Author’s response to reviews

Title: Impact of changing from staining to culture techniques on detection rates of Campylobacter spp. in routine stool samples in Chile

Authors:

Lorena Porte (nenapor@gmail.com)
Carmen Varela (cvarelaa@alemana.cl)
Thomas Haecker (thaeckerl@gmail.com)
Sara Morales (smoralesr@alemana.cl)
Thomas Weitzel (tweitzel@alemana.cl)

Version: 1 Date: 04 Jan 2016

Author’s response to reviews:

Dr. Okeke
Editorial Office
BMC Infectious Diseases

RE: Manuscript INFD-D-15-00381 “Impact of changing from staining to culture techniques on detection rates of Campylobacter spp. in routine stool samples in Chile”

Dear Dr. Okeke!

Thank you for your response and for considering our manuscript for publication.

We appreciate the comments and suggestions made by the reviewers. We added and changed the manuscript substantially according to the reviewers’ observations.

Following you will find our point-by-point response to the reviewers’ comments:
Reviewer #2

1. Methods:

a. Line 95, was only one technician involved in reading of stains, or were these "double-read", even though the technicians involved in the study were experienced. This could have contributed to lower sensitivity?

We agree that „double-checking“ is of importance to evaluate a microscopical method under study conditions. Still, the aim of our study was to compare the performance of staining techniques under routine conditions and not under study conditions. The Chilean recommendation to use staining technique does not include „double-reading“ and a study using such additional measures could enhance the sensitivity in a way that does not represent the performance of the technique in real life. We explained the reading procedure in more detail in lines 99-101 and the possible impact of the reading under routine conditions on the sensitivity in lines 176-177.

The sensitivity reported in study is quite a bit lower than an older study published by Ho et al (Arch Intern Med. 1982 Oct;142(10):1858-60), despite similar prevalences, although the exact staining method used is different.

We agree with that comment. The problem of sensitivity of staining in our study compared to other studies was further discussed and various references such as Ho et al. were included (lines 172-179).

b. Basic fuschin/Carbol-fuschin based stains may pick up the organism better than the Hucker's-Gram stain; the stain utilized may have further diminished sensitivity in the current study. This should be discussed as a possible limitation in the discussion section.

As suggested this problem of different staining procedures was included in the discussion in line 176.
c. Campy plates were only incubated for 48 hours. Typically a minimum of 72 hours is recommended for optimal sensitivity. (Clinical Microbiology Procedures Handbook, 3rd Ed). This could have diminished the sensitivity of culture somewhat.

The optimum incubation time is controversial. As the reviewer mentions, some guidelines recommend reading the plates after 72 h, while others have shown that there is no significant difference between 48 h and 72 h of incubation. We opted to use 48 h of incubation as recommended by the Chilean and other guidelines and included this important but controversial technical detail with respective references in the discussion (lines 189-195).

d. Also the authors should reference previous work outside of South America regarding the use of staining methods for direct detection of Campylobacter from stool and discuss their findings in comparison to these older studies (Ho et al, as referenced before; Sazie ESM, Titus AE: Rapid diagnosis of Campylobacter enteritis. Ann Intern Med 1982;96:62-63).

As suggested, this subject was further discussed in lines 172-179 and the references together with others were included.

e. Lines 87-89: Pre-analytic factors need to be considered, esp for Campylobacter culture - given its fastidious nature. What was the time to processing to stool specimens, transport media used (if any) and were specimens refrigerated/frozen prior to testing? Campylobacters are stable in feces for 3 days at 4 degrees Celsius/2 days at 25 degrees Celsius, and do not tolerate freezing.

As suggested by the reviewer, we added details on transport and processing of samples in lines 90-92.
Minor

1. Abstract: For clarity, Suggest qualifying that culture not available in resource limited settings, upfront. I.e. rephrase to : culture are expensive and often not available in resource limited settings. Therefore, direct staining techniques have been developed as a practical and economical alternative."

The phrase was changed as suggested.

2. Abstract: Why is sensitivity of staining method stated as 7% (since 3/49 = 6.1%)? This also differs from result in text

The reviewer points to a mistake in the abstract that stated 49 positive Campylobacter cases. This number was corrected to 46. The sensitivity of staining was 6.5 (3/46), which was corrected in the abstract and text!

