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Serological Tests for the Diagnosis of Campylobacter Species: A Systematic Review and Meta-Analysis

Research Article

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Abstract

Background: Campylobacteriosis is one of the leading bacterial causes of foodborne illnesses, infections by *Campylobacter* are significant challenge to human health. The diagnosis of campylobacteriosis is difficult, as it requires specific culture techniques and well-established laboratories. But culture-independent serological diagnostic tests can directly detect *Campylobacter* antigen or antibody. This systemic review and meta-analysis aimed to assess the diagnostic accuracy of serological tests used for detection of *Campylobacter* species in different specimens.

Methods: A comprehensive and systematic literature search was done from MEDLINE through PubMed, Scopus, and google scholar on studies published from1999-2021 reporting about the diagnostic test accuracy of serological tests for the diagnosis of *Campylobacter* species. This literature search was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRIMSA) guideline. Articles fulfilling the set selection criteria were included in the meta-analysis. Methodological quality of the included articles was assessed in duplicate using QUADAS-2. The pooled test performance analysis was performed by using MetaDisc 1.4 software.

Results: A total of 13 articles were included in the study. The sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), and Test Efficiency (TE) of the tests was extracted. Then the pooled sensitivity, specificity, positive likelihood ratio, negative likelihood ratio and diagnostic odds ratio of the serological tests for *Campylobacter* species was analyzed. The lowest and highest sensitivity, specificity, PPV, NPV and TE reported was 17.6 and 100, 6 and 100, 36 and 100, 70.3 and 99.8, and 75.8 and 99, respectively. There was significant heterogeneity among the studies. The pooled sensitivity, specificity, LR+, LR- and DOR was 86.7, 93.9, 15.4, 0.12 and 145.3, respectively. The overall diagnostic accuracy of serological tests in detecting *Campylobacter* species from different specimens was excellent with the Area Under the Curve (AUC) value of above 0.97.

Conclusion: The diagnostic test accuracy of serological tests to rule out campylobacteriosis from different specimens is heterogenous. However, the pooled diagnostic test accuracy of these serological tests is very good. Therefore, utilizing serological tests in settings with lack of other culture based or molecular based techniques is recommended.

Keywords: *Campylobacter;* Diagnosis; Sensitivity; Specificity; Serological test

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Introduction

The genus *Campylobacter* is gram negative, non-spore forming microorganism comprising 34 species [1], of which the best-known species are *C. jejuni* and *C. coli*, responsible for gastroenteritis in humans, although other species are also emerging [2]. From metabolic point of view, it is of microaerophilic bacteria that survive and grow best in an environment characterized by a low oxygen tension (5% O2, 10% CO2, and 85% N2) [3]. *Campylobacter* is one of the leading bacterial causes of foodborne illnesses worldwide and primarily it causes gastroenteritis. Most human infections are caused by *C. jejuni* (80-85%), whereas most of the remaining cases are attributed to *C. coli* [4]. It is a global public health concern because it affects both human and animal health. The prevalence of *Campylobacter* species from different sources is highly increasing from time to time due to close contact of humans and animals [4]. Even though epidemiological data from Africa, Asia, and Middle East are still incomplete, available data indicate that *Campylobacter* infection is endemic in these regions [5].

Campylobacter Species (SP) require sophisticated microbiological techniques to grow, and it takes more than 48 hours to get the result. In addition to the microbiological techniques of detection, *Campylobacter* spp can be detected by Polymerase Chain Reaction (PCR) which also requires well established testing laboratories. Because of these reasons, the detection of *Campylobacter* spp. in developing counties is very low as compared to the high-income countries [3]. Serological tests can directly detect *Campylobacter* antigen from different samples. As compared to microbiological and molecular detection of *Campylobacter* spp. serological tests are not time consuming and do not require sophisticated laboratories [6]. Additionally, these serological tests can be easily done without the need of specific laboratory setup. Although serological methods could have diagnostic importance for clinical decision making, there is paucity of data regarding the combined diagnostic accuracy of the tests. Therefore, this study aimed at determining the pooled diagnostic test accuracy of serological tests for *Campylobacter* spp from different specimens which can be of a great importance for policy makers.

Methods

Eligibility Criteria

Articles reporting the sensitivity and specificity of serological tests for *Campylobacter* species were included in this review. Quality indicators such as using culture or a combination of culture and qPCR as a gold standard, sample size and right statistical measurement were noted as quality indicators. Studies with at least 60 samples, cross-sectional studies and surveillances whose response rate was greater than 80% were taken for this review.

