sup>35</sup> in Ain Shams, Egypt, the sensitivity of IC was 86.9%, specificity 98.7%, positive and negative predictive values were 83.3% and 99.7%, respectively.

In agreement with the present study, Gómez-Camarasa et al.<sup>36</sup> reported that the diagnostic accuracy values of the RidaQuick Campylobacter versus culture were: sensitivity of 87%, specificity of 97%, and positive and negative predictive values of 77% and 98%, respectively.

In this study the 18 isolated strains were subjected to 12 antibiotic sensitivity test: 66.7% and 88.9% of them were sensitive to Chloramphenicol (30µg) and Nalidixic acid (30µg) respectively while 77.8%, 88.9%, 94.4% of the isolated strains were resistant to Tetracycline (30µg), Doxycycline (30µg) and Streptomycin (10µg) respectively.

All the isolated strains show 100% resistant to Ampicillin (10µg), Clindamycin (2µg) and Cephalothin (30µg) while they show 77.2% resistant to Gentamycin (10µg) and Trimethoprim-sulfamethoxazole (25µg). 55.6% of the isolated strains were resistant to Ciprofloxacin (5µg) and Erythromycin (15µg).

Girgis et al.<sup>21</sup> in Ain Shams, Egypt agreed with our study. They reported that the isolated strains show 100% resistant to Ampicillin (10µg), 57.9% to Ciprofloxacin (5µg), 53% to Erythromycin (15µg) but disagree with result of resistant to Nalidixic acid (30µg) as the isolated strains in this study show 100% resistant to Nalidixic acid (30µg).

This difference could be attributed to the different rate of use of these antibiotics in different localities and the discovery of the differences in susceptibility patterns in different countries is important for the proper treatment of the patients.

## CONCLUSIONS

Detection of the Campylobacter antigen in pathological feces using RIDAQUICK Campylobacter® is an easily applied and rapid procedure that yields acceptable results alternatives for conventional culture in the laboratory diagnosis of campylobacter enteric infections.

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