3. Line 103: what method was used for susceptibility testing and what interpretive criteria was applied?

As requested, we added the method and interpretation criteria for susceptibility testing (lines 112-115). The information was provided by Roberto Flores and Oscar Duery, Instituto de Salud Pública, who were named in Acknowledgments.

4. Abstract, lines 38-39; methods, results: Please consider applying an appropriate statistical test for trend re: % campylobacter positivity after culture was implemented versus preceding years.

As suggested we included the Z test for comparing two independent proportions to analyze the Campylobacter isolation rates (lines 120-123). Figure 1 was changed accordingly.
5. Discussion: While culture is more costly and involved, the authors do not discuss stool antigen testing, which may be a viable, rapid alternative, although pricing can vary regionally. Have these been used/explored in Chile/South America?

As suggested, we included the usefulness of other culture-independent methods for Campylobacter diagnosis in the discussion (lines 174-182).

6. Table 1, the total number of enteric pathogens cultured is stated to be 99, in reality - should this be 96 (while considering the one case of co-infection with C. jejuni/Yersinia)? Why were the Campylobacter gram stain positive isolates double counted?

The reviewer’s observation is correct. The numbers were corrected!

7. Label Y axes of Figures 1, and 2, and X axes of Figure 2.

Figures 1 and 2 were modified accordingly.

8. Standardize decimal place reporting, sometimes numbers are reported to 0.1%, sometimes it is rounded.

Decimal reporting was standardized within the entire manuscript.
Reviewer #3

- There is an inconsistency in relation to the prevalence of Campylobacter throughout the text. The summary states 6.5% while in the results is reported a 6.1%.

The numbers were corrected (see reviewer #1, point 2).

-The authors state that recent epidemiological studies from Chile are not available. However, this is not true, as recent local data was missing (Collado et al., 2013 Diagn Microbiol Infect Dis 75:434-436). In fact, Collado and colleagues found a prevalence of C. jejuni of 10% in symptomatic persons by either two culture methods (mCCDA and filtration of feces over antibiotic-free blood agar) in the south of Chile; this recent work should be incorporated into the manuscript and discussed.

As suggested, we incorporated this work in the Introduction (line 67) and the Discussion (lines 204-206 and 239-241).

-The methods to generate microaerobic conditions should be stated!

As suggested, the method was included in line 105.

-The specific statistic test should be stated!

The Z test was applied, see reviewer #1, point 4 and lines 120-123.
- Why the samples were incubated just for 48h? This is an inappropriate length of time for Campylobacter isolation and could be an explanation of the low prevalence found in comparison with previous data in Chile. Please discuss!

As mentioned above (Reviewer #2, point 1c), the optimum incubation time is controversial. As the reviewer mentions, some guidelines recommend incubation times of 72 h, while others recommend 48 h. We opted to use 48 h of incubation as recommended by the Chilean and other guidelines and included this important but controversial technical detail in the discussion (lines 189-195). See also Reviewer #1, point 1c.

- Why only a small subset of strains was analyzed for antibiotic resistance? Given the scarce data available, it would be highly recommended to test the antibiotic resistance to the rest of strains!

Antibiotic testing was done in the national reference laboratory (ISP) as part of a recently introduced surveillance. The reason why not all strains were studied are not known to us, but the main reason might be the prolonged transport time of the isolates.

- The method to analyze the susceptibility and the breakpoints of the antibiotic tested should be stated!

As requested, we added the method and interpretation criteria for susceptibility testing (lines 112-115). The information was provided by Roberto Flores and Oscar Duery, Instituto de Salud Pública, who were named in Acknowledgments.

- As the authors state that most Chilean laboratories did not implement Campylobacter culture because is inconvenient and expensive, the cost-effectiveness found in this study should be briefly discussed.
We agree and added a sentence about this subject in the Discussion (lines 225-227).

-Line 71: Change "37° to 42°C" to "37° and/or 42°C"

Changed as suggested.

-Line 203: "Campylobacter" in italics

Changed as suggested.

Reviewer #4

1- The materials and method is very thin in content.