Information Sources and Search Strategy

Article search was done from MEDLINE through PubMed, Scopus, and google scholar from 1999 until March17, 2021. Additionally, manual searching, and the reference lists of some articles were used to retrieve further literature. Two rounds of searches were done; the search was done using Medical Subject Headings (MeSH) terms using key words including sensitivity, specificity, serological tests, *Campylobacter* species.

Study Selection and Data Collection Process

All the identified articles were exported to EndNote 20 library. Screening was done by reading the title followed by reading the abstract and then by reviewing the full work. Articles were independently assessed for inclusion. Similarly, the two authors extracted data from the included articles independently. Disagreements on the data items were resolved by discussion. The extracted data included: name of first author of the article, year of publication, country of study, type of assay, True Positive, True Negative, False Positive, False Negative, and False Negative. The TP, FP, TN, FN results of each studies were extracted separately for each types of assays.

Definitions of Data Items

Index test was any commercial serological test evaluated for the diagnosis of *Campylobacter* species from specimens. Reference test was a standard culture with or without other tests including index test and samples showing positive result with the reference test were considered as true positive otherwise considered as true negative. For detail description and definition of terms related to diagnostic test accuracy such as sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-), diagnostic odds ratio (DOR) and Hierarchical Summary Receiver Operating Characteristic (HSROC) curve [7].

Risk of Bias and Applicability

Methodological quality of the included articles was appraised in duplicate using QUADAS-2 tool: used for the assessment of Diagnostic Test Accuracy (DTA) [7,8]. The tool has four domains for risk of bias judgment and three domains for applicability judgment. If a study is judged as "low" on all domains relating to bias or applicability, it is judged as "low risk of bias" for that study. If a study is judged "high" or "unclear" in more than one domains, it is judged as "high risk or unclear risk of bias/applicability" [8].

Synthesis of Results and Meta-Analysis

The analysis was performed by using MetaDisc 1.4 software. This software is a dedicated and comprehensive test accuracy meta-analysis software [9]. Diagnostic test accuracy summary measures were sensitivity, specificity, diagnostic DOR, LR+, LR- and Summary Receiver Operating Characteristic (SROC) curve. These summary measures were derived at assay level. The summary of sensitivity, specificity, DOR, LR+ and LR- were presented using Tables. Heterogeneity was assessed visually using forest plots. Due to inherent nature of heterogeneity in Diagnostic Test Accuracy (DTA), random effects approach was employed. SROC, curve was also employed to visualize the landscape of the serological tests. Since the I^2 was above 50%, random effects model was used to minimize effect of heterogeneity. The Area Under Curve (AUC) was assessed for determining the DTA of the assays.

Results

Data Selection and Study Characteristics

Articles were selected following the PRIMSA 2009 flow diagram (Figure 1) [10]. Initially, 126 articles were retrieved from different databases and 7 were added through manual search. Then 23 articles were removed due to duplicity. Among the 110 articles screened, 97 articles were removed due to titles and abstracts, irrelevancy of the data and insufficient data. Only 39 articles were eligible for full text review of which 26 were excluded due to failure of meeting the data requirement. Finally, 13 eligible articles were included in the meta-analysis.

The characteristics of the studies included in our meta-analysis is summarized in (Table 1). The 13 articles, published from 1999-2021, reported the DTA of 20 serological assays performed on a total of 4207 specimens of different origin including fecal, preputial wash, sera and skin specimens from humans, animals and the environment. A variety of serological assays with different principles including enzyme immunoassay, immunochromatography and complement fixation principles were included of which enzyme immunoassays comprises majority of the tests. The studies used reference tests to evaluate the DTA of the serological assays. The reference tests include culture

and/or a combination of culture and other assays or a combination of assays other than culture.

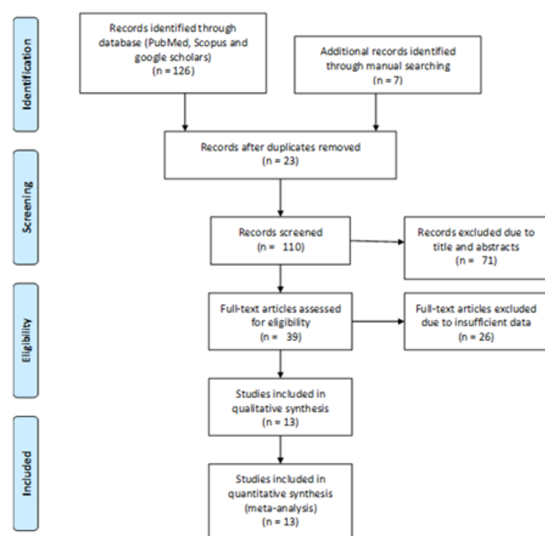

Figure 1: Prisma flow diagram showing the strategy used for article selection.

Results of Individual Studies

The diagnostic accuracy of the individual tests was calculated using a standard formula considering the number of TP, TN, FP and FN specimens. The sensitivity (TP/TP+FN), specificity (TN/TN+FP), positive predictive value (PPV) (TP/TP+FP), negative predictive value (NPV) (TN/TN+FN) and Test Efficiency (TE) (TP+TN/TP+TN+FP+FN) of the individual assays is presented in (Table 1). The lowest and highest sensitivity, specificity, PPV, NPV and TE reported for individual test was 17.6 and 100, 6 and 100, 36 and 100, 70.3 and 99.8, and 75.8 and 99, respectively. The lowest sensitivity, specificity, PPV, NPV and TE for individual tests were reported for complement fixation test, monoclonal antibody ELISA on preputial wash specimens, Ridascreen campylobacter enzyme immunoassay, and EIA-Foss enzyme immunoassay, respectively. On the other hand, the lowest specificity and test efficiency was reported for monoclonal antibody ELISA on preputial wash specimens. On the contrary, this test showed the highest sensitivity (100%) over other tests. ProspecT enzyme immunoassay showed the highest specificity and PPV (100%) while ICA immunochromatography and EIA enzyme immune assay took over the highest NPV. The overall TE was better performed by ProSpecT enzyme immunoassay. The respective average sensitivity, specificity, PPV, NPV and TE of the assays was 84.7, 88.8, 82.2, 90.9 and 90.2 (Table 1).

Bessede et al. [11] evaluated the DTA of two immunochromatography assays on 305 stool specimens collected from patients and reported better performance of Ridaquick *Campylobacter* over ImmunoCard STAT. Granato et al. [12] also evaluated the performance of three assays (Meridian EIA, Remel EIA and Meridian STAT!) employing the principle of enzyme immunoassay. Based on their results, all the tests accurately diagnosed *Campylobacter* species on 485 stool specimens with TE≥96%. In bovine preputial wash specimens, ELISA showed the lowest specificity (6%) and the highest sensitivity (100%) [18] while complement fixation test showed the poorest sensitivity of 17.6% in diagnosing antibodies against *Campylobacter* species from 153 sheep sera [19]. ProspecT enzyme immunoassay showed a perfect specificity (100%) in detecting *Campylobacter jejuni* antibodies in stool specimens [22]. Here the Prospect enzyme immunoassays showed better diagnostic accuracy with TE≥89% [15,20,22] while the EIA-Foss enzyme immunoassay showed the lowest diagnostic accuracy (TE: 70.3%) [17] compared to other immunoassays (Table 1).

Pooled Diagnostic Accuracy of the Serological Tests

The pooled sensitivity, specificity, LR+, LR- and DOR of the included assays was performed. In addition, the area Under Curve (AUC) was shown using SROC curve analysis. Since there is a significant inconsistency and heterogeneity (p<0.001) among the studies, random effects model was used for the meta-analysis. Except for the LR- which has a value below 1, all other pooled results showed good performance of the tests (Table 2). Based on the analysis, the assays show very good ability of identifying true positives with pooled sensitivity of 86.7 (84.8-88.4) (Figure 2). The ability of the tests to identify true negatives is excellent with a pooled specificity of 93.9 (93.2-94.6) (Figure 2). The ratio of the probability of specimens having *Campylobacter* species to be tested positive than negative specimens testing positive is very good with a LR+ value of 15.4 (6.3-37.8) (Figure 2) and a LR- of 0.12 (0.07-0.21) (Figure 2). (Figure 2) shows that positive serological test result for Campylobacter species is significantly associated with increased probability of infection.