There needs to be a specific detailed description of their Hucker stain method, their source of the stains (made in the lab vs purchased already prepared). Their quality testing of the stain. Do they use flooding or immersion? How often are the stain changed and replaced?

As requested, we included details of the staining method and quality control including reference (lines 95-102).

Why Campylosel was chosen?
Before the study, the two Campylobacter media, which were commercially available in Chile at that time, Campylosel (BioMerieux) and Campylobacter Agar (Becton Dickinson), were evaluated in our laboratory with a limited number of strains. Campylosel was chosen since it seemed more selective and supported the growth of all of our control strains. This initial evaluation was not part of the study and therefore not included into the paper.

What is their quality control for that media?

Details plus reference on the quality control of culture were added in lines 105-107.

How are microaerophilic conditions obtained? What is their QC?

Details were included in line 105. QC was performed together with the medium QC as described above.

What are the species of Campylobacter it selects?

According to the manufacturer’s instructions, the medium selects the most common species such as C. jejuni and C. coli. Some stains of other species such as C. fetus, C. laridis, C. sputorum might not grow.

How are the fecal samples plated, how much volume? What was the presentation of fecal samples at arrival in the laboratory?
Details on the plating (by swab) were added in lines 102-105. The exact consistency of the samples at arrival was not recorded and is therefore not available.

What type of training does personnel receive for performing and reading the Hucker stains?

The Campylobacter staining method is part of the laboratory routine in Chile. Technicians are trained during their education. Within our laboratory, young technicians were trained and supervised by experienced technicians until they seem sufficiently experienced. As other microscopical methods, the operator dependency is an inevitable limitation (see Introduction lines 78-79). The problem was further added into the Discussion lines 179-182.

2- There are many articles in the literature that describe the sensitivity of staining as much higher than presented in this article. There is no mention of such literature and no explanation on why this may be the case.

The low sensitivity of staining in our study compared to other studies was further discussed and various references such as Ho et al. were included (lines 172-179). See also comments above.

3- Although, infection with Campylobacter in other countries in South America is mentioned there is a claim that its epidemiological role it is not known, but is prevalence known?

Prevalence data of individual countries in South America can be obtained in the publications of Kaakoush et al. 2015 and Fernandez et al 2011, which are cited in our paper.
How about in ochre countries where culture costs have to be limited. is staining success that low? Pan Afr Med J. 2014; 19: 392. this article thinks otherwise.

As mentioned above, the low sensitivity of staining in our study compared to other studies was further discussed (lines 172-179). The mentioned reference is included. To our opinion, this study might have suffered methodological problems since the isolation rate of culture compared to staining seems very low.

4- The authors noted an increase of Campylobacter presence in stool over a period of time (Jan-April), why this time period only? .

The study aimed to include a sufficient number of consecutive stool samples and was therefore limited to a period of 4 months. The months of January to April were chosen due to practical reasons. The isolation rates were compared to the same period of time of previous years.

This increase is considered to be a methodology issue do to the use of culture. Is this increase statistically significant?

For statistical analysis of the Campylobacter isolation rates within the routine stool samples in our laboratory, we included the Z test for proportions. This test showed that the culture-based rate in 2014 was significantly higher than any of the staining-based rates of each year from 2010-2014 (see Material and Methods and Figure 1).

Was there an issue with the quality of the staining method? Perhaps a lack of interest due to the new culture methodology in the laboratory?
We agree that the staining-based rate during our study period was surprisingly low. Still, it was not significant to previous years (except 2011). A lack of attention due to the additional culture as suggested by the reviewer cannot be excluded. We included this problem in the discussion (lines 175-185).

%- Susceptibility data is interesting but methodology on determination of such susceptibility is not included in the manuscript or at least what type of methodology the reference laboratory follows US or European standards, etc.

As requested, we added the method and interpretation criteria for susceptibility testing (lines 112-115). The information was provided by Roberto Flores and Oscar Duery, Instituto de Salud Pública, who were named in Acknowledgments.

We appreciated the comments and hope you are satisfied with our changes and additions that further clarify some important issues. All authors read and agreed to the revised version. The persons named in Acknowledgments agreed to their inclusion.

Sincerely yours

Lorena Porte