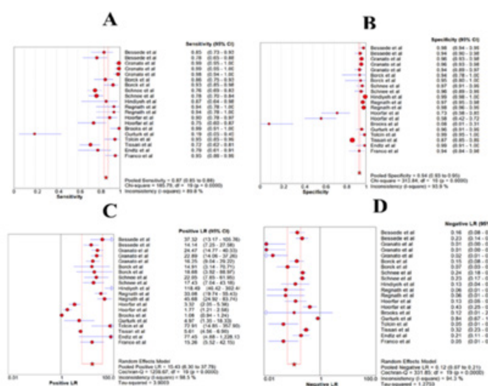

Figure 2: The pooled sensitivity, specificity, LR+ and LR- of serological tests for diagnosis of campylobacter species. (A) Sensitivity (B) Specificity (C) LR+ and (D) LR+.

The odds of truly infected specimens to be positive for *Campylobacter* species using the serological tests is 145.3 times more likely than true negatives to be tested positive by these methods (pooled DOR: 145.3 (61.1-345.3). Overall, the pooled diagnostic accuracy of serological tests in diagnosing *Campylobacter* species from different specimens is excellent with AUC value of above 0.97 (Figure 3). In order to minimize heterogeneity, we performed a subgroup analysis for immunochromatography and enzyme immunoassays but there was no significant difference in the pooled results. Accordingly, although no significant difference was observed on sensitivity, specificity, LR+ and LR-, enzyme immunoassays showed better diagnostic accuracy than the total pooled diagnostic accuracy with a pooled DOR of above 170 compared to a total pooled DOR of 145 (Figure 3). Overall, serological tests for screening *Campylobacter* species can be useful in settings lacking culture and molecular techniques facilities.

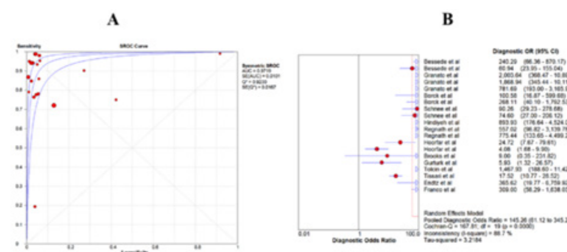

Figure 3: SROC curve with AUC and DOR of serological tests for diagnosis of Campylobacter species. (A) SROC curve and (B) DOR.

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Table 2: Summary pooled sensitivity, specificity, LR+, LR- and DOR of articles included in the study.

Measurement	Pooled value (95% CI)	Inconsistency (I2) (%)	Heterogeneity (X2)	P value
Sensitivity	86.7(84.8-88.4)	89.8	185.8	0
Specificity	93.9(93.2-94.6)	93.9	313.8	0
LR+	15.4(6.3-37.8)	98.5	1259.7	0
LR-	0.12(0.07-0.21)	94.3	331.8	0
DOR	145.3(61.1-345.3)	88.7	167.8	0
EIA-DOR	170.7(60.5-481.3)	90	148	0

Discussion

In our literature search we found only few studies that describe the diagnostic performance of serological tests for *Campylobacter* spp. Out of the 133 articles that were obtained from databases and manual search, only 13 articles were selected for analysis. These articles describe the results of serological tests for *Campylobacter* spp mainly by Enzyme immunoassay, Immunochromatography and Complement fixation technique. *Campylobacter* infection is increasing in most parts of the world. In 2015, Campylobacteriosis was added to the nationally notifiable diseases list [24]. But the real prevalence of *Campylobacter* spp is still not well presented because of the absence of national surveillance program and limited routine availability of culture for *Campylobacter* species in clinical and research settings [4].

There are different serological tests for *Campylobacter* detection. The advantages of these serological test for *Campylobacter* detection over culture-based methods is low cost and faster turnaround time [25]. In a multicenter study based on detection of stool antigen, the sensitivity and specificity ranged from 79.6% to 87.6%, 95.9 to 99.5% of and positive predictive value of 41.3 to 84.3%, respectively [26]. The difference in the sensitivity, specificity and the positive predictive value could arise from the difference in the inherent accuracy of the individual test methods, differences in sample size and differences in types of specimen used. API Campy, Neisseria-Haemophilus (NH) identification card and matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) are used as a promising identification method for *Campylobacter* species [27]. Of these tests the accuracy of MALDI-TOF mass spectrometry was 100% with a sensitivity of 98.3% [27] which is less sensitive than that of monoclonal antibody ELISA test (with a sensitivity of 100%) [18].

The main problem for diagnosis of *campylobacteriosis* is that identification of *Campylobacter* spp is mainly dependent on culture [28]. Culture accuracy is limited by the tendency of *Campylobacter* to die during handling, and by the difficulty of detecting microscopic colonies among competing fecal flora [29]. The minimum amount of *Campylobacter* in stool samples that can be cultured has not been reported yet. This information is necessary for correlation of the numbers of bacteria detected by culture and culture-independent serological tests with clinical diarrheal symptoms [30,31]. Knowing this estimate is helpful for studying asymptomatic carriage of Campylobacter spp. especially in endemic settings. Buss et al. [32] shows that the detection thresholds for *Campylobacter* by culture spanned from 0.3-5 $\times 10^6$ CFU/mL. The detection threshold for an FDA-cleared, rapid, membrane-based EIA the *CAMPYLOBACTER* QUIK CHEK™ test, was 8.4 × 104 CFU/mL for *C. jejuni* and 7.7 $\times 10^5$ CFU/mL for *C. coli* [32]. Fluorescent Microspheres Labeled Immunochromatographic test has limit of 10^6 CFU/Ml [33]. Serologic assays play an important role in epidemiologic studies and surveillance of *Campylobacter* spp., particularly because they can detect minimum amount of *Campylobacter* spp. in the sample [34].

The sensitivity of culture for *Campylobacter* was 72.3% and specificity of 99.9% while EIA had 100% correlation on all discrepant specimens [32]. Similarly, Franco et al. [23] shows the sensitivity and specificity of this EIA to be 96%, 94.5% and test efficiency of 95.4% [23]. A rapid diagnostic test DK14-CA1 has a sensitivity, specificity and PPV of 75.6%, 98.6%, 89and 97.0%, respectively [35] and has similar specificity with Ridaquick *Campylobacter* and EIA tests [16,36]. This test was also corelated with cases defined with their clinical findings with sensitivity, specificity, accuracy and PPV values of 82.1%, 100%, 90.6% and 100% respectively [35]. The diagnostic accuracy values of the RidaQuick were found to have a sensitivity of 87%, specificity of 97%, and positive and negative predictive values of 77% and 98%, respectively [37]. It has similar specificity with Quick check and ICA. ELISA has 100% sensitivity and NPV [18]. An ELISA test was validated by Ang et al. [38] having sensitivity of 93.1% in culture-positive patients, with a specificity of 93.0% with area under the curve value of 0.91 [38]. where as in our analysis the area under the curve value for the pooled tests was 0.97 which is better than the previous study.

Prospect has 100% specificity and PPV with test efficiency of 99% [15,22]. Gold-nanorods-based nano biosensor is a recently developed technique having 88% sensitivity and specificity of 100% [39] similar specificity with Prospect [15] in our study. From this review ELISA showed the highest sensitivity of 100% similarly, a review by Kuhn et al. [40] shows ELISA can be a suitable candidate for a standardized, commercially available method of detecting Campylobacter antibodies [40]. There is difference in sensitivity and specificity of the serological tests in the articles. This is because of the difference in the test protocols used, efficiency of the tests and variability in patient samples. So, validating these serological tests depending on the situation and using them for diagnostic and research laboratories is important as it will increase the detection rate of *Campylobacter* in timely and cost-effective way. Thus, serological tests are important to control and study *Campylobacter* infection at public health level. Although appropriate analysis software for meta-analysis of the diagnostic test accuracy of assays was used, this study has limitations regarding the inclusion of all forms of specimen, inclusion of old articles, and inclusion of assays used to detect any species of *Campylobacter* which resulted in high heterogeneity among the studies. Having these limitations, this study could contribute a lot for stakeholders for the selective use and applicability of serological tests to rule out *campylobacteriosis* in some settings.

The findings of this meta-analysis provide practical information on culture-independent serological methods to detect *Campylobacter* spp. This information will be useful for both small and large diagnostic laboratories as well as provide unanticipated results on under-reported *Campylobacter* species. These culture-independent serological methods have good sensitivity, specificity, negative predictive value, positive predictive value and test efficiency. The findings suggest that serological tests should have a role in clinical decision making. Employing these tests could have importance compared to the culture de-

pendent tests due to low cost, fast turnaround time and requirement of easy laboratory setups especially in low- and middle-income countries where the use of culture and molecular techniques is limited.

Conflict of interest

None.

Ethical statement

None.

Author's contribution

Woinshet Hailu wrote the original draft of the manuscript. Woinshet Hailu, Lulit Hailu were responsible for conception and design of the study. Woinshet Hailu Lulit Hailu, Hylemariam Mihiretie Mengist, Tadesse Eguale were responsible for data curation and interpretation of results, statistical analysis and methodology. All authors read and approved the final manuscript.

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